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Preface

It is rare that so much of the burden of discovery of the physiology of a new hormone has been the domain of a corporate entity—in this case, one that has acquired the name of the hormone.

Several factors have conspired to delay full publication of much of amylin's unique biology. The impetus to write this book was not only to review what has been fully published, but also to summarize much of what has not.

One purpose of this book is to present the totality of what I believe to be true about the physiology of the hormone amylin. I have appraised the peer-reviewed literature. But I have also unashamedly cited lesser publications, particularly the meeting abstracts of learned societies. These often represent the only public record of an amylin-related biology. I have occasionally drawn from the patent literature where this too is the only record. In rare instances, I have included unpublished data, where I have considered them to be necessary.

I apologize in advance if in places I appear to have over-reported the work of my own laboratory. This comes from an intent to inform via a familiar knowledge base and not from a desire to diminish the work of others.

This book is the quintessence of the efforts of so many individuals, that I would offend most to name a few. I have estimated that Amylin Pharmaceuticals, Inc., has expended 300 person-years in exploring amylin's biology, and the effort outside the company must have well exceeded that figure. The fruits of this effort have included not only an understanding of the actions of a single hormone, but the revelation of entirely new physiologies and new modes of therapy, especially in regards to metabolic control. I am privileged to represent here that collective effort.

Andrew Young
October 2005

Foreword

Amylin is a peptide hormone secreted by the pancreatic beta cell along with insulin in response to meal/glucose stimuli. An analog of amylin (pramlintide) is now a pharmaceutical product for treating diabetes. The story of amylin's discovery 20 years ago and the battle to show its therapeutic utility is a fascinating one, and Andrew Young has been in it from the beginning. It begins with the unexpected finding that a strange and probably noxious deposit in the beta cell turned out to be a precipitate of a natural hormone, amylin. This was followed by the steady uncovering of its physiological role. Evolution seems often to derive new functions from old, and amylin, too, is derived from an ancient family of hormones, which includes calcitonin and the powerful neurotransmitter calcitonin-gene related peptide. Although amylin is an islet hormone co-released with insulin, it is also found elsewhere—for example in the central nervous system. Amylin's receptor is a member of a larger family, but here specificity is partly provided by a receptor activity modifying protein (RAMP) that turns the calcitonin receptor into the amylin receptor. This illustrates an important principle: a receptor greatly alters its characteristics according to its environment. The old pharmacological certainty about the specificity of receptors was thus challenged by amylin—specificity in reality depends on the cell environment and can differ from tissue to tissue in major or minor ways even with a completely identical receptor sequence.

The range of amylin's actions is considerable. It is an anorexigen, it has multiple effects on the gastrointestinal tract and digestive processes, it interacts with the actions of other islet hormones, and it affects other peripheral tissues. Its analog, pramlintide, is useful in correcting various metabolic abnormalities in diabetes, where endogenous amylin release is deficient as a consequence of the reduction in beta cell numbers. All of amylin's actions

are described within and add up to an enthralling picture of the intricacy of mammalian control systems.

This extraordinarily readable book takes us through the whole fascinating story with amazing insights into biology and functional control systems. It is a comprehensive treasure of information from a scientist who has been personally involved in every aspect of the amylin world.

Steve R. Bloom
Department of Metabolic Medicine
Division of Investigative Science
Hammersmith Hospital
Imperial College London
London, United Kingdom

Andrew Young

Historical Background

I. Discovery and Nomenclature

In 1900, Opie described a "hyaline" (homogeneous glassy) appearance within the islets of Langerhans from diabetic patients (Opie, 1900a,b) (Fig. 1). The hyaline material present in pancreata from diabetic patients was histologically identified to be amyloid, an extracellular proteinaceous material with characteristic staining properties (Ahronheim, 1943; Ehrlich and Ratner, 1961). Attempts at characterizing the amyloid material were frustrated by its low concentration and insolubility. Some workers suggested that the material within islet amyloid contained insulin or insulin fragments (Nakazato *et al.*, 1989; Pearse *et al.*, 1972; Westermark and Wilander, 1983).

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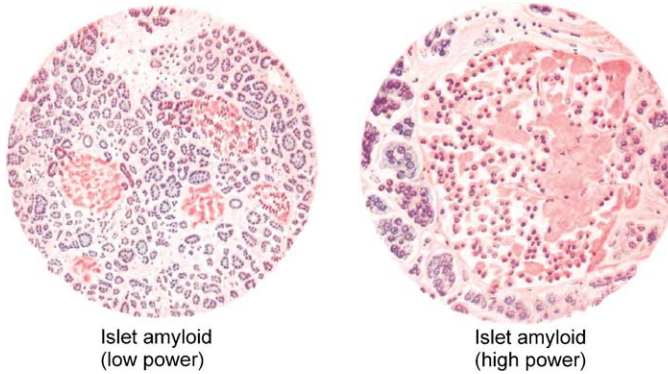

FIGURE 1 Drawings of (left) low-power (Leitz ocular 3, objective 3) and (right) high-power (Leitz ocular 3, objective 6) views showing hyalinization of islets and replacement of islet cells with hyaline (amyloid) material (Opie, 1900b), respectively.

A. Amylin and IAPP

Westermark *et al.*, working with amyloid obtained from resected insulinoma tissue, reported a partial sequence of a material they designated "insulinoma amyloid peptide" (IAP) in November 1986 (Westermark *et al.*, 1986). In 1987, Cooper *et al.* (Clark *et al.*, 1987) reported the full sequence of a 37-amino-acid peptide extracted from amyloid-containing homogenates of pancreata from patients with type 2 diabetes. Westermark *et al.*, in a subsequent publication (Westermark *et al.*, 1987a), and in reporting a more complete sequence of their initial findings (Westermark *et al.*, 1987b), changed their "IAP" designation to "islet amyloid polypeptide" (IAPP). Meanwhile, the initial designation applied by Cooper to his peptide, "diabetes-associated peptide" (DAP), was changed to "amylin" to reflect the historical (amyloid) origin of the peptide, and by removing "diabetes-associated" sought to clarify that its presence was not restricted to the diabetic state (Cooper *et al.*, 1988). IAPP and amylin are the only terms that persist in the literature today; most workers apparently view these terms as synonymous. In a recent survey of 1255 articles in MEDLINE in which either "amylin" or "IAPP" was used in the title or abstract, 1198 (96%) refer to amylin, and of those, 862 (69%) refer only to amylin. Conversely, 27% of articles refer to IAPP, and of those, 4.5% use IAPP exclusively. MEDLINE indexers also apparently recognize the association of "amylin" and "IAPP," since only 4% of articles escape cross-indexing to the other term (see Fig. 2).

The terms amylin and IAPP are not used interchangeably here. Instead, the preferred term amylin is used to mean the 37-amino-acid sequences

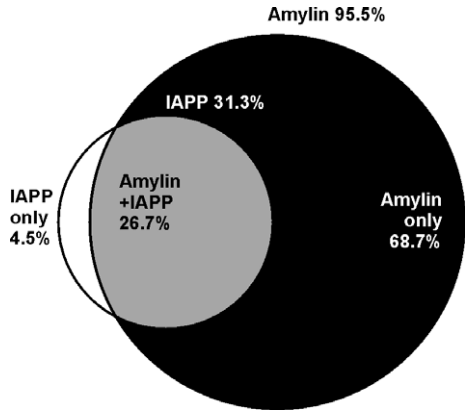

FIGURE 2 Venn diagram of amylin and IAPP nomenclature.

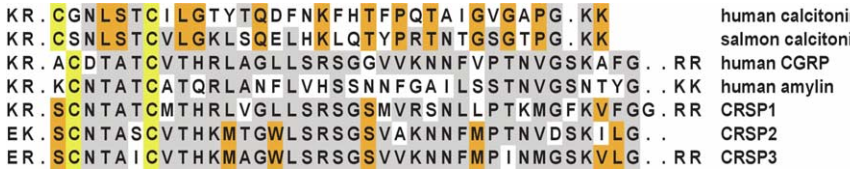

FIGURE 3 Alignment of human and salmon calcitonins with CGRP (human) and CRSPs, illustrating the relatedness of these ligands. Yellow backgrounds anchor the position of disulfide rings. A tan background denotes relatedness within ligand orthologs, while a gray background denotes positional relatedness that transcends ligand groups.

shown in Fig. 3, together with C-terminal amidation and cyclization via a disulfide bond between Cys-2 and Cys-7, posttranslational modifications shown to be necessary for full biological activity (Roberts *et al.*, 1989). This is the structure that when used as a drug adopts the US Adopted Name (USAN) name amlintide (Anonymous, 1997). I refer to works using IAPP when it is clear that the authors are referring to the defined structure of amylin, and to some work in which it is not clear but likely, for example, descriptions of the presence, degree, or localization of immunoreactivity, where the findings are likely to be equally applicable to amylin.

There may be utility in using the term IAPP in its original context (i.e., when referring to material derived from insulinoma tissue), since it is not clear that such material is posttranslationally modified or bioactive. The identification of three further molecular forms of amylin-like molecules (described in the following section) generated by glycosylation *in vivo* (Rittenhouse *et al.*, 1996) exemplifies the need for caution and precision in equating independently described materials.

B. Other Amylin-like Molecular Forms

Approximately 60% of amylin-like immunoreactivity circulating in humans is composed of three glycosylated forms (Rittenhouse *et al.*, 1996). Monosialated pentasaccharides, or similar structures, are linked at Thr-6, Thr-9, or both and are detected by differential reactivity to monoclonal antibodies directed toward different epitopes (Percy *et al.*, 1996). The functional significance of glycosylated forms of human amylin is not fully understood. Glycosylated amylins appear not to bind to amylin receptors in rat nucleus accumbens membranes and are not active in isolated soleus muscle (a bioassay for amylin action; Young *et al.*, 1992b) at concentrations up to 37 nM (Rittenhouse *et al.*, 1996), 2000-fold higher than plasma concentrations.

Yet the glycosylated forms of amylin are likely to arise from an enzymatic (implicitly purposeful) synthetic step. This is in contrast to the accelerated non-enzymatic glycation that occurs with circulating proteins, including hemoglobin, during sustained hyperglycemia. As an example of this latter process, advanced glycosylation endproduct (AGE) amylin has been produced *in vitro* and is reported to accelerate nucleation of amyloid *in vitro* (Kapurniotu *et al.*, 1996). However, it is yet to be identified as a molecular species *in vivo*.

Several studies have addressed the possibility that type 2 diabetes mellitus is associated with a mutation of the amylin gene. A polymorphism has been identified in studies of 155 Caucasians (Cook *et al.*, 1991) and 119 south Indians (McCarthy *et al.*, 1992), but no linkage with disease state was identified. In two studies, no abnormality of the amylin gene or promoter was identified in Japanese patients with NIDDM (Kajio *et al.*, 1992; Tokuyama *et al.*, 1994). In another Japanese study of 540 individuals, a missense mutation was detected in 12 individuals in whom Gly replaced Ser-20 in the amylin molecule (Sakagashira *et al.*, 1996; Sanke *et al.*, 1996). This mutation was significantly overrepresented in patients with early onset NIDDM (10% of patients compared to 4% of all NIDDM patients and 0% of normal individuals). This mutant amylin molecule binds to amylin receptors *in vitro* (Moore *et al.*, unpublished) and is active *in vivo* for inhibition of gastric emptying (Gedulin *et al.*, unpublished). It appears to exhibit increased *in vitro* amyloidogenicity and increased intracellular cytotoxicity compared to wild-type amylin (Sakagashira *et al.*, 2000). These properties may underlie a predisposition to diabetogenesis.

II. Molecular Biology

A. Amylin Gene

The structure and localization of the amylin gene, and the molecular biology of amylin, have been extensively reviewed (Cooper *et al.*, 1989a; Nishi *et al.*, 1990a,b). In brief, the human amylin gene, sequenced by several

groups (Mosselman *et al.*, 1989; Roberts *et al.*, 1989; Sanke *et al.*, 1988), resides as a single copy on chromosome 12 (Mosselman *et al.*, 1988; Roberts *et al.*, 1989). Two exons code for an 89-amino-acid preprohormone (Mosselman *et al.*, 1989; Sanke *et al.*, 1988) (93 amino acids in the rat; Leffert *et al.*, 1989).

Processing of amylin precursors includes cleavage at dibasic sites, probably by prohormone convertases PC2 (Badman *et al.*, 1996) and PC3 (Higham *et al.*, 1999). Posttranslational processing includes amidation at the C terminus (Roberts *et al.*, 1989) and formation of a disulfide loop between Cys-2 and Cys-7 (Cooper *et al.*, 1987).

The primary amino acid sequences for amylins shown in Fig. 4 indicate conservation of structure within mammalian species and chicken. Chicken amylin differs from mammalian amylins, not so much in the mature peptide, but in the N-terminal propeptide, which is 43–46 amino acids longer than in mammals (Fan *et al.*, 1994), and which appears to be more like the prohormone for calcitonin gene-related peptide (CGRP). The observation that DNA code for the flanking peptides accepts mutations at a higher rate than does the code for amylin (Nishi *et al.*, 1989) suggests that those regions are less likely than the defined amylin sequence to code for biologically important molecules.

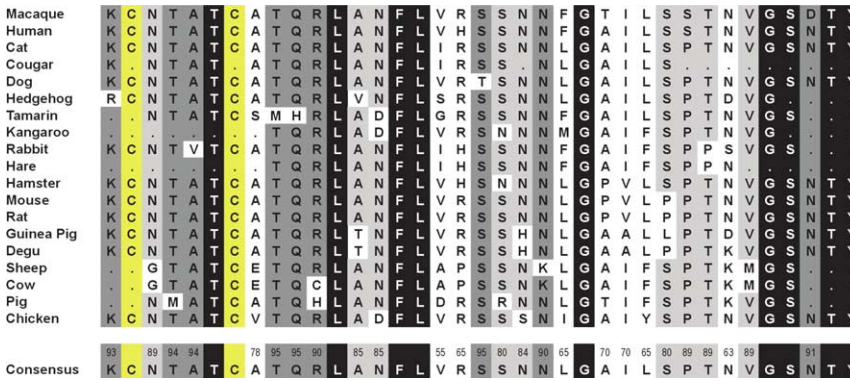

FIGURE 4 Alignment of sequences of amylins from dierent species. Yellow indicates disulfide bonded cysteines. Black columns indicate full amino acid conservation in all species studied. Positions conserved in over 90% of species are indicated by dark gray, and those conserved in over 80% of species by light gray. Sequences are from the following: macaque (Ohagi *et al.*, 1991), human (Sanke *et al.*, 1988), cat (Nishi *et al.*, 1989), cougar (Johnson *et al.*, 1991b; Albrandt *et al.*, 1991), hedgehog (van Dijk, M. A. M. and de Jong, W. W., direct submission), tamarin (Albrandt, K., Sierzega, M. E., Mull, E., and Brady, E. M.G., direct submission), kangaroo (van Dijk, M. A. M., and de Jong, W. W., direct submission), rabbit (Christmanson *et al.*, 1993), hare (Christmanson *et al.*, 1993), hamster (Betsholtz *et al.*, 1989; Nishi *et al.*, 1990), mouse (Betsholtz *et al.*, 1989), rat (van Mansfeld *et al.*, 1990), guinea pig (Nishi *et al.*, 1989), degu (Nishi and Steiner, 1990), sheep, cow, pig (Albrandt, K., Sierzega, M. E., Mull, E., and Brady, E. M. G., direct submission), and chicken (Fan *et al.*, 1994).

B. Similar Peptides

Homology was noted among amylin from pancreatic islets (Clark *et al.*, 1987), amyloid material from insulinoma (Westermark *et al.*, 1987b), and CGRP, a product of alternate splicing of the calcitonin gene (Rosenfeld *et al.*, 1983). Human amylin is 46% identical with human CGRP-2 and rat CGRP, and 43% identical with human CGRP-1. A distant homology is reported between amylin and the insulin/relaxin/IGF superfamily (Cooper *et al.*, 1989b). No statistically significant homology between amylin and calcitonins was noted on Dayhoff analysis (Cooper *et al.*, 1989b), but sequence similarity is clearly apparent between rat amylin and salmon and rat calcitonins, particularly at functionally important segments at N and C termini (Young *et al.*, 1995). The C-terminal portion of adrenomedullin, a 52-amino-acid peptide, shows some (~20%) homology with amylin and CGRPs (Kitamura *et al.*, 1993).

The most recent additions to the above peptide superfamily are calcitonin receptor-stimulating peptides (CRSPs), isolated from porcine brain (Katafuchi *et al.*, 2003b). Three variants have thus far been discovered (Katafuchi *et al.*, 2003a). These ligands appear not to potently stimulate CGRP or adrenomedullin receptors. CRSP-1 stimulates calcitonin and calcitonin-like receptors (Katafuchi *et al.*, 2003b), while CRSP-2 and -3 are weak agonists only, suggesting as-yet-undiscovered pharmacologies in this ligand-receptor superfamily (Katafuchi *et al.*, 2003a).

III. Amyloid and Association with Diabetes

The time course of the appearance of pancreatic amyloid mirrors the appearance of clinical diabetes (Ohsawa *et al.*, 1992). A relatively restricted number of mammalian species exhibit a propensity to form amyloid in pancreatic islets; these are the same species that are susceptible to type 2 diabetes. In addition to humans (Westermark, 1972) and macaque monkeys (Clark *et al.*, 1991; de Koning *et al.*, 1993; Howard, 1988), islet amyloid is found in domestic cats (Betsholtz *et al.*, 1990; Westermark *et al.*, 1987b) as well as in tigers, lions, lynx, raccoons (Jakob, 1970), and cougars (Johnson *et al.*, 1991b). It is not found in islets of dogs or other members of the Canidae (wolf, jackal, fox) (Jakob, 1970). Except for *Octodon degu*, which is a special case (Hellman *et al.*, 1990), amyloid is not found in the islets of rodents. However, human islets transplanted into mice form amyloid (Westermark *et al.*, 1995), suggesting that it is a species-specific characteristic of the peptide itself that leads to amyloid formation (Ashburn and Lansbury, 1993). This idea is supported by the observation that transgenic mice overexpressing human amylin form amyloid (Soeller *et al.*, 1996b; Verchere *et al.*, 1996), but mice overexpressing mouse amylin do not (Soeller *et al.*, 1996b).

Based upon analysis of sequence divergence (Betsholtz *et al.*, 1989) and propensity of subpeptides to form fibrils (Westermark *et al.*, 1990), residues 20–29, especially 24–29, which are common to humans, cats, and raccoons, were predicted by some authors to be amyloidogenic (Johnson *et al.*, 1992; Jordan *et al.*, 1994; Westermark *et al.*, 1990). This prediction does not, however, account for the absence of islet amyloid in dogs (except in insulinoma; Jordan *et al.*, 1990), since dog and cat amylin are identical from residues 20–37. It appears that more than just an amyloidogenic molecule is required, and that some stimulus, perhaps associated with β-cell hypersecretion, may be necessary. For example, mice containing the human amylin transgene usually do not spontaneously develop amyloid (Verchere *et al.*, 1997) but can be induced to do so by such maneuvers as feeding a high-fat diet (Verchere *et al.*, 1996), crossing with an insulin-resistant strain (Hull *et al.*, 2003; Soeller *et al.*, 1996a), induction of insulin resistance with dexamethasone and growth hormone (Couce *et al.*, 1996), or oophorectomy (Kahn *et al.*, 2000). These observations tend to support the idea that β-cell hypersecretion (of an amyloidogenic molecular species) promotes amyloid formation. The resistance of human amylin transgenic mice to amyloid formation when they simultaneously carry β-cell glucokinase deficiency (which limits β-cell secretion) is also consistent with this idea. Some workers in the field conclude, however, that amyloidogenicity involves more than simple β-cell hypersecretion (Marzban *et al.*, 2003) and may include β-cell "strain," in which secretory rate exceeds prohormone convertase capacity, resulting in increased prevalence of prohormone forms of amylin (de Koning *et al.*, 1999) as well as insulin (MacNamara *et al.*, 2000).

The role of amyloid formation, mechanical disruption, and possible cytotoxic effects of amyloid in the pathogenesis of islet secretory failure and diabetes has been covered in numerous reviews (Artozqui *et al.*, 1993; Betsholtz *et al.*, 1993; Butler, 1996; Clark, 1992; Clark *et al.*, 1991, 1995, 1996a,b; Hansen, 1996; Johnson *et al.*, 1988, 1989, 1991a,c; Kamaeva, 1993; O'Brien *et al.*, 1993a,b; Porte *et al.*, 1991; Weir and Bonner-Weir, 1996; Westermark, 1994; Westermark and Johnson, 1988; Westermark *et al.*, 1988, 1991, 1992; Wolffen-Buttel and Van Haeften, 1993) and is not covered in further detail here. These reviews, which constitute ~30% of the ongoing literature, do not generally address a functional (receptor mediated, hormonal) role of amylin.

IV. Properties of Human Amylin

A. A Corrupted Literature

Early confusion regarding the actions of human amylin may have been related to its propensity to self-aggregate, bind to glassware, and result in unpredictable and often negligible peptide concentrations in biological

buffers, leading to widely disparate reports of biological activity of amylin until approximately 1992. For example, concentrations of human amylin measured by radioimmunoassay in soleus incubation buffer were ~1% of those predicted based upon added mass and serial dilution (Young *et al.*, 1992b). Adverse physicochemical properties may also have been responsible for low purity and a 100-fold variability in biological activity of commercially available human amylins (Lehman-deGaeta *et al.*, 1991) and likely contributed to confusion in the literature. An example of such confusion is that prior to 1992, most (15 of 24, 63%) reports (Ahren and Pettersson, 1990; Ar'Rajab and Ahren, 1991; Bretherton-Watt *et al.*, 1990; Broderick and Gold, 1991; Broderick *et al.*, 1991; Fehmann *et al.*, 1990; Ghatei *et al.*, 1990; Gilbey *et al.*, 1989; Gold *et al.*, 1990; Nagamatsu *et al.*, 1990a,b; O'Brien *et al.*, 1990; Pettersson and Ahren, 1990; Tedstone *et al.*, 1989, 1990) concluded that amylin had no impact on insulin secretion. A minority reported an insulinostatic effect (Dégano *et al.*, 1991; Johnson *et al.*, 1990; Kogire *et al.*, 1991; Marco *et al.*, 1990; Murakami *et al.*, 1990; Ohsawa *et al.*, 1989; Peiró *et al.*, 1991; Silvestre *et al.*, 1990a,b). However, after 1992, understanding of the functional role of amylin appeared to progress more rapidly when most workers turned to using rat amylin, which does not exhibit the troublesome physicochemical properties of human amylin (Rodriguez-Gallardo *et al.*, 1995; Young *et al.*, 1992). Since then, 45 of 49 (92%) reports have described an insulinostatic effect (Ahren *et al.*, 1998; Bennet *et al.*, 1993, 1994; Bloom, 1994; Bretherton-Watt *et al.*, 1992a,b; Chuang *et al.*, 1992; Dégano *et al.*, 1992, 1993; Fürnsinn *et al.*, 1992, 1994; Gebre-Medhin *et al.*, 1998; Gedulin *et al.*, 1992, 1993; Göke *et al.*, 1993a,b; Inoue *et al.*, 1993; Kulkarni *et al.*, 1996; Leaming *et al.*, 1995; Lewis *et al.*, 1988; Marco and Silvestre, 1997; O'Harte *et al.*, 1998; Rodriguez-Gallardo *et al.*, 1995; Salas *et al.*, 1994, 1995; Sandler and Stridsberg, 1994; Silvestre *et al.*, 1992, 1993a,b, 1994, 1996, 1997; Smith and Bloom, 1995; Stridsberg *et al.*, 1993; Suzuki *et al.*, 1992; Wagoner *et al.*, 1992, 1993; Wang *et al.*, 1993, 1997; Young and Gedulin; Young *et al.*, 1992a,b, 1993, 1994, 1995). Four reported no effect (Barakat *et al.*, 1994; Nagamatsu *et al.*, 1992; Panagiotidis *et al.*, 1992; Wang *et al.*, 1997).

B. Pramlintide

Although the human hormone is preferred in hormone replacement therapies, this has not always been possible. Development of human amylin as a drug has been impeded by the same physicochemical properties that impeded determination of its biological actions. Extensive testing of multiple full-length analogs of human amylin (Janes *et al.*, 1996) identified the effect of proline substitutions at positions 25, 28, and 29 to promote stability in solution and stability of biological activity. Because it overcame the unfavorable chemical properties (Janes *et al.*, 1996) and yet retained the

spectrum of biological actions of human amylin (Young *et al.*, 1996), [Pro25,28,29]human amylin was chosen for exploration as a therapy for insulin-treated diabetes mellitus (Moyses *et al.*, 1996). It is this molecular structure that has the adopted name pramlintide (Anonymous, 1999) and the proprietary name Symlin (pramlintide acetate injection). Most of the human biology of amylin has therefore been adduced from responses to pramlintide. The actions of amylin in animals have been deduced mainly from studies using rat amylin, with some insights derived from studies using human amylin.

References

Anonymous (1997). USAN council. List No. 392. New names. Amlintide. *Clin. Pharmacol. Ther.* **61**, 500.

Anonymous (1999). USAN Council. List No. 419. New names. Pramlintide acetate. *Clin. Pharmacol. Ther.* **66**, 332.

Ahren, B., and Pettersson, M. (1990). Calcitonin gene-related peptide (CGRP) and amylin and the endocrine pancreas. *Int. J. Pancreatol.* **6**, 1–15.

Ahren, B., Oosterwijk, C., Lips, C. J., and Hoppener, J. W. (1998). Transgenic overexpression of human islet amyloid polypeptide inhibits insulin secretion and glucose elimination after gastric glucose gavage in mice. *Diabetologia* **41**, 1374–1380.

Ahronheim, J. H. (1943). The nature of the hyaline material in the pancreatic islands in diabetes mellitus. *Am. J. Pathol.* **19**, 873–882.

Albrandt, K., Mull, E., Cooper, G. J. S., and Johnson, M. J. (1991). cDNA cloning of canine amylin and detection of tissue transcripts by PCR amplification. *Diabetes* **40**, 35A (abstract 137).

Ar'Rajab, A., and Ahren, B. (1991). Effects of amidated rat islet amyloid polypeptide on glucose-stimulated insulin secretion *in vivo* and *in vitro* in rats. *Eur. J. Pharmacol.* **192**, 443–445.

Artozqui, M. E., Botella, S. M., Camblor, M., Breton, L. I., and Moreno, E. B. (1993). Amylin: its potential role in the etiopathogenicity of diabetes mellitus. *Ann. Med. Interna* **10**, 200–202.

Ashburn, T. T., and Lansbury, P. T. (1993). Interspecies sequence variations affect the kinetics and thermodynamics of amyloid formation—peptide models of pancreatic amyloid. *J. Am. Chem. Soc.* **115**, 11012–11013.

Badman, M. K., Shennan, K. I. J., Jermany, J. L., Docherty, K., and Clark, A. (1996). Processing of pro-islet amyloid polypeptide (proIAPP) by the prohormone convertase PC2. *FEBS Lett.* **378**, 227–231.

Barakat, A., Skoglund, G., Boissard, C., Rosselin, G., and Marie, J. C. (1994). Calcitonin gene-related peptide and islet amyloid polypeptide stimulate insulin secretion in RINm5F cells through a common receptor coupled to a generation of cAMP. *Biosci. Rep.* **14**, 1–13.

Bennet, W. M., Beis, C. S., Ghatei, M. A., Byfield, P. G. H., and Bloom, S. R. (1994). Amylin tonally regulates arginine-stimulated insulin secretion in rats. *Diabetologia* **37**, 436–438.

Betsholtz, C., Christmansson, L., Engstrom, U., Rorsman, F., Svensson, V., Johnson, K. H., and Westermark, P. (1989). Sequence divergence in a specific region of islet amyloid polypeptide (IAPP) explains differences in islet amyloid formation between species. *FEBS Lett.* **251**, 261–264.

Betsholtz, C., Christmanson, L., Engstrom, U., Rorsman, F., Jordan, K., O'Brien, T. D., Murtaugh, M., Johnson, K. H., and Westermark, P. (1990). Structure of cat islet amyloid polypeptide and identification of amino acid residues of potential significance for islet amyloid formation. *Diabetes* **39**, 118–122.

Betsholtz, C., Christmanson, L., Gebre-Medhin, S., and Westermark, P. (1993). Islet amyloid polypeptide—Hen or egg in type 2 diabetes pathogenesis? *Acta Oncol.* **32**, 149–154.

Bloom, S. R. (1994). Paracrine regulation of regulatory peptides. *Biomed. Res.* **15**, 1–4.

Bretherton-Watt, D., Gilbey, S. G., Ghatei, M. A., Beacham, J., and Bloom, S. R. (1990). Failure to establish islet amyloid polypeptide (amylin) as a circulating beta cell inhibiting hormone in man. *Diabetologia* **33**, 115–117.

Bretherton-Watt, D., Ghatei, M. A., Jamal, H., Gilbey, S. G., Jones, P. M., and Bloom, S. R. (1992a). The physiology of calcitonin gene-related peptide in the islet compared with that of islet amyloid polypeptide (amylin). *Ann. N Y Acad. Sci.* **657**, 299–312.

Bretherton-Watt, D., Gilbey, S. G., Ghatei, M. A., Beacham, J., Macrae, A. D., and Bloom, S. R. (1992b). Very high concentrations of islet amyloid polypeptide are necessary to alter the insulin response to intravenous glucose in man. *J. Clin. Endocrinol. Metab.* **74**, 1032–1035.

Broderick, C. L., and Gold, G. (1991). Human and rat amylin have no effect on insulin secretion in isolated rat islets or in HIT cells. *Diabetes* **40**, 233A.

Broderick, C. L., Brooke, G. S., DiMarchi, R. D., and Gold, G. (1991). Human and rat amylin have no effects on insulin secretion in isolated rat pancreatic islets. *Biochem. Biophys. Res. Commun.* **177**, 932–938.

Butler, P. C. (1996). Islet amyloid and its potential role in the pathogenesis of type II diabetes mellitus. *In* "Diabetes Mellitus" (D. LeRoith, S. I. Taylor, and J. M. Olefsky, Eds.), pp. 113–117. Lippincott-Raven Publishers, Philadelphia.

Christmanson, L., Betsholtz, C., Leckstrom, A., Engstrom, U., Cortie, C., Johnson, K. H., Adrian, T. E., and Westermark, P. (1993). Islet amyloid polypeptide in the rabbit and European hare: Studies on its relationship to amyloidogenesis. *Diabetologia* **36**, 183–188.

Chuang, L. M., Wu, H. P., Jou, T. S., Tai, T. Y., and Lin, B. J. I. (1992). Inhibitory effect of islet amyloid polypeptide on glucose-induced proinsulin biosynthesis in rat insulinoma cells. *Pancreas* **7**, 472–476.

Clark, A. (1992). Islet amyloid: An enigma of type 2 diabetes. *Diabet. Met. Rev.* **8**, 117–132.

Clark, A., Cooper, G. J. S., Lewis, C. E., Morris, J. F., Willis, A. C., Reid, K. B., and Turner, R. C. (1987). Islet amyloid formed from diabetes-associated peptide may be pathogenic in type-2 diabetes. *Lancet* **2**, 231–234.

Clark, A., De Koning, E., Hansen, B., Bodkin, N., and Morris, J. F. (1991). Islet amyloid in glucose intolerant and spontaneous diabetic 'Macaca mulatta' monkeys. *In* "Frontiers in Diabetes Research: Lessons from Animal Diabetes III" (E. Shafrir, Ed.), pp. 502–506. Smith-Gordon Limited, London.

Clark, A., Badman, M. K., and deKoning, E. J. P. (1995). Islet amyloid, islet amyloid polypeptide, islet function and glucose metabolism in diabetes. *Diabet. Ann.* **9**, 33–56.

Clark, A., Charge, S. B. P., Badman, M. K., and deKoning, E. J. P. (1996a). Islet amyloid in type 2 (non-insulin-dependent) diabetes. *APMIS* **104**, 12–18.

Clark, A., Charge, S. B. P., Badman, M. K., Mac Arthur, D. A., and deKoning, E. J. P. (1996b). Islet amyloid polypeptide: Actions and role in the pathogenesis of diabetes. *Biochem. Soc. Trans.* **24**, 594–599.

Cook, J. T. E., Patel, P. P., Clark, A., Hoppener, J. W. M., Lips, C. J. M., Mosselman, S., O'Rahilly, S., Page, R. C., Wainscoat, J. S., and Turner, R. C. (1991). Non-linkage of the islet amyloid polypeptide gene with type-2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **34**, 103–108.

Cooper, G. J. S., Willis, A. C., Clark, A., Turner, R. C., Sim, R. B., and Reid, K. B. (1987). Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc. Natl. Acad. Sci. USA* **84**, 8628–8632.

Cooper, G. J. S., Leighton, B., Dimitriadis, G. D., Parry-Billings, M., Kowalchuk, J. M., Howland, K., Rothbard, J. B., Willis, A. C., and Reid, K. B. (1988). Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc. Natl. Acad. Sci. USA* **85**, 7763–7766.

Cooper, G. J. S., Day, A. J., Willis, A. C., Roberts, A. N., Reid, K. B., and Leighton, B. (1989a). Amylin and the amylin gene: Structure, function and relationship to islet amyloid and to diabetes mellitus. *Biochim. Biophys. Acta* **1014**, 247–258.

Cooper, G. J. S., Leighton, B., Willis, A. C., and Day, A. J. (1989b). The amylin superfamily: A novel grouping of biologically active polypeptides related to the insulin A-chain. *Prog. Growth Factor Res.* **1**, 99–105.

Couce, M., Kane, L. A., OBrien, T. D., Charlesworth, J., Soeller, W., McNeish, J., Kreutter, D., Roche, P., and Butler, P. C. (1996). Treatment with growth hormone and dexamethasone in mice transgenic for human islet amyloid polypeptide causes islet amyloidosis and beta-cell dysfunction. *Diabetes* **45**, 1094–1101.

de Koning, E. J. P., Bodkin, N. L., Hansen, B. C., and Clark, A. (1993). Diabetes-mellitus in Macaca-mulatta monkeys is characterised by islet amyloidosis and reduction in beta-cell population. *Diabetologia* **36**, 378–384.

de Koning, E. J. P., Verbeek, J. S., Esapa, C., Laube, B., and Clark, A. (1999). Amyloid fibrils are formed from proislet amyloid polypeptide in transgenic mouse insulinoma cells. *Diabetologia* **42**, A145.

Dégano, P., Peiro, E., Silvestre, R. A., Coma, I., Salas, M., and Marco, J. (1991). Insulin responses of the amylin-infused rat pancreas to glucose vasoactive intestinal polypeptide and arginine. *Diabetologia* **34**, A42.

Dégano, P., Salas, M., Peiro, E., Silvestre, R. A., and Marco, J. (1992). On the mechanism of the inhibitory effect of amylin on insulin secretion: Study in the perfused rat pancreas. *Diabetologia* **35**, A115.

Dégano, P., Silvestre, R. A., Salas, M., Peiró, E., and Marco, J. (1993). Amylin inhibits glucose-induced insulin secretion in a dose-dependent manner. Study in the perfused rat pancreas. *Regul. Pept.* **43**, 91–96.

Ehrlich, J. C., and Ratner, I. M. (1961). Amyloidosis of the islets of langerhans. *Am. J. Pathol.* **38**, 49–59.

Fan, L., Westermark, G., Chan, S. J., and Steiner, D. F. (1994). Altered gene structure and tissue expression of islet amyloid polypeptide in the chicken. *Mol. Endocrinol.* **8**, 713–721.

Fehmann, H. C., Weber, V., Göke, R., Göke, B., Eissele, R., and Arnold, R. (1990). Islet amyloid polypeptide (IAPP;amylin) influences the endocrine but not the exocrine rat pancreas. *Biochem. Biophys. Res. Commun.* **167**, 1102–1108.

Fürnsinn, C., Leuvenink, H., Roden, M., Nowotny, P., and Waldhäusl, W. (1992). Inhibition of glucose induced insulin secretion by amylin in rats *in vivo*. *Diabetologia* **35**, A29.

Fürnsinn, C., Leuvenink, H., Roden, M., Nowotny, P., Schneider, B., Rohac, M., Pieber, T., Clodi, M., and Waldhausl, W. (1994). Islet amyloid polypeptide inhibits insulin secretion in conscious rats. *Am. J. Physiol.* **267**, E300–E305.

Gebre-Medhin, S., Mulder, H., Pekny, M., Westermark, G., Tornell, J., Westermark, P., Sundler, F., Ahren, B., and Betsholtz, C. (1998). Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). *Biochem. Biophys. Res. Commun.* **250**, 271–277.

Gedulin, B., Larson, E., Provost, S., and Koda, J. (1993). The selective amylin antagonist, AC187, enhances the insulin response during intravenous glucose tolerance tests in anesthetized rats. *Diabetes* **42**(Suppl. 1), 229A.

Ghatei, M. A., Datta, H. K., Zaidi, M., Bretherton-Watt, D., Wimalawansa, S. J., MacIntyre, I., and Bloom, S. R. (1990). Amylin and amylin-amide lack an acute effect on blood glucose and insulin. *J. Endocrinol.* **124**, R9–R11.

Gilbey, S. G., Bretherton-Watt, D., Beacham, J., Ghatei, M. A., and Bloom, S. R. (1989). High dose amylin in man: Unexpected failure to affect intravenous glucose tolerance. *BDA Diab. Med.* **6**, 5A.

Göke, R., McGregor, G. P., and Göke, B. (1993a). Amylin alters biological effects of GLP-1 in the ß-cell. *Digestion* **54**, 355–356.

Göke, R., McGregor, G. P., and Göke, B. (1993b). Amylin alters the biological action of the incretin hormone GLP-1 (7–36)amide. *Life Sci.* **53**, 1367–1372.

Gold, G., Dimarchi, R. D., Broderick, C. L., Bue, J. M., Brooke, G. S., and Yen, T. T. (1990). Human amylin has no effect on either rate of insulin release or concentration of glucose in rodents. *Diabetes* **39**, 142A.

Hansen, B. (1996). Primate animal models of non-insulin-dependent diabetes mellitus. *In* (D. LeRoith, S. I. Taylor, and J. M. Olefsky, Eds.), pp. 595–603. Lippincott-Raven Publishers, Philadelphia.

Hellman, U., Wernstedt, C., Westermark, P., O'Brien, T. D., Rathbun, W. B., and Johnson, K. H. (1990). Amino acid sequence from degu islet amyloid-derived insulin shows unique sequence characteristics. *Biochem. Biophys. Res. Commun.* **169**, 571–577.

Higham, C. E., Lawrie, L., Shennan, K. I. J., Birch, N., Docherty, K., and Clark, A. (1999). Synthetic pro-islet amyloid polypeptide is cleaved by recombinant prohormone convertases, PC2 and PC3 *in vitro*. *Diabetologia* **42**, A145.

Howard, C. F. (1988). Spontaneous diabetes in macaca nigra: Relevance to human being with NIDDM. *In* (R. A. Camerini-Davalos, Ed.), pp. 33–41. Plenum Press, New York.

Hull, R. L., Andrikopoulos, S., Verchere, C. B., Vidal, J., Wang, F., Cnop, M., Prigeon, R. L., and Kahn, S. E. (2003). Increased dietary fat promotes islet amyloid formation and beta-cell secretory dysfunction in a transgenic mouse model of islet amyloid. *Diabetes* **52**, 372–379.

Inoue, K., Hiramatsu, S., Hisatomi, A., Umeda, F., and Nawata, H. (1993). Effects of amylin on the release of insulin and glucagon from the perfused rat pancreas. *Horm. Metab. Res.* **25**, 135–137.

Jakob, W. (1970). Unterschungen über die Amyloidose der Karnivoren unter besonderer Berücksichtigung der Altersamyloidose (Studies of amyloidosis of carnivora with special reference to amyloidosis of age). *Zbl. Vet. Med.* **Series A**, 818–829.

Janes, S., Gaeta, L., Beaumont, K., Beeley, N., and Rink, T. (1996). The selection of pramlintide for clinical evaluation. *Diabetes* **45**, 235A.

Johnson, K. H., O'Brien, T. D., Hayden, D. W., Mahoney, W. C., Wernstedt, C., and Westermark, P. (1988). Relationships of islet amyloid polypeptide (IAPP) to spontaneous diabetes in adult cats. *In* (T. Isobe, S. Araki, F. Uchino, S. Kito, and E. Tsubura, Eds.), pp. 673–678. Plenum Press, New York.

Johnson, K. H., O'Brien, T. D., Betsholtz, C., and Westermark, P. (1989). Islet amyloid, islet-amyloid polypeptide, and diabetes mellitus. *N. Engl. J. Med.* **321**, 513–518.

Johnson, K. H., O'Brien, T. D., Jordan, K., Betsholtz, C., and Westermark, P. (1990). The putative hormone islet amyloid polypeptide (IAPP) induces impaired glucose tolerance in cats. *Biochem. Biophys. Res. Commun.* **167**, 507–513.

Johnson, K. H., O'Brien, T. D., and Westermark, P. (1991a). Newly identified pancreatic protein islet amyloid polypeptide. What is its relationship to diabetes? *Diabetes* **40**, 310–314.

Johnson, K. H., Wernstedt, C., O'Brien, T. D., and Westermark, P. (1991b). Amyloid in the pancreatic islets of the cougar (Felis concolor) is derived from islet amyloid polypeptide (IAPP). *Comp. Biochem. Physiol. B* **98**, 115–119.

Johnson, K. H., Jordan, K., O'Brien, T. D., Murtaugh, M. P., Wernstedt, C., Betsholtz, C., and Westermark, P. (1991c). Factors affecting diabetogenesis and amyloidogenesis are provided by studies of IAPP in the dog and cat. *In* (J. B. Natvig, *et al.*, eds.), pp. 445–448. Kluwer Academic Publishers, Norvell, MA.

Johnson, K. H., O'Brien, T. D., Betsholtz, C., and Westermark, P. (1992). Islet amyloid polypeptide: Mechanisms of amyloidogenesis in the pancreatic islets and potential roles in diabetes mellitus. *Lab. Invest.* **66**, 522–535.

Jordan, K., Murtaugh, M. P., O'Brien, T. D., Westermark, P., Betsholtz, C., and Johnson, K. H. (1990). Canine IAPP cDNA sequence provides important clues regarding diabetogenesis and amyloidogenesis in type 2 diabetes. *Biochem. Biophys. Res. Commun.* **169**, 502–508.

Jordan, K. C., O'Brien, T. D., and Johnson, K. H. (1994). Sequence of raccoon IAPP supports importance of a specific structural motif in the development of pancreatic islet amyloidosis. *Amyloid Intl. J. Exp. Clin. Invest.* **1**, 160–164.

Kahn, S. E., Andrikopoulos, S., Verchere, C. B., Wang, F., Hull, R. L., and Vidal, J. (2000). Oophorectomy promotes islet amyloid formation in a transgenic mouse model of type II diabetes. *Diabetologia* **43**, 1309–1312.

Kajio, H., Kobayashi, T., Hara, M., Nakanishi, K., Sugimoto, T., Murase, T., Akanuma, Y., Kosaka, K., Shibasaki, Y., *et al.* (1992). Islet amyloid polypeptide (IAPP) gene analysis in a Japanese diabetic with marked islet amyloid deposition. *Diabet. Res. Clin. Pract.* **15**, 45–48.

Kamaeva, O. I. (1993). Amylin and amyloidosis of the pancreatic islets and their significance in the etiology and pathogenesis of diabetes mellitus type 2. *Ter. Arkh.* **65**, 14–17.

Kapurniotu, A., Bernhagen, J., Al-Abed, Y., and Bucala, R. (1996). Amyloidogenic properties of AGE-modified amylin: Role in the amyloid deposition and islet cell dysfunction of Type II diabetes. *Diabetes* **45**, 39A.

Katafuchi, T., Hamano, K., Kikumoto, K., and Minamino, N. (2003a). Identification of second and third calcitonin receptor-stimulating peptides in porcine brain. *Biochem. Biophys. Res. Commun.* **308**, 445–451.

Katafuchi, T., Kikumoto, K., Hamano, K., Kangawa, K., Matsuo, H., and Minamino, N. (2003b). Calcitonin receptor-stimulating peptide, a new member of the calcitonin gene-related peptide family. Its isolation from porcine brain, structure, tissue distribution, and biological activity. *J. Biol. Chem.* **278**, 12046–12054.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., and Eto, T. (1993). Adrenomedullin: A novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.* **192**, 553–560.

Kogire, M., Ishizuka, J., Thompson, J. C., and Greeley, G. H. (1991). Inhibitory action of islet amyloid polypeptide and calcitonin gene-related peptide on release of insulin from the isolated perfused rat pancreas. *Pancreas* **6**, 459–463.

Kulkarni, R. N., Smith, D. M., Ghatei, M. A., Jones, P. M., and Bloom, S. R. (1996). Investigation of the effects of antisense oligodeoxynucleotides to islet amyloid polypeptide mRNA on insulin release, content and expression. *J. Endocrinol.* **151**, 341–348.

Leaming, R., Johnson, A., Hook, G., Hanley, R., and Baron, A. (1995). Amylin modulates insulin secretion in humans. Studies with an amylin antagonist. *Diabetologia* **38**, A113.

Leffert, J. D., Newgard, C. B., Okamoto, H., Milburn, J. L., and Luskey, K. L. (1989). Rat amylin: Cloning and tissue-specific expression in pancreatic islets. *Proc. Natl. Acad. Sci. USA* **86**, 3127–3310.

Lehman-deGaeta, L. S., Willis, A. C., Young, A. A., Foot, E. A., Albrecht, E., Leighton, B., Rink, T. J., and Cooper, G. J. S. (1991). Variability of chemically synthesized human amylin. *Diabetes* **40**, 236A.

Lewis, C. E., Clark, A., Ashcroft, S. J., Cooper, G. J. S., and Morris, J. F. (1988). Calcitonin gene-related peptide and somatostatin inhibit insulin release from individual rat B cells. *Mol. Cell Endocrinol.* **57**, 41–49.

MacNamara, C. M., Barrow, B. A., Manley, S. E., Levy, J. C., Clark, A., and Turner, R. C. (2000). Parallel changes of proinsulin and islet amyloid polypeptide in glucose intolerance. *Diabet. Res. Clin. Pract.* **50**, 117–126.

Marco, J., and Silvestre, R. (1997). Effect of amylin on islet cell secretion. *Exp. Clin. Endocrinol. Diabet.* **105**, 68.

Marco, J., Peiro, E., Dégano, P., Miralles, P., and Silvestre, R. E. (1990). Amylin inhibits insulin secretion in the perfused rat pancreas. *Diabetes* **39**, 136A.

Marzban, L., Park, K., and Verchere, C. B. (2003). Islet amyloid polypeptide and type 2 diabetes. *Exp. Gerontol.* **38**, 347–351.

McCarthy, M. I., Hitman, G. A., Mohan, V., Ramachandran, A., Snehalatha, C., and Viswanathan, M. (1992). The islet amyloid polypeptide gene and non-insulin-dependent diabetes mellitus in south Indians. *Diabet. Res. Clin. Pract.* **18**, 31–34.

Mosselman, S., Hoppener, J. W. M., Zandberg, J., vanMansfeld, A. D. M., Guerts-van Kessel, A. H. M., Lips, C. J. M., and Jansz, H. S. (1988). Islet amyloid polypeptide: Identification and chromosomal localization of the human gene. *FEBS Lett.* **23**, 227–232.

Mosselman, S., Hoppener, J. W. M., Lips, C. J. M., and Jansz, H. S. (1989). The complete islet amyloid polypeptide precursor encoded by two exons. *FEBS Lett.* **247**, 154–158.

Moyses, C., Thompson, R. G., and Kolterman, O. G. (1996). Pramlintide, a human amylin analogue: A new approach to glycaemic control in patients with diabetes requiring insulin. First World Congress on Prevention of Diabetes and its Complications, April 25–28 1996, Lyngby, Denmark, p. 151.

Murakami, M., Suzuki, S., Sato, Y., Shintami, S., Abe, S., Suzuki, K., Ishuzuka, J., and Toyota, T. (1990). Effects of amylin on insulin secretion from RIN m5F cells. *Diabetes* **39**, 266A.

Nagamatsu, S., Carroll, R., and Steiner, D. F. (1990a). IAPP effects on pancreatic beta cell function in normal rat islets and BTC3 cell line. *Diabetes* **39**, 67A.

Nagamatsu, S., Carroll, R. J., Grodsky, G. M., and Steiner, D. F. (1990b). Lack of islet amyloid polypeptide regulation of insulin biosynthesis or secretion in normal rat islets. *Diabetes* **39**, 871–874.

Nagamatsu, S., Nishi, M., and Steiner, D. F. (1992). Effects of islet amyloid polypeptide (IAPP) on insulin biosynthesis or secretion in rat islets and mouse beta TC3 cells. Biosynthesis of IAPP in mouse beta TC3 cells. *Diabet. Res. Clin. Pract.* **15**, 49–55.

Nakazato, M., Asai, J., Kangawa, K., Matsukura, S., and Matsuo, H. (1989). Establishment of radioimmunoassay for human islet amyloid polypeptide and its tissue content and plasma concentration. *Biochem. Biophys. Res. Commun.* **164**, 394–399.

Nishi, M., and Steiner, D. F. (1990). Cloning the complementary DNA's encoding islet amyloid polypeptide, insulin and glucagon precursors from a new world rodents, the Degu, Octodo. *Mol. Endocrinol.* **4**, 1192–1198.

Nishi, M., Bell, G. I., and Steiner, D. F. (1990a). Sequence of a cDNA encoding syrian hamster islet amyloid polypeptide precursor. *Nucl. Acids Res.* **18**, 6726.

Nishi, M., Sanke, T., Nagamaatsu, S., Bell, G. I., and Steiner, D. F. (1990b). Islet amyloid polypeptide: A new β cell secretory product related to islet amyloid deposits. *J. Biol. Chem.* **265**, 4173–4176.

Nishi, M., Chan, S. J., Nagamatsu, S., Bell, G. I., and Steiner, D. F. (1989). Conservation of the sequence of islet amyloid polypeptide in five mammals is consistent with its putative role as an islet hormone. *Proc. Natl. Acad. Sci. USA* **86**, 5738–5742.

O'Brien, T. D., Westermark, P., and Johnson, K. H. (1990). Islet amyloid polypeptide (IAPP) does not inhibit glucose-stimulated insulin secretion from isolated perfused rat pancreas. *Biochem. Biophys. Res. Commun.* **170**, 1223–1228.

O'Brien, T. D., Butler, P. C., Westermark, P., and Johnson, K. H. (1993a). Islet amyloid polypeptide: A review of its biology and potential roles in the pathogenesis of diabetes mellitus. *Vet. Pathol.* **30**, 317–332.

O'Brien, T. D., Westermark, P., Betsholtz, C., and Johnson, K. H. (1993b). Islet amyloid polypeptide: Biology and role in the pathogenesis of islet amyloidosis and diabetes mellitus. *In* (E. Shafrir, Ed.), p. 117. Biennial review volume: 1992 Smith Gordon/Nixhimura, UK.

O'Harte, F. P. M., AbdelWahab, Y. H. A., Conlon, J. M., and Flatt, P. R. (1998). Glycated IAPP shows a reduced inhibitory action on insulin secretion. *Biochem. Soc. Trans.* **26**, S6.

Ohagi, S., Nishi, M., Bell, G. I., Ensinck, J. W., and Steiner, D. F. (1991). Sequences of islet amyloid polypeptide precursors of an old world monkey, the pig-tailed macaque (macaca-nemestrina), and the dog (canis-familiaris). *Diabetologia* **34**, 555–558.

Ohsawa, H., Kanatsuka, A., Mizuno, Y., Tokuyama, Y., Takada, K., Mikata, A., Makino, H., and Yoshida, S. (1992). Islet amyloid polypeptide-derived amyloid deposition increases along with the duration of type 2 diabetes mellitus. *Diabet. Res. Clin. Pract.* **15**, 17–21.

Ohsawa, H., Kanatsuka, A., Yamaguchi, T., Makino, H., and Yoshida, S. (1989). Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets. *Biochem. Biophys. Res. Commun.* **160**, 961–967.

Opie, E. L. (1900a). On the relation of chronic interstitial pancreatitis to the islands of Langerhans and to diabetes mellitus. *J. Exp. Med.* **5**, 397–428.

Opie, E. L. (1900b). The relation of diabetes mellitus to lesions of the pancreas: Hyaline degeneration of the islands of Langerhans. *J. Exp. Med.* **5**, 527–540.

Panagiotidis, G., Salehi, A. A., Westermark, P., and Lundquist, I. (1992). Homologous islet amyloid polypeptide: Effects on plasma levels of glucagon, insulin and glucose in the mouse. *Diabet. Res. Clin. Pract.* **18**, 167–171.

Pearse, A. G. E., Ewen, S. W. B., and Polak, J. M. (1972). The genesis of apudamyloid in endocrine polypeptide tumours: Histochemical distinction from immunamyloid. *Virchows Arch. B Cell Pathol.* **10**, 93–107.

Peiró, E., Dégano, P., Silvestre, R. A., and Marco, J. (1991). Inhibition of insulin release by amylin is not mediated by changes in somatostatin output. *Life Sci.* **49**, 761–765.

Percy, A. J., Trainor, D. A., Rittenhouse, J., Phelps, J., and Koda, J. E. (1996). Development of sensitive immunoassays to detect amylin and amylin-like peptides in unextracted plasma. *Clin. Chem.* **42**, 576–585.

Pettersson, M., and Ahren, B. (1990). Failure of islet amyloid polypeptide to inhibit basal and glucose-stimulated insulin secretion in model experiments in mice and rats. *Acta Physiol. Scand.* **138**, 389–394.

Porte, D., Howard, C., Westermark, P., and Clark, A. (1991). X-Discussion: Pancreatic islet amyloid polypeptide (IAPP, amylin). *In* (E. Shafrir, Ed.), pp. 516–519. Smith-Gordon Limited, London.

Rittenhouse, J., Chait, B. T., Bierle, J. R., Janes, S. M., Park, D. R., Phelps, J. L., Fineman, M. S., Qin, J., and Koda, J. E. (1996). Heterogeneity of naturally-occurring human amylin due to glycosylation. *Diabetes* **45**, 234A.

Roberts, A., Leighton, B., Todd, J. A., Cockburn, D., Schofield, P. N., Sutton, R., Holt, S., Boyd, Y., Day, A. J., Foot, E. A., Willis, A. C., Reid, K. B. M., and Cooper, G. J. S. (1989). Molecular and functional characterization of amylin, a peptide associated with type 2 diabetes mellitus. *Proc. Natl. Acad. Sci. USA* **86**, 9662–9666.

Rodriguez-Gallardo, J., Silvestre, R. A., Salas, M., and Marco, J. (1995). Rat amylin versus human amylin: Effects on insulin secretion in the perfused rat pancreas. *Med. Sci. Res.* **23**, 569–570.

Rosenfeld, M. G., Mermod, J. J., Amara, S. G., Swanson, L. W., Sawchenko, P. E., Rivier, J., Vale, W. W., and Evans, R. M. (1983). Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature* **304**, 129–135.

Sakagashira, S., Sanke, T., Hanabusa, T., Tabata, H., Jiko, H., Ohagi, S., Kumagaye, K. Y., Nakajima, K., and Nanjo, K. (1996). A missense mutation of amylin gene (Ser20Gly) in Japanese patients with non-insulin-dependent diabetes mellitus. Program and Abstracts, 10th International Congress of Endocrinology, p. 979.

Sakagashira, S., Hiddinga, H. J., Tateishi, K., Sanke, T., Hanabusa, T., Nanjo, K., and Eberhardt, N. L. (2000). S20g mutant amylin exhibits increased *in vitro* amyloidogenicity

and increased intracellular cytotoxicity compared to wild-type amylin. *Am. J. Pathol.* **157**, 2101–2109.

Salas, M., Silvestre, R. A., Gutiérrez, E., Fontels, T., Garcia-Hermida, O., and Marco, J. (1994). Potentiation of the insulin response to glucose by an amylin antagonist (8–32 salmon calcitonin). *Diabetologia* **37**, A116.

Salas, M., Silvestre, R. A., Garcia-Hermida, O., Fontela, T., Rodriguez-Gallardo, J., and Marco, J. (1995). Inhibitory effect of amylin (islet amyloid polypeptide) on insulin response to non-glucose stimuli: Study in perfused rat pancreas. *Diabet. Metab.* **21**, 269–273.

Sandler, S., and Stridsberg, M. (1994). Chronic exposure of cultured rat pancreatic islets to elevated concentrations of islet amyloid polypeptide (IAPP) causes a decrease in islet DNA content and medium insulin accumulation. *Regul. Pept.* **53**, 103–109.

Sanke, T., Bell, G. I., Sample, C., Rubenstein, A. H., and Steiner, D. F. (1988). An islet amyloid peptide is derived from an 89-amino acid precursor by proteolytic processing. *J. Biol. Chem.* **263**, 17243–17246.

Sanke, T., Sakagashira, S., Hanabusa, T., Tabata, H., Jiko, H., Ohagi, S., Nakajima, K., and Nanjo, K. (1996). Significance of a missense mutation (Ser20Gly) of amylin (IAPP) gene in early onset NIDDM in the Japanese population. *Diabetes* **45**, 228A.

Silvestre, R. A., Peiro, E., Dégano, P., Iglesias, A., Miralles, P., and Marco, J. (1990a). Rat amylin inhibits insulin release without affecting glucagon and somatostatin output in the rat pancreas. *Diabetologia* **33**, A40.

Silvestre, R. A., Peiró, E., Dégano, P., Miralles, P., and Marco, J. (1990b). Inhibitory effect of rat amylin on the insulin responses to glucose and arginine in the perfused rat pancreas. *Regul. Pept.* **31**, 23–31.

Silvestre, R. A., Dégano, P., Salas, M., Peiro, E., and Marco, J. (1992). Calcitonin gene-related peptide (CGRP) and amylin inhibition of insulin release is reversed by the 8–37 CGRP fragment. *Diabetologia* **35**, A29.

Silvestre, R. A., Salas, M., Dégaño, P., Fontela, T., García-Hermida, O., and Marco, J. (1993a). Prevention of the inhibitory effect of amylin on GIP-induced insulin release by Pertussis toxin pretreatment. *Diabetologia* **36**, A136.

Silvestre, R. A., Salas, M., Degano, P., Peiro, E., and Marco, J. (1993b). Reversal of the inhibitory effects of calcitonin gene-related peptide (CGRP) and amylin on insulin secretion by the 8–37 fragment of human CGRP. *Biochem. Pharmacol.* **45**, 2343–2347.

Silvestre, R. A., Salas, M., Garcia-Hermida, O., Fontela, T., Degano, P., and Marco, J. (1994). Amylin (islet amyloid polypeptide) inhibition of insulin release in the perfused rat pancreas: Implication of the adenylate cyclase/cAMP system. *Regul. Pept.* **50**, 193–199.

Silvestre, R. A., Salas, M., Rodriguez-Gallardo, J., Garcia-Hermida, O., Fontela, T., and Marco, J. (1996). Effect of (8–32). Salmon calcitonin, an amylin antagonist, on insulin, glucagon and somatostatin release: Study in the perfused pancreas of the rat. *Br. J. Pharmacol.* **117**, 347–350.

Silvestre, R. A., Rodríguez-Gallardo, J., Gutiérrez, E., and Marco, J. (1997). Influence of glucose concentration on the inhibitory effect of amylin on insulin secretion: Study in the perfused rat pancreas. *Regul. Pept.* **68**, 31–35.

Smith, D. M., and Bloom, S. R. (1995). Paracrine/autocrine control of the islet and the amylin family. *Biochem. Soc. Trans.* **23**, 336–340.

Soeller, W. C., Parker, J. C., Emeigh-Hart, S. G., Kreutter, D. K., and Stevenson, R. W. (1996a). Glucose intolerance and late-onset hyperglycemia in A^{vy}/a mice expressing a human islet amyloid polypeptide (huIAPP) transgene. *Diabetes* **45**, 77A.

Soeller, W. C., Parker, J. C., Janson, J., Nelson, R. T., Carty, M. D., Torchia, A. J., Orena, S. J., Stock, J., McNeish, J., Stevenson, R. W., and Kreutter, D. K. (1996b). Two distinct diabetogenic roles for IAPP revealed by elevated expression of human and rodent homologues in transgenic mice. *Diabetes* **45**, 161A.

Stridsberg, M., Berne, C., Sandler, S., Wilander, E., and Oberg, K. (1993). Inhibition of insulin secretion, but normal peripheral insulin sensitivity, in a patient with a malignant endocrine pancreatic tumour producing high amounts of an islet amyloid polypeptide-like molecule. *Diabetologia* **36**, 843–849.

Suzuki, S., Murakami, M., Abe, S., Satoh, Y., Shintani, S., Ishizuka, J., Suzuki, K., Thompson, J. C., and Toyota, T. (1992). The effects of amylin on insulin secretion from Rin m5F cells and glycogen synthesis and lipogenesis in rat primary cultured hepatocytes. *Diabet. Res. Clin. Pract.* **15**, 77–84.

Tedstone, A. E., Nezzer, T., Hughes, S. J., Clark, A., and Matthews, D. R. (1989). The effects of islet amyloid peptide and calcitonin gene-related peptide on insulin secretion in anaesthetised rats and from isolated rat islets. *BDA Diab. Med.* **6**, A38.

Tedstone, A. E., Nezzer, T., Hughes, S. J., Clark, A., and Matthews, D. R. (1990). The effect of islet amyloid polypeptide (amylin) and calcitonin gene-related peptide on glucose removal in the anaesthetized rat and on insulin secretion from rat pancreatic islets in vitro. *Biosci. Rep.* **10**, 339–345.

Tokuyama, Y., Kanatsuka, A., Suzuki, Y., Yamaguchi, T., Taira, M., Makino, H., and Yoshida, S. (1994). Islet amyloid polypeptide gene: No evidence of abnormal promoter region in thirty-five type 2 diabetic patients. *Diabet. Res. Clin. Pract.* **22**, 99–105.

van Mansfeld, A. D. M., Mosselman, S., Hoppener, J. W. M., Zandberg, J., van Teeffelen, H. A. A. M., Baas, P. D., Lips, C. J. M., and Jansz, H. S. (1990). Islet amyloid polypeptide: Structure and upstream sequence of the IAPP gene in rat and man. *Biochim. Biophys. Acta* **1087**, 235–240.

Verchere, C. B., DAlessio, D. A., Palmiter, R. D., Weir, G. C., Bonner Weir, S., Baskin, D. G., and Kahn, S. E. (1996). Islet amyloid formation associated with hyperglycemia in transgenic mice with pancreatic beta cell expression of human islet amyloid polypeptide. *Proc. Natl. Acad. Sci. USA* **93**, 3492–3496.

Verchere, C. B., DAlessio, D. A., Wang, S., Andrikopoulos, S., and Kahn, S. E. (1997). Transgenic overproduction of islet amyloid polypeptide (amylin) is not sufficient for islet amyloid formation. *Horm. Metab. Res.* **29**, 311–316.

Wagoner, P. K., Dukes, I. D., and Worley, J. F., III (1992). Amylin inhibition of glucose-induced changes in electrical activity, intracellular calcium and insulin release in mouse beta-cells. *Diabetes* **41**, 103A.

Wagoner, P. K., Chen, C., Worley, J. F., Dukes, I. D., and Oxford, G. S. (1993). Amylin modulates ß-cell glucose sensing via effects on stimulus-secretion coupling. *Proc. Natl. Acad. Sci. USA* **90**, 9145–9149.

Wang, F., Westermark, G., Gasslander, T., and Permert, J. (1997). Effect of islet amyloid polypeptide on somatostatin inhibition of insulin secretion from isolated rat pancreatic islets. *Regul. Pept.* **72**, 61–67.

Wang, Z.-L., Bennet, W. M., Ghatei, M. A., Byfield, P. G. H., Smith, D. M., and Bloom, S. R. (1993). Influence of islet amyloid polypeptide and the 8–37 fragment of islet amyloid polypeptide on insulin release from perifused rat islets. *Diabetes* **42**, 330–335.

Weir, G. C., and Bonner-Weir, S. (1996). Insulin secretion in non-insulin-dependent diabetes mellitus. *In* (D. LeRoith, S. I. Taylor, and J. M. Olefsky, Eds.), pp. 503–508. Lippincott-Raven Publishers, Philadelphia.

Westermark, P. (1972). Quantitative studies on amyloid in the islets of Langerhans. *Ups. J. Med. Sci.* **77**, 91–94.

Westermark, P. (1994). Amyloid and polypeptide hormones: What is their interrelationship? *Amyloid Intl. J. Exp. Clin. Invest.* **1**, 47–60.

Westermark, P., and Johnson, K. H. (1988). The pathogenesis of maturity-onset diabetes mellitus: Is there a link to islet amyloid polypeptide? *Bioessays* **9**, 30–33.

Westermark, P., and Wilander, E. (1983). Islet amyloid in Type 2 (non-insulin-dependent) diabetes is related to insulin. *Diabetologia* **24**, 342–346.

Westermark, P., Wernstedt, C., Wilander, E., and Sletten, K. (1986). A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. *Biochem. Biophys. Res. Commun.* **140**, 827–831.

Westermark, P., Wernstedt, C., O'Brien, T. D., Hayden, D. W., and Johnson, K. H. (1987a). Islet amyloid in type 2 human diabetes mellitus and adult diabetic cats contains a novel putative polypeptide hormone. *Am. J. Pathol.* **127**, 414–417.

Westermark, P., Wernstedt, C., Wilander, E., Hayden, D. W., O'Brien, T. D., and Johnson, K. H. (1987b). Amyloid fibrils in human insulinoma and islets of Langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells. *Proc. Natl. Acad. Sci. USA* **84**, 3881–3885.

Westermark, P., Johnson, K. H., Sletten, K., Wilander, E., and Wernstedt, C. (1988). The nature of the amyloid in the Islets of Langerhans: A novel polypeptide hormone? *In* (T. Isobe, S. Araki, F. Uchino, S. Kito, and E. Tsubura, Eds.), pp. 667–671. Plenum Press, New York.

Westermark, P., Engstrom, U., Johnson, K. H., Westermark, G. T., and Betsholtz, C. (1990). Islet amyloid polypeptide: Pinpointing amino acid residues linked to amyloid fibril formation. *Proc. Natl. Acad. Sci. USA* **87**, 5036–5040.

Westermark, P., Betsholtz, C., Dominguez, H. L., O'Brien, T. D., and Johnson, K. H. (1991). Islet amyloid polypeptide in humans and cats. *In* (E. Shafrir, Ed.), pp. 499–501. Smith-Gordon Limited, London.

Westermark, P., Johnson, K. H., O'Brien, T. D., and Betsholtz, C. (1992). Islet amyloid polypeptide—A novel controversy in diabetes research. *Diabetologia* **35**, 297–303.

Westermark, P., Eizirik, D. L., Pipeleers, D. G., Hellerstrom, C., and Andersson, A. (1995). Rapid deposition of amyloid in human islets transplanted into nude mice. *Diabetologia* **38**, 543–549.

Wolffen-Buttel, B. H. R., and Van Haeften, T. W. (1993). Review—non-insulin-dependent diabetes-mellitus—Defects in insulin secretion. *Eur. J. Clin. Invest.* **23**, 69–79.

Young, A. A., Carlo, P., Rink, T. J., and Wang, M.-W. (1992a). 8–37hCGRP, an amylin receptor antagonist, enhances the insulin response and perturbs the glucose response to infused arginine in anesthetized rats. *Mol. Cell Endocrinol.* **84**, R1–R5.

Young, A. A., Gedulin, B., Wolfe-Lopez, D., Greene, H. E., Rink, T. J., and Cooper, G. J. S. (1992b). Amylin and insulin in rat soleus muscle: Dose responses for cosecreted noncompetitive antagonists. *Am. J. Physiol.* **263**, E274–E281.

Young, A. A., Gedulin, B., Larson, E., and Rink, T. J. (1993). Evidence from studies using a specific blocker for a metabolic effect of endogenous amylin *in vivo*. *Diabetologia* **36**(suppl. 1), A136.

Young, A. A., Gedulin, B., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., Larson, E., and Rink, T. J. (1994). Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat—Evidence for a metabolic role of endogenous amylin. *FEBS Lett.* **343**, 237–241.

Young, A. A., Wang, M. W., Gedulin, B., Rink, T. J., Pittner, R., and Beaumont, K. (1995). Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. *Metabolism* **44**, 1581–1589.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Andrew Young

Tissue Expression and Secretion of Amylin

I. Summary

Amylin and insulin are co-localized within the same secretory granules of pancreatic β-cells. Acutely, the secreted ratio of amylin:insulin is comparatively invariant, but long-standing hyperglycemia may favor induction of amylin synthesis and secretion over that of insulin. Amylin is also found in much lesser quantities in the gut and other tissues.

In humans, both type 1 diabetes mellitus and the later stages of type 2 diabetes mellitus are characterized by deficiency of both insulin and amylin secretion. The severity of amylin deficiency appears to correlate with the severity of insulin deficiency. This concordance of deficiencies in amylin and insulin secretion observed with the progression of diabetes mellitus is consistent with their co-localization in pancreatic β-cells.

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Amylin is cleared mainly by proteolytic degradation at the kidney. The terminal $t_{½}$ for rat amylin in rats is ~13 min, and that for pramlintide in humans is ~20–45 min.

II. Tissue Expression and Secretion

A. Pancreatic β-Cells and Islets

Since the pancreatic islets were the source of amyloid material from which the full sequence of amylin was derived (Cooper *et al.*, 1987, 1988), initial efforts to localize sites of gene expression were focused there. The amylin gene (also referred to as the IAPP gene by some; Sanke *et al.*, 1988) was found to be localized to pancreas, and specifically in islets (Leffert *et al.*, 1989). Distribution of immunoreactivity included islets (Lutz and Rand, 1997) and coincided with that of insulin (Denijn *et al.*, 1992; Mulder *et al.*, 1993; Röcken *et al.*, 1992; Wang *et al.*, 1990). This coincidence of distribution resolved to not only β-cells (De Vroede *et al.*, 1992), but also β-cell secretory granules (Johnson *et al.*, 1988; Lukinius *et al.*, 1989, 1996).

Additionally in pancreatic islets, some amylin-like immunoreactivity has been described in peripheral regions of the islets dissociated from insulin-like immunoreactivity (Ahren and Sundler, 1992). In those cases, immunoreactive material coincided with that of somatostatin (De Vroede *et al.*, 1992; Mulder *et al.*, 1993) and was localized to δ-cell granules (Lukinius *et al.*, 1996) (Fig. 1).

B. Amylin in the Gut

In rat, mouse, and human, amylin-like immunoreactivity has been described in several places along the gut, predominantly in the pyloric antrum (Asai *et al.*, 1990; Miyazato *et al.*, 1991; Mulder *et al.*, 1994; Nicholl *et al.*, 1992; Ohtsuka *et al.*, 1993; Toshimori *et al.*, 1990). Amylin mRNA has a similar distribution (Mulder *et al.*, 1994). Immunoreactivity is reported in lesser amounts in the body of the stomach (Mulder *et al.*, 1994; Toshimori *et al.*, 1990) and is sparsely scattered from duodenum to colon (Asai *et al.*, 1990; Miyazato *et al.*, 1991). In a phylogenetic study, amylin immunoreactivity was found in the stomach and duodenum of all vertebrate species studied, except for fish (D'Este *et al.*, 1995). The presence of amylin in the stomach is not in association with insulin (Nicholl *et al.*, 1992), but in rat and human, corresponds to gastrin (G) cells (Mulder *et al.*, 1994, 1997a; Ohtsuka *et al.*, 1993). It has been associated with various other neuropeptides, including somatostatin (Mulder *et al.*, 1994, 1997a), peptide YY (Mulder

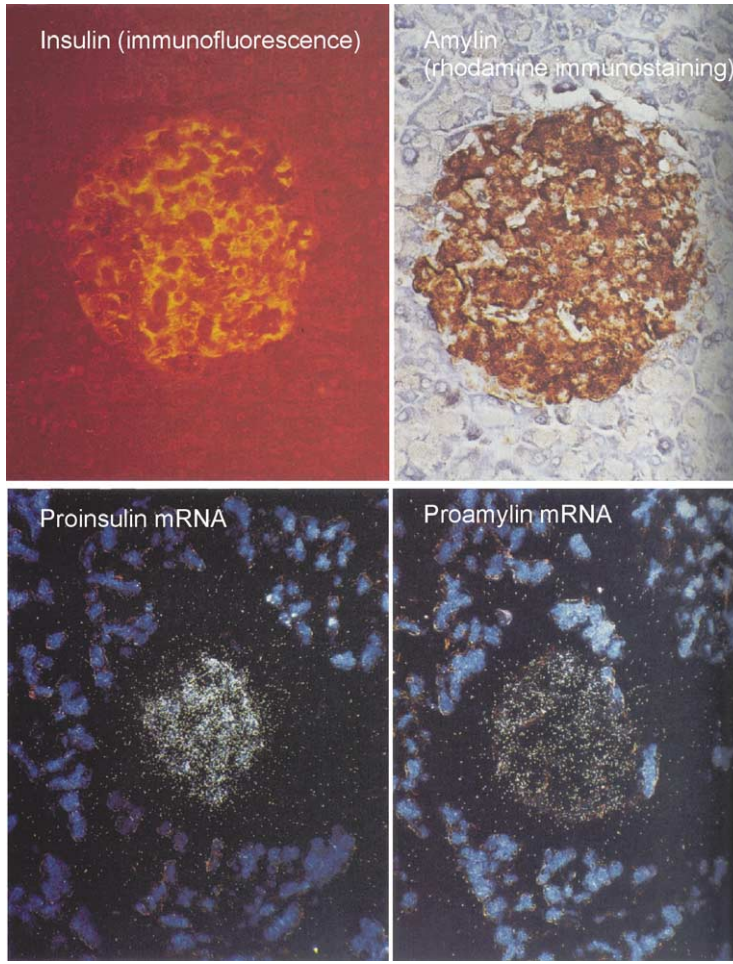

FIGURE 1 Immunofluorescence corresponding to insulin localization (upper left) and rhodamine staining corresponding to amylin localization in pancreatic islets from normal rats. Lower panels show distribution of proinsulin and proamylin mRNA by *in situ* hybridization. Reproduced from Unger and Foster (1992).

et al., 1994, 1997a), serotonin, and chromogranins (D'Este *et al.*, 1994, 1995).

In a histologic study of gut amylin in the neonatal period (days 1, 7, 18, 28, and 45; five animals at each time point), small numbers of amylin-positive cells were consistently found in the basal cell layer of the mucosa, from birth throughout the study. A marked, transient increase in amylin-positive cells was noted in all animals examined on day 18, the day after the introduction of pellet feed (Li *et al.*, 2002).

C. Amylin in the Nervous System

Amylin-like immunoreactivity has been described in a population of small- to medium-sized nerve cell bodies in dorsal root ganglia from all levels and in the jugular-nodose and trigeminal ganglion and in dorsal horn neurones (Mulder *et al*., 1995c), where it co-localized with fibers containing calcitonin gene-related peptide, substance P, and pituitary adenylate cyclase-activating polypeptide. It was present to a lesser extent in peripheral tissues receiving sensory innervation (Mulder *et al*., 1995c), the section of which altered spinal levels (Mulder *et al*., 1997b). Specific amylin-like immunoreactivity is also present in the amygdala (Dilts *et al*., 1995) and other central brain regions (Skofitsch *et al*., 1995).

D. Amylin in Osteoblasts

It has been proposed that amylin is produced by osteoblasts, where it has been hypothesized to act as a local factor within bone (Gilbey *et al*., 1991).

III. Patterns of Amylin Secretion

A. Amylin Secretory Responses

Early descriptions of a concordance of secretory patterns of amylin and insulin in animals (Chou *et al*., 1990; Hammonds *et al*., 1991; Ogawa *et al*., 1990) and in humans (Butler *et al*., 1990b; Mitsukawa *et al*., 1990; Sanke *et al*., 1991) were interpreted as evidence of their co-secretion. Two studies described the parallel regulation of amylin and insulin genes (Koranyi *et al*., 1992; Mulder *et al*., 1996b) (Fig. 2).

B. Control of Secretion

An early indication that amylin and insulin were co-localized in, and were co-secreted from, pancreatic β-cells was the observation that factors modulating insulin secretion also appeared to cause an obligatory modulation of amylin secretion. This was true for stimulation of secretion by glucose (Jamal *et al*., 1993; Kanatsuka *et al*., 1989; Nakazato *et al*., 1990; Ogawa *et al*., 1990; Shiomi *et al*., 1992), arginine (Ogawa *et al*., 1990), or carbachol (Jamal *et al*., 1993) and was true for inhibition of secretion by somatostatin (Jamal *et al*., 1993; Mitsukawa *et al*., 1990). There is a notable similarity of plasma concentration profiles between amylin and insulin (Koda *et al*., 1995).

There is considerable literature, much from the laboratory of Marco *et al*. in Madrid, that addresses the effects of amylin itself on β-cell secretion

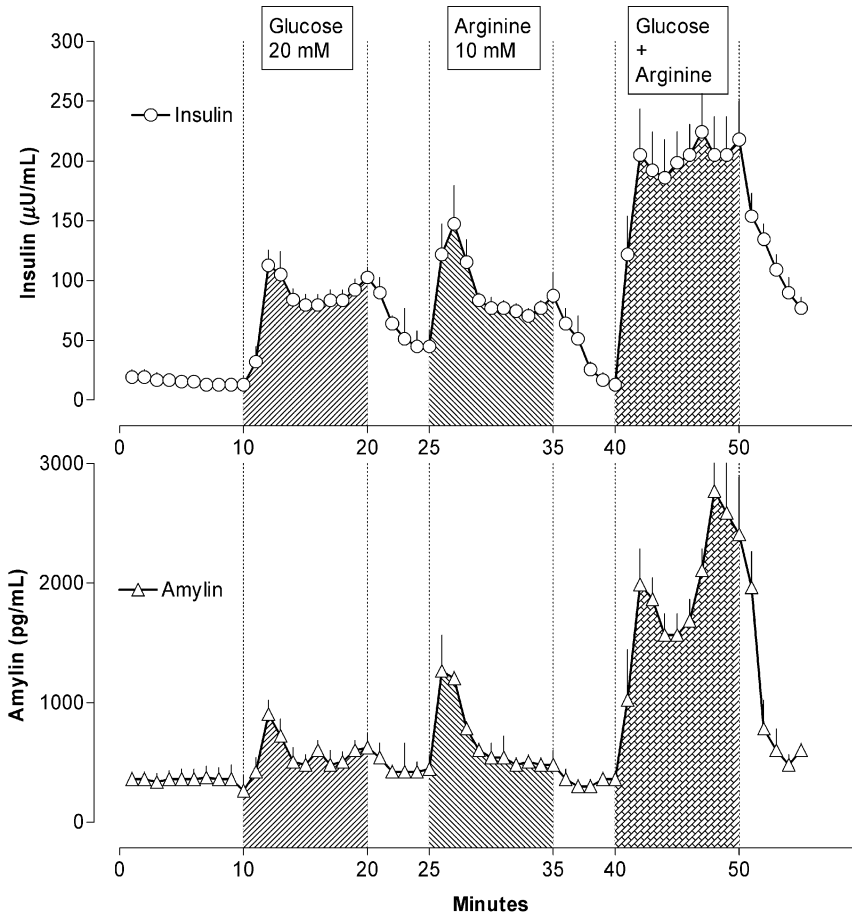

FIGURE 2 Secretory responses from normal isolated perfused rat pancreas showing changes in insulin secretion (upper panel) and amylin secretion (lower panel) with changes in perfusate glucose from 5 mM to 20 mM, changes in arginine from 0 to 10 mM, and the combination, as indicated. Perfusate flow was 2.7 ml/min. Symbols are means ± SEM. $n = 6$ male Wistar rats. Data from Ogawa *et al.* (1990).

(i.e., as a regulator of secretion, in addition to being a secreted product). This is covered in Chapter 9.

C. Differential Control of Amylin versus Insulin Secretion

In contrast to obligatory co-secretion, several papers describe inconstancy in the molar ratio of secreted amylin and insulin (Blackard *et al.*, 1992a,b, 1994; Dunning and Young, 1991; Hiramatsu *et al.*, 1994; Nieuwenhuis *et al.*, 1992a; O'Brien *et al.*, 1991; Pieber *et al.*, 1993, 1994). Several of

these studies did not consider differences in circulating amylin:insulin ratios that may be caused by differences in site of clearance and/or rate of clearance (which is generally faster for insulin than for amylin, and occurs principally at liver for insulin as compared to kidney for amylin). Human studies from Blackard's group (Blackard *et al.*, 1992a,b, 1994) and another group (Ludvik *et al.*, 1996) have addressed secreted ratios directly by measuring arterio-portal concentration differences (or approximations thereof). Such concentration differences are attributable to addition of peptide to splanchnic blood flow. The ratio of amylin:insulin in subjects in which secreted amounts were well correlated was 2.1–2.2% (mole amylin:mole insulin) (Blackard *et al.*, 1994). The ratio was higher in those subjects shown subsequently to be the least glucose tolerant.

In an isolated perfused pancreas preparation (in which kinetics becomes irrelevant), chronic hyperglycemia and treatment with dexamethasone increased the amylin:insulin secreted ratio (O'Brien *et al.*, 1991), while fasting decreased the ratio. Dexamethasone increased amylin mRNA more than it increased insulin mRNA, thus altering the amylin:insulin mRNA ratio (Bretherton-Watt *et al.*, 1989; Mulder *et al.*, 1995a). Mulder has compared the effects of several interventions that increased plasma glucose, including dexamethasone treatment and streptozotocin treatment (Mulder *et al.*, 1996a,c), and concluded that differential effects of these treatments upon amylin versus insulin mRNA were driven by hyperglycemia. There was no evidence that the amylin gene was aberrant in human type 2 diabetes or in MODY (maturity-onset diabetes of the young) (Tokuyama *et al.*, 1994).

In summary, it appears that, acutely, the secreted ratio of amylin:insulin is comparatively invariant, but long-standing hyperglycemia may favor induction of amylin synthesis and secretion over that of insulin.

IV. Circulating Amylin Concentrations

A. Assays

Several groups have published immunoassays that measure amylin and/or IAPP (Nakazato *et al.*, 1989; Pieber *et al.*, 1994; van Hulst *et al.*, 1993, 1994). Many authors, including those using these assays, often have not distinguished between amylin and IAPP, instead using the terms synonymously. Those studies, because they are likely to be measuring amylin immunoreactivity when they describe that of IAPP, are included here.

In a comparison of 10 assay formats from 8 participating laboratories, the inter-quartile range of results obtained using a "low" pooled standard from patients with type 1 diabetes was 0.3–3.6 pM, while with the "high" pooled standard from challenged patients with impaired glucose tolerance, the inter-quartile range was 8.6–25.3 pM (Manley and Hales, 1997).

Comparisons of plasma values reported from different authors should therefore be interpreted with caution.

B. Two-Site (Sandwich) Assays

For assessing plasma amylin concentrations, in this report, greater reliance has been placed upon two-site assays of the type developed at Amylin Pharmaceuticals Inc., in which the specificity of the analyte is assured through its capture at separate epitopes by monoclonal antibodies (Blase *et al.*; Koda *et al.*, 1993; Percy *et al.*, 1994, 1996, 1997; Petry *et al.*, 1995, Vine *et al.*, 1997a,b). With two-site (sandwich) assays, values in non-diabetic subjects range from 3.5 pM while fasting to 31.2 pM following a glucose challenge (Koda *et al.*, 1993); in non-diabetic lean individuals, values ranged from ~10 pM before meals to over 20 pM after meals (Koda *et al.*, 1995) (Fig. 3).

C. Normal Subjects

With standard radioimmunoassays, plasma amylin concentrations in fasted non-diabetic humans have been reported as 1.6 pM (Czyzyk *et al.*, 1996), 2.0 pM (Butler *et al.*, 1990a), 3.1 pM (Hartter *et al.*, 1991), 3.4 pM (Nakazato *et al.*, 1989), 5.0 pM (Mitsukawa *et al.*, 1992), 5.7 pM (van Jaarsveld *et al.*, 1993), 6.0 pM (van Hulst *et al.*, 1994), 6.4 pM (Sanke *et al.*,

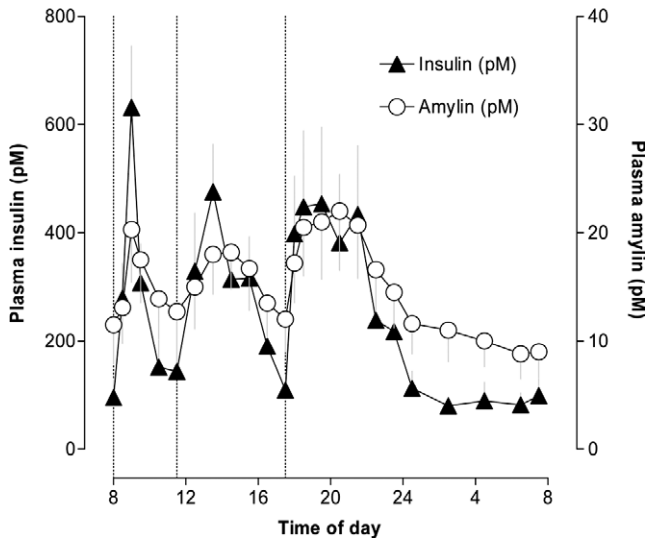

FIGURE 3 Twenty-four-hour concentration profiles (8 a.m. to 8 a.m.) of insulin and amylin in non-diabetic subjects. Standardized timing of standardized meals is indicated by dotted lines. Symbols are means ± SEM. Data from Koda *et al.* (1995).

1991), 6.4 pM (Hanabusa *et al*., 1992), 7.2 pM (Edwards *et al*., 1996), and 8.0 pM (Eriksson *et al*., 1992). Peak (stimulated values) in non-diabetic humans have been reported as 4.4–8.9 pM (Manley and Hales, 1997), 6.5 pM (Czyzyk *et al*., 1996), 10.3 pM (Edwards *et al*., 1996), 14.1 pM (Mitsukawa *et al*., 1992), and 16.8 pM (van Jaarsveld *et al*., 1993).

Basal and stimulated amylin concentrations either are unaffected by age in subjects with similar glucose tolerance (Mitsukawa *et al*., 1992) or are inconsistently associated with age (Edwards *et al*., 1992, 1996).

D. Amylin-like Peptides

A potential confounding influence in the measurement of plasma amylin concentrations was the identification of three additional immunoreactive species, distinguished by the addition of glycosylated moieties (Percy *et al*., 1994), as described in Section I.B in Chapter 1. It appears likely that most of the standard amylin radioimmunoassays will have detected total amylin-like immunoreactivity, since it was only from differences in signal with very selective monoclonal antibody pairs that the presence of these species was identified (Percy *et al*., 1994). Glycosylated forms are elevated relative to non-glycosylated amylin in early non-insulin-dependent diabetes mellitus (Fineman *et al*., 1994; Rittenhouse *et al*., 1996), in insulin resistance (Koda *et al*., 1996), in association with elevated split proinsulin (Wood *et al*., 1990), in hypertension, particularly in African-Americans (Dimsdale *et al*., 1996), and in gestational diabetes mellitus (Wareham *et al*., 1996).

E. Amylin in Insulin Deficiency

Insulin-deficient animals showed reduction or absence of amylin, whether insulin deficiency was invoked chemically with streptozotocin (Bretherton-Watt *et al*., 1989; Inman *et al*., 1990; Jamal *et al*., 1990; Mulder *et al*., 1995b, 1996c; Ogawa *et al*., 1990) or by autoimmune β-cell destruction, as with BB rats (Bretherton-Watt *et al*., 1991; Huang *et al*., 1991a,b). That is, loss of insulin secretion was associated with loss of amylin secretion. This indicates that any source of amylin that is outside of β-cells (for example, pancreatic δ-cells or the gastric antrum) must contribute comparatively little to overall circulating concentrations, or else the disappearance of amylin and insulin following β-cell destruction would be dissimilar.

In human type 1 diabetes, pancreatic amylin content was low (Tasaka *et al*., 1995). Plasma amylin concentrations were described as low (e.g., 0.7 pM, Hartter *et al*., 1991; 1.6 pM, Manley and Hales, 1997) or undetectable (van Hulst *et al*., 1994), and nutrient-stimulated increments in plasma concentration were either low (Hanabusa *et al*., 1992) or unmeasurable (Koda *et al*., 1992). Absence of amylin secretion was similarly observed in children with type 1 diabetes (Akimoto *et al*., 1993) (Fig. 4).

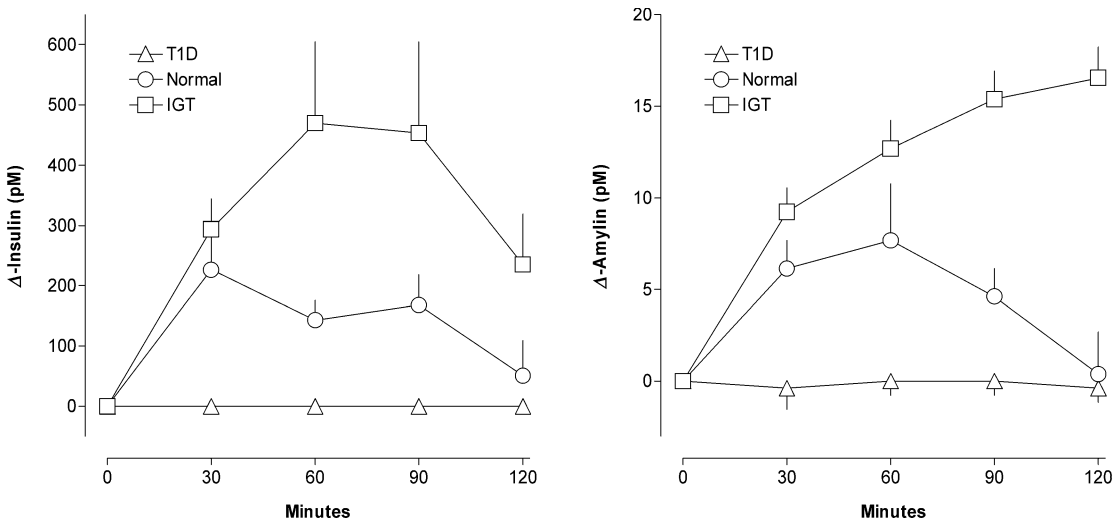

FIGURE 4 Changes in plasma concentration of insulin and amylin secretion following a 75 g oral glucose load in five non-diabetic subjects, five subjects with type 1 diabetes, and six subjects with impaired glucose tolerance. Symbols are means ± SEM. Data from Koda *et al.* (1992).

F. Amylin in Insulin Resistance

In insulin-resistant animals, amylin expression and plasma amylin concentrations were elevated (Bretherton-Watt *et al.*, 1989; Gill and Yen, 1991; Huang *et al.*, 1991b, 1992; Koranyi *et al.*, 1992; O'Brien *et al.*, 1991; Pieber *et al.*, 1994; Tokuyama *et al.*, 1991, 1993). This was especially true if insulin resistance was invoked with dexamethasone (Hiramatsu *et al.*, 1994; Jamal *et al.*, 1990, 1991; Koranyi *et al.*, 1992; Mulder *et al.*, 1995a; O'Brien *et al.*, 1991; Pieber *et al.*, 1993).

In insulin-resistant humans and those with impaired glucose tolerance, plasma amylin concentration was elevated (Enoki *et al.*, 1992; Eriksson *et al.*, 1992; Hanabusa *et al.*, 1992; Koda *et al.*, 1995, 1996; Ludvik *et al.*, 1991, 1996). In Pima Indians with impaired glucose tolerance (characterized by insulin resistance), fasting and peak stimulated plasma concentrations measured using a two-site assay were 6.0 pM and 43.5 pM, respectively (Koda *et al.*, 1993). Values in obese Caucasians with impaired glucose tolerance ranged from ~20 pM fasting to over 50 pM at a post-prandial peak (Koda *et al.*, 1995). Elevated amylin concentrations were present whether insulin resistance was invoked with dexamethasone (Culler *et al.*, 1993; Kautzky-Willer *et al.*, 1996a; Ludvik *et al.*, 1993; Sanke *et al.*, 1991), by normal pregnancy (Kautzky-Willer *et al.*, 1996b), or by gestational diabetes mellitus (Kautzky-Willer *et al.*, 1996b; Zweers *et al.*, 1992).

Thus, amylin excess appears to associate with insulin excess, just as amylin deficiency appears to associate with insulin deficiency, in animals and in humans. An exception to this association arises with insulinoma (Nieuwenhuis *et al.*, 1992a,b; Stridsberg *et al.*, 1995), in which plasma amylin concentrations do not obligatorily track with high insulin concentrations.

G. Amylin in Type 2 Diabetes Mellitus

Rodent models of type 2 diabetes typically exhibit elevations in plasma concentrations of both amylin and insulin (Gill and Yen, 1991; Tokuyama *et al.*, 1993; Pieber *et al.*, 1994). However, rodent type 2 models may differ from humans in the etiopathogenesis of diabetes. They are often characterized by extreme insulin resistance, while the human condition is characterized by a sequence of worsening insulin resistance followed by insulin secretory failure (Saad *et al.*, 1989). This sequence in humans was apparent in cross-sectional studies (Reaven and Miller, 1968) and was also apparent in individuals whose progression into diabetes was followed longitudinally (Saad *et al.*, 1989). Loss of glucose-mediated insulin secretion marked the transition from impaired glucose tolerance into diabetes (Swinburn *et al.*, 1995), consistent with the idea that insulin secretion was no longer sufficient to compensate for insulin resistance.

In accordance with descriptions of insulin secretion in human type 2 diabetes (but in contrast to the pattern in most rodent models), plasma

amylin concentrations and nutrient-stimulated increases in plasma concentration were reduced in diabetic patients compared to non-diabetic subjects (Enoki *et al*., 1992; Fineman *et al*., 1996; Hanabusa *et al*., 1992; Hartter *et al*., 1991; Ludvik *et al*., 1991, 1996; Mitsukawa *et al*., 1991; Rachman *et al*., 1996; Sanke *et al*., 1991; van Jaarsveld *et al*., 1993). The only exception appeared to be gestational diabetes mellitus (Kautzky-Willer *et al*., 1996b; Zweers *et al*., 1992), which may be more mechanistically aligned with the severe insulin resistance of rodent type 2 models.

Thus, in humans, both type 1 diabetes mellitus and the later stages of type 2 diabetes mellitus are characterized by deficiency of both insulin and amylin secretion. It appears that severity of β-cell secretory failure correlates with severity of amylin deficiency. This concordance of deficiencies in amylin and insulin secretion observed with the progression of diabetes mellitus is consistent with their co-localization in pancreatic β-cells.

In human type 2 diabetes, fasting and stimulated plasma amylin concentrations are generally lower in those treated with insulin (typically exhibiting greater β-cell failure) than in those treated with oral hypoglycemic agents. In patients with insulin-treated type 2 diabetes, fasting concentrations were 1.8 pM (Czyzyk *et al*., 1996), 2.1 pM (Hartter *et al*., 1991), and 2.7 pM (van Jaarsveld *et al*., 1993), and showed only a small increase upon stimulation (Koda *et al*., 1992), for example, to 2.3 pM (Czyzyk *et al*., 1996) or 6.1 pM (van Jaarsveld *et al*., 1993). In comparison, patients treated for type 2 diabetes with oral hypoglycemic agents had fasting plasma amylin concentrations that were somewhat higher (e.g., 4.8 pM, Hartter *et al*., 1991; 5.7 pM, van Jaarsveld *et al*., 1993; and 3.2 pM, Czyzyk *et al*., 1996) that became higher upon stimulation (e.g., to 9.4 pM, van Jaarsveld *et al*., 1993, and 9.8 pM, Czyzyk *et al*., 1996).

H. Amylin Concentrations in Other Conditions

Elevated plasma amylin concentrations are observed in hypertension (Dimsdale *et al*., 1996; Kailasam *et al*., 1995; Kautzky-Willer *et al*., 1994; Pacini *et al*., 1993), a condition also associated with insulin resistance (Ferrannini *et al*., 1990) and hyperinsulinemia (Welborn *et al*., 1966). Plasma amylin concentrations are also elevated in primary hyperparathyroidism (Valdemarsson *et al*., 1996), another condition associated with insulin resistance.

Elevations of plasma amylin concentration are reported in patients with renal failure (Ludvik *et al*., 1994; Watschinger *et al*., 1992). In this case, however, the elevation likely reflects reduced renal amylin clearance in those patients rather than increased secretion. Nephrectomy markedly reduces clearance of amylin and pramlintide in rats (Smith *et al*., 1996; Vine *et al*., 1996, 1998), indicating a major role for the kidney in clearance of circulating amylin.

V. Pharmacokinetic Studies

The study of pharmacokinetics of rat amylin and pramlintide in rats and of pramlintide and human amylin in humans has been enabled by the development of sensitive, specific, and linear two-site immunoassay systems.

A. Mechanisms of Amylin Elimination

The kinetics of elimination for insulin and amylin differed, with amylin having a longer half-life. This conclusion was supported by a mathematical model of β-cell peptide kinetics, in which clearance of amylin secreted in response to a glucose challenge was 2.6- to 4-fold lower than that of insulin (0.034–0.053 versus 0.14 min^{-1}, respectively) (Thomaseth *et al.*, 1996). The comparatively rapid elimination of insulin was due in large part to extraction on its first pass through the liver. In an isolated perfused liver preparation, ~50% of insulin was extracted on the first pass, while extraction of amylin was minimal (Nishimura *et al.*, 1992).

In contrast, nephrectomy markedly reduced amylin and pramlintide clearance in rats (Smith *et al.*, 1996; Vine *et al.*, 1996, 1998), indicating a major role for the kidney in clearance. This interpretation concurred with observations that metabolism by the kidney is also a critical route of elimination for many peptide hormones (Ardaillou and Paillard, 1980; Ardaillou *et al.*, 1970; Jorde *et al.*, 1981; Rabkin and Kitaji, 1983). For example, renal metabolism appeared responsible for elimination of up to 60% of the mammalian calcitonins (Ardaillou *et al.*, 1970), which structurally and functionally resemble amylin. The changes in amylin metabolism produced by nephrectomy were similar to those observed for calcitonin (Foster *et al.*, 1972) (Fig. 4).

Renal clearance of amylin inferred from nephrectomy studies was greater than glomerular filtration rate, and instead approached the value for renal plasma flow (Vine *et al.*, 1998) (Fig. 5), the implication being that plasma was cleared of amylin immunoreactivity not merely by filtration, but by renal peptidases associated with the vascular supply.

Amylin concentrations are elevated in renal failure (for example, 15.1 $\pm$ 3.2 versus 3.2 $\pm$ 0.2 pM; Ludvik *et al.*, 1994) (Clodi *et al.*, 1996; Ludvik *et al.*, 1990; Watschinger *et al.*, 1992), as is the case for other hormones metabolized by the kidney.

Enhanced responses in isolated skeletal muscle after application of protease inhibitors led to the proposal that muscle interstitium may also have been a site of degradation (Leighton *et al.*, 1992).

Amylin appeared not to cross the placental barrier at any appreciable rate. After administration of pramlintide to pregnant rats, concentrations in the amniotic fluid and fetal plasma were ~20 pM (1/5000 that of the maternal circulation) and were not different from levels of endogenous rat

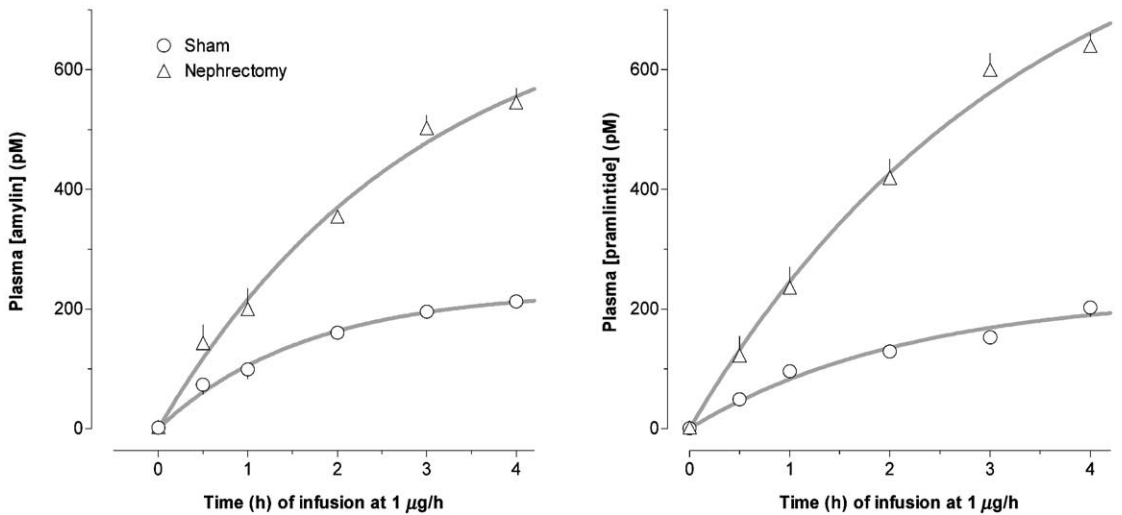

FIGURE 5 Changes in plasma concentrations of rat amylin (left panel) and pramlintide (right panel) in anesthetized rats with and without acute functional nephrectomy. Data from Vine *et al.* (1998).

amylin detected with the same assay prior to administration (Gedulin, unreported data). In the same preparation, human amylin, which was distinguishable from endogenous rat amylin, was undetectable in most fetal samples, despite being present at 8.8 nM (>10,000 times the limit of detection) in the maternal circulation.

In a 2-hr *ex vivo* perfusion of isolated human placental cotyledons, the ratio of maternal to fetal pramlintide concentration was ~500, indicating a low propensity of that amylin analog to cross the placenta (Hiles *et al.*, 2002, 2003).

B. Pharmacokinetics of Amylin in Rats

Subcutaneous bolus injections: Plasma concentrations after different subcutaneous doses of rat amylin resulted in peak concentrations that were approximately linearly dependent upon dose (Young *et al.*, 1996). The dose–concentration relationship following a subcutaneous bolus is quantified here as $C_{max} = 10^{(0.863*\log \text{dose})+2.131}$, where concentration is in pM and dose is in μg. Such relationships have been useful in the interpretation of physiological relevance in a number of dose–response studies. For example, peak plasma concentrations of subcutaneously injected rat amylin at 50% maximally effective gastric inhibitory doses were estimated from this relationship to be ~15 pM (Young *et al.*, 1995), within the circulating range. This supported the conclusion that amylin's effects on gastric emptying were physiological (Fig. 6).

Bioavailability of subcutaneous amylin was assessed as the ratio of the limiting area under the curve (AUC∞) obtained after subcutaneous injection to that obtained after the same dose administered intravenously. At the lowest dose administered (1 μg or ~3 μg/kg), bioavailability of subcutaneous rat amylin was 23.2% (Young *et al.*, 1996). Bioavailability was lower with larger subcutaneous doses.

Intravenous bolus: The terminal decay of plasma amylin concentration after an intravenous bolus injection of rat amylin in rats best fitted $t_{½}$ values between 13.0 and 13.4 min (Young *et al.*, 1996), similar to a previously reported $t_{½}$ of 13.1 min (Young *et al.*, 1993). Values of $t_{½}$ for pramlintide in rats were between 11.5 and 13.8 min, and volumes of distribution were 105 ± 10 ml (332 ± 32 ml/kg) (Young *et al.*, 1996). The dose–concentration relationship presented here for an intravenous bolus is $C_{max} = 10^{(1.04*\log \text{dose})+2.842}$, where concentration is in pM and dose is in μg.

Continuous intravenous infusions: Plasma amylin concentrations during and after continuous infusions at rates of 0.1, 1, 10, 100, or 1000 μg/hr enabled collection of precise estimates of clearance, the infusion rate concentration relation, and terminal (post-equilibration) $t_{½}$. Terminal (mono-component) $t_{½}$ values were between 9.6 and 17.4 min (Young *et al.*, 1996) (Fig. 7). The relationship shown here between steady-state concentration and infusion

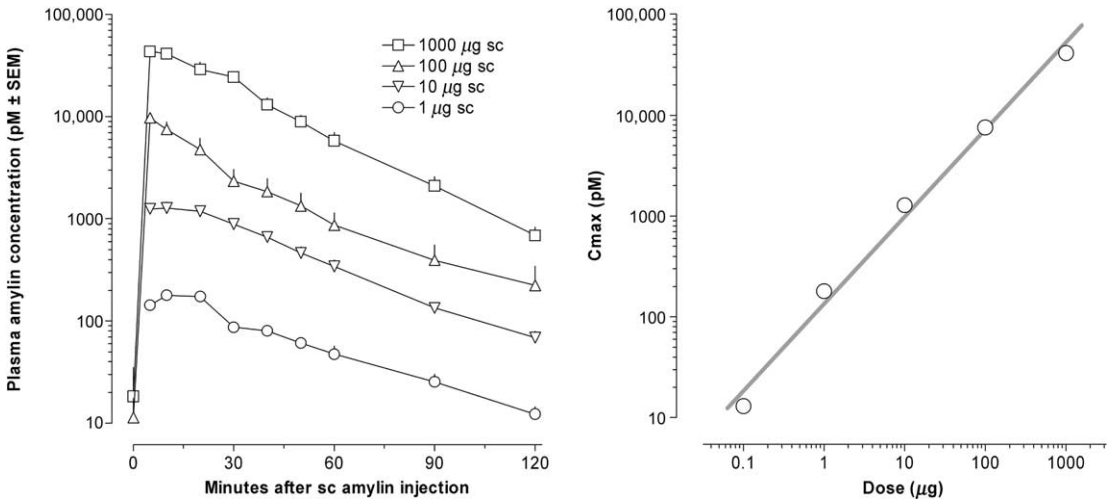

FIGURE 6 Plasma amylin concentrations following subcutaneous injections into rats. Relationship between dose and C_{max}. Data from Young *et al.* (1996).

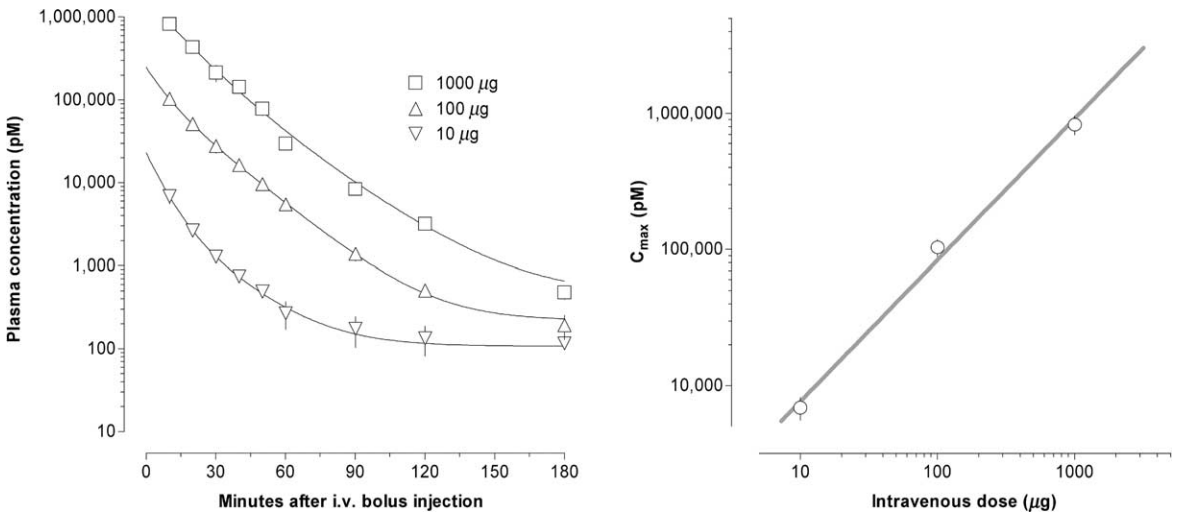

FIGURE 7 Plasma amylin concentrations following intravenous bolus injection into rats. Relationship between intravenous dose and C_{max}. Data from Young *et al.* (1996).

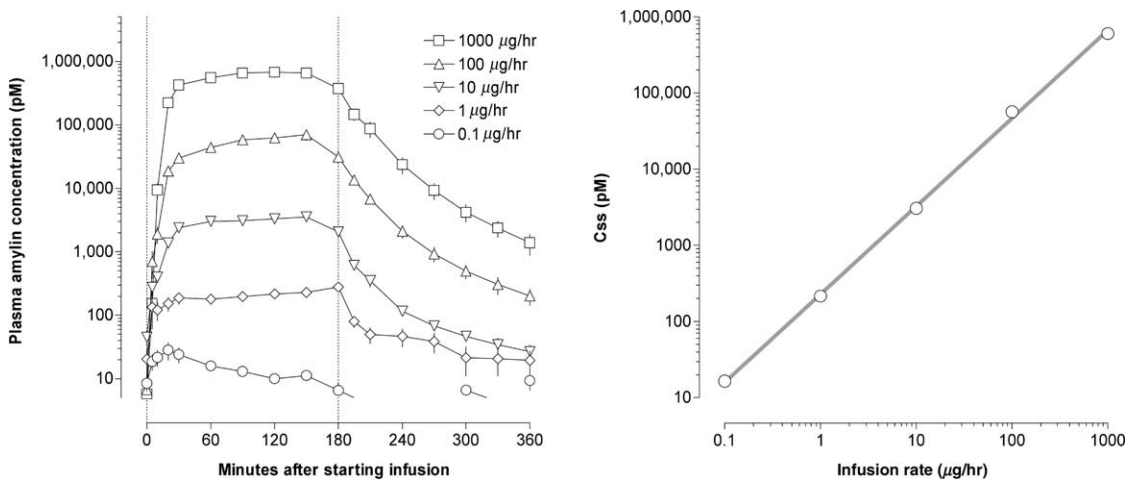

FIGURE 8 Plasma amylin concentrations following continuous intravenous infusions (0–180 min), and following cessation of infusion (180–360 min) in rats. Relationship between infusion rate and steady-state concentration, C_{ss}. Data from Young *et al.* (1996).

rate for amylin in rats was described by $C_{SS} = 10^{(1.155*\log \text{inf rate})+2.359}$, where concentration was in pM and infusion rate was in μg/hr. This relationship has been useful in estimating prevailing plasma amylin concentrations during continuous administration with, for example, Alzet osmotic minipumps.

All pharmacokinetic measures for pramlintide were similar to those of rat amylin at all doses and modes of administration. For example, $t_{½}$ was between 13.2 and 21.4 min after continuous intravenous infusion (Fig. 8).

C. Pharmacokinetics in Humans

Pharmacokinetic studies of exogenous amylinomimetics in humans either have been restricted to pramlintide (Colburn *et al.*, 1996; Moyses *et al.*, 1993; Redalieu *et al.*, 1996) or have reported parameters derived for endogenous amylin by fitting pharmacokinetic models to concentration profiles (Clodi *et al.*, 1996; Kautzky-Willer *et al.*, 1996a; Pacini *et al.*, 1993; Thomaseth *et al.*, 1996). After subcutaneous administration of high doses of pramlintide, $t_{½}$ ranged from 26 ± 8 to 42 ± 2 min (Moyses *et al.*, 1993). After intravenous bolus doses and continuous intravenous infusions, terminal half-lives were 21–47 and 20–46 min, respectively (Colburn *et al.*, 1996), and clearances were ~1 L/min. In one study in which human amylin was injected, modeled $t_{½}$ was 9.5 min and volume of distribution was 45 ml/kg (Clodi *et al.*, 1996) (Fig. 9).

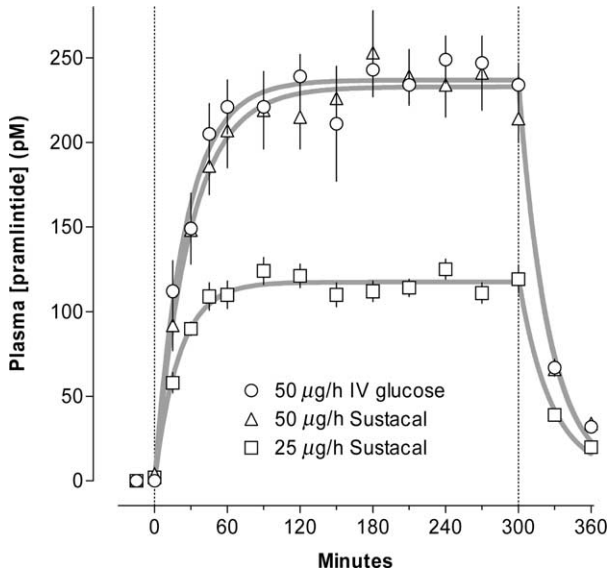

FIGURE 9 Plasma pramlintide concentrations following continuous intravenous infusions into volunteers with type 1 diabetes. Data from Moyses *et al.* (1993).

In summary, amylin is cleared at the kidney, most likely by proteolytic degradation. The terminal $t_{1/2}$ for rat amylin in rats is ~13 min, and that for pramlintide in humans is ~20–45 min.

References

Ahren, B., and Sundler, F. (1992). Localization of calcitonin gene-related peptide and islet amyloid polypeptide in the rat and mouse pancreas. *Cell Tissue Res.* **269**, 315–322.

Akimoto, K., Nakazato, M., Matsukura, S., and Hayakawa, K. (1993). Plasma concentration of islet amyloid polypeptide in healthy children and patients with insulin-dependent diabetes mellitus. *Acta Paediatr.* **82**, 310–311.

Ardaillou, R., and Paillard, F. (1980). Metabolism of polypeptide hormones by the kidney. *Adv. Nephrol. Necker Hosp.* **9**, 247–269.

Ardaillou, R., Sizonenko, P., Meyrier, A., Vallée, G., and Beaugas, C. (1970). Metabolic clearance rate of radioiodinated human calcitonin in man. *J. Clin. Invest.* **49**, 2345–2352.

Asai, J., Nakazato, M., Miyazato, M., Kangawa, K., Matsuo, H., and Matsukura, S. (1990). Regional distribution and molecular forms of rat islet amyloid polypeptide. *Biochem. Biophys. Res. Commun.* **169**, 788–795.

Blackard, W., Clore, J., Schroeder, D., and Kellum, J. (1992a). Amylin to insulin secretory ratios in obese subjects. *Diabetologia* **35**, A115.

Blackard, W., Clore, J., Schroeder, D., and Kellum, J. (1992b). Comparison of amylin and insulin secretory rates in obese non-diabetic man using portal vein catheterization. *Diabetes* **41**.

Blackard, W. G., Clore, J. N., and Kellum, J. M. (1994). Amylin/insulin secretory ratios in morbidly obese man: Inverse relationship with glucose disappearance rate. *J. Clin. Endocrinol. Metab.* **78**, 1257–1260.

Blase, E. K., Koda, J. E., and Phelps, J. L. Monoclonal antibody to the c-terminal end of human amylin—used in assays for the detection of human amylin or amylin analogues. *EP 408294; WO 9211863; WO 9216845.*

Bretherton-Watt, D., Ghatei, M. A., Bloom, S. R., Jamal, H., Ferrier, G. J., Girgis, S. I., and Legon, S. (1989). Altered islet amyloid polypeptide (amylin) gene expression in rat models of diabetes. *Diabetologia* **32**, 881–883.

Bretherton-Watt, D., Ghatei, M. A., Legon, S., Jamal, H., Suda, K., and Bloom, S. R. (1991). Depletion of islet amyloid polypeptide in the spontaneously diabetic (BB) Wistar rat. *J. Mol. Endocrinol.* **6**, 3–7.

Butler, P. C., Chou, J., Carter, W. B., Wang, Y. N., Bu, B. H., Chang, D., Chang, J. K., and Rizza, R. A. (1990a). Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. *Diabetes* **39**, 752–756.

Butler, P. C., Chou, J., Wang, Y. N., Bu, B. H., Chang, D., Carter, B., and Rizza, R. (1990b). Amylin is co-secreted with insulin in man. *Clin. Res.* **38**, 307A.

Chou, J., Butler, P. C., Wang, Y. N., Bu, B. H., Chang, D., Carter, B., Rizza, R., and Chang, J. K. (1990). Co-secretion of amylin and insulin in the rat. *Diabetes* **39**, 133A.

Clodi, M., Hermann, K., Pacini, G., Thomaseth, K., Kautzky-Willer, A., Prager, R., and Ludvik, B. (1996). Islet amyloid polypeptide distribution and kinetics in humans and its relationship to kidney function. *Diabetes* **45**, 232A.

Colburn, W. A., Gottlieb, A. B., Koda, J., and Kolterman, O. G. (1996). Pharmacokinetics and pharmacodynamics of AC137 (25,28,29 tripro-amylin, human) after intravenous bolus and infusion doses in patients with insulin-dependent diabetes. *J. Clin. Pharmacol.* **36**, 13–24.

Cooper, G. J. S., Willis, A. C., Clark, A., Turner, R. C., Sim, R. B., and Reid, K. B. (1987). Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc. Natl. Acad. Sci. USA* **84**, 8628–8632.

Cooper, G. J. S., Leighton, B., Dimitriadis, G. D., Parry-Billings, M., Kowalchuk, J. M., Howland, K., Rothbard, J. B., Willis, A. C., and Reid, K. B. (1988). Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc. Natl. Acad. Sci. USA* **85**, 7763–7766.

Culler, F. L., Koda, J. E., and Meacham, L. R. (1993). Increased amylin secretion is a feature of glucocorticoid induced insulin resistance in man. *Clin. Res.* **41**, 102A.

Czyzyk, A., Zapecka-Dubno, B., and Kasperska-Dworak, A. (1996). Metformin lowers the level of plasma amylin in patients with NIDDM. *Diabetes* **45**, 289A.

D'Este, L., Buffa, R., Pelagi, M., Siccardi, A. C., and Renda, T. (1994). Immunohistochemical localization of chromogranin A and B in the endocrine cells of the alimentary tract of the green frog, Rana esculenta. *Cell Tissue Res.* **277**, 341–349.

D'Este, L., Wimalawansa, S. J., and Renda, T. G. (1995). Amylin-immunoreactivity is co-stored in a serotonin cell subpopulation of the vertebrate stomach and duodenum. *Arch. Histol. Cytol.* **58**, 537–547.

De Vroede, M., Foriers, A., Van de Winkel, M., Madsen, O., and Pipeleers, D. (1992). Presence of islet amyloid polypeptide in rat islet B and D cells determines parallelism and dissociation between rat pancreatic islet amyloid polypeptide and insulin content. *Biochem. Biophys. Res. Commun.* **182**, 886–893.

Denijn, M., De Weger, R. A., Van Mansfeld, A. D., van Unnik, J. A., and Lips, C. J. (1992). Islet amyloid polypeptide (IAPP) is synthesized in the islets of Langerhans. Detection of IAPP polypeptide and IAPP mRNA by combined in situ hybridization and immunohistochemistry in rat pancreas. *Histochemistry* **97**, 33–37.

Dilts, R. P., Phelps, J., Koda, J., and Beaumont, K. (1995). Comparative distribution of amylin and calcitonin gene related peptide (CGRP): Immunoreactivities in the adult rat brain. *Soc. Neurosci. Abstr.* **21**, 1116.

Dimsdale, J. E., Kolterman, O., Koda, J., and Nelesen, R. (1996). Effect of race and hypertension on plasma amylin concentrations. *Hypertension* **27**, 1273–1276.

Dunning, B. E., and Young, D. A. (1991). Plasma and pancreatic amylin to insulin ratios are markedly elevated in obese SHR/N-cp rats. *Diabetes* **40**, 216A.

Edwards, B. J., Perry, H. M., Kaiser, F. E., Morley, J. E., Kraenzle, D., Kreutter, D. K., and Stevenson, R. W. (1996). Age-related changes in amylin secretion. *Mech. Ageing Dev.* **86**, 39–51.

Edwards, B. J. A., Perry, H. M., Kaiser, F. E., Morley, J. E., Kraenzle, D., Kreutter, D., and Stevenson, R. (1992). Age related changes in amylin secretion. *Clin. Res.* **40**, 197A.

Enoki, S., Mitsukawa, T., Takemura, J., Nakazato, M., Aburaya, J., Toshimori, H., and Matsukara, S. (1992). Plasma islet amyloid polypeptide levels in obesity, impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabet. Res. Clin. Pract.* **15**, 97–102.

Eriksson, J., Nakazato, M., Miyazato, M., Shiomi, K., Matsukura, S., and Groop, L. (1992). Islet amyloid polypeptide plasma concentrations in individuals at increased risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **35**, 291–293.

Ferrannini, E., Haffner, S. M., and Stern, M. P. (1990). Essential hypertension—an insulin-resistant state. *J. Cardiovasc. Pharmacol.* **15**, S18–S25.

Fineman, M. S., Koda, J. E., Percy, A., Wareham, N. J., and Hales, C. N. (1994). Elevation of high molecular weight amylin-like peptides along with proinsulin and 32,33 split proinsulin in newly diagnosed diabetics. *Diabetologia* **37**, A52.

Fineman, M. S., Giotta, M. P., Thompson, R. G., Kolterman, O. G., and Koda, J. E. (1996). Amylin response following Sustacal® ingestion is diminished in type II diabetic patients treated with insulin. *Diabetologia* **39**, A149.

Foster, G. V., Clark, M. B., Williams, C., Nathanson, B. M., Horton, R., Buranapong, P., and Glass, H. I. (1972). Metabolic fate of human calcitonin in the dog. *In* "Endocrinology 1971" (S. Taylor, Ed.), pp. 72–78. Heinemann Books, London.

Gilbey, S. G., Ghatei, M. A., Bretherton-Watt, D., Zaidi, M., Jones, P. M., Perera, T., Beacham, J., Girgis, S., and Bloom, S. R. (1991). Islet amyloid polypeptide: Production by an osteoblast cell line and possible role as a paracrine regulator of osteoclast function in man. *Clin. Sci. (Colch)* **81**, 803–808.

Gill, A. M., and Yen, T. T. (1991). Effects of ciglitazone on endogenous plasma islet amyloid polypeptide and insulin sensitivity in obese-diabetic viable yellow mice. *Life Sci.* **48**, 703–710.

Hammonds, P., Palmieri, L., Troge, J., and Mertz, R. (1991). Co-ordinate regulation of insulin and amylin secretion from rat islets of Langerhans and HIT-T15 β-cells. *J. Cell Biochem.* **15B**, 66.

Hanabusa, T., Kubo, K., Oki, C., Nakano, Y., Okai, K., Sanke, T., and Nanjo, K. (1992). Islet amyloid polypeptide (IAPP) secretion from islet cells and its plasma concentration in patients with non-insulin-dependent diabetes mellitus. *Diabet. Res. Clin. Pract.* **15**, 89–96.

Hartter, E., Svoboda, T., Ludvik, B., Schuller, M., Lell, B., Kuenburg, E., Brunnbauer, M., Woloszczuk, W., and Prager, R. (1991). Basal and stimulated plasma levels of pancreatic amylin indicate its co-secretion with insulin in humans. *Diabetologia* **34**, 52–54.

Hiles, R. A., Bawdon, R. E., and Petrella, E. (2002). An evaluation of the potential of two peptides, pramlintide and AC2993 (synthetic exendin-4), to cross the human placental barrier using an *ex vivo* perfusion system. *Toxicologist* **66**, 237.

Hiles, R. A., Bawdon, R. E., and Petrella, E. M. (2003). *Ex vivo* human placental transfer of the peptides pramlintide and exenatide (synthetic exendin-4). *Hum. Exp. Toxicol.* **22**, 623–628.

Hiramatsu, S., Inoue, K., Sako, Y., Umeda, F., and Nawata, H. (1994). Insulin treatment improves relative hypersecretion of amylin to insulin in rats with non-insulin-dependent diabetes mellitus induced by neonatal streptozocin injection. *Metabolism* **43**, 766–770.

Huang, H.-J. S., Cooper, G. J. S., Young, A. A., and Johnson, M. J. (1991a). Deficiency of amylin expression in the pancreas of auto-immune BB/Wor diabetic rats. *J. Cell Biochem.* **15**(Part B), 67.

Huang, H.-J. S., Young, A. A., Johnson, J. M., Gedulin, B., and Cooper, G. J. S. (1991b). Amylin mRNA in the autoimmune diabetic BB/Wor and insulin-resistant LA/N-cp rat. *Diabetes* **40**, 46A.

Huang, H.-J. S., Young, A. A., Koda, J. E., Tulp, O. L., Johnson, M. J., and Cooper, G. J. (1992). Hyperamylinemia, hyperinsulinemia, and insulin resistance in genetically obese LA/N-cp rats. *Hypertension* **19**, I101–I109.

Inman, L., LeChago, J., Roitelman, Y., and Luskey, K. (1990). Pancreatic beta cell expression of amylin in streptozotocin-induced diabetes in rats. *Diabetes* **39**, 143A.

Jamal, H., Bretherton-Watt, D., Suda, K., Ghatei, M. A., and Bloom, S. R. (1990). Islet amyloid polypeptide-like immunoreactivity (amylin) in rats treated with dexamethasone and streptozotocin. *J. Endocrinol.* **126**, 425–429.

Jamal, H., Bretherton-Watt, D., Suda, K., Wang, Z. L., Ghatei, M. A., Williams, S., and Bloom, S. R. (1991). Changes in islet peptide content after dexamethasone treatment. *J. Endocrinol.* **129**, 93.

Jamal, H., Suda, K., Bretherton-Watt, D., Ghatei, M. A., and Bloom, S. R. (1993). Molecular form of islet amyloid polypeptide (amylin) released from isolated rat islets of Langerhans. *Pancreas* **8**, 261–266.

Johnson, K. H., O'Brien, T. D., Hayden, D. W., Jordan, K., Ghobrial, H. K., Mahoney, W. C., and Westermark, P. (1988). Immunolocalization of islet amyloid polypeptide (IAPP) in

pancreatic beta cells by means of peroxidase-antiperoxidase (PAP) and protein A-gold techniques. *Am. J. Pathol.* **130**, 1–8.

Jorde, R., Burhol, P. G., Gunnes, P., and Schulz, T. B. (1981). Removal of IR-GIP by the kidneys in man, and the effect of acute nephrectomy on plasma GIP in rats. *Scand. J. Gastroenterol.* **16**, 469–471.

Kailasam, M. T., Fineman, M. S., Koda, J. E., Parmer, R. J., and O'Connor, D. T. (1995). Circulating amylin in human essential hypertension: Racial differences and early elevations in subjects at genetic risk of hypertension. *J. Investig. Med.* **43**, 266A.

Kanatsuka, A., Makino, H., Ohsawa, H., Tokuyama, Y., Yamaguchi, T., Yoshida, S., and Adachi, M. (1989). Secretion of islet amyloid polypeptide in response to glucose. *FEBS Lett.* **259**, 199–201.

Kautzky-Willer, A., Thomaseth, K., Pacini, G., Clodi, M., Ludvik, B., Streli, C., Waldhausl, W., and Prager, R. (1994). Role of islet amyloid polypeptide secretion in insulin-resistant humans. *Diabetologia* **37**, 188–194.

Kautzky-Willer, A., Thomaseth, K., Clodi, M., Ludvik, B., Waldhausl, W., Prager, R., and Pacini, G. (1996a). Beta-cell activity and hepatic insulin extraction following dexamethasone administration in healthy subjects. *Metab. Clin. Exp.* **45**, 486–491.

Kautzky-Willer, A., Thomaseth, K., Nowotny, P., Ludvik, B., Waldhäusl, W., and Prager, R. (1996b). Elevated proinsulin and islet amyloid pancreatic polypeptide (IAPP) secretion in gestational diabetes. *Diabetes* **45**, 174A.

Koda, J., Fineman, M., Percy, A., Blase, E., and Lillioja, S. (1993). Use of a new two-site immunoassay for amylin to characterize amylin hormone response in Pima Indians. *Diabetologia* **36**, A137.

Koda, J. E., Fineman, M., Rink, T. J., Dailey, G. E., Muchmore, D. B., and Linarelli, L. G. (1992). Amylin concentrations and glucose control. *Lancet* **339**, 1179–1180.

Koda, J. E., Fineman, M. S., Kolterman, O. G., and Caro, J. F. (1995). 24 hour plasma amylin profiles are elevated in IGT subjects vs. normal controls. *Diabetes* **44**(suppl. 1), 238A.

Koda, J. E., Nyholm, B., Fineman, M. S., Hove, K. Y., and Schmitz, O. (1996). Plasma concentrations of amylin reflect insulin sensitivity in relatives of patients with NIDDM and in healthy subjects. *Diabetologia* **39**, A68.

Koranyi, L., Bourey, R., Turk, J., Mueckler, M., and Permutt, M. A. (1992). Differential expression of rat pancreatic islet beta-cell glucose transporter (GLUT 2), proinsulin and islet amyloid polypeptide genes after prolonged fasting, insulin-induced hypoglycaemia and dexamethasone treatment. *Diabetologia* **35**, 1125–1132.

Leffert, J. D., Chick, W. L., and Luskey, K. L. (1989). Islet specific expression of rat amylin. *Clin. Res.* **37**, 571A.

Leighton, B., Chantry, A., and Foot, E. A. (1992). Skeletal muscle may be a site for degradation of CGRP and possibly amylin. *Diabetologia* **35**, A18.

Li, Z., Shi, A., and Karlsson, F. A. (2002). Transient increase of rat gastric amylin in the neonatal period and in experimental ulcers. *J. Gastroenterol.* **37**, 172–176.

Ludvik, B., Berzlanovich, A., Hartter, E., Lell, B., Prager, R., and Graf, H. (1990). Increased amylin levels in patients on chronic hemodialysis. *Nephrol. Dial. Transplant.* **5**, 694–695.

Ludvik, B., Lell, B., Hartter, E., Schnack, C., and Prager, R. (1991). Decrease of stimulated amylin release precedes impairment of insulin secretion in type II diabetes. *Diabetes* **40**, 1615–1619.

Ludvik, B., Clodi, M., Kautzky-Willer, A., Capek, M., Hartter, E., Pacini, G., and Prager, R. (1993). Effect of dexamethasone on insulin sensitivity, islet amyloid polypeptide and insulin secretion in humans. *Diabetologia* **36**, 84–87.

Ludvik, B., Clodi, M., Kautzky-Willer, A., Schuller, M., Graf, H., Hartter, E., Pacini, G., and Prager, R. (1994). Increased levels of circulating islet amyloid polypeptide in patients with chronic renal failure have no effect on insulin secretion. *J. Clin. Invest.* **94**, 2045–2050.

Ludvik, B., Nolan, J. J., Thomaseth, K., Pacini, G., Clodi, M., and Prager, R. (1996). Direct assessment of secretion, kinetics and clearance of beta-cell peptides during hepatic vein catheterization. *Diabetes* **45**, 253A.

Lukinius, A., Wilander, E., Westermark, G. T., Engstrom, U., and Westermark, P. (1989). Co-localization of islet amyloid polypeptide and insulin in the B cell secretory granules of the human pancreatic islets. *Diabetologia* **32**, 240–244.

Lukinius, A., Korsgren, O., Grimelius, L., and Wilander, E. (1996). Expression of islet amyloid polypeptide in fetal and adult porcine and human pancreatic islet cells. *Endocrinology* **137**, 5319–5325.

Lutz, T. A., and Rand, J. S. (1997). Detection of amyloid deposition in various regions of the feline pancreas by different staining techniques. *J. Comp. Pathol.* **116**, 157–170.

Manley, S., and Hales, C. N. (1997). Interim report from an international workshop for the comparison of amylin assays. *Diabetes* **46**, 344A.

Mitsukawa, T., Takemura, J., Asai, J., Nakazato, M., Kangawa, K., Matsuo, H., and Matsukura, S. (1990). Islet amyloid polypeptide response to glucose, insulin, and somatostatin analogue administration. *Diabetes* **39**, 639–642.

Mitsukawa, T., Toshimori, H., Nakazato, M., Takemura, J., and Matsukura, S. (1991). A study of serial plasma islet amyloid polypeptide IAPP levels in an obese NIDDM patient associated with diabetic ketoacidosis. *J. Jpn. Diabet. Soc.* **34**, 543–548.

Mitsukawa, T., Takemura, J., Nakazato, M., Asai, J., Kanagawa, K., Matsuo, H., and Matsukura, S. (1992). Effects of aging on plasma islet amyloid polypeptide basal level and response to oral glucose load. *Diabet. Res. Clin. Pract.* **15**, 131–134.

Miyazato, M., Nakazato, M., Shiomi, K., Aburaya, J., Toshimori, H., Kangawa, K., Matsuo, H., and Matsukura, S. (1991). Identification and characterization of islet amyloid polypeptide in mammalian gastrointestinal tract. *Biochem. Biophys. Res. Commun.* **181**, 293–300.

Moyses, C., Kolterman, O., and Mant, T. (1993). Pharmacokinetics and hyperglycaemic effects of the amylin analogue, AC137, in man. *Diabet. Med.* **10**, S25.

Mulder, H., Lindh, A. C., and Sundler, F. (1993). Islet amyloid polypeptide gene expression in the endocrine pancreas of the rat—a combined in situ hybridization and immunocytochemical study. *Cell Tissue Res.* **274**, 467–474.

Mulder, H., Lindh, A. C., Ekblad, E., Westermark, P., and Sundler, F. (1994). Islet amyloid polypeptide is expressed in endocrine cells of the gastric mucosa in the rat and mouse. *Gastroenterology* **107**, 712–719.

Mulder, H., Ahren, B., Stridsberg, M., and Sundler, F. (1995a). Non-parallelism of islet amyloid polypeptide (amylin) and insulin gene expression in rat islets following dexamethasone treatment. *Diabetologia* **38**, 395–402.

Mulder, H., Ahren, B., and Sundler, F. (1995b). Differential expression of islet amyloid polypeptide (amylin) and insulin in experimental diabetes in rodents. *Mol. Cell Endocrinol.* **114**, 101–109.

Mulder, H., Leckstrom, A., Uddman, R., Ekblad, E., Westermark, P., and Sundler, F. (1995c). Islet amyloid polypeptide (amylin) is expressed in sensory neurons. *J. Neurosci* **15**, 7625–7632.

Mulder, H., Ahren, B., and Sundler, F. (1996a). Islet amyloid polypeptide (amylin) and insulin are differentially expressed in chronic diabetes induced by streptozotocin in rats. *Diabetologia* **39**, 649–657.

Mulder, H., Ahren, B., and Sundler, F. (1996b). Islet amyloid polypeptide and insulin gene expression are regulated in parallel by glucose *in vivo* in rats. *Am. J. Physiol.* **271**, E1008–E1014.

Mulder, H., Myrsen, U., Ahren, B., and Sundler, F. (1996c). Differential expression of islet amyloid polypeptide and insulin correlates with the severity of experimental diabetes. *Diabetes* **45**, 292A.

Mulder, H., Ekelund, M., Ekblad, E., and Sundler, F. (1997a). Islet amyloid polypeptide in the gut and pancreas: Localization, ontogeny and gut motility effects. *Peptides* **18**, 771–783.

Mulder, H., Zhang, Y. Z., Danielsen, N., and Sundler, F. (1997b). Islet amyloid polypeptide and calcitonin gene-related peptide expression are down-regulated in dorsal root ganglia upon sciatic nerve transection. *Mol. Brain Res.* **47**, 322–330.

Nakazato, M., Asai, J., Kangawa, K., Matsukura, S., and Matsuo, H. (1989). Establishment of radioimmunoassay for human islet amyloid polypeptide and its tissue content and plasma concentration. *Biochem. Biophys. Res. Commun.* **164**, 394–399.

Nakazato, M., Miyazato, M., Asai, J., Mitsukawa, T., Kangawa, K., Matsuo, H., and Matsukura, S. (1990). Islet amyloid polypeptide, a novel pancreatic peptide, is a circulating hormone secreted under glucose stimulation. *Biochem. Biophys. Res. Commun.* **169**, 713–718.

Nicholl, C. G., Bhatavdekar, J. M., Mak, J., Girgis, S. I., and Legon, S. (1992). Extra-pancreatic expression of the rat islet amyloid polypeptide (amylin) gene. *J. Mol. Endocrinol.* **9**, 157–163.

Nieuwenhuis, M. G., van Hulst, K. L., Hackeng, W. H. L., and Lips, C. J. M. (1992a). Islet amyloid polypeptide plasma concentrations in patients with insulinoma. *Diabetologia* **35**, A119.

Nieuwenhuis, M. G., van Mansfeld, A. D., van Unnik, J. A., Berends, M. J., and Lips, C. J. (1992b). No constant relationship between islet amyloid polypeptide (IAPP) and insulin expression in insulinomas. *Neth. J. Med.* **41**, 264–271.

Nishimura, S., Sanke, T., Machida, K., Bessho, H., Hanabusa, T., Nakai, K., and Nanjo, K. (1992). Lack of effect of islet amyloid polypeptide on hepatic glucose output in the in situ-perfused rat liver. *Metabolism* **41**, 431–434.

O'Brien, T. D., Westermark, P., and Johnson, K. H. (1991). Islet amyloid polypeptide and insulin secretion from isolated perfused pancreas of fed, fasted, glucose-treated, and dexamethasone-treated rats. *Diabetes* **40**, 1701–1706.

Ogawa, A., Harris, V., McCorkle, S. K., Unger, R. H., and Luskey, K. L. (1990). Amylin secretion from the rat pancreas and its selective loss after streptozotocin treatment. *J. Clin. Invest.* **85**, 973–976.

Ohtsuka, H., Iwanaga, T., Fujino, M. A., and Fujita, T. (1993). Amylin-containing cells in the gastro-entero-pancreatic (GeP) endocrine system of the rat and humans—an immunohistochemical study. *Acta Histochem. Cytochem.* **26**, 405–414.

Pacini, G., Thomaseth, K., Kautzky-Miller, A., Clodi, M., Waldhäusl, W., and Prager, R. (1993). Islet amyloid polypeptide secretion and kinetics in insulin resistant hypertensive patients. *Diabetologia* **36**, A136.

Percy, A. J., Trainor, D., Rittenhouse, J., Janes, S., and Koda, J. E. (1994). Sensitive two-site enzyme immunoassays for amylin and amylin-like peptides in human plasma. *Diabetologia* **37**, A117.

Percy, A. J., Trainor, D. A., Rittenhouse, J., Phelps, J., and Koda, J. E. (1996). Development of sensitive immunoassays to detect amylin and amylin-like peptides in unextracted plasma. *Clin. Chem.* **42**, 576–585.

Percy, A. J., Trainor, D., Blase, E., Redalieu, E., and Koda, J. E. (1997). Development of a sensitive immunoassay for pramlintide and its use in pharmacokinetic studies. *J. Clin. Ligand Assay* **20**, 32–39.

Petry, C. J., Percy, A., Koda, J. E., and Hales, C. N. (1995). Development of sensitive two-site immunoassays for rat amylin. Proceedings of the ACB National Meeting, p. 75.

Pieber, T. R., Stein, D. T., Ogawa, A., Alam, T., Ohneda, M., Mccorkle, K., Chen, L., Mcgarry, J. D., and Unger, R. H. (1993). Amylin-insulin relationships in insulin resistance with and without diabetic hyperglycemia. *Am. J. Physiol.* **265**, E446–E453.

Pieber, T. R., Roitelman, J., Lee, Y., Luskey, K. L., and Stein, D. T. (1994). Direct plasma radioimmunoassay for rat amylin-(1–37): Concentrations with acquired and genetic obesity. *Am. J. Physiol.* **267**, E156–E164.

Rabkin, R., and Kitaji, J. (1983). Renal metabolism of peptide hormones. *Miner. Electrolyte Metab.* **9**, 212–226.

Rachman, J., Payne, M., Levy, J., Barrow, B., Manley, S., Holman, R., and Turner, R. (1996). Post-prandial amylin concentrations are increased by sulphonylurea, but unchanged by basal insulin, in NIDDM. *Diabetologia* **39**, A149.

Reaven, G. M., and Miller, R. (1968). Study of the relationship between glucose and insulin responses to an oral glucose load in man. *Diabetes* **17**, 560–569.

Redalieu, E., Thompson, R. G., Dean, E., Petrella, E., Musunuri, S., and Gottlieb, A. (1996). Pharmacokinetics of pramlintide and free insulin following combined or separate injections in patients with type I diabetes. *Diabetes* **45**, 220A.

Rittenhouse, J., Chait, B. T., Bierle, J. R., Janes, S. M., Park, D. R., Phelps, J. L., Fineman, M. S., Qin, J., and Koda, J. E. (1996). Heterogeneity of naturally-occurring human amylin due to glycosylation. *Diabetes* **45**, 234A.

Röcken, C., Linke, R. P., and Saeger, W. (1992). Immunohistology of islet amyloid polypeptide in diabetes mellitus: Semi-quantitative studies in a post-mortem series. *Virchows Arch. A Pathol. Anat. Histopathol.* **421**, 339–344.

Saad, M. F., Knowler, W. C., Pettitt, D. J., Nelson, R. G., Mott, D. M., and Bennett, P. H. (1989). Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. *Lancet* **1**, 1356–1359.

Sanke, T., Bell, G. I., Sample, C., Rubenstein, A. H., and Steiner, D. F. (1988). An islet amyloid peptide is derived from an 89-amino acid precursor by proteolytic processing. *J. Biol. Chem.* **263**, 17243–17246.

Sanke, T., Hanabusa, T., Nakano, Y., Oki, C., Okai, K., Nishimura, S., Kondo, M., and Nanjo, K. (1991). Plasma islet amyloid polypeptide (Amylin) levels and their responses to oral glucose in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* **34**, 129–132.

Shiomi, K., Nakazato, M., Miyazato, M., Kangawa, K., Matsuo, H., and Matsukura, S. (1992). Establishment of hypersensitive radioimmunoassay for islet amyloid polypeptide using antiserum specific for its N-terminal region. *Biochem. Biophys. Res. Commun.* **186**, 1065–1073.

Skofitsch, G., Wimalawansa, S. J., Jacobowitz, D. M., and Gubisch, W. (1995). Comparative immunohistochemical distribution of amylin-like and calcitonin gene related peptide like immunoreactivity in the rat central nervous system. *J. Physiol. Pharmacol. Can. J. Physiol. Pharmacol.* **73**, 945–956.

Smith, P., La Chappell, R., Blaise, E., Gedulin, B., Young, A., and Vine, W. (1996). Effects of nephrectomy on amylin action and plasma concentration in rats. *Program and Abstracts, 10th International Congress of Endocrinology*, p. 418.

Stridsberg, M., Eriksson, B., Lundqvist, G., Skogseid, B., Wilander, E., and Oberg, K. (1995). Islet amyloid polypeptide (IAPP) in patients with neuroendocrine tumours. *Regul. Pept.* **55**, 119–131.

Swinburn, B. A., Gianchandani, R., Saad, M. F., and Lillioja, S. (1995). *In vivo* beta-cell function at the transition to early non-insulin-dependent diabetes mellitus. *Metabolism* **44**, 757–764.

Tasaka, Y., Nakaya, F., Matsumoto, H., Iwamoto, Y., and Omori, Y. (1995). Pancreatic amylin content in human diabetic subjects and its relation to diabetes. *Pancreas* **11**, 303–308.

Thomaseth, K., Kautzky Willer, A., Ludvik, B., Prager, R., and Pacini, G. (1996). Integrated mathematical model to assess beta-cell activity during the oral glucose test. *Am. J. Physiol.* **33**, E522–E531.

Tokuyama, Y., Kanatsuka, A., Ohsawa, H., Yamaguchi, T., Makino, H., Yoshida, S., Nagase, H., and Inoue, S. (1991). Hypersecretion of islet amyloid polypeptide from pancreatic islets of ventromedial hypothalamic-lesioned rats and obese Zucker rats. *Endocrinology* **128**, 2739–2744.

Tokuyama, Y., Kanatsuka, A., Yamaguchi, T., Ohsawa, H., Makino, H., Nishimura, M., and Yoshida, S. (1993). Islet amyloid polypeptide/amylin contents in pancreata increase in genetically obese and diabetic mice. *Horm. Metab. Res.* **25**, 289–291.

Tokuyama, Y., Kanatsuka, A., Suzuki, Y., Yamaguchi, T., Taira, M., Makino, H., and Yoshida, S. (1994). Islet amyloid polypeptide gene: No evidence of abnormal promoter region in thirty-five type 2 diabetic patients. *Diabet. Res. Clin. Pract.* **22**, 99–105.

Toshimori, H., Narita, R., Nakazato, M., Asai, J., Mitsukawa, T., Kangawa, K., Matsuo, H., and Matsukura, S. (1990). Islet amyloid polypeptide (IAPP) in the gastrointestinal tract and pancreas of man and rat. *Cell Tissue Res.* **262**, 401–406.

Unger, R. H., and Foster, D. W. (1992). Ch 24: Diabetes Mellitus. *In* "Williams Textbook of Endocrinology," (Wilson, J. D. , and Foster, D. W. , Eds.), 8th ed., pp. 1273–1275. W. B. Saunders, Philadelphia.

Valdemarsson, S., Leckstrom, A., Westermark, P., and Bergenfelz, A. (1996). Increased plasma levels of islet amyloid polypeptide in patients with primary hyperparathyroidism. *Eur. J. Endocrinol.* **134**, 320–325.

van Hulst, K. L., Hackeng, W. H. L., Nieuwenhuis, M. G., Höppener, J. W. M., Blankenstein, M. A., and Lips, C. J. M. (1993). An improved method for the determination of islet amyloid polypeptide in plasma: Levels in diabetic patients. *Diabetologia* **36**, A137.

van Hulst, K. L., Hackeng, W. H., Hoppener, J. W., van Jaarsveld, B. C., Nieuwenhuis, M. G., Blankenstein, M. A., and Lips, C. J. (1994). An improved method for the determination of islet amyloid polypeptide levels in plasma. *Ann. Clin. Biochem.* **31**(Pt 2), 165–170.

van Jaarsveld, B. C., Hackeng, W. H., Lips, C. J., and Erkelens, D. W. (1993). Plasma concentrations of islet amyloid polypeptide after glucagon administration in type 2 diabetic patients and non-diabetic subjects. *Diabet. Med.* **10**, 327–330.

Vine, W., Smith, P., LaChappell, R., Blase, E., Lawler, R., and Young, A. (1996). Nephrectomy slows clearance of pramlintide and rat amylin in rats. *Diabetologia* **39**, A235.

Vine, W., Smith, P., LaChappell, R., Blase, E., Percy, A., Koda, J., and Young, A. (1997a). Plasma amylin concentrations in rats quantified by a monoclonal immunoenzymetric assay. 79th Annual Meeting of the Endocrine Society, Program and Abstracts, p. 195.

Vine, W., Blase, E., Smith, P., LaChappell, R., Percy, A., Koda, J., and Young, A. (1997b). Plasma amylin concentrations in rats: Utility of a monoclonal immunoenzymometric assay. *Diabetologia* **40**, A301.

Vine, W., Smith, P., LaChappell, R., Blase, E., Lumpkin, R., and Young, A. (1998). Nephrectomy decreases amylin and pramlintide clearance in rats. *Horm. Metab. Res.* **30**, 514–517.

Wang, Y. N., Chang, D., and Chang, J. K. (1990). Co-localization of amylin and insulin immunoreactivities in porcine pancreatic islets. *FASEB J.* **4**, A402.

Wareham, N. J., Swinn, R., Fineman, M., Koda, J., Taylor, K., and O'Rahilly, S. (1996). The concentration of glycosylated amylin species is increased in women with gestational diabetes mellitus. *Diabet. Med.* **13**, S44.

Watschinger, B., Hartter, E., Traindl, O., Pohanka, E., Pidlich, J., and Kovarik, J. (1992). Increased levels of plasma amylin in advanced renal failure. *Clin. Nephrol.* **37**, 131–134.

Welborn, T. A., Breckenridge, A., Rubinstein, A. H., Dollery, C. T., and Fraser, T. R. (1966). Serum-insulin in essential hypertension and in peripheral vascular disease. *Lancet* **1**, 1336–1337.

Wood, J. M., Mah, S. C., and Schnell, C. (1990). Comparison of the acute hypotensive effects of renin inhibition, converting enzyme inhibition, and angiotensin II antagonism in rats. *J. Cardiovasc. Pharmacol.* **16**(suppl. 4), S60–S64.

Young, A. A., Rink, T. J., and Wang, M. W. (1993). Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-I (CGRP-alpha) in the fasted, anaesthetized rat. *Life Sci.* **52**, 1717–1726.

Young, A. A., Gedulin, B., Vine, W., Percy, A., and Rink, T. J. (1995). Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin. *Diabetologia* **38**, 642–648.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Zweers, E. J. K., Bravenboer, B., van Hulst, K. L., Lips, C. J. M., Christiaens, G. C. M. L., Hackeng, W. H. L., and Erkelens, D. W. (1992). Glucose stimulated islet amyloid polypeptide and gestational diabetes mellitus. *Diabetologia* **35**, A179.

Andrew Young

Receptor Pharmacology

I. Summary

Despite clear evidence for a distinct amylin pharmacology and localization of such pharmacology to sites such as the nucleus accumbens, efforts to clone an amylin receptor were fruitless for over a decade. This enigma led many to doubt the status of amylin as a bona fide hormone. Yet it became apparent during those cloning efforts that, whatever the amylin receptor was, it was somehow similar to a calcitonin receptor.

The enigma of the amylin receptor was solved following the identification of receptor activity modifying proteins (RAMPs). These single transmembrane spanning molecules, when associated with a calcitonin receptor, altered its pharmacology from calcitonin-preferring to amylin-preferring. With at least two forms of the calcitonin receptor and three forms of RAMP, there is the potential for six subtypes of amylin

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receptors. Of these, two appear to predominate. The CTa (shorter form) calcitonin receptor, dimerized with RAMP1 [amylin 1 (a) receptor], appears to represent binding sites at the nucleus accumbens and the subfornical organ. Binding sites at area postrema appear to be composed of CTa + RAMP3 [amylin3 (a) receptors]. Thus far, RAMP proteins have been associated *in vivo* only with the CT/CLR receptor system. It is presently unknown whether RAMPs are more general modulators of receptor function, dynamically modifying responsivity with time or across other receptor classes.

The largest and first identified amylin-binding field was in the nucleus accumbens. The function of these receptors is yet undetermined, but because the nucleus accumbens is within the blood–brain barrier, the cognate ligand is unlikely to be circulating amylin. Dense amylin binding is present at the circumventricular organs, including the subfornical organ, the organum vasculosum lateralis terminalis (OVLT), and the area postrema. There is no diffusional (blood–brain) barrier at these structures, so they most likely respond to circulating (β-cell-derived) amylin. Despite pharmacological evidence of amylin sensitivity in several peripheral tissues, selective amylin binding outside of the brain is observed only in the renal cortex.

The newly designated amylinomimetic drug class was defined on the basis of its unique pharmacology prior to the molecular characterization of amylin receptors. Currently, the class includes any agent that acts as an agonist at characterized amylin receptors.

Several peptides, typically analogs of truncated salmon calcitonin, have been developed as potent and selective amylin antagonists and have been useful in identifying amylinergic responses. Of these, AC187 (30Asn32Tyr[8–32]sCT; Amylin Pharmaceuticals Inc.) is particularly selective and potent, and has been most often cited in studies using amylin antagonists. Antagonism of a response with an order of potency of AC187 > AC66 > CGRP[8–37] is suggestive that it is mediated via amylin receptors. Activation of a response with salmon calcitonin (sCT) > amylin > calcitonin gene-related peptide (CGRP) > mammalian CT suggests activation via the amylin1 (a) receptor, while sCT = amylin >> CGRP > mammalian CT suggests activation via amylin3 (a) receptors. Absence of response to other ligands (e.g., adrenomedullin) is useful for excluding certain pharmacologies.

II. Amylin Receptors

A. Molecular and Biochemical Characterization

The path leading to the molecular identity of the amylin receptor was circuitous. Before its characterization, several studies had suggested a close relationship between the amylin receptor and products of the calcitonin

receptor gene. High-affinity amylin binding sites were reported in MCF-7 human breast carcinoma cells (Chen *et al.*, 1997; Zimmermann *et al.*, 1997). Expression cloning experiments using a peptide antagonist of amylin and calcitonin receptors identified two isoforms of the calcitonin receptor gene in MCF-7 cells. Expression of one of these calcitonin receptor isoforms in cell lines generated a typical calcitonin receptor, as well as small amounts of a high-affinity amylin binding site. However, generation of the amylin binding site was dependent on the cell background in which the gene was expressed. These studies demonstrated that factors related to the cell background appeared to determine whether expression of the calcitonin receptor gene resulted in a receptor able to bind amylin.

High-affinity amylin binding sites were also described in mouse TSH thyrotroph cells (Hanna *et al.*, 1995; Perry *et al.*, 1997). Like the MCF-7 cells, these thyrotroph cells expressed at least two isoforms of the calcitonin receptor gene, generated high-affinity amylin sites, and contained typical calcitonin receptors. Receptors covalently labeled with radio-iodinated amylin in TSH cell membranes were immunoprecipitated with antibodies to the calcitonin receptor, suggesting a shared peptide backbone in the antigenic region. However, amylin receptors differed biochemically from the major calcitonin receptor present in TSH cells in their molecular size and extent of glycosylation.

The highest density of amylin-specific binding sites, described later, is in the nucleus accumbens and area postrema regions of the brain. Numerous attempts to clone a receptor that displayed the pharmacology of these binding sites proved unsuccessful, and the interval between identification of amylin as a ligand and the molecular characterization of amylin receptors was more than a decade. However, several forms of the calcitonin receptor with which amylin could interact were discovered during cloning efforts (Albrandt *et al.*, 1993, 1995; Beaumont *et al.*, 1994). Two of these were splice variants of the calcitonin receptor found in human MCF-7 cells. In addition, a novel receptor was cloned, the calcitonin receptor-like receptor (CRLR) (Njuki *et al.*, 1993), which had 55% amino acid identity to the calcitonin receptor previously identified by Goldring *et al.* (Lin *et al.*, 1991).

Efforts to clone the CGRP receptor were similarly unsuccessful despite clear evidence for a specific pharmacology, a situation that closely paralleled that of amylin. The break came with the discovery by the group of Steven Foord at Glaxo Wellcome (UK) of a family of accessory proteins (RAMPs) (McLatchie *et al.*, 1998) required for the expression of functional CGRP and adrenomedullin receptors. RAMPs were first described as single-transmembrane proteins that were required to transport CRLR (the calcitonin-receptor-like receptor, also referred to as CLR), an otherwise inactive seven-transmembrane protein, to the cell surface (McLatchie *et al.*,

1998). Three distinct RAMPs have thus far been identified. Co-expression of RAMP1 with the CLR gene product resulted in a receptor complex that exhibited the pharmacology of a CGRP receptor. Co-expression of RAMP2 with the CLR gene product resulted in a receptor with adrenomedullin pharmacology. Thus, a single gene product (CLR) was converted into either a CGRP or an adrenomedullin receptor depending on the nature of the associated RAMP accessory protein.

In summary, despite clear evidence for a distinct amylin pharmacology and localization of such pharmacology to sites such as the nucleus accumbens, efforts to clone an amylin receptor were fruitless for over a decade. This enigma led many to doubt the status of amylin as a bona fide hormone. Yet it emerged that whatever the amylin receptor was, it was something like a calcitonin receptor.

B. RAMPs and Calcitonin Receptors

This novel mechanism (RAMPs) for generating receptor specificity was examined in regard to other members of the amylin/CGRP/calcitonin/adrenomedullin peptide group. Jan Fischer (Zurich, Switzerland) (Muff *et al.*, 1999) and Patrick Sexton (Melbourne, Australia) (Sexton *et al.*, 2000; Tilakaratne *et al.*, 1998) independently found that co-expression of RAMP1 with the long form of the calcitonin receptor resulted in a receptor complex with a pharmacology similar to that of the amylin-binding site at the nucleus accumbens (Beaumont *et al.*, 1993). Co-expression of calcitonin receptors with RAMP1 markedly increased amylin binding in transfectants (Christopoulos *et al.*, 1999; Muff *et al.*, 1999), and the resulting binding profile and potency ratios exhibited by salmon calcitonin, amylin, CGRP, and human calcitonin approximated those described for amylin receptors first described in nucleus accumbens (Beaumont *et al.*, 1993) (sCT > amylin > CGRP > hCT). RAMP1 in essence increased sensitivity of expressed receptors to amylin and CGRP, and decreased sensitivity to mammalian calcitonins, modifying them from "calcitonin" receptors to "amylin" receptors. It appeared, therefore, that the amylin receptor could be a result of the interaction of the calcitonin receptor with one or more RAMPs. These findings have been independently confirmed at Amylin Pharmaceuticals Inc., where the therapeutic amylin analog pramlintide has been shown to fully activate reconstituted amylin receptors with a potency similar to that of amylin.

Interestingly, RAMP3 also interacts with calcitonin receptors, producing another amylin receptor of differing pharmacology. The receptor is equally sensitive to amylin and sCT, but not to CGRP, and the receptor

is even less sensitive to mammalian calcitonins than is the CTR + RAMP1 construct (Christopoulos *et al.*, 1999). RAMP2 does not produce an amylin receptor subtype in association with $hCTR_{I1-}$ (also known as hCTa) but can when in association with the $hCTR_{I1+}$ variant of the calcitonin receptor (Tilakaratne *et al.*, 2000) (which associates with all three RAMPS).

In summary, the enigma of the amylin receptor was solved following the identification of RAMPs. These single transmembrane spanning molecules, when associated with a calcitonin receptor, altered its pharmacology from calcitonin-preferring to amylin-preferring. With at least two forms of the calcitonin receptor and three forms of RAMP, there is the potential for six subtypes of amylin receptor (Fig. 1).

C. Distribution of Amylin Receptor Components

The amylin receptor with high CGRP affinity, the CTR + RAMP1 construct, appears to correspond in its pharmacology to binding sites found in the nucleus accumbens and amygdala (Christopoulos *et al.*, 1999). The finding of very abundant RAMP1 mRNA in these sites (Oliver *et al.*, 2001) fits with CTR + RAMP1 being the predominant subtype of amylin receptor at the nucleus accumbens. The CTR + RAMP3 form with lower CGRP affinity appears to correlate with amylin binding sites found in dorsomedial and arcuate hypothalamic nuclei (Christopoulos *et al.*, 1999).

There are two principal forms of the calcitonin receptor. One form was originally identified by Goldring *et al.* (Goldring *et al.*, 1993; Gorn *et al.*,

7TM Structure⇒	CLR	CTa	CTb
Accessory protein			
nil	No pharmacology	calcitonin	calcitonin
RAMP1	CGRP>ADM>amylin> >sCT	sCT>amylin>CGRP>hCT ***nucl. accumbens, muscle, SFO***	
RAMP2	adrenomedullin >>amylin, sCT		
RAMP3		sCT>amylin>>CGRP>hCT ***area postrema***	

FIGURE 1 Pharmacologies resulting from different CTR/CLR + RAMP combinations.

1992) and was similar to calcitonin receptors in the T47D breast carcinoma cell line. A variant form contained a 16-amino-acid insert in the first intracellular loop and/or a 37-amino-acid insert in the first extracellular loop (Albrandt *et al.*, 1995). These were designated as CTR_{I1-} and CTR_{I1+}, respectively, or more simply as CT (a) and CT (b). The combination of each of these with each of the three RAMP subtypes yields six possible dimeric forms, and in principal, six distinct pharmacologies. The possible phenotypes have now been designated amylin 1 (a), 2 (a), 3 (a), 1 (b), 2 (b), and 3 (b) receptors based on CTR and RAMP components, respectively (Hay *et al.*, 2004). In reality, however, two of the six possibilities appear to predominate in the major amylin-sensitive tissues: the nucleus accumbens, the subfornical organ, and the area postrema/nucleus tractus solitarius. For instance, CT (b) is not found at the area postrema, and RAMP3 is not found at the subfornical organ (Barth *et al.*, 2004), limiting possibilities at those sites. It appears that the principal combination at the nucleus accumbens is amylin 1 (a) [the CT (a) + RAMP1 dimer] (Oliver *et al.*, 2001), consistent with the order of affinities of sCT > amylin > CGRP > hCT previously reported for membranes from there (Beaumont *et al.*, 1993). The likely form at the subfornical organ is also amylin 1 (a) (Barth *et al.*, 2004). Skeletal muscle in rats (but not humans) responds to amylin with an amylin 1 (a)-like pharmacology, even though binding has not been observed there.

Area postrema, which is important in metabolic control, appears to contain amylin 3 (a) (the CTa + RAMP3 dimer). The resultant pharmacology displays equally potent amylin and sCT binding, with much lower affinity for CGRP and mammalian calcitonin binding (Barth *et al.*, 2004).

Although one report claims that in the pig RAMPs do not confer amylin sensitivity onto CT receptors (Kikumoto *et al.*, 2003), in another report, area postrema membranes from pig had high-affinity (45 pM) amylin and sCT binding, with lower affinities for CGRP and pig calcitonin (Young *et al.*, 2000), consistent with the amylin 3 (a) profile seen in other species.

In summary, although the potential for six amylin receptor subtypes exists, two appear to predominate. The CTa (shorter) calcitonin receptor, dimerized with RAMP1, termed the amylin 1 (a) receptor, appears to represent binding sites at nucleus accumbens and the subfornical organ. Binding sites at the area postrema appear to be composed of CTa + RAMP3 [amylin 3 (a) receptors] (Fig. 2).

D. Other Properties of RAMP Proteins

The C-terminal (Zumpe *et al.*, 2000) and transmembrane portions (Steiner *et al.*, 2002; Zumpe *et al.*, 2000) of RAMPs 1 and 2 appeared to determine degree of expression, while the N terminus primarily determined the phenotype (specificity for various ligands) (Zumpe *et al.*, 2000).

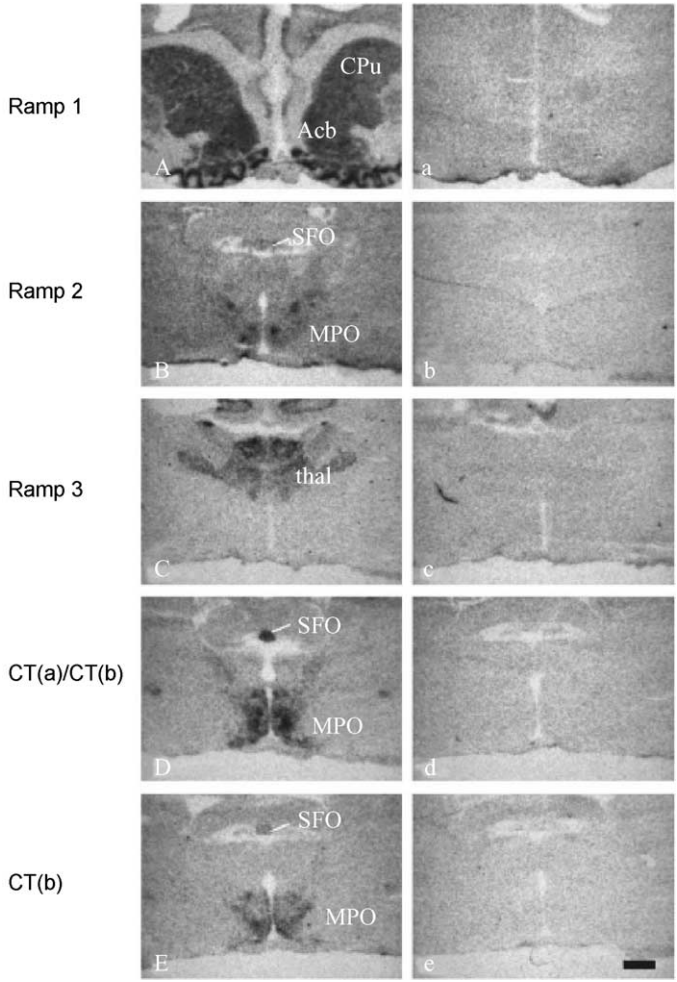

FIGURE 2 Distribution of RAMP and calcitonin receptor variants within rat brain, identifying components present at sites of dense amylin binding. Reproduced from Barth *et al.* (2004).

RAMP proteins could also interact with VIP/PACAP receptors, glucagon, PTH1, and PTH2 receptors when co-expressed in COS-7 cells (Christopoulos *et al.*, 2003), raising the possibility that RAMPs are more general modulators of pharmacological properties than was previously recognized. However, in only a few instances did such co-expression result in a change in pharmacology. It is presently unclear to what extent RAMPs functionally modify *in vivo* pharmacologies beyond the CTR/CLR class.

The pharmacology of some amylinergic responses, for example, the inhibition of gastric emptying, fits better to a mixed model, in which both

amylin and calcitonin sensitivity are present. In rats, the mammalian calcitonins were too potent at inhibiting gastric emptying (Gedulin *et al.*, 1996) for the response to be purely amylinergic. But the pharmacology fitted a model in which, for example, a fraction of calcitonin receptors present was associated with RAMP and a fraction was not. It is presently unclear whether cells differentially express RAMPs or the receptors with which they associate. A further posit of mixed expression is the possibility that cells could dynamically modify (or tune) their own pharmacologies (e.g., in response to their hormonal milieu or other factors) to alter selectivity or sensitivity to certain ligands. It is not known whether this occurs, and if it does, what the consequences are if some of the modifiers (RAMPs) are shared with other signaling systems.

In summary, RAMP proteins thus far have been associated only with the CT/CLR receptor system *in vivo*. It is presently not known whether RAMPs are more general modulators of receptor function, dynamically modifying responsivity with time or across other receptor classes.

III. Amylin Binding

A. Brain Distribution

The distribution of sites corresponding to amylin receptors was first identified by Sexton *et al.* in 1988 (Sexton *et al.*, 1988). They were first termed C3 binding sites, characterized as calcitonin binding sites that also had high affinity to both sCT and CGRP (Sexton *et al.*, 1988). But, since amylin had just been discovered, these authors did not recognize that the C3 sites also bound amylin with high affinity. The discovery that these sites contained amylin receptors was made by Beaumont in 1990 (Beaumont and Rink, 1993a,b) and was reported in 1993 (Beaumont *et al.*, 1993) (Fig. 3).

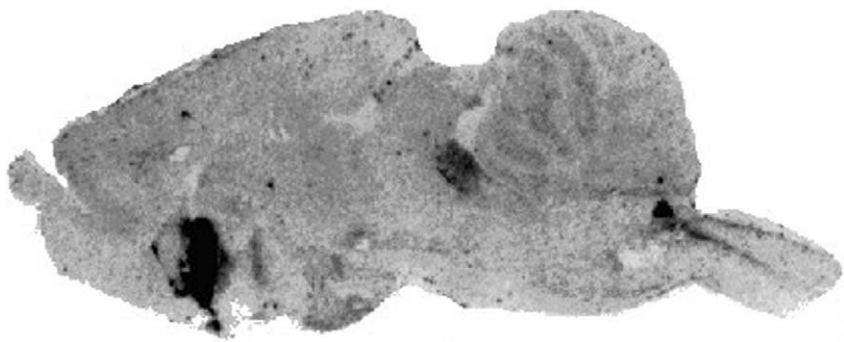

FIGURE 3 Autoradiograph indicating areas of binding of radio-iodinated rat amylin in rat brain. Reproduced from Beaumont *et al.* (1993).

High-affinity amylin binding sites, identified by the specific binding of radio-iodinated rat amylin at low picomolar concentrations, were unevenly distributed in brain (Beaumont *et al.*, 1993; van Rossum *et al.*, 1994). The regions with the highest binding densities in rat brain, as determined by autoradiographic studies, included nucleus accumbens and fundus striati, area postrema, subfornical organ, vascular organ of the lamina terminalis, and locus coeruleus (Sexton *et al.*, 1994). The distribution of amylin receptors was similar in monkey brain, with high densities in area postrema, nucleus of the tractus solitarius (NTS), locus coeruleus, and dorsal raphe (Christopoulos *et al.*, 1995). However, compared to the rat, the density of amylin binding was lower in monkey nucleus accumbens. Highest binding densities were present in monkey hypothalamus.

The pharmacological specificity of these binding sites was initially determined for receptors in rat nucleus accumbens membranes (Beaumont *et al.*, 1993). Amylin had a binding affinity (K_d) of approximately 30 pM for these sites, which matched well with circulating concentrations. The related peptides βCGRP and αCGRP, which share approximately 45% amino acid sequence identity with amylin, had 3- to 12-fold lower affinity than amylin in competitive binding studies. Both rat and human calcitonins, which have 15% amino acid sequence identity with amylin, had quite low affinities. However, salmon calcitonin, which has 30% amino acid sequence identity with amylin, was equipotent with amylin in competitive binding studies (Beaumont *et al.*, 1993) (Fig. 4).

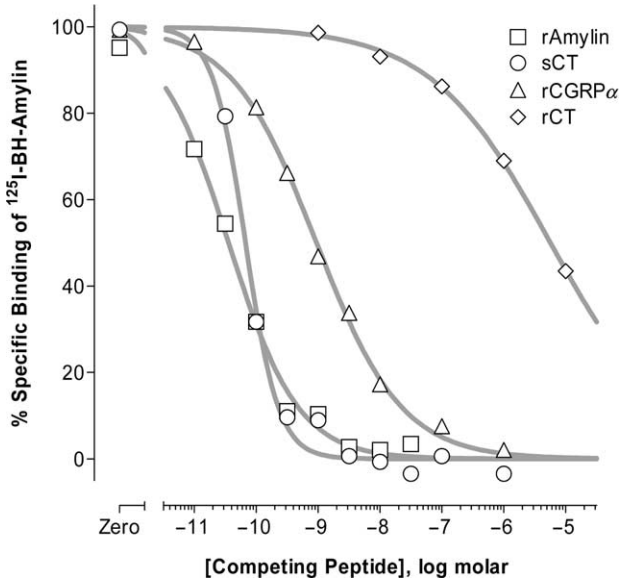

FIGURE 4 Binding isotherms for displacement of radio-iodinated rat amylin from nucleus accumbens membranes. Reproduced from Beaumont *et al.* (1993).

These studies showed that receptors with a high binding affinity for amylin, consistent with its low picomolar circulating concentrations, were present in the brain. These receptors had a characteristic binding specificity and distribution in several species. Amylin binding sites were highly localized to circumventricular organs, notably the area postrema, which have access to amylin circulating in the blood. The pharmacology of amylin in this structure is of particular interest because stimulation of area postrema structure by circulating amylin may drive many, if not all, of its glucoregulatory gut reflexes.

In summary, the largest and first identified amylin receptor field was in the nucleus accumbens. The function of these receptors is yet undetermined, but because the nucleus accumbens is within the blood–brain barrier, the cognate ligand is unlikely to be circulating amylin. Dense amylin binding is present at the circumventricular organs, including the subfornical organ, the OVLT and the area postrema. There is no diffusional (blood–brain) barrier at these structures, so it appears most likely that they respond to circulating (β-cell-derived) amylin.

B. Binding in Area Postrema

The binding of radio-iodinated amylin to membranes from porcine area postrema has recently been characterized (Young *et al.*, 2000). Autoradiographic studies showed that binding sites for [^{125}I]-Bolton-Hunter-labeled rat amylin ([^{125}I]-BH-amylin) were concentrated in the area postrema of rat brain (Sexton *et al.*, 1994). However, due to the limited size of this region in rats, the pharmacological characteristics of these binding sites were not determined. In the pig studies (Young *et al.*, 2000), amylin binding was determined using area postrema tissue dissected from freshly collected brains, and radioligand binding and data analysis were done as in previously described amylin-binding methods (Beaumont *et al.*, 1993).

Saturation binding isotherms indicated that receptors with high binding affinity for [^{125}I]-BH-amylin ($K_d = 25 \pm 4$ pM) were present at high density (87 ± 20 fmol/mg protein). sCT and rat and human amylin were the most potent peptides at competing for [^{125}I]-BH-amylin binding. CGRPs had lower potencies, as did pig calcitonin. Rat and human calcitonin were relatively inactive. Thus, receptors in porcine area postrema were somewhat similar in their binding profile to receptors in rat brain (Beaumont *et al.*, 1993) and in porcine nucleus accumbens (Aiyar *et al.*, 1995), and they were even more consistent with the amylin 3 (a) profile.

The competitive binding potencies of N-terminally truncated peptides and the amylin antagonist AC187 (Young *et al.*, 1994) were determined in pig area postrema (Young *et al.*, 2000). Salmon calcitonin[8–32] and AC187

effectively competed for [^{125}I]-BH-amylin binding at concentrations lower than 100 pM, while CGRP[8–37] was less active.

C. Peripheral Binding

Whole-body autoradiography of rats injected intravenously with [^{125}I]-amylin at intervals between 2 and 30 min before sacrifice revealed apparently specific, early binding in lung and kidney (Stridsberg *et al.*, 1993). The binding was displaceable with non-labeled amylin or CGRP. No binding was noted in other tissues.

Despite clear pharmacological evidence for local amylin action in skeletal muscle and pancreatic islets in rats, no binding has been reported in those tissues. Muscle is methodologically difficult to assess, because of the abundance of intracellular membranes relative to plasma membrane. In islets, it may be that pharmacological effects can be observed in samples in which the number of receptors is too low to register radiographically. Neither is amylin binding reported in bone, despite reports of a distinct amylin pharmacology there (Alam *et al.*, 1993a,b; Zaidi *et al.*, 1991), perhaps for similar reasons. It is unclear whether the binding observed in lung (Stridsberg *et al.*, 1993) was to amylin/calcitonin receptors or to CGRP receptors. There are no reports of pulmonary effects of calcitonins that would support the presence of amylin receptors. On the other hand, CLR (which can give rise to CGRP or adrenomedullin receptors) is abundant there (Han *et al.*, 1997), and it likely mediates the vascular component of ventilation-perfusion matching by CGRP (Janssen and Tucker, 1994; Parsons *et al.*, 1992) (present in lung, Edbrooke *et al.*, 1985; especially in pulmonary nerve fibers, Sonea *et al.*, 1994) and by adrenomedullin. It is probable that lung binding of amylin was to CGRP receptors.

Amylin binding in kidney is readily detectable (Haynes *et al.*, 1994; Wookey *et al.*, 1994). The binding is distinct in its distribution from that of sCT or CGRP. It is displaceable with the antagonists AC413 (Ac-ATQRLANFLVRLQTYPRTNVGANTY) and AC66 (sCT[8–32]), but not with CGRP[8–37]. The binding thus appears related to amylin/calcitonin receptors, but not to CGRP/adrenomedullin receptors (Wookey *et al.*, 1996). In both the spontaneously hypertensive rat (SHR) and in rats in which hypertension was induced with renal ablation, amylin binding was increased in proportion to systolic pressure (Wookey *et al.*, 1997). These changes in binding were reversed with an antihypertensive dose of the angiotensin converting enzyme inhibitor perindopril in the surgical model, but not in the SHR rats (Cao *et al.*, 1997).

In summary, despite pharmacological evidence of amylin sensitivity in several peripheral tissues, selective amylin binding outside of the brain is observed only in the renal cortex.

IV. Identifying Amylinergic Responses

A. Definition of Amylinomimetic Class

Before molecular characterization of amylin receptors, pharmacological and anatomic characterization of an amylin receptor class was apparent, with selective ligands showing distinctive patterns of activities in bioassays, affinities in binding studies, and distributions of selective binding. These distinctions allowed for a definition of an amylinomimetic class of ligands.

Binding potencies at membranes from the nucleus accumbens in rat (Beaumont *et al.*, 1993) indicated a unique pharmacology that was defined in the patent literature as distinctive of amylin receptors (Beaumont and Rink, 1993). The order of potencies of many ligands, both agonists and antagonists, matched the potency for agonism or antagonism to affect glycogen metabolism (stimulate glycogenolysis) in isolated soleus muscle of the rat (U.S. Patent 5,367,052). In addition, an increase in plasma lactate concentration in rats was a feature of amylin action specified in a patent describing the *in vivo* testing of amylinomimetic agents (U.S. Patent 5,234,906) (Cooper *et al.*, 1992). Amylinomimetic agents possess all three properties. Thus far, all molecules that have fulfilled these functional criteria have shown some structural similarity.

Symlin (pramlintide acetate) injection was the first agent to be developed clinically as an amylin receptor agonist, hereafter referred to as amylinomimetic agents. This new drug class includes agents that mimic the actions of amylin at amylin receptors, even though they may differ in other respects, such as amyloidogenicity, solubility, stability, and structure. An amylinomimetic agent binds to and activates amylin receptors, the molecular characterization of these entities having been described previously. Binding and activation can be evidenced by the following:

- activation of an associated signaling cascade, such as the adenylate cyclase system, in a cell-based or membrane-based test system expressing a predominance of amylin receptors

or

- binding to an amylin receptor preparation, this being an expressed receptor or naturally occurring receptor (as has been harvested from membranes at the nucleus accumbens or area postrema)

combined with

- a spectrum of biological activities that can be pharmacologically identified as occurring via amylin receptors.

Note that action at an amylin receptor is a cardinal element of the definition. For example, catecholamines in some bioassay test systems

appear somewhat amylinomimetic in that they oppose insulin-stimulated radioglucose uptake (promote glycogenolysis) in soleus muscle, and will increase plasma lactate and thereafter glucose. However, they will not bind to nucleus accumbens membranes; therefore, they are excluded from this class. Other molecules that are not amylinomimetics may exhibit some, but not all, of these actions. For example, glucagon will increase plasma glucose but not plasma lactate concentration, and will not directly affect glycogen metabolism in rat soleus muscle. Insulin will increase plasma lactate, but will lower plasma glucose and increase rather than decrease radioglucose incorporation into muscle glycogen. Neither glucagon nor insulin will bind to nucleus accumbens, so they are not amylinomimetics.

An amylinomimetic will exhibit all actions that originate from activation of amylin receptors. Salmon calcitonin, human amylin, rat amylin and pramlintide, and many unpublished ligands fulfill these criteria and are therefore amylinomimetics. Some amylinomimetics, for example, sCT and CGRP, also act at other receptors (calcitonin and CGRP receptors). Use of other selective ligands (agonists or antagonists) is often required to determine whether the action is occurring via amylin receptors.

Measurement of effects on soleus muscle glycogen metabolism, plasma glucose, and lactate concentrations in rats are used as an instrument to define, and as a screen to identify, amylinomimetic agents. Paradoxically, these responses are not a prominent feature of the human response to amylin or pramlintide. Effects upon muscle, lactate flux (including Cori cycle activity), and liver are features of amylin's physiological and pharmacological activity in some species, but they appear to be unrelated to pramlintide's therapeutic effect in humans.

In summary, prior to the characterization of amylin receptors, the amylinomimetic drug class had been functionally defined on the basis of its unique pharmacology. Now an amylinomimetic may be defined as any agent with agonist activity at characterized amylin receptors.

B. Amylin Receptor Antagonists

An amylin receptor antagonist will bind to, but not activate, amylin receptors. It will displace and thereby oppose the actions of amylinomimetic agents.

All published work describing blockade of amylin receptors has used truncated peptide agonists or their derivatives. Blockade of an amylin action in skeletal muscle was first described using CGRP[8–37] (Wang *et al.*, 1991), which had previously been described as an antagonist of CGRP (Chiba *et al.*, 1989). Others reported similar findings for CGRP[8–37] and amylin subpeptides (Deems *et al.*, 1991).

The literature on amylin antagonism thus far reports the use of:

- [8–37] fragment of CGRP or amylin (94 citations)
- [8–32] fragment of sCT, AC66 (20 citations)

Other acetylated derivatives of sCT include the following:

- AC253: Ac-11,18Arg,30Asn,32Tyr,9–32sCT (Ac-LGRLSQELHRL-QTYPRTNTGSNTY); five citations
- AC625: Ac-15Glu,18Arg,27Val,30Asn,32Tyr,8–18hAmylin, 19–32sCT (Ac-ATQRLANELVRLQTYPRTNVGSNTY-NH_2); two citations
- AC187: Ac-30Asn,32Tyr,8–32sCT (Ac-VLGKLSQELHKLQ-TYPRTNTGSNTY-NH_2); 39 citations
- AC413: Ac-18Arg,27Val,29Ala,30Asn,32Tyr,8–18human amylin, 19–32sCT (Ac-ATQRLANFLVRLQTYPRTNVGANTY); four citations
- AC512: Ac-LG (Bolton-Hunter monoiodo)LSQELHRLQTYPRTN-TGSNTY); three citations

The AC prefix indicates the compound's identity within the peptide library of Amylin Pharmaceuticals Inc.

Some amylin receptor antagonists will also be antagonists at other receptors. For example, CGRP[8–37], the first used to block effects of exogenous amylin (Wang *et al.*, 1991) and the first used to demonstrate an effect of endogenous amylin (Young *et al.*, 1992), is in fact only a weak amylin receptor antagonist, and high doses were needed to see an effect. It is not selective for amylin receptors and is instead more potent at blocking effects at $CGRP_1$ receptors, for which purpose it is generally used. Similarly, amylin[8–37] is neither especially potent nor selective at amylin receptors.

In contrast, AC66 (sCT[8–32]) is a potent amylin receptor antagonist, but it is 3000-fold less potent at blocking effects at CGRP receptors. Although AC66 blocks calcitonin receptors to some extent, it is far less potent in that action than it is in blocking the amylin 1 (a) and amylin 3 (a) receptors (Kuwasako *et al.*, 2003). AC66 is nonetheless sold for research use as an amylin receptor antagonist (Bachem, Torrance, CA).

AC187 (ac-[Asn^{30},Tyr^{32}]sCT[8–32]) and other peptide antagonists are structurally similar to AC66. AC187 is 400-fold more selective for amylin receptors versus CGRP receptors. This is actually less than the selectivity shown by AC66 for this receptor pair. But, in contrast to AC66 AC187 has the additional advantage of being 40-fold more selective for amylin compared to calcitonin receptors (Young *et al.*, 1994), so is a good general discriminator of amylinergic responses.

In summary, several peptides that are typically analogs of truncated sCTs have been developed as potent and selective amylin antagonists. Of

these, AC187 is among the most selective and potent, and has been most often reported as an amylin antagonist.

C. Use of Selective Ligands

There is a grouping of biological actions for which the order of potency for inhibition is AC187 > AC66 > CGRP[8–37]. These actions include inhibition of radioglucose incorporation into skeletal muscle glycogen (Beaumont *et al.*, 1995), binding to membranes of nucleus accumbens (Beaumont *et al.*, 1995), and inhibition of lactate rises *in vivo* (Beaumont *et al.*, 1995). This order of potency mirrors that observed with reconstituted amylin receptors [especially the amylin 1 (a) profile] and is a hallmark of amylinergic mechanisms. It contrasts with the order of potency in blocking CGRP binding to CGRP receptors at SK-N-MC cells and in blockade of CGRPergic vasoactivity at which CGRP[8–37] > AC187 = amylin[8–37] > AC66 (Howitt and Poyner, 1997).

Because of its good general selectivity, AC187 has been widely used as a tool to determine amylinergic effects. Effects that can be blocked by AC187 in response to an exogenous amylinomimetic, or physiological responses that can be disinhibited by AC187 when it is administered alone, are typically mediated via an amylin pharmacology.

Responses to certain key agonists provide further evidence that such responses are mediated via an amylin pharmacology.

Because sCT is an amylin agonist and a calcitonin agonist but is almost devoid of activity at CGRP receptors, a response to this ligand can almost exclude the possibility that it is mediated via CGRP receptors.

The response to amylin itself is informative. Although amylin can activate CGRP receptors and, for example, evoke vasodilation (Brain *et al.*, 1990; Chin *et al.*, 1994), it is approximately two orders of magnitude less potent than CGRP, so responses observed are more likely to be amylinergic/calcitoninergic, and are less likely to be CGRPergic. CGRPergic responses can be easily identified by the facility with which they are blocked with CGRP[8–37] (Chin *et al.*, 1994).

CGRP is a more promiscuous ligand, activating amylin, calcitonin, and CGRP receptors, and is often less helpful in identifying cognate pharmacologies.

Adrenomedullin, however, can be helpful. Although adrenomedullin activates its own receptor (CLR + RAMP2), it is also an agonist at CGRP receptors (Hall *et al.*, 1995) (CLR + RAMP1) (McLatchie *et al.*, 1998). Adrenomedullin, unlike CGRP, however, is more selective for its own and CGRP receptors than CGRP is for these receptors (Vine *et al.*, 1996), and is virtually devoid of amylinergic and calcitoninergic activity.

In summary, several ligands have proven most useful in identifying amylinergic responses. Antagonism of a response with an order of potency

of AC187 > AC66 > CGRP[8–37] is suggestive of amylin receptor mediation. Generation of a response with sCT > amylin > CGRP > mammalian CT suggests activation via the amylin1 (a) receptor, while sCT = amylin >> CGRP > mammalian CT suggests activation via amylin 3 (a) receptors. Absence of response to other ligands (e.g., adrenomedullin) is useful for excluding certain pharmacologies.

References

Aiyar, N., Baker, E., Martin, J., Patel, A., Stadel, J. M., Willette, R. N., and Barone, F. C. (1995). Differential calcitonin gene-related peptide (CGRP) and amylin binding sites in nucleus accumbens and lung: Potential models for studying CGRP/amylin receptor subtypes. *J. Neurochem.* **65**, 1131–1138.

Alam, A. S. M., Moonga, B. S., Bevis, P. J. R., Huang, C. L. H., and Zaidi, M. (1993a). Amylin inhibits bone resorption by a direct effect on the motility of rat osteoclasts. *Exp. Physiol.* **78**, 183–196.

Alam, A. S. M. T., Bax, C. M. R., Shankar, V. S., Bax, B. E., Bevis, P. J. R., Huang, C. L. H., Moonga, B. S., Pazianas, M., and Zaidi, M. (1993b). Further studies on the mode of action of calcitonin on isolated rat osteoclasts—pharmacological evidence for a 2nd site mediating intracellular Ca2+ mobilization and cell retraction. *J. Endocrinol.* **136**, 7–15.

Albrandt, K., Mull, E., Brady, E. M. G., Herich, J., Moore, C. X., and Beaumont, K. (1993). Molecular cloning of 2 receptors from rat brain with high affinity for salmon-calcitonin. *FEBS Lett.* **325**, 225–232.

Albrandt, K., Brady, E. M. G., Moore, C. X., Mull, E., Sierzega, M. E., and Beaumont, K. (1995). Molecular cloning and functional expression of a third isoform of the human calcitonin receptor and partial characterization of the calcitonin receptor gene. *Endocrinology* **136**, 5377–5384.

Barth, S. W., Riediger, T., Lutz, T. A., and Rechkemmer, G. (2004). Peripheral amylin activates circumventricular organs expressing calcitonin receptor a/b subtypes and receptor-activity modifying proteins in the rat. *Brain Res.* **997**, 97–102.

Beaumont, K., and Rink, T. J. (1993a). Receptor-based screening methods for amylin agonists and antagonists. *U.S. Patent* **5**, 263, 372.

Beaumont, K., and Rink, T. J. (1993b). Receptor-based screening methods for amylin agonists and antagonists. *European Patent EP0529065.*

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Beaumont, K., Moore, C. X., Pittner, R. A., Prickett, K. S., Gaeta, L. S. L., Rink, T. J., and Young, A. A. (1995). Differential antagonism of amylin's metabolic and vascular actions with amylin receptor antagonists. *Can. J. Physiol. Pharmacol.* **73**, 1025–1029.

Beaumont, K., Pittner, R. A., Herich, J., Albrandt, K., and Moore, C. X. (1994). Coupling of two cloned rat calcitonin receptor isoforms to adenylyl cyclase and phospholipase C. Program and Abstracts, Endocrine Society 76th Annual Meeting, June 15–18, Anaheim, CA 424.

Beaumont, K., Pittner, R. A., Moore, C. X., Wolfe-Lopez, D., Prickett, K. S., Young, A. A., and Rink, T. J. (1995). Regulation of muscle glycogen metabolism by CGRP and amylin: CGRP receptors not involved. *Br. J. Pharmacol.* **115**, 713–715.

Brain, S. D., Wimalawansa, S., MacIntyre, I., and Williams, T. J. (1990). The demonstration of vasodilator activity of pancreatic amylin amide in the rabbit. *Am. J. Pathol.* **136**, 487–490.

Cao, Z. M., Wookey, P. J., Wu, L. L., Voskuil, M., vanGeenen, R. C. I., and Cooper, M. E. (1997). Renal amylin binding in normotensive and hypertensive rats: Effects of angiotensin converting enzyme inhibition with perindopril. *J. Hypertension* **15**, 1245–1252.

Chen, W. J., Armour, S., Way, J., Chen, G., Watson, C., Irving, P., Cobb, J., Kadwell, S., Beaumont, K., Rimele, T., and Kenakin, T. (1997). Expression cloning and receptor pharmacology of human calcitonin receptors from MCF-7 cells and their relationship to amylin receptors. *Mol. Pharmacol.* **52**, 1164–1175.

Chiba, T., Yamaguchi, A., Yamatani, T., Nakamura, A., Morishita, T., Inui, T., Fukase, M., Noda, T., and Fujita, T. (1989). Calcitonin gene-related peptide receptor antagonist human CGRP-(8–37). *Am. J. Physiol.* **256**, E331–E335.

Chin, S. Y., Hall, J. M., Brain, S. D., and Morton, I. K. M. (1994). Vasodilator responses to calcitonin gene-related peptide (CGRP) and amylin in the rat isolated perfused kidney are mediated via CGRP (1) receptors. *J. Pharmacol. Exp. Ther.* **269**, 989–992.

Christopoulos, A., Christopoulos, G., Morfis, M., Udawela, M., Laburthe, M., Couvineau, A., Kuwasako, K., Tilakaratne, N., and Sexton, P. M. (2003). Novel receptor partners and function of receptor activity-modifying proteins. *J. Biol. Chem.* **278**, 3293–3297.

Christopoulos, G., Paxinos, G., Huang, X. F., Beaumont, K., Toga, A. W., and Sexton, P. M. (1995). Comparative distribution of receptors for amylin and the related peptides calcitonin gene related peptide and calcitonin in rat and monkey brain. *Can. J. Physiol. Pharmacol.* **73**, 1037–1041.

Christopoulos, G., Perry, K. J., Morfis, M., Tilakaratne, N., Gao, Y., Fraser, N. J., Main, M. J., Foord, S. M., and Sexton, P. (1999). Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product. *Mol. Pharmacol.* **56**, 235–242.

Cooper, G. J. S., Rink, T. J., and Young, A. A. (1992). Identifying cpds. which affect amylin activity—using an in-vivo biological model in which amylin increases levels of lactate and glucose. *World Patent Application*.

Deems, R. O., Cardinaux, F., Deacon, R. W., and Young, D. A. (1991). Amylin or CGRP (8–37) fragments reverse amylin-induced inhibition of 14C-glycogen accumulation. *Biochem. Biophys. Res. Commun.* **181**, 116–120.

Edbrooke, M. R., Parker, D., McVey, J. H., Riley, J. H., Sorenson, G. D., Pettengill, O. S., and Craig, R. K. (1985). Expression of the human calcitonin/CGRP gene in lung and thyroid. *EMBO J.* **4**, 715–724.

Gedulin, B. R., Jodka, C. M., Green, D. L., and Young, A. A. (1996). Comparison of 21 peptides on inhibition of gastric emptying in conscious rats. *Dig Dis. Week* A742.

Goldring, S. R., Gorn, A. H., Yamin, M., Krane, S. M., and Wang, J. T. (1993). Characterization of the structural and functional properties of cloned calcitonin receptor cDNAs. *Horm. Metab. Res.* **25**, 477–480.

Gorn, A. H., Flannery, M. S., Manning, C. A., and Goldring, S. R. (1992). Expression of a cloned high affinity calcitonin (CT) receptor cDNA confers binding and cAMP responsiveness for amylin (AMY) and calcitonin gene related peptide (CGRP). *Clin. Res.* **40**, 209A.

Hall, J. M., Siney, L., Lippton, H., Hyman, A., Jaw, K. C., and Brain, S. D. (1995). Interaction of human adrenomedullin (13–52) with calcitonin gene-related peptide receptors in the microvasculature of the rat and hamster. *J. Br. J. Pharmacol.* **114**, 592–597.

Han, Z. Q., Coppock, H. A., Smith, D. M., VanNoorden, S., Makgoba, M. W., Nicholl, C. G., and Legon, S. (1997). The interaction of CGRP and adrenomedullin with a receptor expressed in the rat pulmonary vascular endothelium. *J. Mol. Endocrinol.* **18**, 267–272.

Hanna, F. W. F., Smith, D. M., Johnston, C. F., Akinsanya, K. O., Jackson, M. L., Morgan, D. G. A., Bhogal, R., Buchanan, K. D., and Bloom, S. R. (1995). Expression of a novel receptor for the calcitonin peptide family and a salmon calcitonin-like peptide in the alpha-thyrotropin thyrotroph cell line. *Endocrinology* **136**, 2377–2382.

Hay, D. L., Christopoulos, G., Christopoulos, A., and Sexton, P. M. (2004). Amylin receptors: Molecular composition and pharmacology. *Biochem. Soc. Trans.* **32**, 865–867.

Haynes, J. M., Wookey, P. J., Tikellis, C. M., Du, H. C., Sexton, P. M., and Cooper, M. E. (1994). Amylin and calcitonin gene related peptide in the rat kidney: Functional and autoradiographic studies. *Can. J. Physiol. Pharmacol.* **72**, 566.

Howitt, S. G., and Poyner, D. R. (1997). The selectivity and structural determinants of peptide antagonists at the CGRP receptor of rat, L6 myocytes. *J. Br. J. Pharmacol.* **121**, 1000–1004.

Janssen, P. L., and Tucker, A. (1994). Calcitonin gene-related peptide modulates pulmonary vascular reactivity in isolated rat lungs. *J. Appl. Physiol.* **77**, 142–146.

Kikumoto, K., Katafuchi, T., and Minamino, N. (2003). Specificity of porcine calcitonin receptor and calcitonin receptor-like receptor in the presence of receptor-activity-modifying proteins. *Hypertens. Res.* **26**(Suppl.), S15–S23.

Kuwasako, K., Kitamura, K., Nagoshi, Y., and Eto, T. (2003). Novel calcitonin-(8–32)-sensitive adrenomedullin receptors derived from co-expression of calcitonin receptor with receptor activity-modifying proteins. *Biochem. Biophys. Res. Commun.* **301**, 460–464.

Lin, H. Y., Harris, T. L., Flannery, M. S., Aruffo, A., Kaji, E. H., Gorn, A., Kolakowski, L. F., Jr., Lodish, H. F., and Goldring, S. R. (1991). Expression cloning of an adenylate cyclase-coupled calcitonin receptor. *Science* **254**, 1022–1024.

McLatchie, L. M., Fraser, N. J., Main, M. J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M. G., and Foord, S. M. (1998). RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature* **393**, 333–339.

Muff, R., Bühlmann, N., Fischer, J. A., and Born, W. (1999). An amylin receptor is revealed following co-transfection of a calcitonin receptor with receptor activity modifying proteins-1 or -3. *Endocrinology* **140**, 2924–2927.

Njuki, F., Nicholl, C. G., Howard, A., Mak, J. C. W., Barnes, P. J., Girgis, S. I., and Legon, S. (1993). A new calcitonin-receptor-like sequence in rat pulmonary blood vessels. *Clin. Sci.* **85**, 385–388.

Oliver, K. R., Kane, S. A., Salvatore, C. A., Mallee, J. J., Kinsey, A. M., Koblan, K. S., Keyvan-Fouladi, N., Heavens, R. P., Wainwright, A., Jacobson, M., Dickerson, I. M., and Hill, R. G. (2001). Cloning, characterization and central nervous system distribution of receptor activity modifying proteins in the rat. *Eur. J. Neurosci.* **14**, 618–628.

Parsons, G. H., Nichol, G. M., Barnes, P. J., and Chung, K. F. (1992). Peptide mediator effects on bronchial blood velocity and lung resistance in conscious sheep. *J. Appl. Physiol.* **72**, 1118–1122.

Perry, K. J., Quiza, M., Myers, D. E., Morfis, M., Christopoulos, G., and Sexton, P. M. (1997). Characterization of amylin and calcitonin receptor binding in the mouse alpha-thyroid-stimulating hormone thyrotroph cell line. *Endocrinology* **138**, 3486–3496.

Sexton, P. M., McKenzie, J. S., and Mendelsohn, F. A. O. (1988). Evidence for a new subclass of calcitonin/calcitonin gene-related peptide binding site in rat brain. *Neurochem. Int.* **12**, 323–335.

Sexton, P. M., Paxinos, G., Kenney, M. A., Wookey, P. J., and Beaumont, K. (1994). *In vitro* autoradiographic localization of amylin binding sites in rat brain. *Neuroscience* **62**, 553–567.

Sexton, P. M., Perry, K. J., Christopoulos, G., Morfis, M., and Tilakaratne, N. (2000). What makes an amylin receptor? *In* (D. Poyner, I. Marshall, and S. Brain, Eds.), pp. 79–89. Landes Bioscience, Georgetown, TX.

Sonea, I. M., Bowker, R. M., Robinson, N. E., and Broadstone, R. V. (1994). Substance P and calcitonin gene-related peptide-like immunoreactive nerve fibers in lungs from adult equids. *Am. J. Vet. Res.* **55**, 1066–1074.

Steiner, S., Muff, R., Gujer, R., Fischer, J. A., and Born, W. (2002). The transmembrane domain of receptor-activity-modifying protein 1 is essential for the functional expression of a calcitonin gene-related peptide receptor. *Biochemistry* **41**, 11398–11404.

Stridsberg, M., Tjalve, H., and Wilander, E. (1993). Whole-body autoradiography of 123I-labelled islet amyloid polypeptide (IAPP). Accumulation in the lung parenchyma and in the villi of the intestinal mucosa in rats. *Acta Oncol.* **32**, 155–159.

Tilakaratne, N., Christopoulos, G., Perry, K., Morfis, M., Foord, S. M., and Sexton, P. M. (1998). Multiple amylin receptor phenotypes arise from RAMP interaction with the calcitonin receptor gene product. *Proc. Aust. Soc. Clin. Exp. Pharmacol. Toxicol.* **5**, 37.

Tilakaratne, N., Christopoulos, G., Zumpe, E. T., Foord, S. M., and Sexton, P. M. (2000). Amylin receptor phenotypes derived from human calcitonin receptor/RAMP coexpression exhibit pharmacological differences dependent on receptor isoform and host cell environment. *J. Pharmacol. Exp. Ther.* **294**, 61–72.

van Rossum, D., Menard, D. P., Fournier, A., St Pierre, S., and Quirion, R. (1994). Autoradiographic distribution and receptor binding profile of [I-125]Bolton Hunter-rat amylin binding sites in the rat brain. *J. Pharmacol. Exp. Ther.* **270**, 779–787.

Vine, W., Beaumont, K., Gedulin, B., Pittner, R., Moore, C. X., Rink, T. J., and Young, A. A. (1996). Comparison of the *in vitro* and *in vivo* pharmacology of adrenomedullin, calcitonin gene-related peptide and amylin in rats. *Eur. J. Pharmacol.* **314**, 115–121.

Wang, M. W., Young, A. A., Rink, T. J., and Cooper, G. J. S. (1991). 8–37h-CGRP antagonizes actions of amylin on carbohydrate metabolism *in vitro* and *in vivo*. *FEBS Lett.* **291**, 195–198.

Wookey, P., Tikellis, C., Du, C., Sexton, P., Young, A. A., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., and Cooper, M. E. (1994). Identification, characterisation and localisation of amylin binding sites in rat kidney using specific amylin antagonists. *J. Hypertension* **12**, S9.

Wookey, P. J., Tikellis, C., Du, H. C., Qin, H. F., Sexton, P. M., and Cooper, M. E. (1996). Amylin binding in rat renal cortex, stimulation of adenylyl cyclase, and activation of plasma renin. *Am. J. Physiol.* **39**, F289–F294.

Wookey, P. J., Cao, Z., vanGeenen, R. C. I., Voskuil, M., Darby, I. A., Komers, R., and Cooper, M. E. (1997). Increased density of renal amylin binding sites in experimental hypertension. *Hypertension* **30**, 455–460.

Young, A., Moore, C., Herich, J., and Beaumont, K. (2000). Neuroendocrine actions of amylin. *In* "The CGRP Family: Calcitonin Gene-Related Peptide (CGRP), Amylin, and Adrenomedullin" (D. Poyner, I. Marshall, and S. D. Brain, Eds.), pp. 91–102. Landes Bioscience, Georgetown, TX.

Young, A. A., Carlo, P., Rink, T. J., and Wang, M.-W. (1992). 8–37hCGRP, an amylin receptor antagonist, enhances the insulin response and perturbs the glucose response to infused arginine in anesthetized rats. *Mol. Cell Endocrinol.* **84**, R1–R5.

Young, A. A., Gedulin, B., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., Larson, E., and Rink, T. J. (1994). Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat—Evidence for a metabolic role of endogenous amylin. *FEBS Lett.* **343**, 237–241.

Zaidi, M., Alam, A. S. M. T., Soncini, R., Avaldi, F., and Moonga, B. S. (1991). Calcitonin acts upon two receptor subtypes on the osteoclast: The 'amylin-site' and the 'calcitonin-site'. *J. Bone Miner. Res.* **6**, S198.

Zimmermann, U., Fluehmann, B., Born, W., Fischer, J. A., and Muff, R. (1997). Coexistence of novel amylin-binding sites with calcitonin receptors in human breast carcinoma MCF-7 cells. *J. Endocrinol.* **155**, 423–431.

Zumpe, E. T., Tilakaratne, N., Fraser, N. J., Christopoulos, G., Foord, S. M., and Sexton, P. M. (2000). Multiple ramp domains are required for generation of amylin receptor phenotype from the calcitonin receptor gene product. *Biochem. Biophys. Res. Commun.* **267**, 368–372.

Andrew Young

Amylin and the Integrated Control of Nutrient Influx

I. Summary

The most potent actions of amylin that occur at physiological plasma concentrations include inhibition of food intake, gastric emptying, acid and digestive enzyme secretion, and glucagon secretion. These actions share a common outcome; they each help regulate the rate at which nutrients (including glucose) appear in the blood (R_a). Amylin physiologically orchestrates, via several parallel processes, the rate of entry of nutrient into the circulation, as shown schematically in Fig. 1. In this way, amylin's function may be viewed as complementary to that of insulin (secreted from the same pancreatic β-cells), which orchestrates the exit of nutrient from blood and its storage in peripheral tissues.

The following discussion addresses the emerging picture that, although amylin is co-secreted with an endocrine hormone from endocrine

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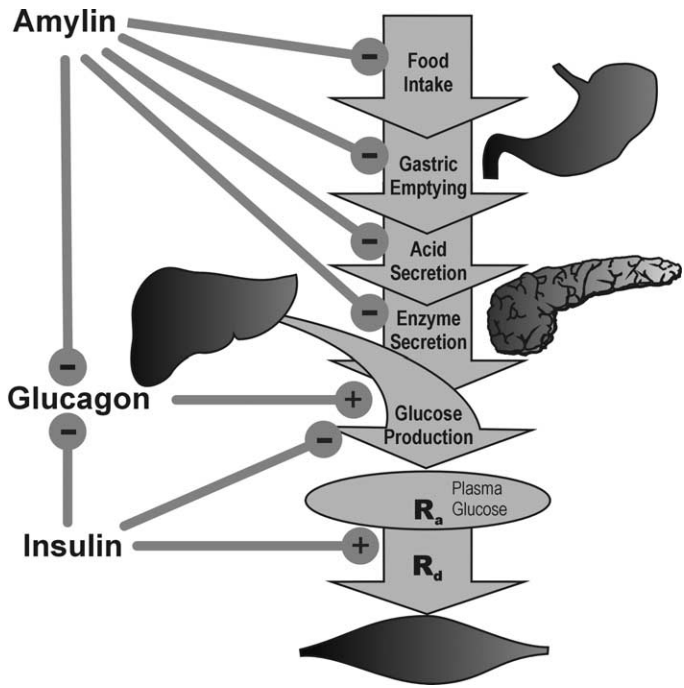

FIGURE 1 Schema of amylin actions that conspire to control rate of nutrient entry into plasma, in contrast with actions of insulin to accelerate nutrient disposal (e.g., into muscle and other insulin-sensitive tissues).

tissue (the pancreatic islets), the target for its most potent and physiologically relevant effects appears to be the central nervous system. Amylin thus may be primarily regarded as a neuroendocrine hormone (Young *et al.*, 2000).

II. Overview of Reported Actions

Nearly 60 different effects have been reported in various experiments using amylin or pramlintide in a variety of species (see Table I). Clearly, not all of these effects are physiologic. Some of them likely represent artifacts present only via non-relevant receptors at pharmacological concentrations. Some of them are a feature in animals but not in humans, and some of the reports are possibly wrong.

Our understanding of the physiological role of amylin has been obtained comparatively recently, and has been advanced using selective antagonists to

TABLE I Reported Actions of Amylin

Action	*Reference*
Inhibition of muscle glycogen synthesis	Leighton and Cooper, 1988
Activation of muscle glycogenolysis	Young *et al.*, 1991c
Inhibition of peripheral glucose uptake	Molina *et al.*, 1990; Young *et al.*, 1990
Increase in plasma glucose	Young *et al.*, 1991a
Increase in plasma lactate	Young *et al.*, 1991a
Stimulation of endogenous glucose production	Molina *et al.*, 1990
Stimulation of glucose release from muscle	Young *et al.*, 1993a
Increase in liver glycogen content	Young *et al.*, 1991d
Increase in Cory cycling	Young *et al.*, 1991b
Inhibition of insulin secretion	Dégano *et al.*, 1993
Inhibition of arginine-stimulated glucagon secretion	Gedulin *et al.*, 1997
Stimulation of exocrine pancreatic metabolism	Iwamoto *et al.*, 1992
Inhibition of CCK-stimulated pancreatic secretion	Gedulin *et al.*, 1998
Amelioration of pancreatitis	Young *et al.*, 2005
Inhibition of gastric emptying	Young *et al.*, 1995a
Reduction of post-prandial hyperglycemia	Kolterman *et al.*, 1994
Inhibition of gastric acid secretion	Guidobono *et al.*, 1994
Stimulation of gastrin secretion	Funakoshi *et al.*, 1992
Reduction of antral gastrin	Makhlouf *et al.*, 1996
Stimulation of somatostatin in fundus	Zaki *et al.*, 2002
Inhibition of gastric histamine secretion	Zaki *et al.*, 2002
Inhibition of ethanol-induced gastritis	Jodka *et al.*, 1997
Inhibition of indomethacin-induced gastritis	Guidobono *et al.*, 1997
Gut relaxation	Mulder *et al.*, 1997a
Reduction of plasma calcium	MacIntyre, 1989
Reduction of plasma potassium	Young *et al.*, 1996
Inhibition of osteoclasts	Alam *et al.*, 1993
Selective inhibition of resorption	Dacquin *et al.*, 2004
Stimulation of osteoblasts	Romero *et al.*, 1993
Stimulation of calciuria	Miles *et al.*, 1994
Central inhibition of food intake	Chance *et al.*, 1991b
Reduction in hypothalamic orexin, MCH	Barth *et al.*, 2003
Inhibition of hypothalamic dopamine release	Brunetti *et al.*, 2002
Peripheral inhibition of food intake	Morley and Flood, 1991
Increase in water intake	Rauch *et al.*, 1997
Inhibition of ethanol intake	Wolfe *et al.*, 2003
Reduction in fat:protein ratio	Roth *et al.*, 2004
Centrally, increase in body temperature	Chance *et al.*, 1991a
Modulation of learning/memory	Flood and Morley, 1992
Opiate-sparing analgesia	Young, 1997
Centrally, decrease in locomotion	Bouali *et al.*, 1995
Stimulation of renin secretion	Young *et al.*, 1994c
Stimulation of aldosterone secretion	Nuttall *et al.*, 1995
Increase in tubular sodium reabsorption	Harris *et al.*, 1997
Increase in urine volume	Vine *et al.*, 1998
Increase in urinary sodium excretion	Vine *et al.*, 1998
Stimulation of cutaneous vasodilation	Brain *et al.*, 1990

(continues)

TABLE I (*continued*)

Action	*Reference*
Stimulation of pulmonary vasodilation	Dewitt *et al.*, 1994
Stimulation of tracheal mucus secretion	Wagner *et al.*, 1995
Relaxation of airway smooth muscle	Bhogal *et al.*, 1994
Reduction in blood pressure	Young *et al.*, 1993c
Aqueous humor outflow	Alajuuma *et al.*, 2003
Umbilical venous endothelial proliferation	Datta *et al.*, 1990
Stimulation of cardiocyte growth	Bell *et al.*, 1995
Increase in renal thiazide receptor	Blakely *et al.*, 1997
Stimulation of CNS tyrosine and tryptophan transport	Balasubramaniam *et al.*, 1991
Anti-inflammatory action	Clementi *et al.*, 1995
Amplification of eosinophil responses	Hom *et al.*, 1995
Reduction of plasma fructosamine	Thompson *et al.*, 1996
Reduction in glucose fluctuations	Kovatchev *et al.*, 2004
Increase in cardiocyte contractility	Bell and McDermott, 1995
Stimulation of atrial contractility	Piao *et al.*, 2004
Inhibition of ANP secretion	Piao *et al.*, 2004
Inhibition of growth hormone release	Netti *et al.*, 1995
Apoptosis in cultured nerve cells	May *et al.*, 1993
Apoptosis in cultured ß-cells	Lorenzo *et al.*, 1994
Protective eect in islets	Mulder *et al.*, 1997b
Growth factor in kidney	Wookey *et al.*, 1998
Inhibition of ghrelin secretion	Gedulin *et al.*, 2004

subtract (and thereby determine) the effect of endogenous amylin. Other indications of physiological relevance have been obtained in dose–response studies in which responses have been observed at doses that result in changes in plasma concentration that are comparable to those observed with endogenous peptide.

III. Prior Theories of Pathogenic and Physiological Roles

Several hypotheses preceded the currently favored view of amylin's physiology. Although they are less tenable in view of current evidence, they still pervade some of the current literature, and so are addressed here in the context in which they originally arose. Plausible at the time, these hypotheses were the instrument through which the clinical utility of amylin was sought, and they have resulted in an accelerated emergence of new physiology and identification of new therapeutic modalities. Historic views of amylin's role are presented here, since they were the framework upon which many informative physiological experiments were performed.

A. Insulin Resistance

The hypothesis that excess amylin action was implicated in the pathogenesis of insulin resistance and obesity-related hypertension lead to the filing of investigational new drug applications for two amylin receptor antagonists, AC253 and AC625. The first of these was explored clinically by Glaxo PLC in insulin-resistant subjects. The hypothesis that amylin was pathogenic in insulin resistance (Cooper *et al.*, 1988), and that antagonists would ameliorate insulin resistance, arose partly as a consequence of the historical order in which biological actions were discovered. Several of the features of insulin resistance corresponded with aspects of amylin action observed in rodents:

- A potent effect to inhibit insulin-stimulated glycogen formation in isolated skeletal muscle in rats (Leighton and Cooper, 1988; Young *et al.*, 1992) fitted with the identification of impaired muscle glycogen synthesis as an early event in insulin resistance (Bogardus and Lillioja, 1990; Lillioja *et al.*, 1986; Shulman *et al.*, 1990; Young *et al.*, 1988).
- A spared sensitivity to the antilipolytic action of insulin in fat (versus resistance to effects on glucose disposal) (Yki-Järvinen *et al.*, 1987) generated a tissue-specific heterogeneity of insulin resistance that fitted with the tissue-specific effects of amylin. An absence of effect of amylin in fat (Lupien and Young, 1993), but presence of effects in muscle, concurred with distribution of effects in rodents.
- An effect of amylin to increase lactate turnover in rats (Young, 1993) also matched the increased Cori cycle activity seen in type 2 diabetic patients (Zawadzki *et al.*, 1988).
- Amylin's effect to blunt first-phase insulin secretion (Dégano *et al.*, 1993) concurred with such blunting being a feature of insulin resistance and an early predictor of type 2 diabetes (Eriksson *et al.*, 1989).
- Amylin stimulation of the renin-angiotensin system (Young *et al.*, 1994c, 1995a) promoted consideration of excess amylin action as a factor that associated insulin resistance with essential hypertension in syndrome-X (metabolic syndrome) (Young *et al.*, 1994b).

Instead of being due to an inhibition of insulin effect, effects in rat muscle were identified as due to cAMP-mediated activation of glycogen phosphorylase (Pittner *et al.*, 1995), resulting in release of lactate into plasma (Vine *et al.*, 1995a; Young *et al.*, 1991a) and substrate-mediated gluconeogenesis (Young *et al.*, 1993b). These actions on muscle glycogen metabolism and lactate flux (Cori cycle) appeared to occur at physiological amylin concentrations in the rat (Vine *et al.*, 1995a,c; Young *et al.*, 1994a) and pointed toward an activity that was more than simply the inhibition of insulin action.

In humans, the amylin antagonist AC253 amplified nutrient-stimulated insulin secretion (Leaming *et al.*, 1995), consistent with the disinhibition of

local feedback control at the β-cell (discussed below). But AC253 did not otherwise alter insulin action in those clinical studies (Leaming *et al.*, 1995; Mather *et al.*, 2002).

B. Syndrome-X

A second amylin receptor antagonist, AC625, was explored at Amylin Pharmaceuticals Inc. in relation to its potential effect on the renin-angiotensin system and to a possible utility in the treatment of obesity-related hypertension. An investigational new drug application was filed for human amylin (amlintide; AC001) to explore the effects of the endogenous hormone on the renin-angiotensin system in humans. In dose–response studies in rats and humans, the effects of amylin to stimulate the renin-angiotensin-aldosterone system (Cooper *et al.*, 1995; Vine *et al.*, 1995b) appeared sufficiently potent to entertain the idea that, at least at pathophysiological concentrations, amylin could be involved in hypertension associated with β-cell hypersecretion (Young *et al.*, 1995a). However, 4-day administration of AC625 did not affect blood pressure in mildly hypertensive humans, and continuously infused AC253 had no effects on continuously monitored blood pressure in dogs made hypertensive and insulin resistant by fat feeding (Young *et al.*, 1999). Conversely, chronic administration of pramlintide to people with diabetes did not result in elevations of blood pressure (Young *et al.*, 1999), leading to the conclusion that amylin per se was not involved in the pathogenesis of essential hypertension. Clinical exploration of amylin antagonists in metabolic syndrome was subsequently abandoned.

C. Counterregulation during Hypoglycemia

In rats, amylin and pramlintide stimulated glycogenolysis in skeletal muscle, leading to an increase in plasma lactate concentration, that then supplied substrate for gluconeogenesis. It was found that the acute glucose-elevating effects of glucagon were enhanced when given in association with amylin (Young *et al.*, 1993b). In addition, amylin administered to rats made diabetic with streptozotocin dose-dependently restored liver glycogen content (Young *et al.*, 1991d). These effects suggested that an amylin agonist may have utility in protecting diabetic individuals from hypoglycemia (Beaumont *et al.*, 1992). Initial studies following the filing of an investigational new drug application for pramlintide focused upon a potential benefit in recovery from insulin-induced hypoglycemia. However, the spectrum of actions present in rodents was different from those in humans, and this indication was not pursued. However, observations made during those clinical studies partly led to the eventual elucidation of the physiological role of amylin and prediction of the clinical utility of pramlintide.

References

Alajuuma, P., Oksala, O., and Uusitalo, H. (2003). Amylin competes for binding sites of CGRP in the chamber angle and uvea of monkey, cat, and pig eye. *J. Ocul. Pharmacol. Ther.* **19**, 555–567.

Alam, A. S. M., Moonga, B. S., Bevis, P. J. R., Huang, C. L. H., and Zaidi, M. (1993). Amylin inhibits bone resorption by a direct effect on the motility of rat osteoclasts. *Exp. Physiol.* **78**, 183–196.

Balasubramaniam, A., Zhang, F. S., Thomas, I., and Chance, W. T. (1991). Amylin increases transport of tyrosine and tryptophan into brain. *Soc. Neurosci. Abstr.* **17**, 976.

Barth, S. W., Riediger, T., Lutz, T. A., and Rechkemmer, G. (2003). Differential effects of amylin and salmon calcitonin on neuropeptide gene expression in the lateral hypothalamic area and the arcuate nucleus of the rat. *Neurosci. Lett.* **341**, 131–134.

Beaumont, K., Rink, T. J., and Young, A. A., inventors (1992). Compsns. comprising calcitonin and insulin or glucagon—useful as a hypo- or hyperglycaemic agent for treating types I and II diabetes mellitus and other insulin-requiring conditions. WO 9216222.

Bell, D., and McDermott, B. J. (1995). Activity of amylin at CGRP (1)-preferring receptors coupled to positive contractile response in rat ventricular cardiomyocytes. *Regul. Pept.* **60**, 125–133.

Bell, D., Schluter, K. D., Zhou, X. J., McDermott, B. J., and Piper, H. M. (1995). Hypertrophic effects of calcitonin gene-related peptide (CGRP) and amylin on adult mammalian ventricular cardiomyocytes. *J. Mol. Cell Cardiol.* **27**, 2433–2443.

Bhogal, R., Sheldrick, R. L. G., Coleman, R. A., Smith, D. M., and Bloom, S. R. (1994). The effects of IAPP and CGRP on guinea pig tracheal smooth muscle *in vitro*. *Peptides* **15**, 1243–1247.

Blakely, P., Vaughn, D. A., and Fanestil, D. D. (1997). Amylin, calcitonin gene-related peptide, and adrenomedullin: Effects on thiazide receptor and calcium. *Am. J. Physiol.* **41**, F410–F415.

Bogardus, C., and Lillioja, S. (1990). Where all the glucose doesn't go in non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **322**, 262–263.

Bouali, S. M., Wimalawansa, S. J., and Jolicoeur, F. B. (1995). *In vivo* central actions of rat amylin. *Regul. Pept.* **56**, 167–174.

Brain, S. D., Wimalawansa, S., MacIntyre, I., and Williams, T. J. (1990). The demonstration of vasodilator activity of pancreatic amylin amide in the rabbit. *Am. J. Pathol.* **136**, 487–490.

Brunetti, L., Recinella, L., Orlando, G., Michelotto, B., Di Nisio, C., and Vacca, M. (2002). Effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release in the hypothalamus. *Eur. J. Pharmacol.* **454**, 189–192.

Chance, W. T., Balasubramaniam, A., Zhang, F.-S., and Fischer, J. E. (1991a). Hyperthermia following the intrahypothalamic administration of amylin. *Surg. Forum* **42**, 84–86.

Chance, W. T., Balasubramaniam, A., Zhang, F. S., Wimalawansa, S. J., and Fischer, J. E. (1991b). Anorexia following the intrahypothalamic administration of amylin. *Brain Res.* **539**, 352–354.

Clementi, G., Caruso, A., Cutuli, V. M. C., Prato, A., Debernardis, E., Fiore, C. E., and Amicoroxas, M. (1995). Anti-inflammatory activity of amylin and CGRP in different experimental models of inflammation. *Life Sci.* **57**, PL193–PL197.

Cooper, G. J. S., Leighton, B., Dimitriadis, G. D., Parry-Billings, M., Kowalchuk, J. M., Howland, K., Rothbard, J. B., Willis, A. C., and Reid, K. B. (1988). Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc. Natl. Acad. Sci. USA* **85**, 7763–7766.

Cooper, M. E., McNally, P. G., Phillips, P. A., and Johnston, C. I. (1995). Amylin stimulates plasma renin concentration in humans. *Hypertension* **26**, 460–464.

Dacquin, R., Davey, R. A., Laplace, C., Levasseur, R., Morris, H. A., Goldring, S. R., Gebre-Medhin, S., Galson, D. L., Zajac, J. D., and Karsenty, G. (2004). Amylin inhibits bone resorption while the calcitonin receptor controls bone formation *in vivo*. *J. Cell Biol.* **164**, 509–514.

Datta, H., Rafter, P., Chen, Z., Wimalawansa, S., and Macintyre, I. (1990). Amylin-amide displays a proliferative effect on human umbilical vein endothelial cells. *Biochem. Soc. Trans.* **18**, 1276.

Dégano, P., Silvestre, R. A., Salas, M., Peiró, E., and Marco, J. (1993). Amylin inhibits glucose-induced insulin secretion in a dose-dependent manner. Study in the perfused rat pancreas. *Regul. Pept.* **43**, 91–96.

Dewitt, B. J., Cheng, D. Y., Caminiti, G. N., Nossaman, B. D., Coy, D. H., Murphy, W. A., and Kadowitz, P. J. (1994). Comparison of responses to adrenomedullin and calcitonin gene-related peptide in the pulmonary vascular bed of the cat. *Eur. J. Pharmacol.* **257**, 303–306.

Eriksson, J., Franssila-Kallunki, A., Ekstrand, A., Saloranta, C., Widen, E., Schalin, C., and Groop, L. (1989). Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. *New Engl. J. Med.* **321**, 337–343.

Flood, J. F., and Morley, J. E. (1992). Differential effects of amylin on memory processing using peripheral and central routes of administration. *Peptides* **13**, 577–580.

Funakoshi, A., Miyasaka, K., Kitani, K., Nakamura, J., Funakoshi, S., Fukuda, H., and Fujii, N. (1992). Stimulatory effects of islet amyloid polypeptide (amylin) on exocrine pancreas and gastrin release in conscious rats. *Regul. Pept.* **38**, 135–143.

Gedulin, B., Smith, P., Gedulin, G., Baron, A., and Young, A. (2004). Amylin potently inhibits ghrelin secretion in rats. *Diabetes* **53**(2), A340(abstract 1411–P).

Gedulin, B. R., Rink, T. J., and Young, A. A. (1997). Dose-response for glucagonostatic effect of amylin in rats. *Metabolism* **46**, 67–70.

Gedulin, B. R., Jodka, C., Lawler, R., Hoyt, J. A., and Young, A. A. (1998). Amylin inhibits lipase and amylase secretion from the exocrine pancreas in rats. *Diabetes* **47**, A280 (abstract 1086).

Guidobono, F., Coluzzi, M., Pagani, F., Pecile, A., and Netti, C. (1994). Amylin given by central and peripheral routes inhibits gastric acid secretion. *Peptides* **15**, 699–702.

Guidobono, F., Pagani, F., Ticozzi, C., Sibilia, V., Pecile, A., and Netti, C. (1997). Protection by amylin of gastric erosions induced by indomethacin or ethanol in rats. *Br. J. Pharmacol.* **120**, 581–586.

Harris, P. J., Cooper, M. E., Hiranyachattada, S., Berka, J. L., Kelly, D. J., Nobes, M., and Wookey, P. J. (1997). Amylin stimulates proximal tubular sodium transport and cell proliferation in the rat kidney. *Am. J. Physiol.* **41**, F13–F21.

Hom, J. T., Estridge, T., Pechous, P., and Hyslop, P. A. (1995). The amyloidogenic peptide human amylin augments the inflammatory activities of eosinophils. *J. Leukocyte Biol.* **58**, 526–532.

Iwamoto, Y., Takahashi, Y., Sakuma, N., Shiraishi, I., Inooka, G., Kumakura, S., Awata, T., and Kuzuya, T. (1992). Effect of islet amyloid polypeptide (IAPP/amylin) on 2-deoxyglucose uptake in mouse pancreatic acini. *Diabet. Res. Clin. Pract.* **15**, 71–75.

Jodka, C., Gedulin, B., Lawler, R., Grazzini, M., and Young, A. (1997). Amylin protects against ethanol-induced gastric mucosal damage and inhibits pentagastrin-stimulated gastric acid secretion in rats. *Diabetes* **46**, 365A (abstract 1386).

Kolterman, O., Kisicki, J. C., Peltier, L., Gottlieb, A., and Moyses, C. (1994). Infusion of amylin agonist AC-0137 reduces postprandial hyperglycemia in subjects with type 1 diabetes (IDDM). *Clin. Res.* **42**, 87A.

Kovatchev, B., Cox, D. J., McCall, A., Crean, J., Gloster, M., Maggs, D., and Whitehouse, F. (2004). Effects of pramlintide on the magnitude and speed of postprandial blood glucose fluctuations in patients with type 1 diabetes. *Diabetologia* **47**(Suppl. 1), A283 (abstract 783).

Leaming, R., Johnson, A., Hook, G., Hanley, R., and Baron, A. (1995). Amylin modulates insulin secretion in humans. Studies with an amylin antagonist. *Diabetologia* **38**, A113.

Leighton, B., and Cooper, G. J. S. (1988). Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle *in vitro*. *Nature* **335**, 632–635.

Lillioja, S., Mott, D. M., Zawadzki, J. K., Young, A. A., Abbott, W. G., and Bogardus, C. (1986). Glucose storage is a major determinant of *in vivo* "insulin resistance" in subjects with normal glucose tolerance. *J. Clin. Endocrin. Metab.* **62**, 922–927.

Lorenzo, A., Razzaboni, B., Weir, G. C., and Yankner, B. A. (1994). Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* **368**, 756–760.

Lupien, J. R., and Young, A. A. (1993). No measurable effect of amylin on lipolysis in either white or brown isolated adipocytes from rats. *Diabet. Nutr. Metab.* **6**, 13–18.

MacIntyre, I. (1989). Amylinamide, bone conservation, and pancreatic beta cells. *Lancet* **2**, 1026–1027.

Makhlouf, P. C., Zaki, M., Harrington, L., McCuen, R., and Schubert, M. L. (1996). Endogenous amylin stimulates somatostatin (SST) and inhibits gastrin secretion from the antrum of human, dog and rat stomach. *Gastroenterology* **110**, PA1096.

Mather, K. J., Paradisi, G., Leaming, R., Hook, G., Steinberg, H. O., Fineberg, N., Hanley, R., and Baron, A. D. (2002). Role of amylin in insulin secretion and action in humans: Antagonist studies across the spectrum of insulin sensitivity. *Diabet. Metab. Res. Rev.* **18**, 118–126.

May, P. C., Boggs, L. N., and Fuson, K. S. (1993). Neurotoxicity of human amylin in rat primary hippocampal cultures—similarity to Alzheimer's disease amyloid-beta neurotoxicity. *J. Neurochem.* **61**, 2330–2333.

Miles, P. D. G., Deftos, L. J., Moossa, A. R., and Olefsky, J. M. (1994). Islet amyloid polypeptide (amylin) increases the renal excretion of calcium in the conscious dog. *Calcif. Tissue Int.* **55**, 269–273.

Molina, J. M., Cooper, G. J. S., Leighton, B., and Olefsky, J. M. (1990). Induction of insulin resistance *in vivo* by amylin and calcitonin gene-related peptide. *Diabetes* **39**, 260–265.

Morley, J. E., and Flood, J. F. (1991). Amylin decreases food intake in mice. *Peptides* **12**, 865–869.

Mulder, H., Ekelund, M., Ekblad, E., and Sundler, F. (1997a). Islet amyloid polypeptide in the gut and pancreas: Localization, ontogeny and gut motility effects. *Peptides* **18**, 771–783.

Mulder, H., Gebre-Medhin, S., Betsholtz, C., Ahren, B., and Sundler, F. (1997b). Islet amyloid polypeptide knock out mice develop a more severe form of alloxan-induced diabetes. *Diabetologia* **40**, A135 (abstract 527).

Netti, C., Sibilia, V., Pagani, F., Lattuada, N., Coluzzi, M., Pecile, A., and Guidobono, F. (1995). Inhibitory effect of amylin on growth hormone responsiveness to growth-hormone-releasing hormone in the rat. *Neuroendocrinology* **62**, 313–318.

Nuttall, A., Bryan, G. L., and Moyses, C. (1995). Administration of human amylin increases plasma renin activity and plasma aldosterone in man. *Am. J. Hypertens.* **8**, 108A (abstract B16).

Piao, F. L., Cao, C., Han, J. H., Kim, S. Z., Cho, K. W., and Kim, S. H. (2004). Amylin-induced suppression of ANP secretion through receptors for CGRP1 and salmon calcitonin. *Regul. Pept.* **117**, 159–166.

Pittner, R., Beaumont, K., Young, A., and Rink, T. (1995). Dose-dependent elevation of cyclic AMP, activation of glycogen phosphorylase, and release of lactate by amylin in rat skeletal muscle. *Biochim. Biophys. Acta* **1267**, 75–82.

Rauch, M., Schmid, H. A., Koch, J., Riediger, T., and Simon, E. (1997). Blood-borne amylin stimulates water intake via central action on neurons of the subfornical organ. *Pflugers Arch.* **433**, 618.

Romero, D., Bryer, H. P., Rucinski, B., Cvetkovic, M., Liu, C. C., and Epstein, S. (1993). Amylin stimulates osteoblast number in the diabetic rat independent of serum IGF1. *J. Bone Miner. Res.* **8**, S369.

Roth, J., Hughes, H., and Anderson, C. (2004). Amylin-induced changes in body weight and body composition in high-fat fed female rats: Effects of prior or concurrent food restriction. *Obes. Res.* **12**(Suppl.), A109–A110 (abstract 423–P).

Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., DeFronzo, R. A., and Shulman, R. G. (1990). Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N. Engl. J. Med.* **322**, 223–228.

Thompson, R. G., Pearson, L., Gottlieb, A., and Kolterman, O. G. (1996). Pramlintide, an analog of human amylin, reduced fructosamine in patients with Type I diabetes. *Diabetes* **45**, 222A (abstract 818).

Vine, W., Smith, P., Lachappell, R., Rink, T. J., and Young, A. A. (1995a). Lactate production from the rat hindlimb is increased after glucose administration and is suppressed by a selective amylin antagonist: Evidence for action of endogenous amylin in skeletal muscle. *Biochem. Biophys. Res. Commun.* **216**, 554–559.

Vine, W., Smith, P., Percy, A., and Young, A. (1995b). Concentration-response for amylin stimulation of plasma renin activity in rats. *J. Am. Soc. Nephrol.* **6**, 750.

Vine, W., Percy, A., Gedulin, B., Moore, C., Smith, P., Crocker, L., Koda, J., and Young, A. (1995c). Evidence for metabolic action of endogenous amylin from effects of a neutralizing monoclonal antibody in rats. *Diabetes* **44**, 251A (abstract 925).

Vine, W., Smith, P., LaChappell, R., Blase, E., and Young, A. (1998). Effects of rat amylin on renal function in the rat. *Horm. Metab. Res.* **30**, 518–522.

Wagner, U., Bredenbroker, D., Barth, P. J., Fehmann, H. C., and Vonwichert, P. (1995). Amylin immunoreactivity in the rat trachea and characterization of the interaction of amylin and somatostatin on airway mucus secretion. *Res. Exp. Med.* **195**, 289–296.

Wolfe, A., Massi, M., and Geary, N. (2003). Exogenous amylin inhibits ethanol intake in Sardinian alcohol-preferring rats. *Appetite* **40**, 370.

Wookey, P. J., Tikellis, C., Nobes, M., Casley, D., Cooper, M. E., and Darby, I. A. (1998). Amylin as a growth factor during fetal and postnatal development of the rat kidney. *Kidney Int.* **53**, 25–30.

Yki-Järvinen, H., Kubo, K., Zawadzki, J., Lillioja, S., Young, A., Abbott, W., and Foley, J. E. (1987). Dissociation of *in vitro* sensitivities of glucose transport and antilipolysis to insulin in NIDDM. *Am. J. Physiol.* **253**, E300–E304.

Young, A. A., Bogardus, C., Wolfe-Lopez, D., and Mott, D. M. (1988). Muscle glycogen synthesis and disposition of infused glucose in humans with reduced rates of insulin-mediated carbohydrate storage. *Diabetes* **37**, 303–308.

Young, A. A., Wang, M. W., and Cooper, G. J. S. (1991a). Amylin injection causes elevated plasma lactate and glucose in the rat. *FEBS Lett.* **291**, 101–104.

Young, A. A., Wang, M.-W., Cooper, G. J. S., and Mott, D. M. (1991b). Amylin and insulin exert complementary control over Cori cycle activity. *J. Cell Biochem.* **15**(Part B), 68.

Young, A. A., Mott, D. M., Stone, K., and Cooper, G. J. S. (1991c). Amylin activates glycogen phosphorylase in the isolated soleus muscle of the rat. *FEBS Lett.* **281**, 149–151.

Young, A. A., Crocker, L. B., Wolfe-Lopez, D., and Cooper, G. J. S. (1991d). Daily amylin replacement reverses hepatic glycogen depletion in insulin-treated streptozotocin diabetic rats. *FEBS Lett.* **287**, 203–205.

Young, A. A., Gedulin, B., Wolfe-Lopez, D., Greene, H. E., Rink, T. J., and Cooper, G. J. S. (1992). Amylin and insulin in rat soleus muscle: Dose responses for cosecreted noncompetitive antagonists. *Am. J. Physiol.* **263**, E274–E281.

Young, A. A. (1993). *In* "Amylin and Its Effects on the Cori Cycle," pp. 15–21. Synergy Medical Education, Surrey, UK.

Young, A. A., Carlo, P., Smith, P., Wolfe-Lopez, D., Pittner, R., Wang, M. W., and Rink, T. (1993a). Evidence for release of free glucose from muscle during amylin-induced glycogenolysis in rats. *FEBS Lett.* **334**, 317–321.

Young, A. A., Cooper, G. J. S., Carlo, P., Rink, T. J., and Wang, M. W. (1993b). Response to intravenous injections of amylin and glucagon in fasted, fed, and hypoglycemic rats. *Am. J. Physiol.* **264**, E943–E950.

Young, A. A., Rink, T. J., and Wang, M. W. (1993c). Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-I (CGRP-alpha). in the fasted, anaesthetized rat. *Life Sci.* **52**, 1717–1726.

Young, A. A., Gedulin, B., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., Larson, E., and Rink, T. J. (1994a). Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat—Evidence for a metabolic role of endogenous amylin. *FEBS Lett.* **343**, 237–241.

Young, A. A., Rink, T. J., Vine, W., and Gedulin, B. (1994b). Amylin and syndrome-X. *Drug Dev. Res.* **32**, 90–99.

Young, A. A., Vine, W., Carlo, P., Smith, P., Rink, T. J., Rumble, J., and Cooper, M. E. (1994c). Amylin stimulation of renin activity in rats: A possible link between insulin resistance and hypertension. *J. Hypertension* **12**, S152.

Young, A., Nuttall, A., Moyses, C., Percy, A., Vine, W., and Rink, T. (1995a). Amylin stimulates the renin-angiotensin-aldosterone axis in rats and man. *Diabetologia* **38**, A225 (abstract 872).

Young, A. A., Gedulin, B., Vine, W., Percy, A., and Rink, T. J. (1995b). Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin. *Diabetologia* **38**, 642–648.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Young, A. A. (1997). Amylin Pharmaceuticals Inc.. inventor, Methods and compositions for treating pain with amylin or agonists thereof. U.S. Patent 5 667 279.

Young, A. A., Jodka, C., Pittner, R., Parkes, D., and Gedulin, B. R. (2005). Dose-response for inhibition by amylin of cholecystokinin-stimulated secretion of amylase and lipase in rats. *Regul. Pept.* **130**, 19–26.

Young, A., Kolterman, O., and Hall, J. (1999). Amylin innocent in essential hypertension? *Diabetologia* **42**, 1029.

Young, A., Moore, C., Herich, J., and Beaumont, K. (2000). Neuroendocrine actions of amylin. *In* "The CGRP Family: Calcitonin Gene-Related Peptide (CGRP), Amylin, and Adrenomedullin" (D. Poyner, I. Marshall, and S. D. Brain, Eds.), pp. 91–102. Landes Bioscience, Georgetown, TX.

Young, D. A., Deems, R. O., Deacon, R. W., McIntosh, R. H., and Foley, J. E. (1990). Effects of amylin on glucose metabolism and glycogenolysis *in vivo* and *in vitro*. *Am. J. Physiol.* **259**, E457–E461.

Zaki, M., Koduru, S., McCuen, R., Vuyyuru, L., and Schubert, M. L. (2002). Amylin, released from the gastric fundus, stimulates somatostatin and thus inhibits histamine and acid secretion in mice. *Gastroenterology* **123**, 247–255.

Zawadzki, J. K., Wolfe, R. R., Mott, D. M., Lillioja, S., Howard, B. V., and Bogardus, C. (1988). Increased rate of Cori cycle in obese subjects with NIDDM and effect of weight reduction. *Diabetes* **37**, 154–159.

Andrew Young

Inhibition of Food Intake

I. Summary

Over 100 publications, principally from five groups, describe an effect of amylin and amylin analogs in inhibition of food intake in animals and humans. The major groups contributing to this area are those of the following:

- Chance and Balasubramaniam (Balasubramaniam *et al.*, 1991a,b; Chance *et al.*, 1991a,b, 1992a,b, 1993)
- Morley, Flood, and Edwards (Edwards and Morley, 1992; Flood and Morley, 1992; Macintosh *et al.*, 2000; Morley and Flood, 1991, 1994; Morley *et al.*, 1992, 1993, 1994, 1995, 1996, 1997)
- Lutz, Geary, and others (Barth *et al.*, 2003; Del Prete *et al.*, 2002; Lutz *et al.*, 1994, 1995a,b, 1996a,b, 1997a,b, 1998a,b,c, 2000a,b, 2001a, b,c, 2003; Mollet *et al.*, 2001, 2003a,b, 2004; Riediger *et al.*, 2002, 2004; Rushing *et al.*, 2000a,b, 2001, 2002)

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- Workers at Amylin Pharmaceuticals Inc., or their collaborators (Bhavsar *et al.*, 1995, 1996, 1997a, 1998; Birkemo *et al.*, 1995; Chapman *et al.*, 2004a,b; Edwards *et al.*, 1998; Feinle *et al.*, 2002; Mack *et al.*, 2003; Riediger *et al.*, 1999; Roth *et al.*, 2004; Watkins *et al.*, 1996; Weyer *et al.*, 2004; Young, 1997; Young and Bhavsar, 1996)
- Arnelo, Reidelberger, and others (Arnelo *et al.*, 1996a,b, 1997a,b, 1998, 2000; Fruin *et al.*, 1997; Granqvist *et al.*, 1997; Reidelberger *et al.*, 2001, 2002, 2004).

The magnitude of amylin inhibition of food intake, and its potency for this effect when delivered peripherally, suggests a physiological role in satiogenesis. Increases in food intake following disruption of amylin signal-signaling (e.g., with amylin receptor blockade, or with amylin gene knock-out mice) further support a role of endogenous amylin to tonically restrict nutrient intake. In addition, synergies with other endogenous satiety agents may be present, and convey greater physiological importance than is conveyed by single signals.

The anorectic effect of amylin is consistent with a classic amylin pharmacology. The anorectic effect of peripheral amylin appears principally due to a direct action at the area postrema/nucleus tractus solitarius, and is not merely a consequence of gastric fullness, for example.

Circulating amylin appears to physiologically inhibit secretion of ghrelin, an orexigenic peptide from the stomach.

In contrast to the actions of many other anorexigens, amylin appears to stimulate drinking. This disposgenic effect is likely mediated via amylin-sensitive neurones in the subfornical organ, a circumventricular structure, that like the area postrema does not present a blood–brain barrier. Amylin's dipsogenic effect may explain prandial drinking, which has heretofore been regarded as a learned behavior.

II. Food Intake

A. Magnitude of Effect

Early descriptions indicated that amylin's effect of reducing food intake was sizeable compared to other peptides (Chance *et al.*, 1991b; Morley and Flood, 1991). Amylin-treated mice exhibited 53% (Watkins *et al.*, 1996) to 57% (Bhavsar *et al.*, 1998) reductions in food intake. Intravenous injections of 100 μg reduced food intake for 1 hr in rats (Chance *et al.*, 1993). Intravenous infusions of amylin for 3 hr in rats reduced cumulative food intake by up to 78% (Reidelberger *et al.*, 2001) (Fig. 1).

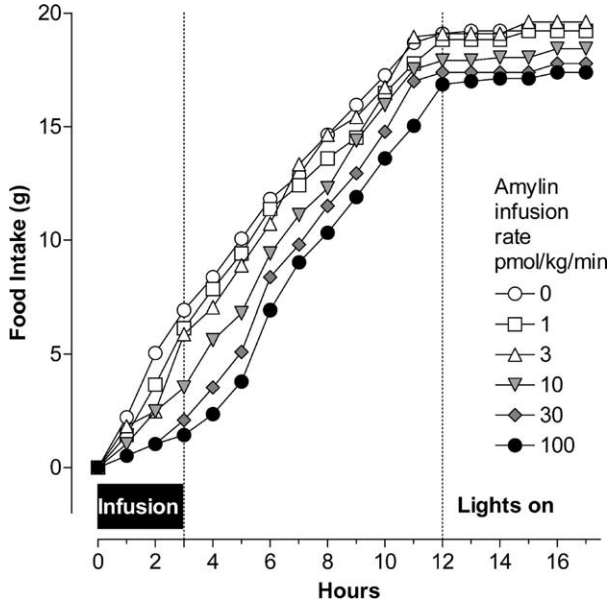

FIGURE 1 Effect of amylin infused via chronic jugular venous cannulae at different rates for 3 hr on subsequent cumulative food intake. Rats were non-fasted. Infusions began 15 min prior to the onset of the dark (feeding) cycle. Data from Reidelberger *et al.* (2001).

B. Potency of Effect

In fasted mice, the ED_{50} for amylin's satiogenic effect following intraperitoneal injection was around 3.5–5 μg/kg (~1 nmol/kg) (Bhavsar *et al.*, 1998; Watkins *et al.*, 1996), making it approximately equipotent with cholecystokinin octapeptide (CCK-8) in the same model (ED_{50} ~1 nmol/kg) (Bhavsar *et al.*, 1998). Doses 3-fold higher (10 μg/kg) resulted in 30-min plasma concentrations of ~80 pM, implying that effective doses were associated with near-physiological increments in plasma amylin concentration (Watkins *et al.*, 1996). In rats, the ED_{50} was ~10 μg/rat (~25 μg/kg, ~6 nmol/kg) (Watkins *et al.*, 1997). Effects of amylin on food intake were detected in young rats at doses as low as (0.1–1 μg/kg) if rats were food-deprived for 24 hr, but not when deprived for 12 hr (Lutz *et al.*, 1994, 1995b). The C_{max} for these doses was in the 10–100 pM range (Young *et al.*, 1996), suggesting that satiety might be a physiological effect of amylin. During a 3-hr amylin infusion in which food intake was inhibited by up to 78% (Reidelberger *et al.*, 2001), significant effects, associated with 1- and 2-hr food intake reductions of 37–26%,

were evoked with an infusion of 1 pmol/kg/min. With this infusion rate, the increment in plasma amylin concentration observed in other pharmaceutical studies was ~10 pM (Young *et al.*, 1996), a concentration within the circulating range, which further supports satiogenesis being a physiological response. The ED_{50} for amylin inhibition of food intake in that study was 8 pmol/kg/min, similar to that of CCK-8 (14 pmol/kg/min) (Reidelberger *et al.*, 2001), supporting a previous report that these peptides were equipotent when administered by intraperitoneal injection (Bhavsar *et al.*, 1998).

Continuous infusion of amylin at 8 pmol/kg/min (~5 μg/hr) by miniosmotic pump inhibited food intake and weight gain over an 8-day period (Arnelo *et al.*, 1996b). In that study, infusion of rat amylin at 2, 7, and 25 pmol/kg/min increased plasma amylin concentrations from a basal level of 10 pM to 35, 78, and 236 pM, respectively, values that are close to physiological and within pathophysiological ranges reported in some animal models. The lowest infusion rate (2 pmol/kg/min) was associated with a plasma amylin concentration of ~35 pM and a statistically significant 14% inhibition of food intake that lasted 5 days. The highest dose administered (25 pmol/kg/min) had the greatest effect, with inhibition of up to 44%, which endured throughout the 8 days (Arnelo *et al.*, 1996b).

To further explore the physiological significance of amylin's satiogenic effect, post-prandial increments in plasma concentration were matched to effective doses in the same model (Arnelo *et al.*, 1998). Food intake increased plasma amylin levels from a fasting level of 11 pM to a peak level of 19 pM after ~2 hr in rats with jugular vein and aortic catheters. The threshold intravenous dose for amylin suppression of feeding was between 1 and 3 pmol/kg/min, the latter dose decreasing 4-hr intake by ~25% and increasing plasma amylin by ~24 pM. These results suggested that postprandial plasma levels of amylin were close to those required to independently reduce food intake (Arnelo *et al.*, 1998).

The anorectic effect of amylin appeared more potent than other effects, such as a putative suppression of insulin action in rats. The latter were thereby less likely to be physiologic. Infusion rates of 7 and 2 pmol/kg/min, which reduced food intake from 44 g (control) to 36 g (amylin; $P < 0.01$) and from 34 g (control) to 29 g (amylin; $P = 0.07$), respectively, had no effect on the glucose metabolic rate (GMR) (18.5 ± 0.6 mmol/kg hr [control] versus 18.7 ± 0.9 mmol/kg hr [amylin]; 14.4 ± 0.7 mmol/kg hr [control] versus 15.6 ± 0.7 mmol/kg hr [amylin], respectively) (Arnelo *et al.*, 1997b).

C. Effect of Disrupting Amylin Signaling

Although initial studies did not detect the effect (Lutz *et al.*, 1997b; Watkins *et al.*, 1996), later studies reported that the selective amylin antagonist AC187, when administered alone, increased food intake (Arnelo *et al.*,

1997a; Granqvist *et al.*, 1997; Reidelberger *et al.*, 2004; Rushing *et al.*, 2001). These latter findings supported the idea that endogenous amylin exerted a tonic (physiological) effect to restrain food intake (Fig. 2).

Test meals have been shown to induce activity (as measured by cFos induction) at the area postrema in rats (Emond *et al.*, 2001; Phifer and Berthoud, 1998; Yamamoto and Sawa, 2000b). The finding that AC187 suppressed such cFos induction (Riediger *et al.*, 2004) indicated that amylin signaling contributed to area postrema *activation*. Importantly, since AC187 blocks only amylin signaling, and not that of other satiogens, it further implied that amylin signaling constituted a distinguishably large portion of the total neuronal activation.

Continuous intracerebroventricular infusion of AC187 increased food intake in rats, and although body weight was not different from controls, there was a 30% increase in body fat (Rushing *et al.*, 2001).

A role of endogenous amylin has been inferred from mice in which the amylin gene was knocked out (Gebre-Medhin *et al.*, 1996). Male mice became ~30% overweight in one study (Gebre-Medhin *et al.*, 1997a,b), and both sexes were heavier in another (Devine and Young, 1998).

D. Amylin/CCK Synergy

A powerful synergy has been reported between amylin and cholecystokinin octapeptide (CCK-8) to inhibit food intake (Bhavsar *et al.*, 1998). The amylin:CCK combination was more effective and 16- to 31-fold more potent in inhibiting short-term food intake than either peptide alone, and the synergy was formally identified using isobolar analysis (Bhavsar *et al.*, 1998). In this analysis, as a true test of additivity, it is asked whether two agents, each dosed to obtain a given level of effect (the isobole), can be

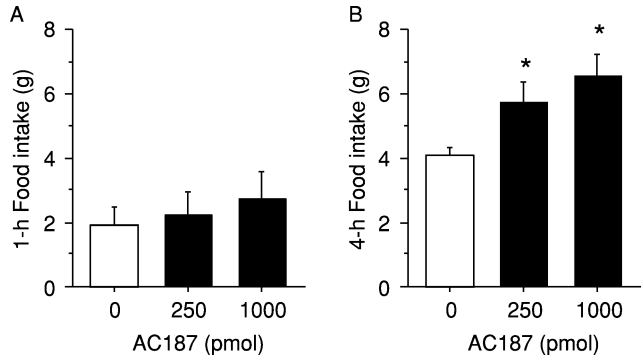

FIGURE 2 Effect of the amylin antagonist AC187 to increase food intake and adiposity, without effect on lean mass. From Rushing *et al.* (2001).

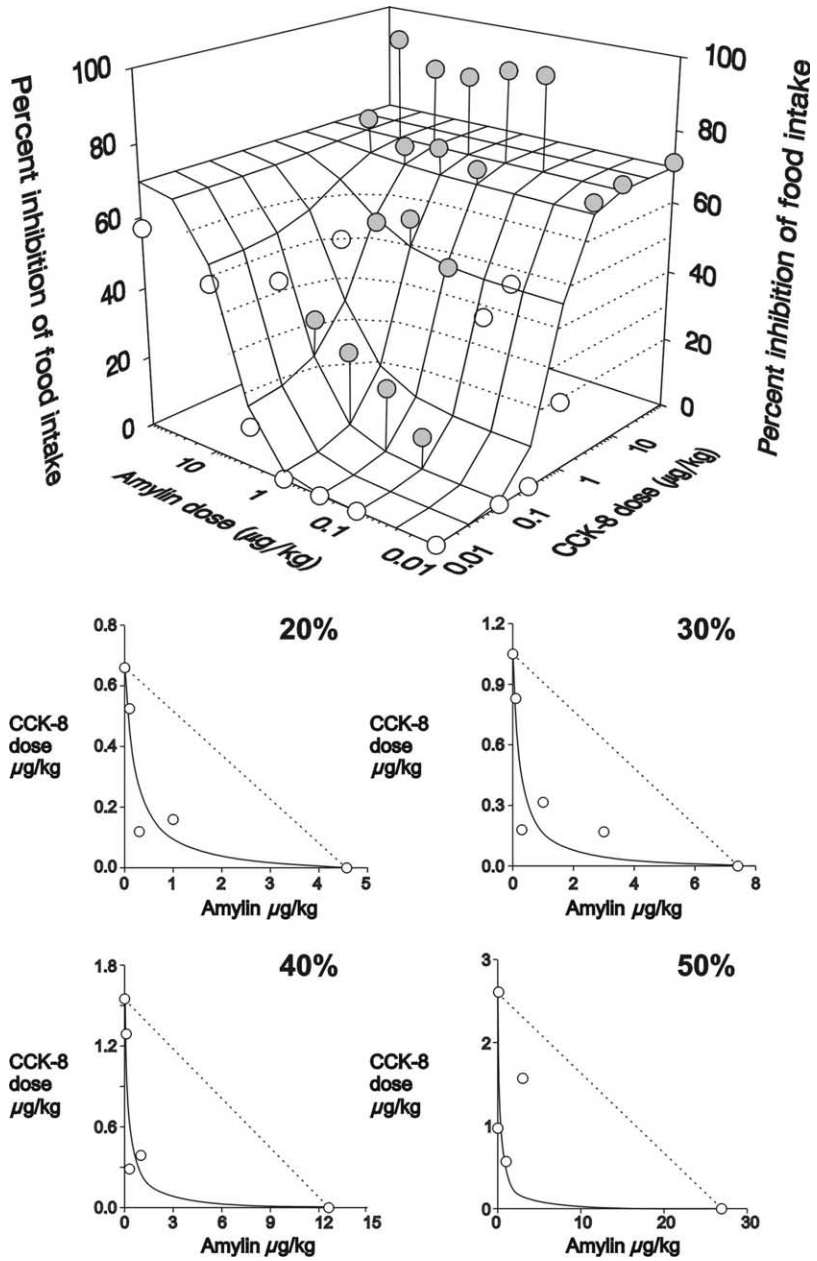

FIGURE 3 Response surface (upper panel) describing the interaction of amylin and CCK on inhibition of food intake in mice. Lower panels are isobolograms for 20, 30, 40, and 50% inhibition of food intake, illustrating non-substitutability (synergism) between amylin and CCK for anorectic effect. From Bhavsar *et al.* (1998).

proportionately exchanged for each other and yield the same effect. If proportionate exchange results in a greater effect, then a synergy has been demonstrated; if a lesser effect is obtained, then some form of antagonism has been demonstrated (Berenbaum, 1981, 1985). Statistically ineffective individual doses of amylin and CCK, when combined, evoked near-maximal inhibition of food intake (Bhavsar *et al.*, 1998). In the isobolar analysis, mixtures of both peptides gave effects that were obtainable only with single agents present at many times the mass. On some parts of the response surface, responses to the combination exceeded those obtainable with single agents at any dose.

Synergy for inhibition of food intake has also been proposed for the combination of insulin and amylin (Rushing *et al.*, 2000b) (Fig. 3).

Synergies of this type between short-term satiety agents may be physiologically relevant since it is a mixture of satiety hormones, rather than any single hormone, that is typically secreted in response to meals (Bhavsar *et al.*, 1996). It has been reported that the selective amylin antagonist AC253 attenuated CCK-induced anorexia (Lutz *et al.*, 2000b). Could it be that this phenomenon represented a disengagement of a physiological satiogenic synergy? That is, the normal amylin-mediated amplification of the CCK drive had been reduced. It may be that a consensus of satiety signals, each associated with different macronutrient drives, is necessary to fully inhibit feeding.

In summary, the magnitude of amylin inhibition of food intake, and its potency for this effect when delivered peripherally, suggests a physiological role in satiogenesis. Increases in food intake following disruption of amylin signaling (e.g., with amylin receptor blockade or with amylin gene knockout) further support a role of endogenous amylin to tonically restrict nutrient intake. In addition, synergies with other endogenous satiety agents may be present and may convey greater physiological import than is conveyed by single signals.

E. Pharmacology of Effect

The order of potency of various ligands at identified amylin receptors is salmon calcitonin > amylin $\geq$ CGRP $\gg$ mammalian calcitonins (Beaumont *et al.*, 1993). These same ligands exhibit a similar order of potency when tested for effects on food intake (Reidelberger *et al.*, 2002). For example, salmon but not mammalian calcitonins administered intracerebroventricularly reduced food intake (Freed *et al.*, 1979; Yamamoto *et al.*, 1982), and amylin was more effective than CGRP in reducing food intake, both centrally (Chance *et al.*, 1992a; Lutz *et al.*, 1998b) and peripherally (Lutz *et al.*, 1998). The idea that the effects of these ligands may be mediated via amylin receptors was supported by the observation that pretreatment with AC187 abolished the amylin-mediated decrease in food intake (Watkins *et al.*, 1996). Others (Arnelo *et al.*, 1997a; Granqvist *et al.*, 1997) (but not all;

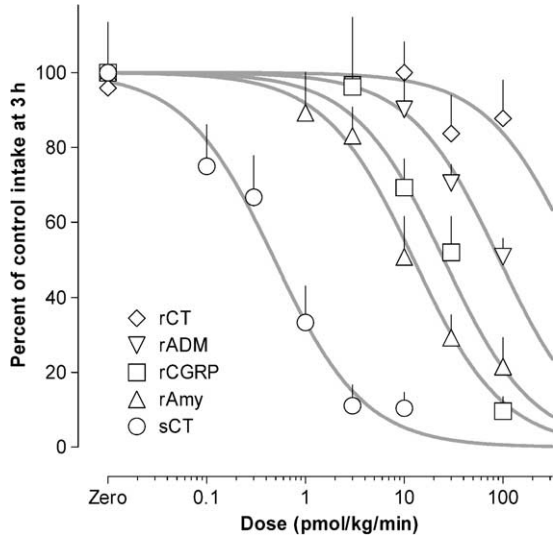

FIGURE 4 Food intake measured over 3 hr in non-fasted rats continuously infused via the jugular vein with salmon calcitonin, rat amylin, rat CGRP, rat adrenomedullin, and rat calcitonin at the indicated infusion rates. The infusion rate response does not take into account differences in clearance of the respective peptides (does not equate to a concentration response). The order of potency is nonetheless similar to that of a classic amylin pharmacology, as described by Beaumont *et al.* (1993). Data from Reidelberger *et al.* (2002).

Lutz *et al.*, 1997b) confirmed this observation, and reported that in contrast to AC187, the CGRP antagonist CGRP[8–37] was comparatively ineffective at blocking amylin's anorectic effect. The conclusion is that the receptors mediating this action were more amylin/calcitonin responsive than CGRP responsive (Fig. 4).

In aging animals, in which plasma amylin concentrations tend to be elevated (Pieber *et al.*, 1994), amylin's anorectic effect tends to weaken (Morley *et al.*, 1993). This suggests the possibility of "amylin resistance" for this particular response in such animals. The possibility of amylin resistance is examined in greater detail in Chapter 6 of this volume.

F. Further Characterization of Effect

Amylin injections of 1 μg/kg in food-deprived rats reduced the size of the first postdeprivation meal without affecting intrameal feeding rate or the size or timing of subsequent meals. These results suggest that amylin inhibits feeding by facilitating meal-ending satiety processes (Lutz *et al.*, 1995b). The observations that amylin did not cause a conditioned taste aversion indicated that reduction in food intake was not secondary to malaise, but likely represented a (pleasurable) meal-ending satiety (Chance *et al.*, 1992a; Lutz *et al.*, 1995b; Rushing *et al.*, 2002).

It was apparent that effects on food intake were not obligatorily tied to effects on gastric emptying, as exemplified by disparities in potencies of amylin, GLP-1, and CCK for these two actions (Birkemo *et al.*, 1995; Bhavsar *et al.*, 1995).

In short-term (7 day) studies amylin administration evoked a greater weight loss and a less severe catabolic response (as assessed by blood urea nitrogen) than occurred in animals pair fed the same quantity of food consumed by those receiving amylin (Fruin *et al.*, 1997). In chronic studies, amylin (Mack *et al.*, 2003; Roth and Anderson, 2004; Roth *et al.*, 2004; Rushing *et al.*, 2000a) and salmon calcitonin (Lutz *et al.*, 2001b) administration reduced body fat content, it spared, and even augmented, protein content (Roth *et al.*, 2004). This apportionment of weight loss differs from that seen after caloric restriction in lean humans, in whom lean tissue loss is significant, and can even exceed that of fat tissue (Forbes, 2000).

Mention of weight loss here introduces the topic, but does not necessarily imply that the satiogenic effect of amylin is the only (or dominant) driver of weight loss. Several other potentially contributing mechanisms exist, and they are described in later chapters.

III. Localization of Effect to Area Postrema

Dissection of the abdominal vagus nerve (Lutz *et al.*, 1995a) or the common hepatic vagus branch (Lutz *et al.*, 1994) did not block the anorectic effect of amylin (Lutz *et al.*, 1994). Similarly, treatment with capsaicin to destroy splanchnic afferents did not eliminate amylin's anorectic effect, despite attenuating CCK anorexia (Edwards *et al.*, 1998; Lutz *et al.*, 1998a). Neither did the anorectic effect appear to be a consequence of effects on gastric emptying (Lutz *et al.*, 1995a). These results pointed instead to the anorexic action being centrally mediated. A profound effect of amylin, when administered directly into the brain (Chance *et al.*, 1991b), tended to support this, as did the observation that, in a dose–response study, amylin was over 50-fold more potent as an anorexigen when delivered by intracerebroventricular versus by intraperitoneal injection (Bhavsar *et al.*, 1997a,b; Watkins *et al.*, 1997).

The expression of cFos, a 55 kDa nuclear protein, is useful as a general marker of cellular activation and can indicate brain regions in which neural traffic increases in response to a putative ligand. The pattern of expression of Fos-like immunoreactivity was determined in brain of rats treated with peripheral injections of amylin. Such treatment produced a strong cFos signal in the area postrema and caudal nucleus tractus solitarius, as well as in the bed nucleus of the stria terminalis and central nucleus of the amygdala, but not in the hypothalamic paraventricular nucleus (Rowland *et al.*, 1997). Riediger *et al.*, (Riediger *et al.*, 2004) similarly observed activation

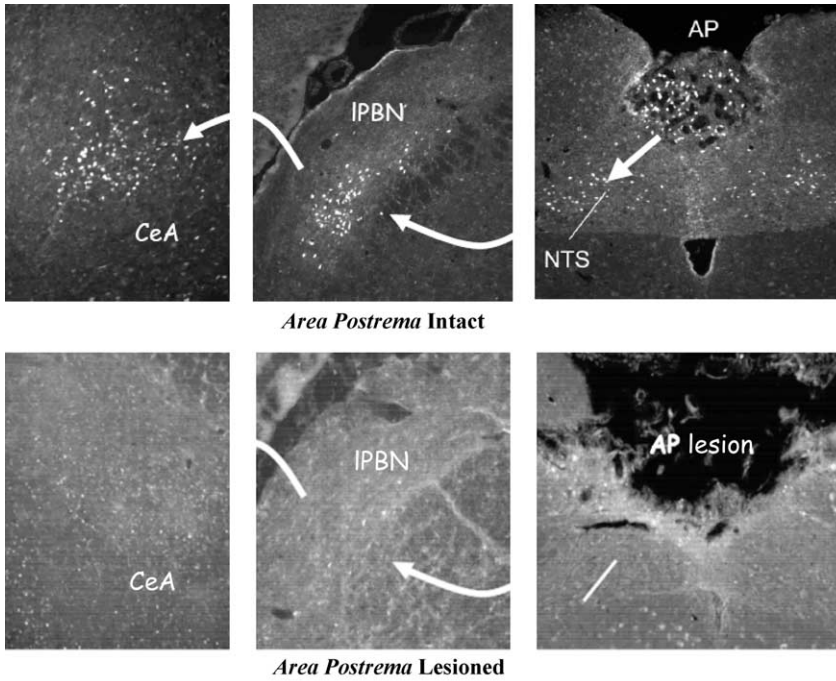

FIGURE 5 Effect of area postrema lesions on appearance of rostral Fos signals after peripheral injections of amylin in rats. Images courtesy of Thomas Lutz, Zurich.

at the area postrema, the lateral parabrachial nucleus, and the central amygdala following peripheral amylin injection.

Some of the activation of these regions could be secondary to signaling from a prime responsive area, such as the area postrema. To test if this was the case, the induction of Fos-like immunoreactivity (Fos-ir) following amylin was examined in rats in which the area postrema was aspirated (Rowland and Richmond, 1999). In these animals, the signal not only was absent in the area postrema (confirming accuracy of the lesioning), but also was absent or reduced in many rostral brain regions, supporting the idea that the area postrema is a primary activated site, which then projects signals to other sites. Riediger *et al.*, (Riediger *et al.*, 2004) observed that Fos signal resulting from peripheral amylin injection was attenuated at the nucleus tractus solitarius, parabrachial nucleus, and central amygdala when the area postrema is lesioned, and proposed an interconnection between these nuclei (Fig. 5).

Examples of secondarily activated sites may be found in experiments in which intraperitoneal injections of amylin and salmon calcitonin were found to alter expression of orexigenic neuropeptides. Messenger RNA (mRNA) expression for orexin and the orexigenic melanin concentrating hormone (MCH) was suppressed in the lateral hypothalamic area, whereas

mRNA levels of neuropeptide Y (NPY), cocaine, and amphetamine-regulated transcript (CART), agouti-gene-related protein (AGRP), and pro-opiomelanocortin (POMC) were unaffected (Barth *et al.*, 2003).

The anorectic/satiogenic effects of amylin were not apparent in rats when the area postrema was lesioned by aspiration (Edwards *et al.*, 1998; Miller *et al.*, 1998). Other studies also showed that aspiration lesions of the area postrema either completely eliminated (Lutz *et al.*, 2001a; Rowland and Richmond, 1999) or markedly attenuated (Lutz *et al.*, 1998c) the anorectic effect of amylin. In contrast, the anorectic effect of dexfenfluramine and rostral expression of cFOS after its administration was maintained after area postrema lesions (Rowland and Richmond, 1999). That is, although anorectic effects of amylin had been eliminated, lesions of the area postrema had not destroyed the capacity of all agents to mediate satiety, implying that satiogenic circuits were substantially intact.

In rats with lesions of the area postrema, both amylin and CGRP still evoked an anorectic effect when delivered into the lateral ventricle, indicating the presence of amylin-responsive rostral sites (Lutz *et al.*, 1998b). And neurons responsive to amylin have been identified in brain slices from the arcuate nucleus (Davidowa *et al.*, 2004). However, it is not clear to what extent these rostral sites participate in the response to circulating peptide. The antagonist AC187 increased spontaneous food intake when infused locally at the area postrema (Mollet *et al.*, 2004). Such a result is more consistent with amylinergic suppression of food intake being predominantly from the area postrema than with it being from the confluence of multiple amylin-responsive areas.

Different meal-related stimuli (mechanical, nutrient, and hormonal cues) evoke cFos expression at the area postrema (Emond *et al.*, 2001; Fraser *et al.*, 1995; Phifer and Berthoud, 1998; Yamamoto and Sawa, 2000a,b). The area postrema has been promoted as a site for integrated metabolic control of feeding behavior (Horn *et al.*, 1999). Meal-related activation of cFos at the area postrema is attenuated by peripheral pretreatment with the antagonist AC187 (Riediger *et al.*, 2004). The implications of this observation are profound. If a general feeding-related response is blocked by a selective amylin receptor antagonist, it indicates that a large fraction of the signal driving that response is amylinergic (Fig. 6).

It has been proposed, since the area postrema is implicated in the vomiting reflex, that amylin's anorexic/satiogenic effects are simply a manifestation of sickness behavior. However, in clinical studies with pramlintide, weight loss occurred independently of reports of nausea (Fineman *et al.*, 2001; Weyer *et al.*, 2001). In a clinical physiology study, energy intake was reduced by 16% in 15 obese subjects without any report of nausea, and by 23% in subjects with diabetes, 10/11 of whom did not report nausea (Chapman *et al.*, 2004a; Weyer *et al.*, 2004). In a patient satisfaction survey, the effects of pramlintide on appetite control were reported as beneficial (Marrero *et al.*, 2004). In animal studies from four different groups using

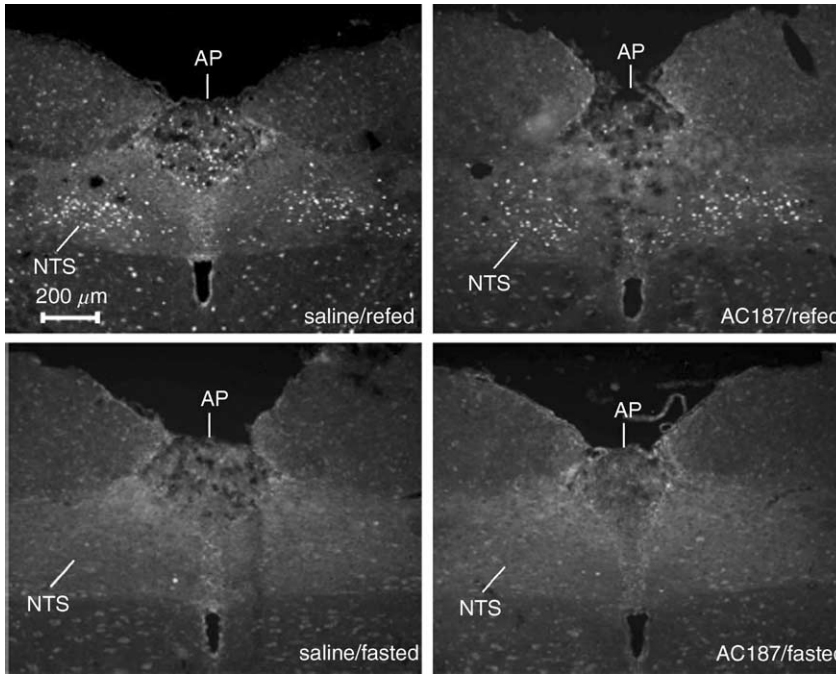

FIGURE 6 Similarly to peripheral injections of amylin, refeeding of fasted rats induced Fos expression at the area postrema (upper left panel) relative to controls (lower left). Pre-administration of the amylin antagonist AC187 markedly attenuated the Fos response at the area postrema (upper right), indicating that a significant fraction of meal-induced activity there is amylinergic. From Riediger *et al.* (2004).

the conditioned taste aversion paradigm (Chance *et al.*, 1992a; Lutz *et al.*, 1995b; Morley *et al.*, 1997; Rushing *et al.*, 2002), amylin inhibition of food intake was judged non-aversive.

The importance of the hindbrain in ingestive control has likely been underestimated due to overemphasis on the hypothalamus. The classical hypothalamic model has been challenged by Grill and Kaplan, who have demonstrated the integrative achievements of the chronically maintained, supracollicular decerebrate rat (Grill and Kaplan, 2002). Decerebrate rats show discriminative responses to taste stimuli and gut-mediated meal termination that is similar to those in intact rats. It appears that the caudal brain stem, in neural isolation from the forebrain, is sufficient to mediate inhibitory ingestive responses to a range of visceral afferent and hormonal signals. The hypothalamic circuits appear more necessary in generating hyperphagic responses to food deprivation.

In summary, the anorectic effect of amylin is consistent with a classic amylin pharmacology. The anorectic effect of peripheral amylin appears due principally to a direct action at the area postrema/nucelus tractus solitarius (Fig. 7).

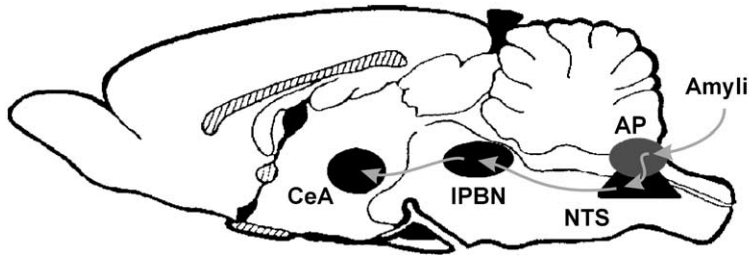

FIGURE 7 Proposed amylinergic pathway of neuronal transmission following meal-related stimulation of the area postrema. Images courtesy of Thomas Lutz, Zurich.

IV. Amylin Interaction at Other Appetite Control Circuits

Cross-talk between several pathways mediating ingestive control is apparent. For example, food intake in *ob/ob* (leptin-deficient) mice was suppressed by 98% with an amylin dose that suppressed it by only 53% in wild-type mice (Young and Bhavsar, 1996). In experiments using antagonists, the histaminergic system appeared to be involved in transduction of amylin's inhibitory effect on feeding in rats, but the serotoninergic system did not (Lutz *et al.*, 1996b). And while the anorectic effects of peripheral amylin and leptin appear to be mediated via histamine (H1) receptors, the same is not true for CCK (Mollet *et al.*, 2001).

Dopamine-3 receptor knockout mice were also hyper-responsive to the anorectic effects of both amylin and leptin (Benoit *et al.*, 2003). Dopaminergic pathways have been implicated in amylin satiogenesis (Lutz *et al.*, 2001c), and amylin inhibited *in vitro* release of dopamine from hypothalamic synaptosomes (Brunetti *et al.*, 2002).

A. Amylin and Ghrelin Secretion

The endogenous growth hormone secretagogue ghrelin (Kojima *et al.*, 1999) has recently generated attention through reports that it increases food intake (Wren *et al.*, 2000) and adiposity (Tschop *et al.*, 2000) in rodents. As such, it is a rare example of a circulating orexigen. Its pattern of secretion is opposite to that of most meal-related peptides and, indeed, reciprocates that of the β-cell. But insulin had only a minor effect to inhibit ghrelin secretion. It instead appears that amylin, rather than insulin, is the dominant β-cell inhibitor of ghrelin secretion. The 50% suppression of active ghrelin in the presence of amylin was comparable in magnitude to meal-induced suppression (Gedulin *et al.*, 2004). Dose–response analysis indicated the effect could occur with physiological amylin concentrations, and this was supported by an increase in plasma ghrelin concentrations immediately after administration of AC187 (Gedulin *et al.*, 2004). As with several other effects, amylin

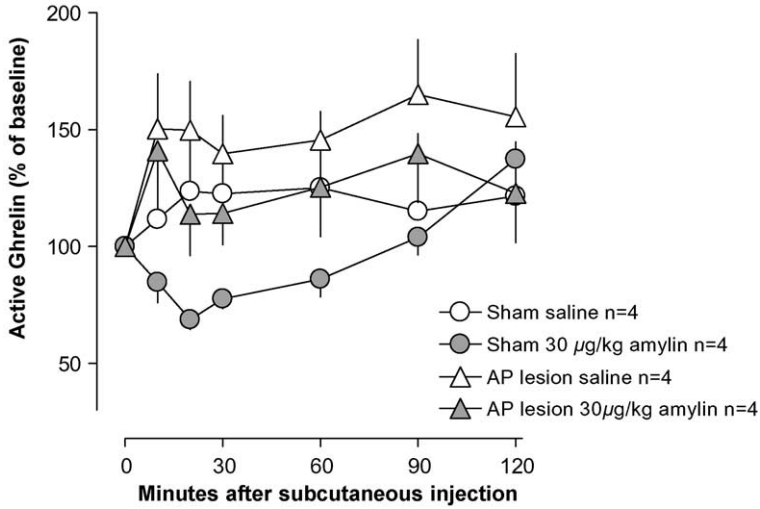

FIGURE 8 Amylin suppression of active ghrelin secretion is annulled following lesions of area postrema. From Young *et al.* (2004).

inhibition of ghrelin secretion appeared to be mediated via the area postrema, since the effect was entirely eliminated in lesioned animals (Young *et al.*, 2004). It is presently unknown how much, if any, of the effects of amylin to reduce food intake and adiposity are due to reduction of ghrelin-related orexigenic and adipogenic drives (Fig. 8).

B. Effect of Amylin on Drinking Behavior

Many peptides that reduce food consumption will concomitantly reduce water consumption. This does not appear to necessarily be the case for amylin where a reduction in water intake is typically only observed in the presence of food. The opposite effect is usually observed in fasted animals (Lutz, personal communication). Peripheral amylin injections stimulated drinking in 13/17 rats (cf. 6/33 controls), similar to the 16/20 response to administration of the dipsogenic hormone angiotensin-II (Rauch *et al.*, 1997; Riediger *et al.*, 1999a). Others have also reported a behavioral specificity, in that feeding is inhibited with amylin, without there being inhibition of drinking (Asarian *et al.*, 1998; Baldo and Kelley, 1999). In pygmy goats, amylin and sCT were both anorexigenic, yet sCT was dipsogenic at low dose (Del Prete *et al.*, 2002). CGRP, which can also act as an amylin agonist, reduced food intake but not water intake (Morley *et al.*, 1996).

The dipsogenic effect of amylin has been proposed as an explanation for prandial drinking (Rauch *et al.*, 1997; Riediger *et al.*, 1999a), which had hitherto been regarded as a learned behavior. The effect of amylin on drinking behavior is likely mediated via action at the subfornical organ,

which, like the area postrema, is a circumventricular structure devoid of a blood–brain barrier. Amylin activated over 70% of neurons in subfornical organ recordings. The pharmacology was amylin-like in that sCT and CGRP were stimulatory and AC187 was inhibitory (Rauch *et al.*, 1997; Riediger *et al.*, 1999a,b).

Amylin at low peripheral doses was reported to reduce ethanol intake in alcohol-preferring rats (Wolfe *et al.*, 2003). It is presently unknown whether this effect is alcohol specific, or whether it is related to effects on ingestive and drinking control.

In summary, it appears that circulating amylin inhibits food intake via action at one circumventricular organ, the area postrema, and stimulates drinking via action at another, the subfornical organ (Simon, 2000).

References

Arnelo, U., Blevins, J. E., Larsson, J., Permert, J., Westermark, P., Reidelberger, R. D., and Adrian, T. E. (1996a). Effects of acute and chronic infusion of islet amyloid polypeptide on food intake in rats. *Scand. J. Gastroenterol.* **31**, 83–89.

Arnelo, U., Permert, J., Adrian, T. E., Larsson, J., Westermark, P., and Reidelberger, R. D. (1996b). Chronic infusion of islet amyloid polypeptide causes anorexia in rats. *Am. J. Physiol.* **40**, R1654–R1659.

Arnelo, U., Permert, J., Granqvist, L., Adrian, T., Westermark, P., Smith, D., and Reidelberger, R. (1997a). Effects of AC187, IAPP 8–37, and CGRP 8–37 on IAPP-induced anorexia in rats. *Proc. Neurosci. Abstr.* **23**, 102.37.

Arnelo, U., Permert, J., Larsson, J., Reidelberger, R. D., Arnelo, C., and Adrian, T. E. (1997b). Chronic low dose islet amyloid polypeptide infusion reduces food intake, but does not influence glucose metabolism, in unrestrained conscious rats: Studies using a novel aortic catheterization technique. *Endocrinology* **138**, 4081–4085.

Arnelo, U., Reidelberger, R., Adrian, T. E., Larsson, J., and Permert, J. (1998). Sufficiency of postprandial plasma levels of islet amyloid polypeptide for suppression of feeding in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **275**, R1537–R1542.

Arnelo, U., Herrington, M. K., Theodorsson, E., Adrian, T. E., Reidelberger, R., Larsson, J., Marcusson, J., Strommer, L., Ding, X. Z., and Permert, J. (2000). Effects of long-term infusion of anorexic concentrations of islet amyloid polypeptide on neurotransmitters and neuropeptides in rat brain. *Brain Res.* **887**, 391–398.

Asarian, L., Eckel, L. A., and Geary, N. (1998). Behaviorally specific inhibition of sham feeding by amylin. *Peptides* **19**, 1711–1718.

Balasubramaniam, A., Renugopalakrishnan, V., Stein, M., Fischer, J. E., and Chance, W. T. (1991a). Syntheses, structures and anorectic effects of human and rat amylin. *Peptides* **12**, 919–924.

Balasubramaniam, A., Zhang, F. S., Thomas, I., and Chance, W. T. (1991b). Amylin increases transport of tyrosine and tryptophan into brain. *Soc. Neurosci. Abstr.* **17**, 976.

Baldo, B. A., and Kelley, A. E. (1999). Effects of insulin or amylin infusion into the nucleus accumbens shell on unconditioned exploratory and ingestive behaviors. *Soc. Neurosci. Abstr.* **25**, 2141.

Barth, S. W., Riediger, T., Lutz, T. A., and Rechkemmer, G. (2003). Differential effects of amylin and salmon calcitonin on neuropeptide gene expression in the lateral hypothalamic area and the arcuate nucleus of the rat. *Neurosci. Lett.* **341**, 131–134.

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Benoit, S. C., McQuade, J. A., Clegg, D. J., Xu, M., Rushing, P. A., Woods, S. C., and Seeley, R. J. (2003). Altered feeding responses in mice with targeted disruption of the dopamine-3 receptor gene. *Behav. Neurosci.* **117**, 46–54.

Berenbaum, M. C. (1981). Criteria for analyzing interactions between biologically active agents. *Adv. Cancer Res.* **35**, 269–335.

Berenbaum, M. C. (1985). The expected effect of a combination of agents: The general solution. *J. Theor. Biol.* **114**, 413–431.

Bhavsar, S. P., Gedulin, B. R., Beaumont, K., Rink, T. J., and Young, A. A. (1995). Inhibition of gastric emptying and of food intake appear to be independently controlled in rodents. *Soc. Neurosci. Abstr.* **21**, 460.

Bhavsar, S., Watkins, J., and Rink, T. (1996). Synergistic effect of amylin and cholecystokinine octapeptide on food intake in mice. *Diabetes* **45**, 333A.

Bhavsar, S., Watkins, J., and Young, A. (1997a). Comparison of central and peripheral administration of amylin on reduction of food intake in rats. *Diabetologia* **40**(suppl 1), A302.

Bhavsar, S., Watkins, J., and Young, A. (1997b). The effect of amylin on food intake appears to be centrally mediated in rats. *Diabetes* **40**, 254A.

Bhavsar, S., Watkins, J., and Young, A. (1998). Synergy between amylin and cholecystokinin for inhibition of food intake in mice. *Physiol. Behav.* **64**, 557–561.

Birkemo, L. S., Johnson, M. F., and Ervin, G. N. (1995). CCK-8 and amylin produce different patterns of effects on anorexia and delayed gastric emptying. *Proc. Neurosci. Abstr.* **21**, 459.

Brunetti, L., Recinella, L., Orlando, G., Michelotto, B., Di Nisio, C., and Vacca, M. (2002b). Effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release in the hypothalamus. *Eur. J. Pharmacol.* **454**, 189–192.

Chance, W. T., Balasubramaniam, A., Zhang, F.-S., and Fischer, J. E. (1991a). Hyperthermia following the intrahypothalamic administration of amylin. *Surg. Forum* **42**, 84–86.

Chance, W. T., Balasubramaniam, A., Zhang, F. S., Wimalawansa, S. J., and Fischer, J. E. (1991b). Anorexia following the intrahypothalamic administration of amylin. *Brain Res.* **539**, 352–354.

Chance, W. T., Balasubramaniam, A., Chen, X., and Fischer, J. E. (1992a). Tests of adipsia and conditioned taste aversion following the intrahypothalamic injection of amylin. *Peptides* **13**, 961–964.

Chance, W. T., Balasubramaniam, A., Thomas, I., and Fischer, J. E. (1992b). Amylin increases transport of tyrosine and tryptophan into the brain. *Brain Res.* **593**, 20–24.

Chance, W. T., Balasubramaniam, A., Stallion, A., and Fischer, J. E. (1993). Anorexia following the systemic injection of amylin. *Brain Res.* **607**, 185–188.

Chapman, I., Parker, B., Doran, S., Feinle-Bisset, C., Wishart, J., Wang, Y., Gao, H.-Y., McIntyre, S., Burrell, T., Deckhut, D., Weyer, C., and Horowitz, M. (2004a). Effect of pramlintide on ad-libitum food intake in obese subjects and subjects with type 2 diabetes: A randomized, double-blind, placebo-controlled, cross-over study. *Diabetes* **53**(suppl. 2), A82.

Chapman, I., Parker, B., Doran, S., Feinle-Bisset, C., Wishart, J., Wang, Y., Lush, C., McIntyre, S., Burrell, T., Deckhut, D., Weyer, C., and Horowitz, M. (2004b). Effect of pramlintide on ad-libitum food intake in obese subjects and subjects with type 2 diabetes: A randomized, double-blind, placebo-controlled, cross-over study. *Diabetologia* **47** (suppl. 1), A283–A284.

Davidowa, H., Ziska, T., and Plagemann, A. (2004). Arcuate neurons of overweight rats differ in their responses to amylin from controls. *Neuroreport* **15**, 2801–2805.

Del Prete, E., Schade, B., Riediger, T., Lutz, T. A., and Scharrer, E. (2002a). Effects of amylin and salmon calcitonin on feeding and drinking behavior in pygmy goats. *Physiol. Behav.* **75**, 593–599.

Devine, E., and Young, A. A. (1998). Weight gain in male and female mice with amylin gene knockout. *Diabetes* **47**(suppl. 1), A317.

Edwards, B. J. A., and Morley, J. E. (1992). Amylin. *Life Sci.* **51**, 1899–1912.

Edwards, G. L., Power, J. D., and Young, A. A. (1998). Attenuation of the satiogenic effects of amylin by lesions of the *area postrema*, but not by intraperitoneal capsaicin. *Proc. Neurosci. Abstr.* **24**, 406.3.

Emond, M., Schwartz, G. J., and Moran, T. H. (2001). Meal-related stimuli differentially induce c-Fos activation in the nucleus of the solitary tract. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R1315–R1321.

Feinle, C., O'Donovan, D., and Horowitz, M. (2002). Carbohydrate and satiety. *Nutr. Rev.* **60**, 155–169.

Fineman, M., Maggs, D., Burrell, T., Velte, M., and Kolterman, O. (2001). Addition of pramlintide to insulin therapy in type 1 diabetes: Impact on glycemic and weight control stratified by BMI. *Diabetes* **50**(suppl. 2), A112.

Flood, J. F., and Morley, J. E. (1992). Differential effects of amylin on memory processing using peripheral and central routes of administration. *Peptides* **13**, 577–580.

Fraser, K. A., Raizada, E., and Davison, J. S. (1995). Oral-pharyngeal-esophageal and gastric cues contribute to meal-induced c-fos expression. *Am. J. Physiol.* **268**, R223–R230.

Freed, W. J., Perlow, M. J., and Wyatt, R. J. (1979). Calcitonin: Inhibitory effect on eating in rats. *Science* **206**, 850–852.

Fruin, A. B., Arnelo, U., Granqvist, L., Strommer, L., Larsson, J., and Permert, J. (1997). Weight loss induced by islet amyloid polypeptide (IAPP) is not fully explained by reduction in food intake. *Digestion* **58**, 55.

Gebre-Medhin, S., Mulder, H., Ahren, B., Pekny, M., Tornell, J., Sundler, F., Westermark, P., and Betsholtz, C. (1996). Mice lacking IAPP develop and reproduce normally and appear metabolically unaffected. *Diabetologia* **39**, A181.

Gebre-Medhin, S., Mulder, H., Pekny, M., Zhang, Y., Tornell, J., Westermark, P., Sundler, F., Ahren, B., and Betsholtz, C. (1997a). Altered glucose homeostasis, body weight and nociception in IAPP (amylin) null mutant mice. *Diabetes* **40**, 29A.

Gebre-Medhin, S., Mulder, H., Pekny, M., Zhang, Y. Z., Tornell, J., Westermark, P., Sundler, F., Ahren, B., and Betsholtz, C. (1997b). IAPP (amylin) null mutant mice; plasma levels of insulin and glucose, body weight and pain responses. *Diabetologia* **40**, A26.

Gedulin, B., Smith, P., Gedulin, G., Baron, A., and Young, A. (2004). Amylin potently inhibits ghrelin secretion in rats. *Diabetes* **53**(suppl. 2), A340.

Granqvist, L., Permert, J., Arnelo, U., Adrian, T., Westermark, P., Smith, D., and Reidelberger, R. (1997). Effects of AC187, IAPP (8–37). and CGRP (8–37). on IAPP induced anorexia in rats. *Digestion* **58**, 55.

Grill, H. J., and Kaplan, J. M. (2002). The neuroanatomical axis for control of energy balance. *Front. Neuroendocrinol.* **23**, 2–40.

Horn, C. C., Addis, A., and Friedman, M. I. (1999). Neural substrate for an integrated metabolic control of feeding behavior. *Am. J. Physiol.* **45**, R113–R119.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**, 656–660.

Lutz, T. A., Del Prete, E., and Scharrer, E. (1994). Reduction of food intake in rats by intraperitoneal injection of low doses of amylin. *Physiol. Behav.* **55**, 891–895.

Lutz, T. A., Del Prete, E., and Scharrer, E. (1995a). Subdiaphragmatic vagotomy does not influence the anorectic effect of amylin. *Peptides* **16**, 457–462.

Lutz, T. A., Geary, N., Szabady, M. M., DelPrete, E., and Scharrer, E. (1995b). Amylin decreases meal size in rats. *Physiol. Behav.* **58**, 1197–1202.

Lutz, T. A., Del Prete, E., Szabady, M. M., and Scharrer, E. (1996a). Attenuation of the anorectic effects of glucagon, cholecystokinin, and bombesin by the amylin receptor antagonist CGRP (8–37). *Peptides* **17**, 119–124.

Lutz, T. A., Del Prete, E., Walzer, B., and Scharrer, E. (1996b). The histaminergic, but not the serotoninergic, system mediates amylin's anorectic effect. *Peptides* **17**, 1317–1322.

Lutz, T. A., Pieber, T. R., Walzer, B., Del Prete, E., and Scharrer, E. (1997a). Different influence of CGRP (8–37), an amylin and CGRP antagonist, on the anorectic effects of cholecystokinin and bombesin in diabetic and normal rats. *Peptides* **18**, 643–649.

Lutz, T. A., Rossi, R., Althaus, J., Del Prete, E., and Scharrer, E. (1997b). Evidence for a physiological role of central calcitonin gene-related peptide (CGRP) receptors in the control of food intake in rats. *Neurosci. Lett.* **230**, 159–162.

Lutz, T. A., Althaus, J., Rossi, R., and Scharrer, E. (1998a). Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers. *Am. J. Physiol.* **274**, R1777–R1782.

Lutz, T. A., Rossi, R., Althaus, J., Del Prete, E., and Scharrer, E. (1998b). Amylin reduces food intake more potently than calcitonin gene-related peptide (CGRP) when injected into the lateral brain ventricle in rats. *Peptides* **19**, 1533–1540.

Lutz, T. A., Senn, M., Althaus, J., Del Prete, E., Ehrensperger, F., and Scharrer, E. (1998c). Lesion of the area postrema nucleus of the solitary tract (AP/NTS) attenuates the anorectic effects of amylin and calcitonin gene-related peptide (CGRP) in rats. *Peptides* **19**, 309–317.

Lutz, T. A., Tschudy, S., Rushing, P. A., and Scharrer, E. (2000a). Amylin receptors mediate the anorectic action of salmon calcitonin (sCT). *Peptides* **21**, 233–238.

Lutz, T. A., Tschudy, S., Rushing, P. A., and Scharrer, E. (2000b). Attenuation of the anorectic effects of cholecystokinin and bombesin by the specific amylin antagonist AC253. *Physiol. Behav.* **70**, 533–536.

Lutz, T. A., Mollet, A., Rushing, P. A., Riediger, T., and Scharrer, E. (2001a). The anorectic effect of a chronic peripheral infusion of amylin is abolished in area postrema/nucleus of the solitary tract (AP/NTS) lesioned rats. *Int. J. Obes. Relat. Metab. Disord.* **25**, 1005–1011.

Lutz, T. A., Rushing, P. A., and Riediger, T. (2001b). Repeated salmon calcitonin injection lowers body weight and body fat. *Sci. World J.* **1**, 25.

Lutz, T. A., Tschudy, S., Mollet, A., Geary, N., and Scharrer, E. (2001c). Dopamine D (2). receptors mediate amylin's acute satiety effect. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R1697–R703.

Lutz, T. A., Edwards, G. L., Grabler, V., Becskei, C., and Riediger, T. (2003). Amylin's anorectic effect depends on an intact lateral parabrachial nucleus (IPBN). *Appetite* **40**, 346–347.

Macintosh, C., Morley, J. E., and Chapman, I. M. (2000). The anorexia of aging. *Nutrition* **16**, 983–995.

Mack, C., Hoyt, J., Moore, C., Jodka, C., and Sams-Dodd, F. (2003). Sustained reduction in food intake and body weight in high fat-fed rats during 28-day amylin infusion. *Diabetes* **52**(suppl. 1), A389.

Marrero, D., Kruger, D., Burrell, T., Gloster, M., Crean, J., Herrmann, K., and Kolterman, O. (2004). Patients with type 1 diabetes: Perceptions associated with pramlintide as an adjunctive treatment to insulin. *Diabetes* **53**(suppl. 2), A137–A138.

Miller, C. C., Dilts, R. P., Young, A. A., and Edwards, G. L. (1998). Lesion of the area postrema (AP) attenuates hindbrain cFOS expression after amylin treatment. *Proc. Neurosci. Abstr.* **24**, 847.10.

Mollet, A., Lutz, T. A., Meier, S., Riediger, T., Rushing, P. A., and Scharrer, E. (2001). Histamine H (1). receptors mediate the anorectic action of the pancreatic hormone amylin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**, R1442–R1448.

Mollet, A., Meier, S., Grabler, V., Gilg, S., Scharrer, E., and Lutz, T. A. (2003a). Endogenous amylin contributes to the anorectic effects of cholecystokinin and bombesin. *Peptides* **24**, 91–98.

Mollet, A., Meier, S., Riediger, T., and Lutz, T. A. (2003b). Histamine H (1). receptors in the ventromedial hypothalamus mediate the anorectic action of the pancreatic hormone amylin. *Peptides* **24**, 155–158.

Mollet, A., Gilg, S., Riediger, T., and Lutz, T. A. (2004). Infusion of the amylin antagonist AC 187 into the area postrema increases food intake in rats. *Physiol. Behav.* **81**, 149–155.

Morley, J. E., and Flood, J. F. (1991). Amylin decreases food intake in mice. *Peptides* **12**, 865–869.

Morley, J. E., and Flood, J. F. (1994). Effects of amylin and CGRP on appetite regulation and memory. *Can. J. Physiol. Pharmacol.* **72**, 32.

Morley, J. E., Flood, J., and Silver, A. J. (1992). Effects of peripheral hormones on memory and ingestive behaviors. *Psychoneuroendocrinology* **17**, 391–399.

Morley, J. E., Morley, P. M., and Flood, J. F. (1993). Anorectic effects of amylin in rats over the life span. *Pharmacol. Biochem. Behav.* **44**, 577–580.

Morley, J. E., Flood, J. F., Horowitz, M., Morley, P. M. K., and Walter, M. J. (1994). Modulation of food intake by peripherally administered amylin. *Am. J. Physiol.* **267**, R178–R184.

Morley, J. E., Flood, J. F., Farr, S. A., Perry, H. J., Kaiser, F. E., and Morley, P. M. K. (1995). Effects of amylin on appetite regulation and memory. *Can. J. Physiol. Pharmacol.* **73**, 1042–1046.

Morley, J. E., Farr, S. A., and Flood, J. F. (1996). Peripherally administered calcitonin gene-related peptide decreases food intake in mice. *Peptides* **17**, 511–516.

Morley, J. E., Suarez, M. D., Mattamal, M., and Flood, J. F. (1997). Amylin and food intake in mice: Effects on motivation to eat and mechanism of action. *Pharmacol. Biochem. Behav.* **56**, 123–129.

Phifer, C. B., and Berthoud, H. R. (1998). Duodenal nutrient infusions differentially affect sham feeding and Fos expression in rat brain stem. *Am. J. Physiol.* **274**, R1725–R1733.

Pieber, T. R., Roitelman, J., Lee, Y., Luskey, K. L., and Stein, D. T. (1994). Direct plasma radioimmunoassay for rat amylin-(1–37): Concentrations with acquired and genetic obesity. *Am. J. Physiol.* **267**, E156–E164.

Rauch, M., Schmid, H. A., Koch, J., Riediger, T., and Simon, E. (1997). Blood-borne amylin stimulates water intake via central action on neurons of the subfornical organ. *Pflugers Arch.* **433**, 618.

Reidelberger, R. D., Arnelo, U., Granqvist, L., and Permert, J. (2001). Comparative effects of amylin and cholecystokinin on food intake and gastric emptying in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R605–R611.

Reidelberger, R. D., Kelsey, L., and Heimann, D. (2002). Effects of amylin-related peptides on food intake, meal patterns, and gastric emptying in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, R1395–R1404.

Reidelberger, R. D., Haver, A. C., Arnelo, U., Smith, D. D., Schaffert, C. S., and Permert, J. (2004). Amylin receptor blockade stimulates food intake in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R568–R574.

Riediger, T., Rauch, M., and Schmid, H. A. (1999a). Actions of amylin on subfornical organ neurons and on drinking behavior in rats. *Am. J. Physiol.* **276**, R514–R521.

Riediger, T., Schmid, H. A., Young, A. A., and Simon, E. (1999b). Pharmacological characterisation of amylin-related peptides activating subfornical organ neurones. *Brain Res.* **837**, 161–168.

Riediger, T., Schmid, H. A., Lutz, T. A., and Simon, E. (2002). Amylin and glucose co-activate area postrema neurons of the rat. *Neurosci. Lett.* **328**, 121–124.

Riediger, T., Zuend, D., Becskei, C., and Lutz, T. A. (2004). The anorectic hormone amylin contributes to feeding-related changes of neuronal activity in key structures of the gut-brain axis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286**, R114–R122.

Roth, J. D., and Anderson, C. (2004). Amylin induces weight loss and decreases body fat through both anorexigenic and metabolic effects, edn., p. 213. Keystone Symposia.

Roth, J., Hughes, H., and Anderson, C. (2004). Amylin-induced changes in body weight and body composition in high-fat fed female rats: Effects of prior or concurrent food restriction. *Obes. Res.* **12**(Suppl.), A109–A110.

Rowland, N. E., Crews, E. C., and Gentry, R. M. (1997). Comparison of Fos induced in rat brain by GLP-1 and amylin. *Regul. Pept.* **71**, 171–174.

Rowland, N. E., and Richmond, R. M. (1999). Area postrema and the anorectic actions of dexfenfluramine and amylin. *Brain Res.* **820**, 86–91.

Rushing, P. A., Hagan, M. M., Seeley, R. J., Lutz, T. A., and Woods, S. C. (2000a). Amylin: A novel action in the brain to reduce body weight. *Endocrinology* **141**, 850–853.

Rushing, P. A., Lutz, T. A., Seeley, R. J., and Woods, S. C. (2000b). Amylin and insulin interact to reduce food intake in rats. *Horm. Metab. Res.* **32**, 62–65.

Rushing, P. A., Hagan, M. M., Seeley, R. J., Lutz, T. A., D'Alessio, D. A., Air, E. L., and Woods, S. C. (2001). Inhibition of central amylin signaling increases food intake and body adiposity in rats. *Endocrinology* **142**, 5035–5038.

Rushing, P. A., Seeley, R. J., Air, E. L., Lutz, T. A., and Woods, S. C. (2002). Acute 3rd-ventricular amylin infusion potently reduces food intake but does not produce aversive consequences. *Peptides* **23**, 985–988.

Simon, E. (2000). Interface properties of circumventricular organs in salt and fluid balance. *News Physiol. Sci.* **15**, 61–67.

Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* **407**, 908–913.

Watkins, J., Bhavsar, S., and Young, A. A. (1996). Effect of amylin to inhibit food intake in rats can be blocked with the selective amylin receptor antagonist, AC187. *In* Program and Abstracts, 10th International Congress of Endocrinology 419.

Watkins, J., Bhavsar, S., and Young, A. (1997). Amylin's effect on reduction of food intake in rats may be centrally mediated. International Behavioral Neuroscience Society, Apr. 24–27, San Diego, CA. 1997.

Weyer, C., Maggs, D. G., Fineman, M., Gottlieb, A. D., Shen, L. Z., and Kolterman, O. G. (2001). Amylin replacement with pramlintide as an adjunct to insulin therapy facilitates a combined improvement in glycemic and weight control in type 1 diabetes. *Diabetologia* **44**(suppl. 1), A237.

Weyer, C., Chapman, I., Parker, B., Doran, S., Feinle-Bisset, C., Wishart, J., Wang, Y., Burns, C., Lush, C., and Horowitz, M. (2004). Effect of pramlintide on ad-libitum food intake in obese subjects and subjects with type 2 diabetes: A randomized, double-blind, placebo-controlled, crossover study. *Obes. Res.* **12**(suppl.), A28.

Wolfe, A., Massi, M., and Geary, N. (2003). Exogenous amylin inhibits ethanol intake in Sardinian alcohol-preferring rats. *Appetite* **40**, 370.

Wren, A. M., Small, C. J., Ward, H. L., Murphy, K. G., Dakin, C. L., Taheri, S., Kennedy, A. R., Roberts, G. H., Morgan, D. G., Ghatei, M. A., and Bloom, S. R. (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* **141**, 4325–4328.

Yamamoto, T., and Sawa, K. (2000a). c-Fos-like immunoreactivity in the brainstem following gastric loads of various chemical solutions in rats. *Brain Res.* **866**, 135–143.

Yamamoto, T., and Sawa, K. (2000b). Comparison of c-fos-like immunoreactivity in the brainstem following intraoral and intragastric infusions of chemical solutions in rats. *Brain Res.* **866**, 144–151.

Yamamoto, Y., Nakamuta, H., Koida, M., Seyler, J. K., and Orlowski, R. C. (1982). Calcitonin-induced anorexia in rats: A structure-activity study by intraventricular injections. *Jpn. J. Pharmacol.* **32**, 1013–1017.

Young, A. (1997). Role of amylin in nutrient intake: Animal studies. *Diabet. Med.* **14**, S14–S18.

Young, A. A., and Bhavsar, S. (1996). Genetically obese (ob/ob). Mice are more sensitive to amylin and endotoxin-induced suppression of food intake. *In* Program and Abstracts, 10th International Congress of Endocrinology 419.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Young, A. A., Smith, P., Gedulin, G., Baron, A., and Gedulin, B. R. (2004). Amylin inhibition of ghrelin secretion depends upon an intact area postrema. *Diabetologia* **47**(suppl. 1), A237.

Andrew Young

Inhibition of Gastric Emptying

I. Summary

In studies aimed at defining the role of amylin in glucose control, elevations of postprandial glucose concentration were blunted in subjects infused with the human amylin analog, pramlintide (Kolterman *et al.*, 1995, 1996). An effect similar to blunt glucose excursions was observed by Brown and others during infusions of amylin in dogs trained to drink glucose (Brown *et al.*, 1994). The effect of pramlintide in humans was present when glucose was administered orally, but not when administered intravenously, suggesting that the effect was due to a deceleration of glucose uptake from the meal, rather than an acceleration of its metabolism (Kolterman *et al.*, 1995). Since amylin did not affect the rate of glucose transit across exteriorized gut loops (Young and Gedulin, 2000), it was proposed that blunting of postprandial glucose profiles could reflect effects on gastric emptying. Rates of gastric emptying have been determined using three different approaches: (1) by

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measurement of remnant dye found in acutely excised stomachs, (2) by the systemic appearance of labels that are not significantly absorbed until they leave the stomach (e.g., labeled glucose, acetaminophen, ^{13}C-labeled volatiles), and (3) by following the passage of radiolabeled meal components scintigraphically, with a γ-camera. Amylin and/or pramlintide were shown to potently inhibit gastric emptying by the first method in animals (Clementi *et al.*, 1996; Young *et al.*, 1995a, 1996b), by the second method in animals (Gedulin *et al.*, 1995; Young *et al.*, 1995a, 1996a) and in humans, including those with type 1 and type 2 diabetes (Burrell *et al.*, 2003b; Hücking *et al.*, 2000; Kong *et al.*, 1998; Lee *et al.*, 2000; Vella *et al.*, 2002), and by scintigraphy in patients with type 1 diabetes (Kong *et al.*, 1997, 1998) and in nondiabetic subjects (Samsom *et al.*, 2000). Depending upon dose, responses ranged from a slowing of emptying rate (e.g., by ~50%) to a complete cessation. In rats, amylin was 15-fold more potent on a molar basis than glucagon-like peptide-1 (GLP-1) and 20-fold more potent than cholecystokinin octapeptide (CCK-8) for inhibition of gastric emptying (Young *et al.*, 1996b). It was the most potent mammalian peptide of 21 tested for this action (Gedulin *et al.*, 1996b). Amylin inhibition of gastric emptying appears to be mediated by a central mechanism (Clementi *et al.*, 1996; Dilts *et al.*, 1997; Young *et al.*, 2000). An intact vagus nerve (Jodka *et al.*, 1996) and an intact area postrema (Edwards *et al.*, 1998) are required for the effect. In rats that underwent total subdiaphragmatic vagotomy or surgical ablation of the area postrema, amylin was no longer effective at inhibiting gastric emptying (Edwards *et al.*, 1998). The effect of amylin and amylin agonists (including pramlintide) to inhibit gastric emptying was reversed by insulin-induced hypoglycemia (Gedulin and Young, 1998; Gedulin *et al.*, 1997b,c,d; Young *et al.*, 1996a). This suggests the existence of a glucose-sensitive "fail-safe" mechanism that safeguards against severe hypoglycemia; nutrients ingested in response to the hunger that accompanies hypoglycemia can pass rapidly through the stomach for immediate digestion and absorption, unimpaired by the physiological restraint of amylin that would normally prevail at normal glucose concentrations. It seems likely that amylinergic control of gastric emptying is mediated via neurons in the area postrema shown in brain slices to be activated by amylin, and inhibited by low glucose (Riediger *et al.*, 1999). Such neurons have been proposed to mediate glucoprivic gut reflexes (Adachi *et al.*, 1995).

II. Background

A. Metabolic Significance of Control of Gastric Emptying

A meal or other glucose challenge can easily contain 10 times the amount of free glucose typically present in the adult human. Digestion of carbohydrate and absorption of glucose occur very rapidly and efficiently

once food passes from stomach to intestine. Unrestricted digestion and absorption of an unrestricted flow of carbohydrate into the small bowel could easily lead to an influx that exceeded the capacity to dispose of the carbohydrate load, resulting in large excursions in plasma glucose concentration. The modest glycemic excursions, despite large fluctuations in ingested load and a limited capacity to metabolize such a load, point instead to the presence of controls on nutrient influx. Such control is apparent for carbohydrate, which is released from the stomach into the intestine at a rate of ~1.6 (Horowitz *et al.*, 1993) to 2.1 (Brener *et al.*, 1983) kcal/min (~400–530 mg glucose/min) in nondiabetic humans. The rigidity with which this release rate was maintained, despite a range of ingested glucose concentrations and loads (Brener *et al.*, 1983) is evidence of active (negative feedback) control. This controlled rate of carbohydrate release from the stomach approximates the rate at which it can be stored in peripheral tissues in response to physiological insulin concentrations (Young *et al.*, 1988). It appears that gastric emptying is a major gatekeeper of glucose appearance in the blood (R_a) after meals.

B. Gastric Emptying in Diabetes

Disturbances of gastric emptying are reported to be a common feature of diabetes, although the literature regarding the nature of disturbances is described as confused, with reports of both delayed and accelerated gastric emptying (Horowitz and Fraser, 1994). Many reports have not distinguished between type 1 and type 2 diabetes. Others have not distinguished between cases in which autonomic neuropathy was and was not present (gastroparesis being associated with the former; Cavallo-Perin *et al.*, 1991), and very few studies have been conducted during euglycemia, which is especially significant, since plasma glucose concentration is a major determinant of gastric emptying (Fraser *et al.*, 1990; Green *et al.*, 1996; Ishiguchi *et al.*, 2002; MacGregor *et al.*, 1976; McCann and Stricker, 1986; Morgan *et al.*, 1988; Oster-Jorgensen *et al.*, 1990; Samsom *et al.*, 1997; Schvarcz *et al.*, 1993, 1995b, 1997). The field of gastrointestinal disturbance in diabetes has recently been extensively reviewed (Anonymous, 2004), including in animal models of diabetes (Young and Edwards, 2004).

In type 1 diabetic subjects without evidence of autonomic dysfunction, gastric emptying has been reported to be accelerated (Nakanome *et al.*, 1983; Nowak *et al.*, 1990). Emptying is also accelerated in several animal models of type 1 diabetes, including BB (BioBreeding) rats (Nowak *et al.*, 1994; Young *et al.*, 1995a) and streptozoticin (STZ)-treated rats (Edens and Friedman, 1988; Granneman and Stricker, 1984; Nowak *et al.*, 1994; Ogata *et al.*, 1996; Stricker and McCann, 1985). In a particularly well-controlled clinical study, Pehling *et al.* (Pehling *et al.*, 1984) observed the appearance of ingested isotopic glucose in patients with type 1 diabetes whose endogenous

glucose production, fasting glucose concentration, and rate of glucose utilization had been normalized by continuous subcutaneous insulin infusion. Despite the best normalization of metabolic fluxes attainable with insulin alone, diabetic subjects had a 60% increase in the initial appearance of meal-derived glucose relative to nondiabetic controls. This was accompanied by comparable increases in total glucose appearance and the postprandial glucose increment. They proposed that these disturbances were due to accelerated gastric emptying (Pehling *et al.*, 1984).

Accelerated gastric emptying is more robustly associated with human insulin-resistant states, including type 2 diabetes (Bertin *et al.*, 2001; Frank *et al.*, 1995; Phillips *et al.*, 1991, 1992; Schwartz *et al.*, 1996), hyperinsulinemia (Schwartz *et al.*, 1995), and hypertension (Phillips *et al.*, 1997). Most studies performed in animal models of insulin resistance and type 2 diabetes also report an acceleration of gastric emptying. The diabetic Fatty Zucker model of type 2 diabetes (Green *et al.*, 1997), the LA/N corpulent rat (Gedulin *et al.*, 1994a), and a surgically induced hyperphagic model (Black *et al.*, 1990) exhibit accelerated emptying. Thus, gastric emptying appears accelerated in insulin-resistant states in rats and humans.

III. Effects of Amylin on Gastric Emptying

A. Magnitude of Effect

Exploration of amylin's effect on gastric emptying was prompted by blunting of postprandial plasma glucose excursions subjects with type 1 diabetes (Kolterman *et al.*, 1994a, 1995) and in nondiabetic dogs trained to drink glucose (Brown *et al.*, 1994) during infusions of amylin or pramlintide. The lowering of post-challenge glucose excursions could have been due to an acceleration of glucose disposal, or to an effect to slow glucose transport across the gut wall, but amylin affected neither of these mechanisms in animal or clinical studies. The observation that plasma glucose profiles were unchanged by pramlintide when glucose was delivered intravenously indicated that glucose disposal was unaffected, and there was no effect of intravenous amylin on glucose uptake from *in situ* gut loops perfused via the lumen with labeled glucose (Young and Gedulin, 2000). The conclusion that glucose appearance was reduced was consistent with a slowed release from the stomach.

Explorations of amylin's effect on rate of gastric emptying have used three different approaches: (1) measurement of remnant dye (typically phenol red) found in acutely excised stomachs (Plourde *et al.*, 1993), (2) measurement of the systemic appearance of labels that are not significantly absorbed until they leave the stomach (e.g., labeled glucose, acetaminophen,

^{13}C-labeled volatiles), and (3) following the passage of radiolabeled meal components by γ-scintigraphy.

The phenol red dye retention method (Plourde *et al.*, 1993) was used to determine amylin's effect in normal and insulin-treated (insulin- and amylin-deficient) BB rats. The phenol red dye remaining in stomachs excised 20 min after gavage was compared to the amount found in stomachs excised immediately after gavage. In nondiabetic Sprague Dawley rats, 51 $\pm$ 5% remained, and in nondiabetic BB rats, the fraction was somewhat similar ($\sim$40 $\pm$ 9%) (Young *et al.*, 1995a). Rat amylin at certain doses could fully inhibit gastric emptying in both normal and diabetic rats (Young *et al.*, 1994, 1995a).

An alternate technique, similar to that used in humans, was developed that did not involve sacrifice of animals, and that allowed better characterization of the time course of gastric slowing. Emptying of the stomach was signaled by the appearance in plasma of gavaged tritiated glucose (either [3-^{3}H]glucose or tritiated 3-O-methylglucose). Prior experiments in animals in which the pylorus had been ligated confirmed that labeled glucose was not absorbed to any significant degree by the stomach. Appearance in the plasma of label was therefore a reliable marker that material had passed the stomach. By this technique, amylin was also shown to potently slow gastric emptying, as indicated by the slower rates at which gavaged label appeared in the plasma (Young *et al.*, 1995a). Salmon calcitonin, an amylin agonist, showed a similar slowing in the same rodent model (Young *et al.*, 1995b).

Using γ-scintigraphy to follow the passage of a labeled meal, a similar response was noted with pramlintide. In subjects with type 1 diabetes, at a plasma concentration of $\sim$160 pM (representing somewhat supraphysiological amylin activity), pramlintide delayed the onset of liquid emptying to 69 min (from 7 min with placebo). Similarly, the lag in solid emptying was extended to 150 min (cf. 44 min with placebo) (Kong *et al.*, 1997; Macdonald *et al.*, 1995). Near-maximal effects of pramlintide on scintigraphically measured gastric emptying were without effect on colonic transit (Samsom *et al.*, 2000).

Similar results were obtained in a separate study of subjects with type 1 diabetes using plasma appearance of 3-ortho-methylglucose (3-OMG) to signal passage beyond the stomach (Kong *et al.*, 1998), and similar results were obtained in a study in which appearance in the breath of ^{13}CO2 derived from ingested ^{13}C-octanoic acid was used as a marker of gastric passage (Hücking *et al.*, 2000). Gastric emptying of solids, measured by the ^{13}C-spirulina breath test, was equally delayed by pramlintide in patients with type 1 or type 2 diabetes (Vella *et al.*, 2002). Pramlintide delayed the plasma appearance of acetaminophen (a marker of emptying) in patients with type 2 diabetes (Burrell *et al.*, 2003a,b).

B. Potency of Effect

In both nondiabetic rats and in the BB rat model of type 1 diabetes, rat amylin inhibition gastric emptying occurred at low doses. By the phenol red technique, the ED_{50} for inhibition of emptying was 0.43 μg, a dose calculated to raise plasma amylin concentrations by ~20 pM, a concentration range observed *in vivo* (Young *et al.*, 1995a). The ED_{50} for amylin inhibition of gastric emptying determined by gavaged label was 0.35 μg, similar to that determined by the phenol red technique (Gedulin *et al.*, 1994b; Young *et al.*, 1995a) (Fig. 1).

These experiments had a special significance in that they were the first to demonstrate an effect of systemic amylin at concentrations clearly within the physiological range. In the 7 years prior to this demonstration, a hormonal role of amylin had been presumed, but hitherto unproved.

Dose-finding experiments in human subjects typically used doses of 30, 60, and 90 μg pramlintide. In several studies, the 30 μg s.c. dose elicited near-maximal effect (Hücking *et al.*, 2000; Kong *et al.*, 1998; Samsom *et al.*, 2000; Vella *et al.*, 2002). These doses result in peak plasma concentrations of ~35 pM, decaying to ~20 pM within 1 hr, and represent near-physiological excursions of plasma amylin activity.

A rigorous test of the physiological significance of a ligand is a change in physiological state when its action is ablated. Several studies have shown an acceleration of gastric emptying when amylin action is negated. In amylin-deficient BB rats, the rate of appearance of gavaged label (gastric emptying) was accelerated 3.3-fold (Gedulin *et al.*, 1995). Another study showed a 2.2-fold acceleration of emptying in BB rats (Nowak *et al.*, 1994) and a 1.5-fold acceleration in STZ-treated rats (Nowak *et al.*, 1994). It was thus possible that the accelerated gastric emptying seen in these amylin-deficient animals represented the absence of normally present tonic inhibition.

Effects of negating an existing amylin signal have been explored using the selective amylin antagonist AC187. Effects of AC187 have been most marked when amylin tone was high, such as in the fed state and in hyperamylinemic animals. In normal fed rats, pre-injection of 3 mg AC187 accelerated emptying 1.7-fold (Gedulin *et al.*, 1995). Using tracer appearance in a crossover study, AC187 had no detectable effect on gastric emptying rate in fasted lean LA/N rats, but in corpulent (hyperamylinemic; Huang *et al.*, 1992) rats, AC187 pre-injection accelerated gastric emptying (Gedulin *et al.*, 1994a).

Thus, several lines of clinical and animal evidence point to modulation of gastric emptying being a physiological action of amylin secreted in response to meals. This evidence includes (1) a dose response wherein active doses were associated with physiological concentration changes, (2) an accelerated

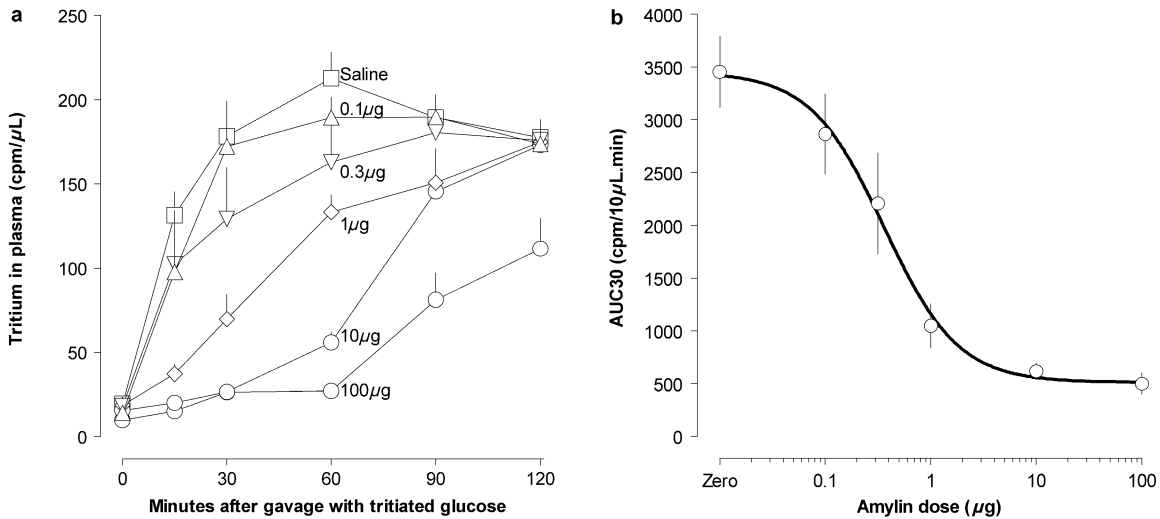


FIGURE 1 Dose response for amylin inhibition of gastric emptying in nondiabetic rats, measured by appearance in the plasma of tritium from labeled glucose gavaged at $t = 0$. Data from Young *et al.* (1995).

gastric emptying in amylin-deficient models, and (3) an accelerated gastric emptying during amylin receptor blockade.

C. Amylin Resistance

In human studies using pramlintide, enduring glycemic responses appear to require higher doses in subjects with type 2 diabetes (480 μg/day) who secrete endogenous amylin than in subjects with type 1 diabetes (who secrete negligible or no amylin; dose of 90–180 μg/day). Similarly, obese non-insulin-treated (including nondiabetic) subjects, who exhibit the highest plasma amylin concentrations (Koda *et al.*, 1995), tolerate (and require) even higher doses (720 μg/day) in weight loss studies (Weyer *et al.*, 2003). Resistance to the effects of circulating hormones (for example, insulin and leptin) is often observed when these hormones are present at higher concentrations. Gastric emptying dose and concentration responses for pramlintide inhibition of gastric emptying were compared in lean and obese Zucker rats to test whether amylin resistance was a feature in chronical hyperamylinemia.

Fasting plasma concentrations of amylin in obese Zucker rats were 5.5-fold higher than in lean littermate controls (16 versus 2.8 pM). Using the tracer appearance method, the ED_{50} for pramlintide inhibition of gastric emptying was 3.1-fold higher in obese than in lean Zucker rats (0.40 μg ± 0.07 log versus 0.13 μg ± 0.05 log; $P < 0.03$). However, to compensate for differences in endogenous secretion and for kinetic differences, mean plasma amylin immunoreactivity was used as the basis for concentration response analysis. The EC_{50} for inhibition of gastric emptying thus derived was 8.3-fold higher in obese Zucker rats than in lean controls (24 versus 2.9 pM; $P < 0.0001$). That is, chronically hyperamylinemic Zucker rats showed a reduction in amylin sensitivity that was commensurate with their relative elevation of plasma amylin concentration (Gedulin *et al.*, 1999).

This observation suggests, but does not establish, a causal relationship between hyperamylinemia and amylin resistance. Nor does it prove that there is a compensatory reduction in amylin sensitivity for all responses. In this regard, it is notable that heterogeneity of response is a feature of insulin resistance, where stimulation of fat storage is somewhat preserved in the face of diminished glucose transport (Yki-Järvinen *et al.*, 1987).

D. Pharmacology

Using the phenol red method, ED_{50}s for the gastric inhibitory effects of rat amylin, human amylin, and pramlintide were 0.13 μg ± 0.14 log, 0.18 μg ± 0.10 log, and 0.11 μg ± 0.17 log, respectively, and were not statistically distinguishable from each other (Young *et al.*, 1996c). In a comparative

study of the potencies of amylin-related peptides and other gut peptides, salmon calcitonin, rat calcitonin, and rat CGRP each emerged as potent inhibitors of emptying (ED_{50}s of 0.19, 0.64, and 1.62 μg, respectively) (Gedulin *et al.*, 1996b). The order of potency for these peptides is consistent with an amylin-like (Beaumont *et al.*, 1993) or calcitonin-like pharmacology. In particular, the potency of the teleost calcitonins, which do not interact at CGRP receptors, indicates that inhibition of emptying with these ligands cannot be predominantly CGRPergic. Adrenomedullin, which has been identified as a CGRP agonist with greater selectivity for CGRP versus amylin/calcitonin receptors than CGRP itself (Vine *et al.*, 1996), was also without effect on gastric emptying (Vine *et al.*, 1996) in preparations in which amylin and salmon calcitonin were effective (Young *et al.*, 1995b). This observation negates a role for CGRP-responsive (or adrenomedullin-responsive) pathways in regulation of gastric emptying.

The spectrum of effects of the selective amylin antagonist AC187 offers further clues. AC187 displaces amylin from its receptors with ~500 times the potency that it displaces CGRP from its receptors, and with ~25 times the potency that it displaces salmon calcitonin from calcitonin receptors (Beaumont *et al.*, 1995). AC187 is reported to block several effects of exogenous amylin (Beaumont *et al.*, 1995; Gedulin *et al.*, 2004; Watkins *et al.*, 1996). Administered alone, it accelerated gastric emptying in nonfasted (Gedulin *et al.*, 1995) or hyperamylinemic rats (Gedulin *et al.*, 1994a). These results fit with the presence of a postprandial amylin/calcitonin tone that contributes to restraint of gastric emptying. While the receptors mediating that tone clearly appear not to be CGRP receptors, the pharmacology also does not appear to exactly fit calcitoninergic or amylinergic types. For the latter, mammalian calcitonins should be much less potent than they were shown to be. The comparative potencies are instead consistent with a mixed pharmacology, driven by amylin and calcitonin receptors in parallel. The formation of amylin receptors as heterodimers of an otherwise functional calcitonin receptor with a receptor activity modifying protein (RAMP) could explain a mixed pharmacology, if expression of each component was unequal. Undertitration of RAMPs versus calcitonin receptors in cells driving gastric responses would result in the presence of both amylin and calcitonin receptors on the cell surface and would account for the preservation to some extent of calcitonin sensitivity for this response. Both components, functional calcitonin receptors and RAMP, are present in the brain structure central to amylin's gastric effect, the area postrema (Barth *et al.*, 2004).

E. Localization of Effect

The major brain site regulating gastric motility is the dorsal vagal complex of the brain stem, composed of the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus (DMV), and area postrema. The

DMV receives information originating in visceral afferents projecting to the NTS and area postrema, and integrates this information to regulate efferent nerve activity to the stomach and other viscera (Rogers *et al.*, 1996). The area postrema is one of the circumventricular organs, which are characterized by their proximity to cerebral ventricles, the lack of a blood–brain barrier, and the richness of their receptors, especially for circulating peptides. Activity arising in the area postrema projects to the NTS and higher brain sites, including hypothalamic nuclei involved in appetite control.

Several studies of amylin's effects on gastric emptying in rats support the idea that these effects are mediated by the central nervous system. First, neither amylin nor amylin agonists affect motility of gastric fundus *ex vivo* (Katsoulis *et al.*, 1989). Second, it is apparent that an intact vagus nerve is necessary for amylin to slow gastric emptying. Although there was a change in general gastric tone following subdiaphragmatic vagotomy, subcutaneous amylin had no effect on the course of gastric emptying (Jodka *et al.*, 1996). In contrast, the same doses of amylin in sham-operated control animals produced a prolonged delay in gastric emptying (Fig. 2).

Third, the potency of amylin to inhibit gastric emptying depended upon the intracranial location of injection (Dilts *et al.*, 1997). Injection of 0.1 to 1 μg amylin into the lateral ventricle was little more effective than equivalent subcutaneous doses at delaying gastric emptying. However, when injected into the 4th ventricle, amylin was approximately 10-fold more potent at inhibiting gastric emptying. These results are consistent with a site of action for amylin in the brain stem near the 4th ventricle.

Fourth, in rats with aspiration ablation of the area postrema, amylin was no longer effective at inhibiting gastric emptying (Edwards *et al.*, 1998). Fifth, the area postrema exhibits a high density of amylin binding (Sexton *et al.*, 1994), contains the necessary elements for the heterodimeric amylin receptor (Barth *et al.*, 2004), and contains neurons activated by low concentrations of amylin. In a brain slice preparation of the area postrema of the rat, superfused rat amylin in a 1–100 nM range excited nearly half of all spontaneously active neurons (Riediger *et al.*, 1999) (Fig. 3).

These findings led to the interpretation that circulating amylin acts at the area postrema, and from there via the vagus, to regulate gastric motility.

IV. Effects on Postprandial Nutrient Profiles

Brown and others observed in dogs trained to drink glucose an effect of co-infused amylin to blunt plasma glucose excursions (Brown *et al.*, 1994). Similarly, elevations of postprandial glucose concentration were blunted

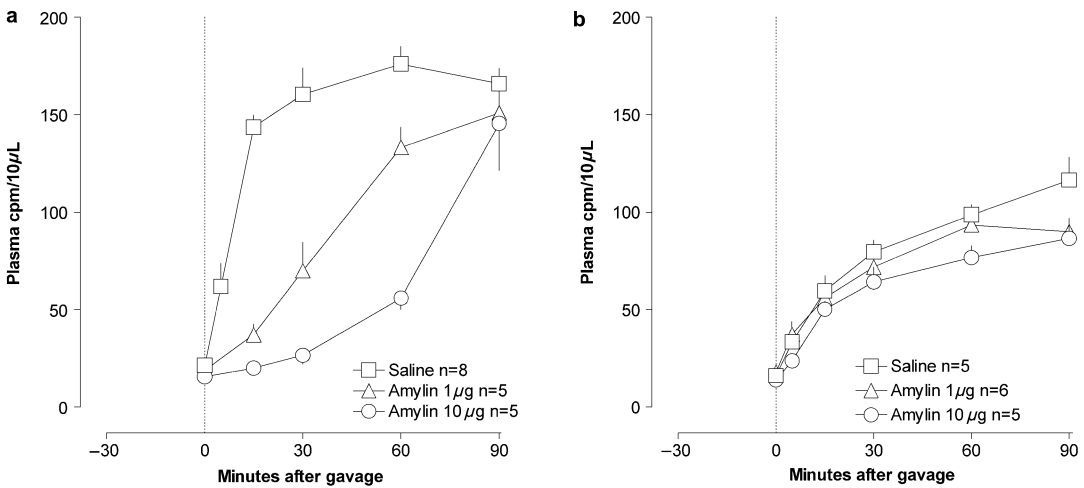


FIGURE 2 Dose-dependent effect of exogenous amylin (a) to slow gastric emptying in intact animals, and absence of such effect in rats with subdiaphragmatic vagotomy (b).

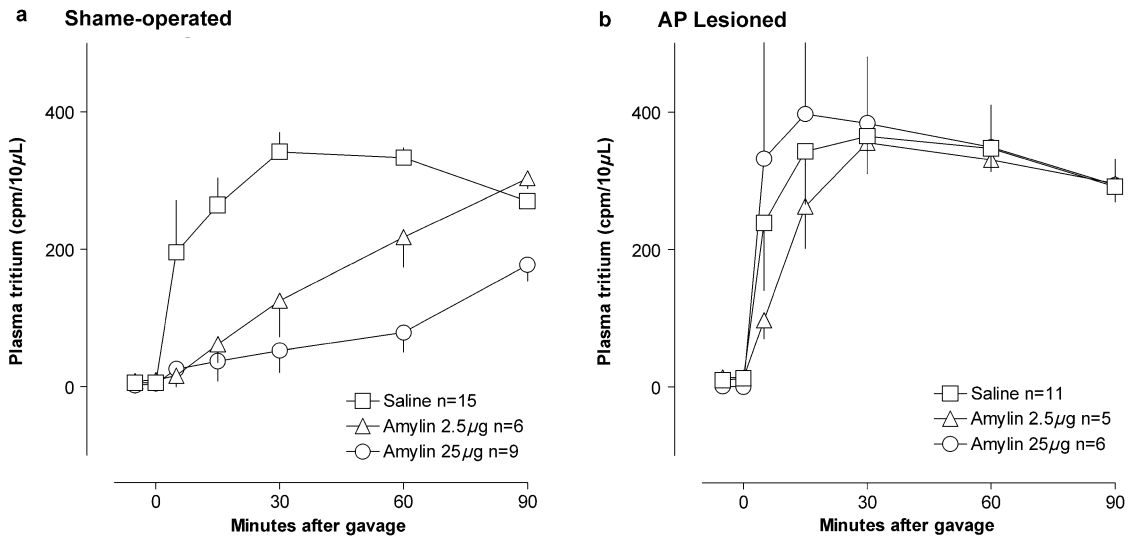


FIGURE 3 Dose dependent effect of exogenous amylin to slow gastric emptying in intact rats (a), and absence of such effect in animals with lesions of the area postrema (b).

in subjects with type 1 diabetes infused with the human amylin analog pramlintide (Kolterman *et al.*, 1994b). The effect of pramlintide has been observed in numerous clinical studies, and in numerous contexts (Hücking *et al.*, 2000; Kolterman *et al.*, 1995, 1996; Levetan *et al.*, 2003; Maggs *et al.*, 2002; Nyholm *et al.*, 1999; Want *et al.*, 2002), including in type 2 diabetes (Thompson *et al.*, 1997; Weyer *et al.*, 2003, 2005).

A similar effect was observed experimentally in nondiabetic rats. Preinjection with pramlintide (1 μg s.c.) reduced the plasma glucose excursion after gavage with 1 ml of 50% dextrose (Young *et al.*, 1996c).

There was less fluctuation in glycemic profiles in patients with type 1 diabetes after 4 weeks of pramlintide therapy. The period spent in the euglycemic range increased by 32%, and the period spent in hyperglycemic and/or hypoglycemic ranges decreased (Levetan *et al.*, 2003).

V. Hypoglycemic Override

Oral carbohydrate, preferably glucose, is the standard mode of rescue from insulin-induced hypoglycemia. Since the amylin analog pramlintide was proposed as a treatment in patients at risk from insulin-induced hypoglycemia, it was important to determine the effects of amylin agonists on gastric emptying during hypoglycemia.

Hypoglycemia accelerates gastric emptying (Green *et al.*, 1996; Schvarcz *et al.*, 1993, 1995a,b), likely as a counterregulatory gastrointestinal response aimed at maximizing nutrient availability and minimizing glycopenia. Treatments that delay nutrient absorption, including those that work by delaying gastric emptying, could present a hazard during hypoglycemia, since they could interfere with efforts to restore plasma glucose by oral supplementation.

Because insulin was to be used to invoke reductions in plasma glucose concentration, the effect of insulin *per se* (independently of glycemic change) on gastric emptying needed to be determined. This was done in normal Sprague Dawley rats given recombinant human insulin (Humulin-R, Eli Lilly, Indianapolis, IN) at doses of 0 (saline), 0.1, 1, 10, or 100 μg immediately before gavage (Gedulin and Young, 1998; Green *et al.*, 1996). At the two higher doses, parallel groups were also administered glucose to preempt a fall in plasma glucose, creating insulin dose responses for gastric emptying, with and without glycemic change. Where there was no effect on plasma glucose, insulin also had no effect on gastric emptying. Where there was insulin-induced hypoglycemia (to 1.8 and 2.2 mM), there was acceleration of gastric emptying. That is, insulin invoked gastric acceleration that was secondary to reductions in glucose concentration (Fig. 4).

The permissive effect of glucose on gastric emptying has been quantified in a concentration response. In normoglycemic rats, ~50% of gastric contents

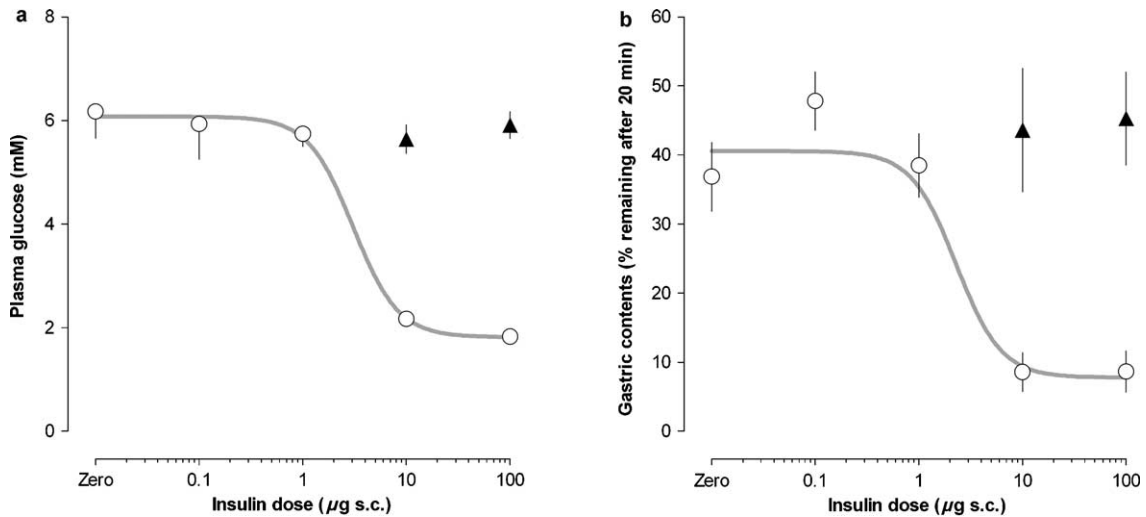


FIGURE 4 Insulin dose response for plasma glucose (a) and gastric emptying expressed as percent of gavaged contents remaining after 20 min (b). Supplementation with glucose to prevent insulin-induced changes (black triangles) also prevented changes in rate of gastric emptying. Data from Gedulin and Young (1998).

remained 20 min after gavage. By pretreatment with various doses of insulin and/or glucose, a range of plasma glucose concentrations was attained in rats in which gastric emptying was measured by the (terminal) phenol red method. Analysis of the plasma glucose concentration at which gastric retention was 50% of that observed during euglycemia returned an EC_{50} of 4.1 ± 0.4 mM in saline-pretreated controls (Gedulin and Young, 1998).

Effects of hypoglycemia on amylinergic control of gastric emptying (or conversely, effects of amylin agonists on hypoglycemic acceleration of gastric emptying) were studied in rats using two experimental designs: the above-mentioned phenol red method (Young *et al.*, 1995a) that quantitated the fraction of gastric contents passed 20 min after gavage, and plasma appearance of gavaged 3-O-[methyl-^{3}H]glucose (Gedulin and Young, 1998; Gedulin *et al.*, 1996a; Young *et al.*, 1996a), a method that allowed some assessment of the time course of events.

Using the phenol red method, pretreatment with amylin (or pramlintide) 1 μg s.c. during normoglycemia resulted in essentially total retention of gastric contents 20 min after gavage. When amylin treatment was coupled to insulin pretreatment to obtain plasma glucose concentrations that exended into the hypoglycemic range, gastric emptying was accelerated, and to a similar extent as in rats administered insulin alone. That is, the effect of hypoglycemia to accelerate gastric emptying was unencumbered by the presence of high amylin concentrations; with a plasma glucose concentration of

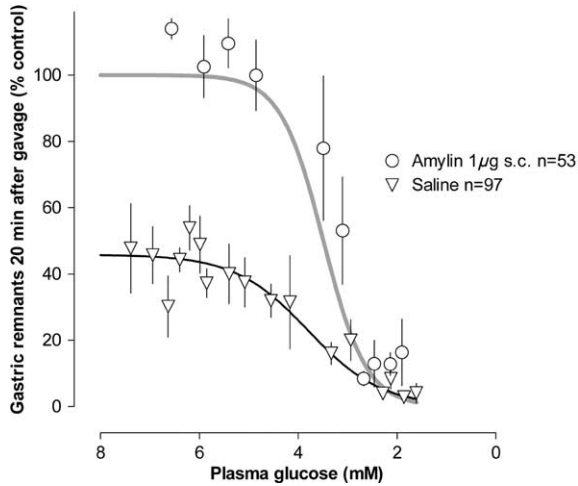
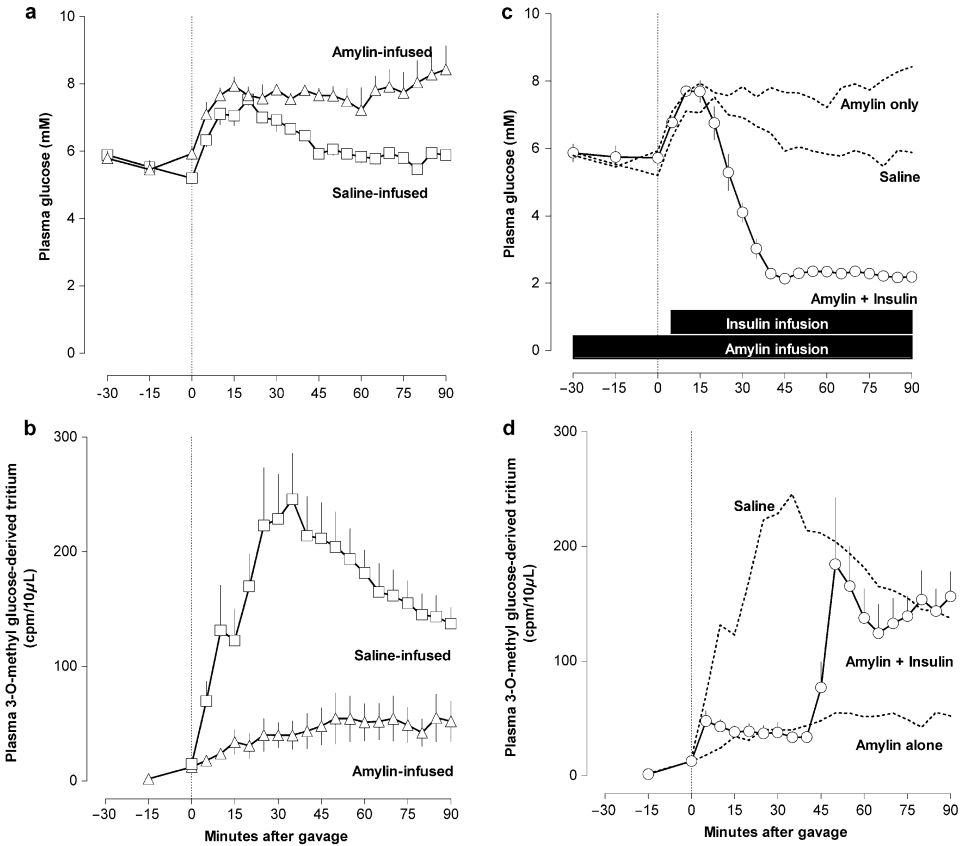


FIGURE 5 Glucose concentration response for acceleration of gastric emptying (contents at 20 min approaching zero) with decreasing plasma glucose (black curve). At normoglycemia, gastric emptying was slowed following administration of 1 μg amylin, but hypoglycemic override was still present (gray curve). Data from Gedulin and Young (1998).


a
Plasma glucose (mM)
Amylin-infused
Saline-infused
c
Plasma glucose (mM)
Amylin only
Saline
Amylin + Insulin
Insulin infusion
Amylin infusion
b
Plasma 3-O-methyl glucose-derived tritium (cpm/10μL)
Saline-infused
Amylin-infused
Minutes after gavage
d
Plasma 3-O-methyl glucose-derived tritium (cpm/10μL)
Saline
Amylin + Insulin
Amylin alone
Minutes after gavage

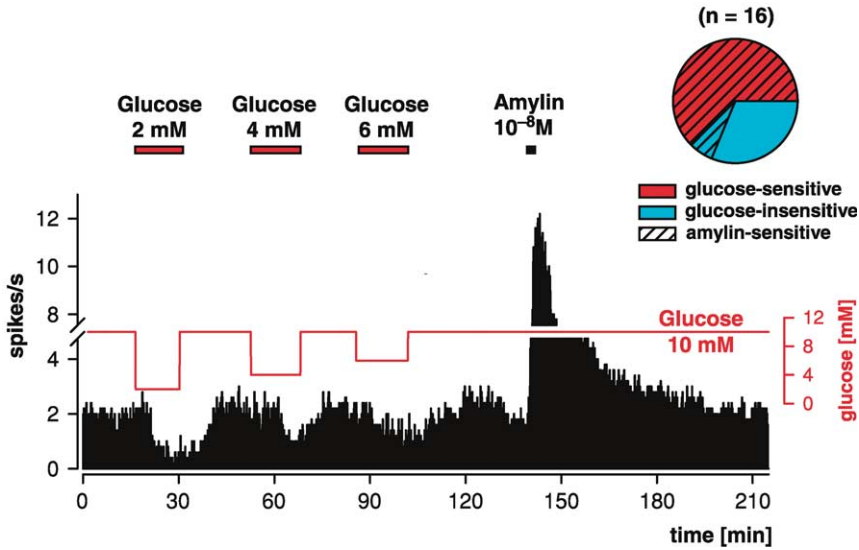


FIGURE 7 Single unit extracellular recording of a spontaneously active neuron from a brain slice preparation of area postrema. The neuron is responsive to both, perfusate glucose concentration (red line), and to applied amylin. Almost all amylin-sensitive neurons were also glucose sensitive. Data from Riediger *et al.* (1999).

1.75 mM, retention of gastric contents was only 3% in both amylin- and saline-treated rats (Gedulin and Young, 1998) (Fig. 5).

The glycemic threshold below which gastric emptying was accelerated was further explored using the tracer appearance method. In conscious rats in which gastric emptying had been inhibited by prior continuous infusion of amylin or pramlintide, inhibition of emptying was affirmed by the markedly reduced (versus saline controls) appearance in plasma of label (3-O-[methyl-^{3}H]-glucose) gavaged at $t = 0$ min. Hypoglycemia was thereafter induced by a 5 mU/min insulin infusion in half the rats. The time and glucose concentration at the onset of gastric emptying (indicated by the appearance in plasma of gavaged label deviated from the normoglycemic baseline) were noted. In amylin-treated rats subsequently rendered hypoglycemic with insulin, onset of gastric emptying occurred when plasma glucose had fallen to 2.1 $\pm$ 0.1 mM (Gedulin and Young, 1998). Experiments performed using

FIGURE 6 Experiments illustrating dynamics of the hypoglycemic acceleration of gastric emptying. Plasma glucose (a and c) was maintained or elevated in saline- and amylin-infused rats (a and b), and was reduced to hypoglycemic levels with insulin infusion (c and d). As plasma glucose approached 2 mM, gavaged label, retained in the stomach following amylin infusion, was abruptly released and appeared in the plasma (d). Data from Gedulin and Young (1998).

pramlintide in the same experimental model yielded a similar result (Gedulin *et al.*, 1997a,b) (Fig. 6).

These data support the idea of a central "fail-safe" mechanism whereby hypoglycemia can override amylinergic slowing of gastric emptying. Similar results have been obtained when other peptide inhibitors of gastric emptying have been used (GLP-1, Gedulin and Young, 1998; exenatide, Jodka *et al.*, 2000; and CCK octapeptide, Gedulin and Young, 1998) (Fig. 7).

In a brain slice preparation of the area postrema of the rat, superfused rat amylin excited nearly half of all spontaneously active neurons (Riediger *et al.*, 1999). Interestingly, 91% of all neurons identified as amylin sensitive were also glucose sensitive in that they markedly changed firing rate with changes of ambient glucose in the 2–6 mM range. Conversely, all amylin-insensitive neurons were also glucose insensitive. A similar concordance between glucose sensitivity and CCK sensitivity has been noted in area postrema neurons (Funahashi and Adachi, 1993). Previously recognized (Adachi *et al.*, 1995) glucose sensitivity in neurons from this brain region was proposed to play a fail-safe role in glycemic homeostasis (Adachi *et al.*, 1995). It is possible that glucose sensitivity of amylinergic neurons at the area postrema underlies the hypoglycemic override of the gastric actions of amylin and pramlintide in intact animals.

References

Adachi, A., Kobashi, M., and Funahashi, M. (1995). Glucose-responsive neurons in the brainstem. *Obes. Res.* **3**, 735S–740S.

Barth, S. W., Riediger, T., Lutz, T. A., and Rechkemmer, G. (2004). Peripheral amylin activates circumventricular organs expressing calcitonin receptor a/b subtypes and receptor-activity modifying proteins in the rat. *Brain Res.* **997**, 97–102.

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Beaumont, K., Moore, C. X., Pittner, R. A., Prickett, K. S., Gaeta, L. S. L., Rink, T. J., and Young, A. A. (1995). Differential antagonism of amylin's metabolic and vascular actions with amylin receptor antagonists. *Can. J. Physiol. Pharmacol.* **73**, 1025–1029.

Bertin, E., Schneider, N., Abdelli, N., Wampach, H., Cadiot, G., Loboguerrero, A., Leutenegger, M., Liehn, J. C., and Thiefin, G. (2001). Gastric emptying is accelerated in obese type 2 diabetic patients without autonomic neuropathy. *Diabet. Metab.* **27**, 357–364.

Black, R. M., Conover, K. L., and Weingarten, H. P. (1990). Accelerated gastric emptying in VMH-lesioned rats is secondary to excess weight gain. *Am. J. Physiol.* **259**, R658–R661.

Brener, W., Hendrix, T. R., and McHugh, P. R. (1983). Regulation of the gastric emptying of glucose. *Gastroenterology* **85**, 76–82.

Brown, K., Menius, A., Sandefer, E., Edwards, J., and James, M. (1994). The effects of amylin on changes in plasma glucose and gastric emptying following an oral glucose load in conscious dogs. *Diabetes* **43**(suppl. 1), 172A(abstract 0536).

Burrell, T. A., Fineman, M. S., Deckhut, D., Weyer, C., McIntyre, S., Wang, Y., Lutz, K., Nielsen, L. L., and Kolterman, O. G. (2003). Mealtime subcutaneous (SC) injection of pramlintide slows without arresting gastric emptying in patients with type 2 diabetes. *Diabetes* **52**(suppl. 1), A113(abstract 483–P).

Burrell, T. A., Fineman, M. S., Deckhut, D., Weyer, C., McIntyre, S., Wang, Y., Lutz, K., Nielsen, L. L., and Kolterman, O. G. (2003). Mealtime subcutaneous (SC) injection of pramlintide slows without arresting gastric emptying in patients with type 2 diabetes. *Diabet. Metab.* **29**, 4S124–4S125(abstract 1780).

Cavallo-Perin, P., Aimo, G., Mazzillo, A., Riccardini, F., and Pagano, G. (1991). Gastric emptying of liquids and solids evaluated by acetaminophen test in diabetic patients with and without autonomic neuropathy. *Riv. Eur. Sci. Med. Pharmacol.* **13**, 205–209.

Clementi, G., Caruso, A., Cutuli, V., de Bernardis, E., Prato, A., and Amico-Roxas, M. (1996). Amylin given by central or peripheral routes decreases gastric emptying and intestinal transit in the rat. *Experientia* **52**, 677–679.

Dilts, R., Gedulin, B., Jodka, C., Beaumont, K., and Young, A. (1997). Central infusion of amylin delays gastric emptying in the rat. *Pharmacologist* **39**, 32(abstract 64).

Edens, N. K., and Friedman, M. I. (1988). Satiating effect of fat in diabetic rats: Gastrointestinal and postabsorptive factors. *Am. J. Physiol.* **255**, R123–R127.

Edwards, G. L., Gedulin, B. R., Jodka, C., Dilts, R. P., Miller, C. C., and Young, A. (1998). Area postrema (AP)-lesions block the regulation of gastric emptying by amylin. *Neurogastroenterol. Motil.* **10**, 26.

Frank, J. W., Saslow, S. B., Camilleri, M., Thomforde, G. M., Dinneen, S., and Rizza, R. A. (1995). Mechanism of accelerated gastric emptying of liquids and hyperglycemia in patients with type II diabetes mellitus. *Gastroenterology* **109**, 755–765.

Fraser, R. J., Horowitz, M., Maddox, A. F., Harding, P. E., Chatterton, B. E., and Dent, J. (1990). Hyperglycaemia slows gastric emptying in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* **33**, 675–680.

Funahashi, M., and Adachi, A. (1993). Glucose-responsive neurons exist within the area postrema of the rat: In vitro study on the isolated slice preparation. *Brain Res. Bull.* **32**, 531–535.

Gedulin, B. R., and Young, A. A. (1998). Hypoglycemia overrides amylin-mediated regulation of gastric emptying in rats. *Diabetes* **47**, 93–97.

Gedulin, B., Jodka, C., Green, D., and Young, A. (1994a). Effect of amylin receptor antagonist, AC187, on labelled glucose absorption and oral glucose tolerance in corpulent LA/N rats. Program and Abstracts. American Diabetes Association 29th Research Symposium and the International Congress on Obesity Satellite Conference, August 25–27, Boston, Mass, 40(abstract 45).

Gedulin, B., Vine, W., and Rink, T. (1994b). Accelerated gastric emptying in insulin-treated BB/W (IDDM) rats. *Diabetes* **43**, 134A(abstract 423).

Gedulin, B., Green, D., Jodka, L., and Young, A. (1995). Endogenous amylin and gastric emptying in rats: Comparison with GLP-1 and CCK-8. *Diabetologia* **38**, A244(abstract 945).

Gedulin, B. R., Jodka, C. M., Green, D. E., Rink, T. J., and Young, A. A. (1996a). Reversal of amylin-inhibition of gastric emptying by insulin-induced hypoglycemia. *J. Investig. Med.* **44**, 160A.

Gedulin, B. R., Jodka, C. M., Green, D. L., and Young, A. A. (1996b). Comparison of 21 peptides on inhibition of gastric emptying in conscious rats. *Dig Dis. Week* A742.

Gedulin, B., Jodka, C., and Young, A. (1997a). Insulin-induced hypoglycaemia reverses pramlintide-inhibition of gastric emptying in rats. *Diabet. Med.* **14**, S44(P103).

Gedulin, B., Jodka, C., and Young, A. (1997b). Insulin-induced hypoglycemia reverses pramlintide inhibition of gastric emptying in rats. *Can. J. Diabet. Care* **21**, 4(abstract 14).

Gedulin, B., Jodka, C., and Young, A. (1997c). Insulin-induced hypoglycemia reverses pramlintide-inhibition of gastric emptying in rats [in French]. *Diabet. Metab.* **23**, P055.

Gedulin, B., Jodka, C., and Young, A. (1997d). Insulin-induced hypoglycemia reverses pramlintide-inhibition of gastric emptying in rats [in German]. *Diabetes und Stoffwechsel* **6**, 57.

Gedulin, B., Jodka, C., Hoyt, J., and Young, A. (1999). Evidence for amylin resistance for inhibition of gastric emptying in hyperamylinemic Fatty Zucker rats. *Endocrine Society 81st Annual Meeting Program and Abstracts*, p. 217(abstract P1–388).

Gedulin, B., Smith, P., Gedulin, G., Baron, A., and Young, A. (2004). Amylin potently inhibits ghrelin secretion in rats. *Diabetes* **53**(suppl. 2), A340(abstract 1411–P).

Granneman, J. G., and Stricker, E. M. (1984). Food intake and gastric emptying in rats with streptozotocin-induced diabetes. *Am. J. Physiol.* **247**, R1054–R1061.

Green, D., Jodka, C., Young, A., and Gedulin, B. (1996). Gastric emptying in rats is accelerated by insulin-induced hypoglycemia, but is unaffected by insulin per se. *Diabetes* **45**, 188A.

Green, G. M., Guan, D., Schwartz, J. G., and Phillips, W. T. (1997). Accelerated gastric emptying of glucose in Zucker type 2 diabetic rats: Role in postprandial hyperglycaemia. *Diabetologia* **40**, 136–142.

Horowitz, M., and Fraser, R. (1994). Disordered gastric motor function in diabetes mellitus. *Diabetologia* **37**, 543–551.

Horowitz, M., Edelbroek, M. A., Wishart, J. M., and Straathof, J. W. (1993). Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects. *Diabetologia* **36**, 857–862.

Huang, H.-J. S., Young, A. A., Koda, J. E., Tulp, O. L., Johnson, M. J., and Cooper, G. J. (1992). Hyperamylinemia, hyperinsulinemia, and insulin resistance in genetically obese LA/N-cp rats. *Hypertension* **19**, I101–I109.

Hücking, K., Brüggehofe, J., Pox, C., Holst, J. J., Petrella, E., Fineman, M., Kolterman, O., Schmiegel, W., and Nauck, M. (2000). Effects of pramlintide on gastric emptying and the temporal pattern of insulin secretory responses. *Diabetologia* **43**(suppl. 1), A140.

Ishiguchi, T., Tada, H., Nakagawa, K., Yamamura, T., and Takahashi, T. (2002). Hyperglycemia impairs antro-pyloric coordination and delays gastric emptying in conscious rats. *Auton. Neurosci.* **95**, 112–120.

Jodka, C., Green, D., Young, A., and Gedulin, B. (1996). Amylin modulation of gastric emptying in rats depends upon an intact vagus nerve. *Diabetes* **45**(suppl. 2), 235A.

Jodka, C., Parkes, D., and Young, A. (2000). Hypoglycemic override of inhibition of gastric emptying by exendin-4. *Diabetes* **49**, A285.

Katsoulis, S., Conlon, J. M., Schmidt, W. E., and Creutzfeldt, W. (1989). Effects of calcitonin gene related peptides (CGRP), calcitonin (CT) and islet amyloid polypeptide (IAPP) on gastric motility of guinea-pig and rat. *Regul. Pept.* **26**, 77.

Koda, J. E., Fineman, M. S., Kolterman, O. G., and Caro, J. F. (1995). 24 hour plasma amylin profiles are elevated in IGT subjects vs. normal controls. *Diabetes* **44**(suppl. 1), 238A.

Kolterman, O., Gottlieb, A., and Moyses, C. (1994). Amylin agonist, AC-137, reduces postprandial hyperglycemia in subjects with insulin dependent diabetes mellitus (IDDM). *Diabetes* **43**, 78A.

Kolterman, O., Kisicki, J. C., Peltier, L., Gottlieb, A., and Moyses, C. (1994b). Infusion of amylin agonist AC-0137 reduces postprandial hyperglycemia in subjects with type 1 diabetes (IDDM). *Clin. Res.* **42**, 87A.

Kolterman, O. G., Gottlieb, A., Moyses, C., and Colburn, W. (1995). Reduction of postprandial hyperglycemia in subjects with IDDM by intravenous infusion of AC137, a human amylin analogue. *Diabet. Care* **18**, 1179–1182.

Kolterman, O. G., Schwartz, S., Corder, C., Levy, B., Klaff, L., Peterson, J., and Gottlieb, A. (1996). Effect of 14 days' subcutaneous administration of the human amylin analogue,

pramlintide (AC137), on an intravenous insulin challenge and response to a standard liquid meal in patients with IDDM. *Diabetologia* **39**, 492–499.

Kong, M. F., King, P., Macdonald, I. A., Stubbs, T. A., Perkins, A. C., Blackshaw, P. E., Moyses, C., and Tattersall, R. B. (1997). Infusion of pramlintide, a human amylin analogue, delays gastric emptying in men with IDDM. *Diabetologia* **40**, 82–88.

Kong, M. F., Stubbs, T. A., King, P., Macdonald, I. A., Lambourne, J. E., Blackshaw, P. E., Perkins, A. C., and Tattersall, R. B. (1998). The effect of single doses of pramlintide on gastric emptying of two meals in men with IDDM. *Diabetologia* **41**, 577–583.

Lee, J. S., Vella, A., Camilleri, M., Szarka, L. A., Daniel, D. A., Burton, D., Zinsmeister, A. R., and Rizza, R. A. (2000). Vagal inhibition in the retardation of gastric emptying of solids by the amylin analog, pramlintide, in diabetes mellitus. *Gastroenterology* **118**, A–624.

Levetan, C., Want, L. L., Weyer, C., Strobel, S. A., Crean, J., Wang, Y., Maggs, D. G., Kolterman, O. G., Chandran, M., Mudaliar, S. R., and Henry, R. R. (2003). Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglycerides excursions among patients with type 1 diabetes intensively treated with insulin pumps. *Diabet. Care* **26**, 1–8.

Macdonald, I., King, P., Kong, M.-F., Stubbs, T., Perkins, A., Moyses, C., and Tattersall, R. (1995). Infusion of the human amylin analogue, AC137, delays gastric emptying in men with IDDM. *Diabetologia* **38**(Suppl. 1), A32.

MacGregor, I. L., Gueller, R., Watts, H. D., and Meyer, J. H. (1976). The effect of acute hyperglycemia on gastric emptying in man. *Gastroenterology* **70**, 190–196.

Maggs, D., Weyer, C., Crean, J., Wang, Y., Burrell, T., Fineman, M., Kornstein, J., Schwartz, S., Guiterrez, M., and Kolterman, O. (2002). Mealtime amylin replacement with pramlintide markedly improves postprandial glucose excursions when added to insulin lispro in patients with type 2 diabetes: A dose-timing study. *Diabetologia* **45**(suppl. 2), A264.

McCann, M. J., and Stricker, E. M. (1986). Gastric emptying of glucose loads in rats: Effects of insulin-induced hypoglycemia. *Am. J. Physiol.* **251**, R609–R613.

Morgan, L. M., Tredger, J. A., Hampton, S. M., French, A. P., Peake, J. C., and Marks, V. (1988). The effect of dietary modification and hyperglycaemia on gastric emptying and gastric inhibitory polypeptide (GIP) secretion. *J. Nutr. Br. J. Nutr.* **60**, 29–37.

Nakanome, C., Akai, H., Hongo, M., Imai, N., Toyota, T., Goto, Y., Okuguchi, F., and Komatsu, K. (1983). Disturbances of the alimentary tract motility and hypermotilinemia in the patients with diabetes mellitus. *Tohoku J. Exp. Med.* **139**, 205–215.

Nowak, T. V., Johnson, C. P., Wood, C. M., Adams, M. B., Roza, A. M., Kalbfleisch, J. H., Palmer, D. W., and Soergel, K. H. (1990). Evidence for accelerated gastric emptying in asymptomatic patients with insulin-dependent diabetes mellitus. *Gastroenterology* **98**, A378.

Nowak, T. V., Roza, A. M., Weisbruch, J. P., and Brosnan, M. R. (1994). Accelerated gastric emptying in diabetic rodents: Effect of insulin treatment and pancreas transplantation. *J. Lab. Clin. Med.* **123**, 110–116.

Nyholm, B., Orskov, L., Hove, K., Gravholt, C., Moller, N., Alberti, K., Moyses, C. K. O., and Schmitz, O. (1999). The amylin analog pramlintide improves glycemic control and reduces postprandial glucagon concentrations in patients with type 1 diabetes mellitus. *Metabolism* **48**, 935–941.

Ogata, M., Iizuka, Y., Murata, R., and Hikichi, N. (1996). Effect of streptozotocin-induced diabetes on cyclosporin A disposition in rats. *Biol. Pharm. Bull.* **19**, 1586–1590.

Oster-Jorgensen, E., Pedersen, S. A., and Larsen, M. L. (1990). The influence of induced hyperglycaemia on gastric emptying rate in healthy humans. *Scand. J. Clin. Lab. Invest.* **50**, 831–836.

Pehling, G., Tessari, P., Gerich, J. E., Haymond, M. W., Service, F. J., and Rizza, R. A. (1984). Abnormal meal carbohydrate disposition in insulin-dependent diabetes. Relative contributions of endogenous glucose production and initial splanchnic uptake and effect of intensive insulin therapy. *J. Clin. Invest.* **74**, 985–991.

Phillips, W. T., Salman, U. A., McMahan, C. A., and Schwartz, J. G. (1997). Accelerated gastric emptying in hypertensive subjects. *J. J. Nucl. Med.* **38**, 207–211.

Phillips, W. T., Schwartz, J. G., and McMahan, C. A. (1991). Rapid gastric emptying in patients with early non-insulin-dependent diabetes mellitus [letter]. *N. Engl. J. Med.* **324**, 130–131.

Phillips, W. T., Schwartz, J. G., and McMahan, C. A. (1992). Rapid gastric emptying of an oral glucose solution in type 2 diabetic patients. *J. Nucl. Med.* **33**, 1496–1500.

Plourde, V., St-Pierre, S., Fournier, A., and Tache, Y. (1993). CGRP [8–37] blocks the inhibition of gastric emptying induced by intravenous injection of αCGRP in rats. *Life Sci.* **52**, 857–862.

Riediger, T., Rauch, M., Jurat, G., and Schmid, H. A. (1999). Central nervous targets for pancreatic amylin. *Pflugers Arch.* **437**, R142.

Rogers, R. C., McTigue, D. M., and Hermann, G. E. (1996). Vagal control of digestion: Modulation by central neural and peripheral endocrine factors. *Neurosci. Biobehav. Rev.* **20**, 57–66.

Samson, M., Akkermans, L. M., Jebbink, R. J., van Isselt, H., vanBerge-Henegouwen, G. P., and Smout, A. J. (1997). Gastrointestinal motor mechanisms in hyperglycaemia induced delayed gastric emptying in type I diabetes mellitus. *Gut* **40**, 641–646.

Samson, M., Szarka, L. A., Camilleri, M., Vella, A., Zinsmeister, A. R., and Rizza, R. A. (2000). Pramlintide, an amylin analog, selectively delays gastric emptying: Potential role of vagal inhibition. *Am. J. Physiol.* **278**, G946–G951.

Schvarcz, E., Palmer, M., Aman, J., Lindkvist, B., and Beckman, K. W. (1993). Hypoglycaemia increases the gastric emptying rate in patients with type 1 diabetes mellitus. *Diabet. Med.* **10**, 660–663.

Schvarcz, E., Palmer, M., Aman, J., and Berne, C. (1995a). Atropine inhibits the increase in gastric emptying during hypoglycemia in humans. *Diabet. Care* **18**, 1463–1467.

Schvarcz, E., Palmer, M., Aman, J., and Berne, C. (1995b). Hypoglycemia increases the gastric emptying rate in healthy subjects. *Diabet. Care* **18**, 674–676.

Schvarcz, E., Palmer, M., Aman, J., Horowitz, M., Stridsberg, M., and Berne, C. (1997). Physiological hyperglycemia slows gastric emptying in normal subjects and patients with insulin-dependent diabetes mellitus. *Gastroenterology* **113**, 60–66.

Schwartz, J. G., Green, G. M., Guan, D. F., McMahan, C. A., and Phillips, W. T. (1996). Rapid gastric emptying of a solid pancake meal in type II diabetic patients. *Diabet. Care* **19**, 468–471.

Schwartz, J. G., McMahan, C. A., Green, G. M., and Phillips, W. T. (1995). Gastric emptying in Mexican Americans compared to non-Hispanic whites. *Dig. Dis. Sci.* **40**, 624–630.

Sexton, P. M., Paxinos, G., Kenney, M. A., Wookey, P. J., and Beaumont, K. (1994). *In vitro* autoradiographic localization of amylin binding sites in rat brain. *Neuroscience* **62**, 553–567.

Stricker, E. M., and McCann, M. J. (1985). Visceral factors in the control of food intake. *Brain Res. Bull.* **14**, 687–692.

Thompson, R. G., Gottlieb, A., Organ, K., Koda, J., Kisicki, J., and Kolterman, O. G. (1997). Pramlintide: A human amylin analogue reduced postprandial plasma glucose, insulin, and C-peptide concentrations in patients with type 2 diabetes. *Diabet. Med.* **14**, 547–555.

Vella, A., Lee, J. S., Camilleri, M., Szarka, L. A., Burton, D. D., Zinsmeister, A. R., Rizza, R. A., and Klein, P. D. (2002). Effects of pramlintide, an amylin analogue, on gastric emptying in type 1 and 2 diabetes mellitus. *Neurogastroenterol. Motil.* **14**, 123–131.

Vine, W., Beaumont, K., Gedulin, B., Pittner, R., Moore, C. X., Rink, T. J., and Young, A. A. (1996). Comparison of the *in vitro* and *in vivo* pharmacology of adrenomedullin, calcitonin gene-related peptide and amylin in rats. *Eur. J. Pharmacol.* **314**, 115–121.

Want, L. L., Levetan, C., Weyer, C., Maggs, D. G., Crean, J., Wang, Y., Strobel, S., Schoenamsgruber, E., Kolterman, O. G., Chandran, M., Henry, R. R., and Mudaliar, S.

(2002). Reduced postprandial glucose, glucagon and triglyceride excursions following 4 weeks of pramlintide treatment in patients with type 1 diabetes treated intensively with insulin pumps. *Diabetes* **51**(Suppl. 2), A117.

Watkins, J., Bhavsar, S., and Young, A. A. (1996). Effect of amylin to inhibit food intake in rats can be blocked with the selective amylin receptor antagonist, AC187. *Program and Abstracts, 10th International Congress of Endocrinology*, p. 419.

Weyer, C., Aronne, L., Fujoika, K., Aroda, V., Edelman, S., Chen, K., Lush, C., Wang, Y., Burns, C., Lutz, K., McIntyre, S., Kornstein, J., Wintle, M., and Baron, A. (2005). Safety, dose tolerance, and weight-related effects of pramlintide in obese subjects with or without type 2 diabetes. *Obes Rev* **6**(Suppl. 1), 21.

Weyer, C., Kim, D., Burrell, T., Wang, Y., Kornstein, J., Bicsak, T., Fineman, M., Ruggles, J., Schwartz, S., and Kolterman, O. (2003). Mealtime amylin replacement with pramlintide markedly reduced postprandial glucose excursions when added to insulin lispro in patients with type 1 or type 2 diabetes: A dose-timing study. *Diabetes* **52**(Suppl. 1), A16.

Yki-Järvinen, H., Kubo, K., Zawadzki, J., Lillioja, S., Young, A., Abbott, W., and Foley, J. E. (1987). Dissociation of *in vitro* sensitivities of glucose transport and antilipolysis to insulin in NIDDM. *Am. J. Physiol.* **253**, E300–E304.

Young, A. A., and Edwards, G. M. (2004). Effects of diabetes mellitus on gastrointestinal function in animal models. *In* "Gastrointestinal Function in Diabetes Mellitus" (M. Horowitz, and M. Samson, Eds.), pp. 29–95. John Wiley and Sons, Ltd, Chichester, UK.

Young, A. A., and Gedulin, B. (2000). Effect of amylin on intestinal glucose transport. *Diabetologia* **43**, A179.

Young, A., Carlo, P., Smith, P., Vine, W., and Gedulin, B. (1994). Dose dependent inhibition of gastric emptying by subcutaneous injection of amylin in non-diabetic and BB/W (IDDM) rats. *Diabetes* **43**, 174A.

Young, A. A., Bogardus, C., Stone, K., and Mott, D. M. (1988). Insulin response of components of whole-body and muscle carbohydrate metabolism in humans. *Am. J. Physiol.* **254**, E231–E236.

Young, A. A., Gedulin, B., Vine, W., Percy, A., and Rink, T. J. (1995a). Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin. *Diabetologia* **38**, 642–648.

Young, A. A., Wang, M. W., Gedulin, B., Rink, T. J., Pittner, R., and Beaumont, K. (1995b). Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. *Metabolism* **44**, 1581–1589.

Young, A. A., Gedulin, B. R., Jodka, C., and Green, D. (1996a). Insulin-induced hypoglycemia reverses amylin-inhibition of gastric emptying in rats. *Diabetes* **45**, 187A.

Young, A. A., Gedulin, B. R., and Rink, T. J. (1996b). Dose-responses for the slowing of gastric emptying in a rodent model by glucagon-like peptide (7–36)NH2, amylin, cholecystokinin, and other possible regulators of nutrient uptake. *Metabolism* **45**, 1–3.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996c). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Young, A., Moore, C., Herich, J., and Beaumont, K. (2000). Neuroendocrine actions of amylin. *In* "The CGRP Family: Calcitonin Gene-Related Peptide (CGRP), Amylin, and Adrenomedullin" (D. Poyner, I. Marshall, and S. D. Brain, Eds.), pp. 91–102. Landes Bioscience, Georgetown, TX.

Andrew Young

Effects on Digestive Secretions

I. Summary

Rat amylin subcutaneously injected into rats dose-dependently inhibits pentagastrin-stimulated gastric acid secretion and protects the stomach from ethanol-induced gastritis. The ED_{50}s for these actions (0.050 and 0.036 μg, respectively) are the lowest for any dose-dependent effect of amylin thus far described, and their similar potencies are consistent with a mechanistic (causal) association. At higher amylin doses, inhibition of gastric acid secretion was almost complete (93.4%). Gastric injury (measured by a subjective analog scale) was inhibited by up to 67%. The observation that effective doses of amylin result in plasma concentrations of 7–10 pM (i.e., within the reported range; Pieber *et al.*, 1994) supports the interpretation that inhibition of gastric acid secretion and maintenance of gastric mucosal integrity are physiological actions of endogenous amylin. The pharmacology of these responses fits with one mediated via amylin-like receptors.

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Rat amylin inhibited CCK-stimulated secretion of pancreatic enzymes, amylase, and lipase by up to ~60% without having significant effect in the absence of CCK. ED_{50}s for the effect were in the 0.1–0.2 μg range, calculated to produce plasma amylin excursions within the physiological range. Effects of informative ligands are consistent with the concept of amylin receptor mediation. Amylin was effective in ameliorating the severity of pancreatitis in a rodent model.

The amylin analog pramlintide inhibited gallbladder emptying in mice as measured by total weight of acutely excised gallbladders.

Amylin inhibition of gastric acid secretion, pancreatic enzyme secretion, and bile secretion likely represents part of an orchestrated control of nutrient appearance. Modulation of digestive function fits with a general role of amylin in regulating nutrient uptake. Rate of ingestion, rate of release from the stomach, and rate of digestion of various food groups appear to be under coordinate control.

II. Gastric Acid Secretion

A. Background

Complex carbohydrates, proteins, and triglycerides, comprising the three major food groups, are each formed in condensation (water-forming) reactions. Digestion of these foods into absorbable moieties (e.g., monosaccarides, amino acids, and fatty acids) essentially involves the reversal of this process, hydrolysis (Guyton and Hall, 1996b). Gastric acid participates in this action, especially with respect to protein and triglyceride digestion, and may therefore be regarded as a contributor to the aggregate rate of nutrient uptake (Alpers, 1994).

Amylin is the most potent endogenous inhibitor of gastric emptying so far identified in mammals (Gedulin *et al.*, 1996; Young *et al.*, 1996a), being more potent, per molar subcutaneous dose, than other physiological inhibitors of gastric emptying, secretin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) (Young *et al.*, 2002). Control of gastric emptying appears to be a physiological function of amylin, occurring at normal plasma concentrations (Pieber *et al.*, 1994; Young *et al.*, 1995). Gut peptides that slow gastric emptying at physiological concentrations also typically inhibit gastric acid secretion (e.g., secretin, MacLellan *et al.*, 1988; Rhee *et al.*, 1991; CCK, Burckhardt *et al.*, 1994; Konturek *et al.*, 1992; GLP-1, Schjoldager *et al.*, 1989; PYY, Adrian *et al.*, 1985; Guo *et al.*, 1987; Pappas *et al.*, 1985, 1986;). In view of this association, and because of the potential role of acid secretion in control of nutrient availability, the effect of amylin on gastric acid secretion is of interest.

B. Effect of Amylin on Gastric Acid Secretion in Rats

The effects of amylin or pramlintide on acid secretion have not been studied in humans. Several methods have been used to study effects of amylin gastric acid secretion in rats. In a modification of the Shay test in rats (Shay *et al.,* 1945), gastric contents were measured 3 hr after pyloric ligation. Amylin injected peripherally in high doses (up to 100 μg/kg) inhibited gastric acid secretion (Guidobono *et al.,* 1994). Such pharmacological doses did not, however, identify this action as physiological. Intracerebroventricular administration of amylin was ~2 orders of magnitude more potent than intravenous administration in inhibiting gastric acid secretion in a similar preparation (Guidobono *et al.,* 1994).

Studies aimed at determining the physiological relevance of amylin inhibition of acid secretion were performed in rats chronically fitted with double gastric fistulae (Zivic Miller). A grommet-shaped double lumen plug was sutured into the stomach wall, and separate entry and exit cannulae communicating with the gastric lumen were exteriorized at the interscapular region, allowing frequent flushing and assessment of gastric acid secretion by titration of the gastric aspirate. Gastric acid secretion was stimulated with pentagstrin (125 μg/kg s.c.) and followed 40 min later with a range of amylin (or pramlintide doses). Pentagastrin stimulated gastric acid secretion 4.6-fold. Amylin injected 40 min later dose-dependently inhibited gastric acid production by up to 94% with a $t_{½}$ of ~8 min and an ED_{50} of 0.05 μg/rat. In fact, the inhibitory effect was sufficiently profound to reduce acid secretion to approximately one-third of the basal (unstimulated) rate. The ED_{50} dose was estimated from previously determined pharmacokinetic analyses for this animal model (Young *et al.,* 1996b) to produce a peak plasma amylin concentration of 10 pM (using the relation concentration $[\text{pM}] = 10^{0.86 \log \text{dose in } \mu\text{g} + 2.13}$) and a concentration 60 min after injection of 7 pM, well within the endogenous circulating range. That is, the *in vivo* dose response for inhibition of gastric acid secretion indicated this was a physiological action of amylin. The *in vivo* potency of amylin's effect of inhibiting pentagastrin-stimulated gastric acid secretion in the presently described rat model was compared with that of GLP-1 in the same model, and it was 180-fold greater (Gedulin *et al.,* 1997b) (Fig. 1).

C. Localization of Amylinergic Inhibition of Gastric Acid Secretion

Central control of gastric acid secretion involves a cholinergic pathway involving the nucleus tractus solitarius, area postrema, and dorsal motor nucleus of the vagus (Okuma and Osumi, 1986a,b) as well as capsaicin-sensitive vagal afferents (Sakaguchi and Sato, 1987). The area postrema can respond to locally applied agents with changes in gastric acid secretion

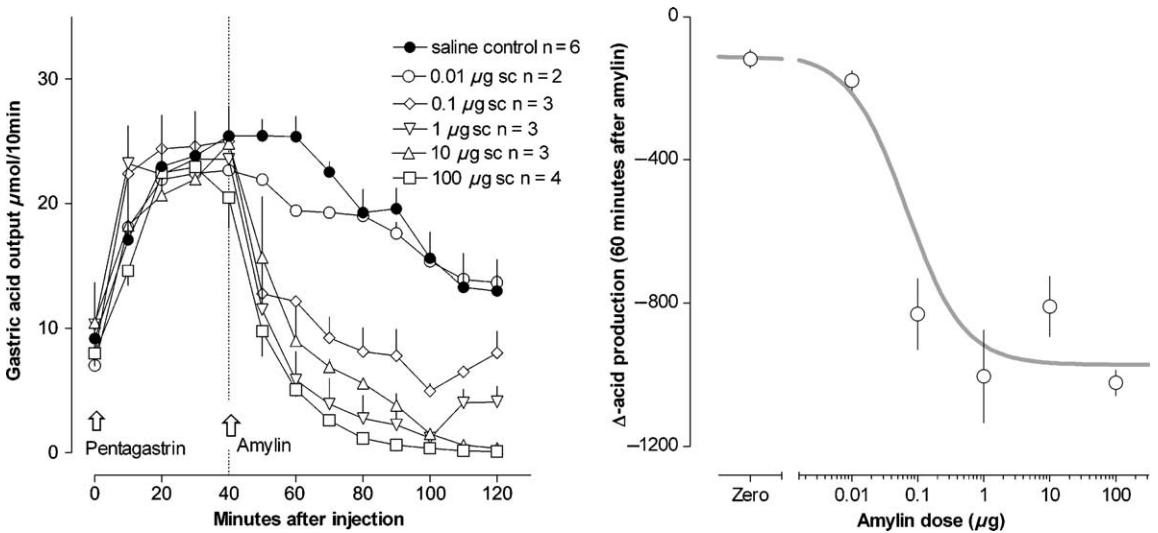

FIGURE 1 Dose response for amylin inhibition of pentagstrin-stimulated secretion of acid from fistulated stomachs of conscious rats. Data from Gedulin *et al.* (2005).

(Okuma and Osumi, 1986; Okuma *et al.*, 1987; Tache *et al.*, 1989; Zhang and Huang, 1993).

Several authors have concluded that the dominant acid-inhibitory actions of calcitonin gene-related peptide (CGRP) are specific, are central, and are not explained by direct parietal cell effects (Helton *et al.*, 1989; Tache, 1992). As with the effects of infused cholecystokinin and peptide YY to inhibit gastric acid secretion (Lloyd *et al.*, 1997), the effect of CGRP required an intact vagus nerve (Lenz *et al.*, 1985; Tache *et al.*, 1984). Acid-inhibitory effects of centrally administered CGRP were unaffected by systemic antibody that neutralized its peripheral effects (Lenz *et al.*, 1984). These and other studies (Lenz and Brown, 1987; Lenz *et al.*, 1984; Morley *et al.*, 1981; Okimura *et al.*, 1986; Tache *et al.*, 1984, 1991) led many to infer a central site of CGRPergic inhibition of gastric acid secretion. As discussed later, central acid inhibitory effects previously ascribed to CGRP are likely to be mediated via an amylin-like pharmacology.

The involvement of central amylin receptors in the control of gastric acid secretion is supported by the dense localization of such receptors in the area postrema/nucleus tractus solitarius (Sexton *et al.*, 1994). This circumventricular brain stem structure, which modulates other gastric actions of amylin (Edwards *et al.*, 1998) (described elsewhere), is sensitive to circulating peptides, receives much gastric vagal input (Ewart *et al.*, 1988; Yuan and Barber, 1993), and communicates directly with the dorsal motor nucleus of the vagus (Rogers *et al.*, 1996), whence central acid secretory drive emanates (Guyton and Hall, 1996a). Clementi *et al.*, proposed that the gastroprotective effect of amylin was central and that pathways involved dopamine-2 receptors (Clementi *et al.*, 1996); this proposal was supported by reports that amylin's effect could be blocked with domperidone, a DA_2 receptor antagonist (Clementi *et al.*, 1997).

The central effects of amylin in inhibition of gastric acid secretion appeared not to depend upon a somatostatinergic mechanism in the stomach. Pretreatment with cysteamine, which depletes somatostatin in the stomach, did not prevent centrally administered amylin from inhibiting gastric acid secretion in pylorus-ligated rats (Guidobono *et al.*, 1994).

D. Peripheral (Local) Gastric Acid Inhibitory Effect of Amylin

The identification of central mechanisms mediating amylin-inhibition of gastric acid secretion do not preclude the existence of direct preipheral effects. For example, in addition to central mechanisms, secretin inhibits acid secretion through a local effect independently of central connections (Lloyd *et al.*, 1997).

A local somatostatin-dependent action of amylin in inhibiting acid secretion from mouse stomach *in vitro* was reported (Zaki *et al.*, 1996)

and cannot be excluded as a contributory mechanism. Moreover, this local effect was blocked with AC187, but not CGRP[8–37], indicating it was likely to be specifically amylinergic (Makhlouf *et al.,* 1996; Zaki *et al.,* 1996). In the same preparation, amylin inhibited gastrin secretion as well as somatostatin secretion (Makhlouf *et al.,* 1996).

Past descriptions of possible sites of action of CGRP's inhibition of gastric acid secretion provide clues as to how amylin may operate. Mechanisms have included direct peripheral effects (Holzer *et al.,* 1991; Tache *et al.,* 1991). At the stomach, CGRP is reported to modulate the antral mucosal response to acid (Manela *et al.,* 1995), to locally stimulate somatostatin secretion (Zdon *et al.,* 1988), and to directly stimulate parietal cells (Umeda and Okada, 1987). In regard to a local acid inhibitory effect, it may be significant that amylin-like immunoreactivity in the gut of rats and humans is predominantly in the pyloric antrum (Asai *et al.,* 1990; Miyazato *et al.,* 1991; Mulder *et al.,* 1994; Nicholl *et al.,* 1992; Ohtsuka *et al.,* 1993; Toshimori *et al.,* 1990), where it is localized with gastrin in G-cells (Mulder *et al.,* 1994, 1997; Ohtsuka *et al.,* 1993).

E. Amylin Inhibition of Gastric Acid Secretion During Hypoglycemia

Insulin stimulation of gastric acid secretion (Isenberg *et al.,* 1969) appears to be secondary to its hypoglycemic effect. For example, increases in plasma glucose concentration inhibit gastric acid secretion (Lam *et al.,* 1993; Moore, 1980), including that stimulated by insulin (Stacher *et al.,* 1976). Increases in glucose also inhibit amino acid-stimulated acid secretion (Lam *et al.,* 1995). Studies using microinjection of D-glucose into different brain regions indicate that glucose-induced inhibition of gastric acid secretion appears to be localized to structures around the nucleus tractus solitarius (Sakaguchi and Sato, 1987). Amylin inhibition of gastric acid secretion was not associated with (explained by) changes in plasma glucose concentration (Gedulin *et al.,* 1997b).

Several amylinergic effects, for example, inhibition of gastric emptying (Gedulin and Young, 1998; Gedulin *et al.,* 1997c) and inhibition of glucagon secretion (Parkes *et al.,* 1999), are overridden by hypoglycemia. These patterns suggests "fail-safe" glucose counterregulatory reflexes in which the restraint that amylin exerts on nutrient availability is lifted during hypoglycemia. Whether hypoglycemia overrides amylinergic inhibition of gastric acid secretion has not been directly addressed, although there are clues from the literature that such a mechanism indeed exists.

In a Shay test of gastric acid secretion in pylorus-ligated rats, Guidobono *et al.* (Guidobono *et al.,* 1994) compared the acid inhibitory effect of intracerebroventricular amylin in saline-treated rats with that in rats administered 1 U of insulin intravenously. Whereas amylin inhibited acid secretion by 87%

in saline-treated rats, its inhibition of insulin-stimulated acid secretion was much attenuated (27% inhibition). Indeed, acid secretion in the presence of both insulin and amylin was 2.2-fold greater than basal. Although plasma glucose was not reported, the 1 U intravenous dose is likely, from historical responses, to have produced hypoglycemia. That is, as with other central amylinergic responses, amylin inhibition of gastic acid secretion may also be overridden by hypoglycemia.

F. Gastroprotective Effect of Amylin

Amylin at elevated doses was reported to protect against erosions and mucosal damage in rats administered ethanol, indomethacin (Guidobono *et al.*, 1997), reserpine, and serotonin (Clementi *et al.*, 1997). Its gastroprotective effect in those studies was not explored at doses that would mimic physiological fluctuations in plasma concentration. One study reported a gastroprotective effect only when amylin was given centrally, and not when given subcutaneously at doses of 10 and 40 μg/kg (Guidobono *et al.*, 1997). The authors discounted a mechanistic link between acid-inhibitory and gastroprotective effects. Other work reported here found that the dose responses for these two actions were indistinguishable, and thus could well support a causal association.

One study (Clementi *et al.*, 1997) reported gastroprotective effects of amylin with doses likely to result in plasma amylin concentrations of $\sim$1 nM, around 100-fold higher than concentrations of endogenous amylin in rats (Pieber *et al.*, 1994; Vine *et al.*, 1998).

G. Physiological Relevance of Amylin Gastroprotection

In a study designed to probe the physiological relevance of amylin in maintenance of the gastric mucosa, fasted male rats were administered various s.c. doses of amylin 20 min before gavage with 1 ml absolute ethanol (Gedulin *et al.*, 1997a). Thirty minutes later, their stomachs were excised and the everted mucosae were immediately graded for severity of mucosal damage by observers blinded to the experimental treatment. They used scores of 0 (no observable damage) to 5 (100% of mucosal surface covered by hyperemia, ulceration, or sloughing), comparable to those developed by Guidobono and others (Guidobono *et al.*, 1997). Amylin given 5 min before the ethanol gavage profoundly and potently protected the stomach from mucosal injury. The injury score was reduced 67%, and the ED_{50} (0.036 μg/rat) was statistically indistinguishable from that obtained for inhibition of gastric acid secretion. The ED_{50} dose was estimated to have resulted in a peak plasma amylin concentration of 8 pM (Gedulin *et al.*, 1997a) (that is, within the physiological range). It is therefore possible that endogenous amylin may play a role in the maintenance of gastric mucosal integrity (Fig. 2).

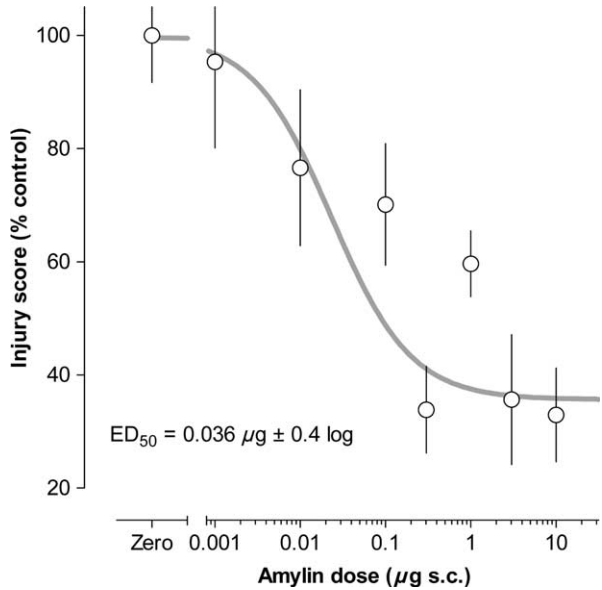

FIGURE 2 Dose response for gastroprotective effect of amylin in rats gavaged with ethanol. Data from Gedulin *et al.* (1997a,b).

A physiological role of endogenous hormones has often been inferred from events following their negation. Treatment of rats with the β-cell toxin streptozotocin results in amylin deficiency. Streptozotocin is reported to induce gastric mucosal lesions in rodents (Goldin *et al.*, 1997; Hung and Huang, 1995; Piyachaturawat *et al.*, 1991; Takeuchi *et al.*, 1997). This condition is not reversed by insulin replacement (Piyachaturawat *et al.*, 1991) and therefore appears unlikely to be due to an absence of insulin. In explaining these findings, some have proposed that streptozotocin may be directly toxic at the gastric mucosa. However, such a mechanism does not explain why NOD (non-obese type 1 diabetic) mice with autoimmune β-cell destruction also exhibit gastric erosions (Nishimura *et al.*, 1983). The absence of a factor from the β-cell, such as amylin, could underlie the propensity of both of these models to gastric erosion. The susceptibility of amylin-deficient adult humans to gastric injury is unclear. Type 1 diabetic children, however, have a 3- to 4-fold elevation in rate of peptic disease (Burghen *et al.*, 1992).

An indirect probe of whether endogenous amylin exerted a gastroprotective effect would be to examine the effects of amylin secretagogues, such as glucose. Prior administration of 0.25 g D-glucose, shown to increase endogenous plasma amylin concentrations in fasted Sprague Dawley rats to 5 pM (Vine *et al.*, 1998), significantly decreased blinded gastric injury score (by 19 $\pm$ 5%, $P < 0.0005$; Gedulin *et al.*, unpublished). One

interpretation of this result is that glucose-stimulated endogenous amylin could be protective.

H. Pharmacology of Acid-Inhibitory and Gastroprotective Effects

The literature on the effects of structurally related peptides assists in the interpretation of the pharmacology of amylin-mediated effects on gastric acid secretion and gastric injury. Calcitonin gene-related peptide (Beglinger *et al.,* 1988; Holzer *et al.,* 1991; Lenz and Brown, 1987; Lenz *et al.,* 1984; Okimura *et al.,* 1986; Tache *et al.,* 1991; Zanelli *et al.,* 1992) and teleost calcitonins (eel and salmon) (Doepfner, 1976; Guidobono *et al.,* 1991; Okimura *et al.,* 1986) are reported to inhibit gastric acid secretion and gastric lesions with high potency. When directly compared, they were found to be generally more potent than mammalian calcitonins (Lenz and Brown, 1987; Okimura *et al.,* 1986).

Amylin administered i.c.v. (Guidobono *et al.,* 1994) was more potent than CGRP in the same model (Hughes *et al.,* 1984). The pharmacology, in which the gastric-inhibitory potency of teleost calcitonins = amylin > CGRP > mammalian calcitonins, fits that described for amylin receptors (Beaumont *et al.,* 1993) and cannot accommodate a purely CGRP-like pharmacology; CGRP receptors are not significantly activated by teleost or mammalian calcitonins (Beaumont *et al.,* 1993). An observation that CGRP [8–37] (a CGRP antagonist; Chiba *et al.,* 1989) reverses inhibition of gastric acid secretion (Clementi *et al.,* 1997), does not identify this as a CGRPergic action; at appropriately high doses, CGRP[8–37] can also block amylinergic responses (Young *et al.,* 1992). Instead, blockade of acid inhibitory and gastroprotective effects with AC187 would indicate that this mechanism was likely to be mediated via amylin- or calcitonin-like receptors, since AC187 is 500-fold more selective for amylin versus CGRP receptors, and 25-fold more selective versus calcitonin receptors (Beaumont *et al.,* 1995). Pre-administration of AC187 (3 mg i.v.) negated the gastroprotective effect of rat amylin (0.3 μg s.c.) in ethanol-gavaged rats (87% of control injury score versus 34% in amylin-treated rats). In separate experiments, pre-administration of AC187 negated amylin inhibition of pentagastrin-stimulated acid secretion (Gedulin *et al.,* 2005) (Fig. 3).

III. Pancreatic Enzyme Secretion

Exocrine secretion of digestive enzymes from the pancreas could be a further determinant of rate of nutrient uptake from meals and was therefore examined as a potential control point in amylinergic influence on nutrient assimilation. Effects of amylin on exocrine secretion of pancreatic enzymes

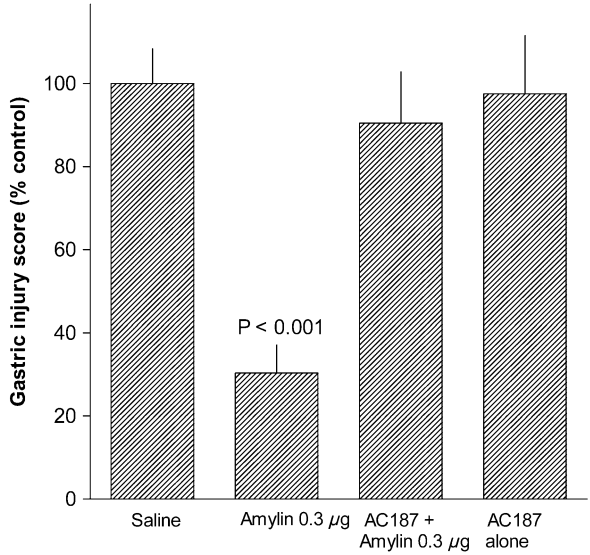

FIGURE 3 Reversal of gastroprotective effect of amylin in ethanol-gavaged rats with the selective amylin antagonist AC187. Data from Gedulin *et al.* (2005).

were examined *in vivo* in rats and *in vitro* in isolated pancreatic acini and the pancreatic acinar cell line Ar42j. To date, no *in vivo* studies of amylin actions in this system have been conducted in humans.

A. Effects of Amylin on Pancreatic Exocrine Secretion *In Vivo*

One study has investigated effects of amylin on pancreatic enzyme secretion in intact rats (Gedulin *et al.*, 1998). The pancreatic duct was cannulated under anesthesia, and secretions were collected every 15 min for assay of amylase and lipase activity, as well as for measurement of secreted volume. Effects of cholecystokinin octapeptide (CCK-8; 1 μg s.c.) or rat amylin alone (0.1–1 μg s.c.) were assessed. CCK-8 increased 60-min secretion of amylase and lipase activity 7.7- and 6.4-fold over basal, respectively. Two-thirds of this increase was attributable to an increased flow, and one-third to an increased enzyme concentration in the secretion. In contrast, amylin had no significant effect on unstimulated enzyme secretion.

When amylin was administered in association with CCK-8, secretion of amylase was suppressed by up to 58%, two-thirds of which was attributable to a reduction in secretory flow, and one-third to a reduction in enzyme concentration. A similar decrease was observed in secreted lipase (Fig. 4).

ED_{50}s for the inhibition of CCK-stimulated juice flow, amylase secretion, and lipase secretion were 0.11 μg, 0.21 μg, and 0.11 μg, respectively.

These ED_{50}s were not statistically different from each other and were calculated from separate kinetic studies (Young *et al.*, 1996b) to have resulted in peak plasma concentrations of 15–26 pM, comparable to the 9 pM (Vine *et al.*, 1998) to 15 pM (Pieber *et al.*, 1994) range reported to circulate in fed rats. This potency is consistent with the concept that inhibition of pancreatic enzyme secretion is a physiological effect of amylin.

Pramlintide produced similar effects on CCK-stimulated pancreatic enzyme secretion.

B. Pharmacology of Exocrine Inhibitory Action of Amylin

As with some other amylinergic responses, the literature on the effects of structurally related peptides (CGRP and teleost and mammalian calcitonins) can assist in the interpretation of the pharmacology of amylin-mediated effects on exocrine pancreatic secretion. The inhibition by amylin of stimulated secretion of pancreatic enzymes is similar to patterns reported for both CGRP (Bunnett *et al.*, 1991) and calcitonins (Funovics *et al.*, 1981; Hotz *et al.*, 1977; Nakashima *et al.*, 1977; Nealon *et al.*, 1990), including salmon calcitonin (Paul, 1975). Stimulated (Mulholland *et al.*, 1989; Nakashima *et al.*, 1977) but not basal (Funovics *et al.*, 1981) secretion was inhibited with calcitonin or CGRP. The observation that effects of CGRP and calcitonin are additive (Nealon *et al.*, 1990) could be consistent with their acting via a common receptor. If so, this could not be at CGRP receptors, since calcitonins do not significantly interact with them. But CGRP and, especially, salmon calcitonin interact with amylin receptors (Beaumont *et al.*, 1993). These previously reported effects of CGRP and calcitonins to inhibit stimulated pancreatic exocrine secretion *in vivo* would instead support an effect mediated via an amylinergic pathway.

C. Effects of Amylin in Ar42j Cells

Ar42j cells, a model of pancreatic acinar cells derived from a pancreatic carcinoma line, exhibit many aspects of pancreatic acinar behavior, including secretion of amylase in response to stimulation with pituitary adenylate cyclase activating peptide (PACAP38) (Kashimura *et al.*, 1993; Raufman *et al.*, 1991; Schmidt *et al.*, 1993). PACAP38-mediated signaling in acinar and Ar42j cells appeared to occur via other than cAMP (Kashimura *et al.*, 1993), and is now recognized as occurring via second messengers activation of phospholipase C and mobilization of intracellular calcium (Barnhart *et al.*, 1997). Using the response to PACAP27 and PACAP38 (125 nM) as positive controls (to indicate that cells and signaling pathways were intact), effects of amylin on phospholipase C activation were tested in Ar42j cells. Ar42j cells grown to confluence were incubated overnight with [2-^{3}H]-myo-inositol and

Amylase secreted (U/15 min)
350
300
250
200
150
100
50
0
–30
0
30
60
90
Minutes
Saline
CCK + Saline
CCK + 0.1 μg Amylin
CCK + 0.3 μg Amylin
CCK + 1 μg Amylin
Amylase secretion (U/60min)
500
400
300
200
Amylase
Zero
0.1
0.3
1
Amylin dose (μg s.c.)
Lipase secreted (U/15min)
500
400
300
200
100
0
–30
0
30
60
90
Minutes
Saline
CCK + Saline
CCK + 0.1 μg Amylin
CCK + 0.3 μg Amylin
CCK + 1 μg Amylin
Lipase secretion (U/60min)
800
600
400
200
Lipase
Zero
0.1
0.3
1
Amylin dose (μg s.c.)

then for an initial 20 min before exposure to PACAP27, PACAP38, and rat amylin. Inositol phosphates were extracted (Pittner and Fain, 1989), and rates of inositol monophosphate production were assessed as a measure of phospholipase C activation (Young *et al.*, 2005). Although Ar42j cells responded to PACAP27 or PACAP38 with ~4-fold increases in phospholipase C activity, there was no significant change in activity following application of rat amylin (1 μM) (Young *et al.*, 2005). These results indicated that effects on exocrine secretion were indirect (e.g., centrally mediated). Although Huang *et al.*, (Huang *et al.*, 1996) reported effects of amylin in Ar42j cells, they saw no effects with CGRP or salmon calcitonin, which would tend to support the absence of an amylinergic effect (Fig. 5).

To probe whether amylin had an effect on signaling pathways other than phospholipase C, responses were assessed in a microphysiometer. Rates of acid production, as measured with a cytosensor microphysiometer (Molecular Devices, Menlo Park, CA) can be used as an indicator of general cellular response, independent of knowledge of a second messenger system (Owicki *et al.*, 1990; Parce *et al.*, 1990; Pitchford *et al.*, 1995). Using the acidification rate response to PACAP38 (125 nM) to verify that cell signaling was intact, the effect of amylin (1 μM) was tested in Ar42j cells. While exposure to PACAP38 for 6 min evoked a characteristically prolonged activation of Ar42j cells, with activity increasing to 215% of basal, the activity following application of rat amylin over the same period was 89% of basal. That is, there was no direct cellular activation by amylin in this cell line.

D. Effects of Amylin in Isolated Acinar Cells

Effects of signaling molecules in derived cell lines can be misleading if such cell lines do not contain the full complement of biologies as the tissues they are purported to imitate. Effects of amylin were tested in primary pancreatic acini isolated by collagenase digestion methods (Amsterdam and Jamieson, 1974; Gardner and Jackson, 1977). Resulting dispersed acini were suspended in agarose and entrapped onto a microphysiometer capsule (Molecular Devices). Isolated acini exposed to PACAP38 (100 nM) for 10 min, used as a positive control, increased their activity to 163% of basal. Exposure to the same concentration of rat amylin for the same period of time had no significant effect (88% of basal activity) (Young *et al.*, 2005).

Fehmann *et al.*, (Fehmann *et al.*, 1990) and Kikuchi *et al.*, (Kikuchi *et al.*, 1991) affirmed that amylin had no direct effect on isolated pancreatic acini, as assessed by release of amylase *in vitro*. To the extent that Ar42j cells mimic pancreatic acinar cells, there are four independent findings that support the conclusion that pancreatic acinar cells are not amylin responsive.

FIGURE 4 Dose response for amylin inhibition of CCK-stimulated secretion of amylase and lipase from catheterized pancreas in anesthetized rats. Data from Young *et al.* (2005).

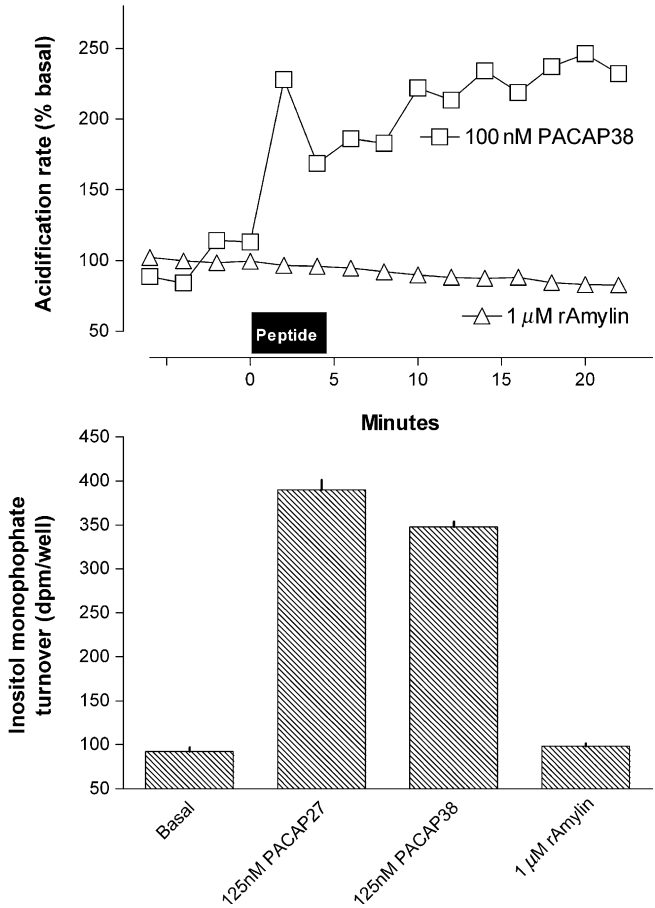

FIGURE 5 Absence of effect of amylin Ar42j cells on acidification rate, a generalized response (upper panel), or on phosphoinositol turnover (lower panel). In each case, the positive control response to PACAP was present. Data from Young *et al.* (2005).

In one study, when both were measured, CGRP inhibited CCK-stimulated pancreatic enzyme secretion *in vivo* but not in isolated perfused pancreas or dispersed acini (Mulholland *et al.*, 1989). Those findings pointed to an extrapancreatic (central) control that has been interpreted as vagal cholinergic (Owyang, 1994).

The report that amylin inhibits enzyme secretion *in vivo* concurs with the literature for calcitonins and CGRP, and in the simplest interpretation, points to an amylin-like pharmacology. The report that amylin inhibits stimulated pancreatic exocrine secretion *in vivo* but has no detectable effect *in vitro* in pancreatic acini or a derivative cell line is consistent with the parallel literature for CGRP. Both literatures are consistent with a central (indirect) amylinergic control of pancreatic enzyme secretion.

E. Physiological Implications of Modulating Enzyme Secretion

Amylin modulation of digestive enzyme secretion aligns with a general effect of regulating digestive function (as exemplified by influence on gastric acid secretion). This general effect further fits with an overall physiological role to regulate nutrient assimilation and rate of glucose appearance. Because surgical patients tolerate excision of large fractions of absorptive gut remarkably well, without obvious malabsorption, many have presumed that digestive and absorptive capacity is present in abundant excess. It may therefore be questioned whether the 24–67% suppression of pancreatic enzyme secretion obtainable with amylin agonists appreciably contributes to control of nutrient assimilation. An attempt to quantify limiting fluxes in absorption (Weber and Ehrlein, 1998) examined the kinetics of absorptive capacity of hydrolysates of each of the major food groups and enabled calculation of the fraction of available gut length required for complete absorption of nutrient entering at the prevailing rate of gastric emptying. Even when prior hydrolysis eliminated intraluminal digestion as a rate-limiting step, at least 55% of gut length was required (indicating only an 80% reserve), dispelling the notion that absorptive capacity is present in great excess. Decreases in rate of intraluminal digestion (by decreasing enzyme activity in the lumen, for example) can only decrease this reserve. A separate report showed that decreases of exocrine secretory capacity to one-third of normal were sufficient to cause steatorrhea (Cole *et al.*, 1987) and affirmed that digestive and absorptive capacity is not in great excess. Indeed, although safety factors (the relation between digestive/aborptive capacity and load) are initially high in suckling rat pups, they approach 1.0 as individuals enter adulthood (O'Connor and Diamond, 1999). These studies (Cole *et al.*, 1987; O'Connor and Diamond, 1999; Weber and Ehrlein, 1998) support the notion that digestive enzymes are secreted parsimoniously, in amounts titrated to be just sufficient for complete digestion and absorption.

Not only does digestive capacity seem parsimoniously distributed, proteolytic activity secreted from the pancreas is subject to feedback control, and by an interesting mechanism. Luminal CCK-releasing factor (LCRF), a peptide secreted from proximal gut, presumptively acts at (as-yet-unidentified) intraluminal receptors to amplify the release of CCK from I-cells at the duodenal and jejunal mucosa. Increased CCK secretion thence stimulates pancreatic enzyme secretion, which then digests LCRF and decreases its signal. If there is insufficient protease to digest and neutralize LCRF, more CCK (and protease) is secreted (Spannagel *et al.*, 1996).

These examples illustrate that modulating digestive capacity may have physiological significance as an influx effector in control of fuel balance. A pharmacological example is provided by the clinical experience with enzyme

inhibitors. Slowing digestion and absorption of complex carbohydrates by blocking α-glucosidase lowers glycemic indices (Balfour and McTavish, 1993; Coniff *et al.*, 1995), and inhibiting pancreatic lipase results in weight loss (James *et al.*, 1997). Pramlintide is reported to lower plasma glucose (Ratner *et al.*, 1998; Rosenstock *et al.*, 1998) and body weight (Whitehouse *et al.*, 1998) in type 1 and type 2 diabetic patients. The extent to which effects on gut amylase and lipase activity contribute to these effects is not presently established. Such effects, if present in humans, differ from those of irreversible digestive enzyme inhibitors in that they are not associated with an increased incidence of side effects such as flatulence and steatorrhoea.

F. Effects of Amylin on Experimental Pancreatitis in Mice

Agents that inhibit pancreatic enzyme secretion, for example, somatostatin, have the potential to limit severity of disease in acute pancreatitis, a severe condition that in the United States has a prevalence of ~0.5% and claims ~4000 lives annually (Greenberger *et al.*, 1991). In a mouse model of pancreatitis, a frog skin CCK agonist, caerulein, was injected (0.01 μg i.p.) on three occasions, 2 hr apart, and blood was taken 5 hr later for measurement of amylase as an assessment of pancreatic damage (Warzecha *et al.*, 1997). The 2.6-fold elevation in amylase in saline-treated control mice was dose-dependently ameliorated with amylin (0.1 μg doses and above) injected 5 min before the caerulein (Fig. 6).

CGRP was also effective in a caerulein-induced model of pancreatitis (Warzecha *et al.*, 1997). In a study of 94 patients with pancreatitis, salmon calcitonin significantly improved pain and normalization of serum amylase (Goebell *et al.*, 1979). The concordance of effects of amylin, salmon calcitonin, and CGRP is consistent with the involvement of an amylin-like pharmacology in the amelioration of pancreatitis.

IV. Effects of Amylin on Gallbladder Contraction

Amylin control of nutrient appearance includes regulation of several digestive functions, including some (acid and lipase secretion) that affect digestion and absorption of fats. In addition to these latter secretions, digestion and absorption of dietary fats are also influenced by secretion of bile into the intestinal lumen following contraction of the gallbladder. Rats do not possess a gallbladder and are thus unsuited for studies of this mechanism. However, mice have a gallbladder, and control of emptying can be studied by comparing weights of acutely excised gallbladders, bile included (Bignon *et al.*, 1999).

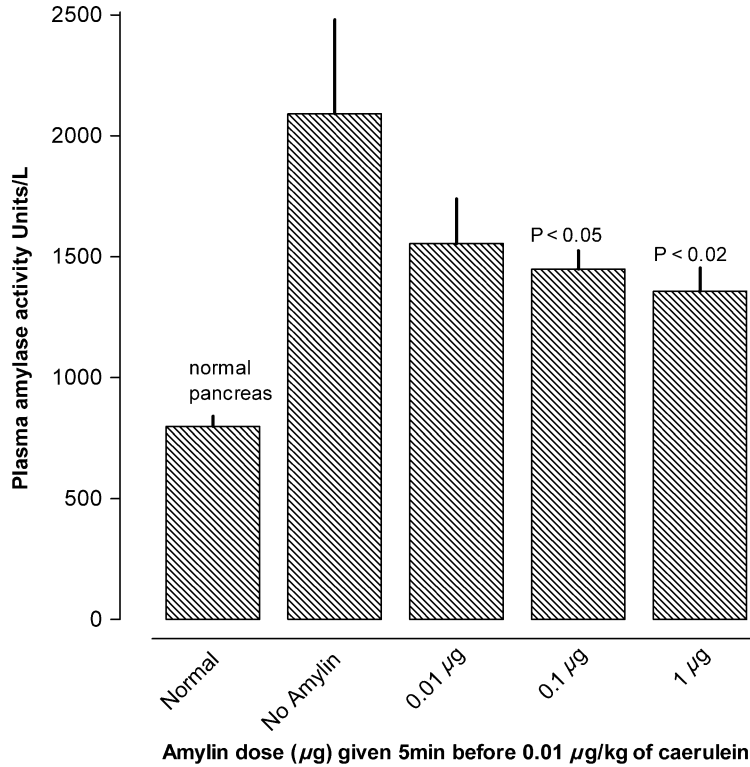

FIGURE 6 Amylin inhibition of caerulein-induced pancreatitis (assessed by plasma amylase concentration) in mice. Data from Young *et al.* (2005).

Following food deprivation for 3 hr, mice were injected s.c. with saline or CCK-8, with or without various s.c. doses of pramlintide. Thirty minutes later, mice were euthanized by cervical dislocation and the gallbladder was excised and weighed. CCK-8 itself evoked gallbladder contraction, as inferred by a 77% decrease in gallbladder weight. Pramlintide alone dose-dependently inhibited gallbladder emptying, as inferred by a doubling in weight of the gallbladder plus bile. The effect of pramlintide (10 μg) to inhibit gallbladder emptying was reversed with co-administration of the selective amylin antagonist AC187 (300 μg s.c.), pointing to an amylin-like pharmacology. Pramlintide did not prevent CCK-stimulated emptying of the gallbladder (Gedulin *et al.*, in press) (Fig. 7).

CGRP infusions in guinea pigs are reported to inhibit CCK-induced gallbladder contraction (Hashimoto *et al.*, 1988) and can cause relaxation of gallbladder smooth muscle *in vitro* (Hashimoto *et al.*, 1988; Kline *et al.*, 1991). CGRP also inhibited CCK-induced and meal-induced gallbladder contraction in conscious beagle dogs (Lenz *et al.*, 1993). CGRP halved bile

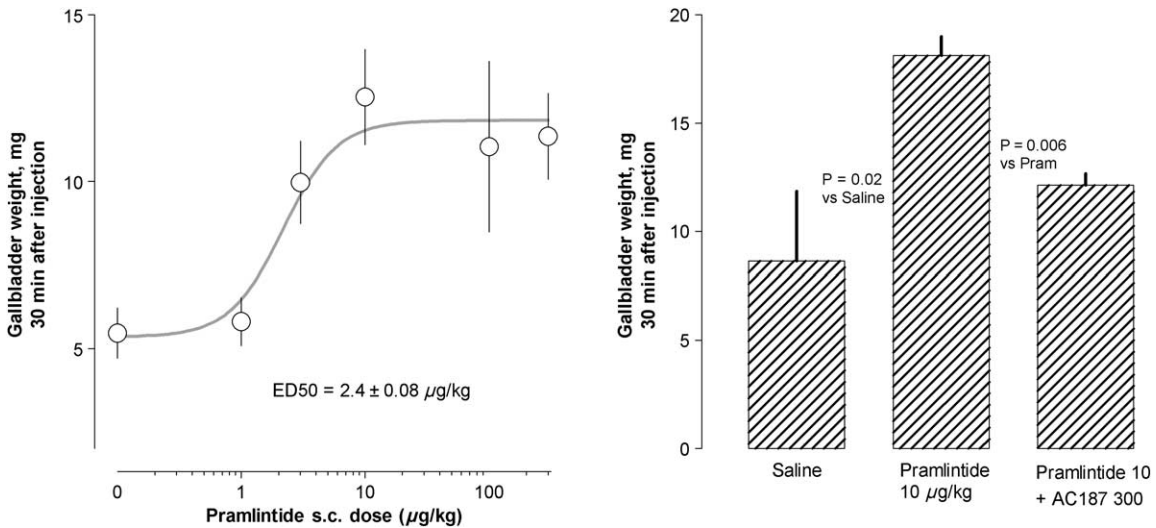

FIGURE 7 Effect of the amylin agonist pramlintide on inhibition of gall bladder emptying (assessed as gall bladder weight) in mice. The effect was blocked with the selective amylin antagonist AC187. Unpublished data from Gedulin *et al.*

flow into the duodenum in pigs (Rasmussen *et al.,* 1997) via a cholinergic, CCK-independent mechanism.

In human studies, salmon calcitonin potently inhibited meal-induced contraction of the gallbladder (Jonderko *et al.,* 1989b), increasing interdigestive volume (Jonderko *et al.,* 1989a). Salmon calcitonin had no direct effect *in vitro* on guinea pig gallbladder contraction (Portincasa *et al.,* 1989), consistent with an extrapancreatic autonomic effect. Concordance of the relaxive effects of amylin agonists CGRP and salmon calcitonin and annulment of the effect with the selective amylin antagonist AC187 suggests that these actions are mediated via an amylin-like pharmacology.

Effects of amylin agonists to inhibit bile ejection are similar to those described for PYY (Hoentjen *et al.,* 2001), which is similarly CCK-independent and is proposed to be vagally mediated.

Hyperglycemia causes a reduction in gallbladder contraction in healthy individuals, via a mechanism that is distinct from CCK (De Boer *et al.,* 1993, 1994). Hyperglycemic reduction of gallbladder contraction was absent in subjects with type 1 diabetes (De Boer *et al.,* 1994). The absence of modulation of contraction was associated with a similar absence of modulation of vagal activity (as inferred from pancreatic polypeptide measurements) (De Boer *et al.,* 1994). An amylinergic mechanism, acting via the vagus as it does for other responses, could partly underlie the inhibition by hyperglycemia of meal-induced gallbladder contraction. The absence of amylin could similarly account for the absence of hyperglycemic effect on contractility in type 1 diabetic individuals.

Physiologically, control of bile ejection is one of the cascade of controls that moderates nutrient assimilation from the meal. For agents that physiologically restrict nutrient appearance, R_a (e.g., amylin and PYY; Hoentjen *et al.,* 2001), it is fitting that in addition to limiting other digestive secretions, they also limit bile ejection. Conversely, for agents that enhance R_a (e.g., glucagon), it is similarly consistent that they additionally augment gallbladder contraction (Jansson *et al.,* 1978).

V. Effects of Amylin on Intestinal Glucose Transport

A further mechanism by which nutrient assimilation might be controlled is control of absorption, independent of effects on gut motility or digestion. While nutritional state can affect brush border enzyme and transporter expression, the evidence that this function can be acutely controlled is sparse. There is one report that insulin may affect this process (Argiles *et al.,* 1992). The possibility that amylin might modulate glucose transport from the gut lumen was tested in an *in situ* gut loop preparation in anesthetized rats in which the vascular supply was intact but a 25 cm section of jejunum was exteriorized to enable perfusion of the lumen. Phloridzin, an

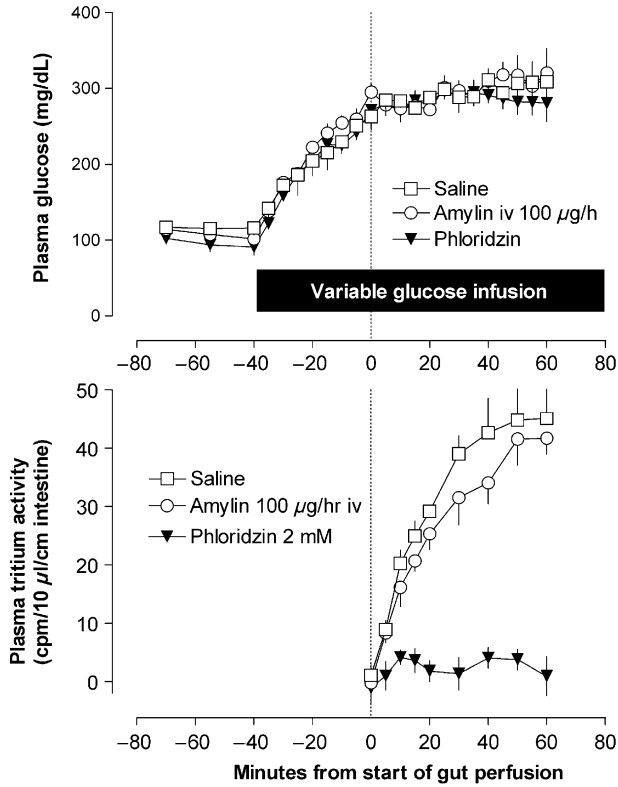

FIGURE 8 Absence of effect of intravenously infused amylin on uptake of labeled glucose from the *in situ* perfused gut lumen in anesthetized rats. Plasma glucose was clamped during gut perfusion. Inhibition of glucose uptake was inhibited in the presence of phloridzin, a positive control. Data from Young and Gedulin (2000).

inhibitor of the sodium-glucose co-transporter (Rodriguez *et al.*, 1982), was used as a positive control (Debnam and Levin, 1975), and when present in the perfused gut lumen, decreased labeled glucose appearance in the vascular circuit by 92% (Young and Gedulin, 2000).

Amylin perfused via the systemic circulation did not, however, affect the rate of appearance in the circulation of labeled glucose perfused via the gut lumen (Young and Gedulin, 2000).

In summary, it appears that amylin's moderation of nutrient uptake from the meal is restricted to effects on gastrointestinal motility and secretion and, not residing locally in the gut, is instead extrinsic and primarily mediated via autonomic control (Fig. 8).

References

Adrian, T. E., Savage, A. P., Sagor, G. R., Allen, J. M., Bacarese-Hamilton, A. J., Tatemoto, K., Polak, J. M., and Bloom, S. R. (1985). Effect of peptide YY on gastric, pancreatic, and biliary function in humans. *Gastroenterology* **89**, 494–499.

Alpers, D. H. (1994). Digestion and absorption of carbohydrates and proteins. *In* "Physiology of the Gastrointestinal Tract" (L. R. Johnson, Ed.), 3rd ed., pp. 1723–1750. Raven Press, New York.

Amsterdam, A., and Jamieson, J. D. (1974). Studies on dispersed pancreatic exocrine cells. I. Dissociation technique and morphologic characteristics of separated cells. *J. Cell Biol.* **63**, 1037–1056.

Argiles, J. M., Zegri, A., Arbos, J., Garcia, C., and Lopez-Soriano, F. J. (1992). The role of insulin in the intestinal absorption of glucose in the rat. *Int. J. Biochem.* **24**, 631–636.

Asai, J., Nakazato, M., Miyazato, M., Kangawa, K., Matsuo, H., and Matsukura, S. (1990). Regional distribution and molecular forms of rat islet amyloid polypeptide. *Biochem. Biophys. Res. Commun.* **169**, 788–795.

Balfour, J. A., and McTavish, D. (1993). Acarbose: An update of its pharmacology and therapeutic use in diabetes mellitus. *Drugs* **46**, 1025–1054.

Barnhart, D. C., Sarosi, G. A., Jr., and Mulholland, M. W. (1997). PACAP-38 causes phospholipase C-dependent calcium signaling in rat acinar cell line. *Surgery* **122**, 465–475.

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Beaumont, K., Moore, C. X., Pittner, R. A., Prickett, K. S., Gaeta, L. S. L., Rink, T. J., and Young, A. A. (1995). Differential antagonism of amylin's metabolic and vascular actions with amylin receptor antagonists. *Can. J. Physiol. Pharmacol.* **73**, 1025–1029.

Beglinger, C., Born, W., Hildebrand, P., Ensinck, J. W., Burkhardt, F., Fischer, J. A., and Gyr, K. (1988). Calcitonin gene-related peptides I and II and calcitonin: Distinct effects on gastric acid secretion in humans. *Gastroenterology* **95**, 958–965.

Bignon, E., Alonso, R., Arnone, M., Boigegrain, R., Brodin, R., Gueudet, C., Heaulme, M., Keane, P., Landi, M., Molimard, J. C., Olliero, D., Poncelet, M., Seban, E., Simiand, J., Soubrie, P., Pascal, M., Maffrand, J. P., and Le Fur, G. (1999). SR146131: A new potent, orally active, and selective nonpeptide cholecystokinin subtype 1 receptor agonist. II. *In vivo* pharmacological characterization. *J. Pharmacol. Exp. Ther.* **289**, 752–761.

Bunnett, N. W., Mulvihill, S. J., and Debas, H. T. (1991). Calcitonin gene-related peptide inhibits exocrine secretion from the rat pancreas by a neurally mediated mechanism. *Exp. Physiol.* **76**, 115–123.

Burckhardt, B., Delco, F., Ensinck, J. W., Meier, R., Bauerfeind, P., Aufderhaar, U., Ketterer, S., Gyr, K., and Beglinger, C. (1994). Cholecystokinin is a physiological regulator of gastric acid secretion in man. *Eur. J. Clin. Invest.* **24**, 370–376.

Burghen, G. A., Murrell, L. R., Whitington, G. L., Klyce, M. K., and Burstein, S. (1992). Acid peptic disease in children with type I diabetes mellitus. A complicating relationship. *Am. J. Dis. Child* **146**, 718–722.

Chiba, T., Yamaguchi, A., Yamatani, T., Nakamura, A., Morishita, T., Inui, T., Fukase, M., Noda, T., and Fujita, T. (1989). Calcitonin gene-related peptide receptor antagonist human CGRP(8–37). *Am. J. Physiol.* **256**, E331–E335.

Clementi, G., Valerio, C., Emmi, I., Prato, A., and Drago, F. (1996). Behavioral effects of amylin injected intracerebroventricularly in the rat. *Peptides* **17**, 589–591.

Clementi, G., Caruso, A., Cutuli, V. M. C., Prato, A., deBernardis, E., and Amico-Roxas, M. (1997). Effect of amylin in various experimental models of gastric ulcer. *Eur. J. Pharmacol.* **332**, 209–213.

Cole, S. G., Rossi, S., Stern, A., and Hofmann, A. F. (1987). Cholesteryl octanoate breath test. Preliminary studies on a new noninvasive test of human pancreatic exocrine function. *Gastroenterology* **93**, 1372–1380.

Coniff, R. F., Shapiro, J. A., Seaton, T. B., Hoogwerf, B. J., and Hunt, J. A. (1995). A double-blind placebo-controlled trial evaluating the safety and efficacy of acarbose for the treatment of patients with insulin-requiring type II diabetes. *Diabet. Care* **18**, 928–932.

De Boer, S. Y., Masclee, A. A. M., Lam, W. F., Schipper, J., Jansen, J. B. M. J., and Lamers, C. B. H. W. (1993). Hyperglycemia modulates gallbladder motility and small intestinal transit time in man. *Dig. Dis. Sci.* **38**, 2228–2235.

De Boer, S. Y., Masclee, A. A. M., Lam, W. F., Lemkes, H. H. P. J., Schipper, J., Frohlich, M., Jansen, J. B. M. J., and Lamers, C. B. H. W. (1994). Effect of hyperglycaemia on gallbladder motility in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* **37**, 75–81.

Debnam, E. S., and Levin, R. J. (1975). An experimental method of identifying and quantifying the active transfer electrogenic component from the diffusive component during sugar absorption measured *in vivo*. *J. Physiol. (Lond.)* **246**, 181–196.

Doepfner, W. (1976). Effects of synthetic salmon calcitonin on gastric secretion and ulcer formation in conscious cats and rats. *In* (H. Goebell and J. Hotz, Eds.), pp. 60–70. Demeter Verlag, Grafelfing.

Edwards, G. L., Gedulin, B. R., Jodka, C., Dilts, R. P., Miller, C. C., and Young, A. (1998). Area postrema (AP)-lesions block the regulation of gastric emptying by amylin. *Neurogastroenterol. Motil.* **10**, 26.

Ewart, W. R., Jones, M. V., and King, B. F. (1988). Central origin of vagal nerve fibres innervating the fundus and corpus of the stomach in rat. *J. Auton. Nerv. Syst.* **25**, 219–231.

Fehmann, H. C., Weber, V., Göke, R., Göke, B., Eissele, R., and Arnold, R. (1990). Islet amyloid polypeptide (IAPP; amylin) influences the endocrine but not the exocrine rat pancreas. *Biochem. Biophys. Res. Commun.* **167**, 1102–1108.

Funovics, J., Holbling, N., Rauhs, R., Pointner, H., Niebauer, G., Walde, I., and Kopf, N. (1981). The effect of SST, glucagon, calcitonin and PGE1 on exocrine pancreatic secretion in the unrestrained dog in long-term experiments. *Eur. Surg. Res.* **13**, 213–226.

Gardner, J. D., and Jackson, M. J. (1977). Regulation of amylase release from dispersed pancreatic acinar cells. *J. Physiol. (Lond.)* **270**, 439–454.

Gedulin, B. R., and Young, A. A. (1998). Hypoglycemia overrides amylin-mediated regulation of gastric emptying in rats. *Diabetes* **47**, 93–97.

Gedulin, B. R., Jodka, C. M., Green, D. L., and Young, A. A. (1996). Comparison of 21 peptides on inhibition of gastric emptying in conscious rats. *Dig. Dis. Week* A742.

Gedulin, B. R., Lawler, R. L., Jodka, C. M., Grazzini, M. L., and Young, A. A. (1997a). Amylin inhibits pentagastrin-stimulated gastric acid secretion and protects against ethanol-induced gastric mucosal damage in rats. *Diabetologia* **40**, A299.

Gedulin, B. R., Lawler, R. L., Jodka, C. M., and Young, A. A. (1997b). Comparison of effects of amylin, glucagon-like peptide-1 and exendin-4 to inhibit pentagastrin-stimulated gastric acid secretion. *Diabetologia* **40**, A300.

Gedulin, B., Jodka, C., and Young, A. (1997c). Insulin-induced hypoglycemia reverses pramlintide inhibition of gastric emptying in rats. *Can. J. Diabet. Care* **21**, 4.

Gedulin, B. R., Jodka, C., Lawler, R., Hoyt, J. A., and Young, A. A. (1998). Amylin inhibits lipase and amylase secretion from the exocrine pancreas in rats. *Diabetes* **47**, A280.

Gedulin, B. R., Lawler, R., Jodka, C., and Young, A. A. (2005). Amylin inhibition of pentagastrin-stimulated gastric acid secretion and ethanol-induced gastritis in rats. [unpublished].

Goebell, H., Ammann, R., Herfarth, C., Horn, J., Hotz, J., Knoblauch, M., Schmid, M., Jaeger, M., Akovbiantz, A., Linder, E., Abt, K., Nuesch, E., and Barth, E. (1979). A double-blind

trial of synthetic salmon calcitonin in the treatment of acute pancreatitis. *Scand. J. Gastroenterol.* **14**, 881–889.

Goldin, E., Ardite, E., Elizalde, J. I., Odriozola, A., Panes, J., Pique, J. M., and Fernandez-Checa, J. C. (1997). Gastric mucosal damage in experimental diabetes in rats: Role of endogenous glutathione. *Gastroenterology* **112**, 855–863.

Greenberger, N. J., Toskes, P. P., and Isselbacher, K. J. (1991). Acute and chronic pancreatitis. *In* (J. D. Wilson, E. Braunwald, K. J. Isselbacher, R. G. Petersdorf, J. B. Marting, A. S. Fauci and R. K. Root, Eds.), 12th edn., pp. 1372–1383. McGraw-Hill, New York.

Guidobono, F., Netti, C., Pagani, F., Bettica, P., Sibilia, V., Pecile, A., and Zanelli, J. (1991). Effect of unmodified eel calcitonin on gastric acid secretion and gastric ulcers in the rat. *Farmaco.* **46**, 555–563.

Guidobono, F., Coluzzi, M., Pagani, F., Pecile, A., and Netti, C. (1994). Amylin given by central and peripheral routes inhibits gastric acid secretion. *Peptides* **15**, 699–702.

Guidobono, F., Pagani, F., Ticozzi, C., Sibilia, V., Pecile, A., and Netti, C. (1997). Protection by amylin of gastric erosions induced by indomethacin or ethanol in rats. *Br. J. Pharmacol.* **120**, 581–586.

Guo, Y. S., Singh, P., Gomez, G., Greeley, G. H., Jr., and Thompson, J. C. (1987). Effect of peptide YY on cephalic, gastric, and intestinal phases of gastric acid secretion and on the release of gastrointestinal hormones. *Gastroenterology* **92**, 1202–1208.

Guyton, A. C., and Hall, J. E. (1996a). Secretory functions of the alimentary tract. *In* (A. C. Guyton and J. E. Hall, Eds.), 9th edn., pp. 815–832. W.B. Saunders, Philadelphia.

Guyton, A. C., and Hall, J. E. (1996b). Digestion and absorption in the gastrointestinal tract. *In* (A. C. Guyton and J. E. Hall, Eds.), 9th edn., pp. 833–844. W.B. Saunders, Philadelphia.

Hashimoto, T., Poston, G. J., Greeley, G. H., Jr., and Thompson, J. C. (1988). Calcitonin gene-related peptide inhibits gallbladder contractility. *Surgery* **104**, 419–423.

Helton, W. S., Mulholland, M. M., Bunnett, N. W., and Debas, H. T. (1989). Inhibition of gastric and pancreatic secretion in dogs by CGRP: Role of somatostatin. *Am. J. Physiol.* **256**, G715–G720.

Hoentjen, F., Hopman, W. P., and Jansen, J. B. (2001). Effect of circulating peptide YY on gallbladder emptying in humans. *Scand. J. Gastroenterol.* **36**, 1086–1091.

Holzer, P., Lippe, I. I., Raybould, H. E., Pabst, M. A., Livingston, E. H., Amann, R., Peskar, B. M., Peskar, B. A., Tache, Y., and Guth, P. H. (1991). Role of peptidergic sensory neurons in gastric mucosal blood flow and protection. *Ann. N Y Acad. Sci.* **632**, 272–282.

Hotz, J., Goebell, H., and Ziegler, R. (1977). Calcitonin and exocrine pancreatic secretion in man: Inhibition of enzymes stimulated by CCK-pancreozymin, caerulein, or calcium—No response to vagal stimulation. *Gut* **18**, 615–622.

Huang, Y., Fischer, J. E., and Balasubramaniam, A. (1996). Amylin mobilizes [Ca2+] (i) and stimulates the release of pancreatic digestive enzymes from rat acinar AR42J cells: Evidence for an exclusive receptor system of amylin. *Peptides* **17**, 497–502.

Hughes, J. J., Levine, A. S., Morley, J. E., Gosnell, B. A., and Silvis, S. E. (1984). Intraventricular calcitonin gene-related peptide inhibits gastric acid secretion. *Peptides* **5**, 665–667.

Hung, C. R., and Huang, E. Y. (1995). Role of acid back-diffusion in the formation of mucosal ulceration and its treatment with drugs in diabetic rats. *J. Pharm. Pharmacol.* **47**, 493–498.

Isenberg, J. I., Stening, G. F., Ward, S., and Grossman, M. I. (1969). Relation of gastric secretory response in man to dose of insulin. *Gastroenterology* **57**, 395–398.

James, W. P., Avenell, A., Broom, J., and Whitehead, J. (1997). A one-year trial to assess the value of orlistat in the management of obesity. *Int. J. Obes. Relat. Metab. Disord.* **21**, S24–S30.

Jansson, R., Steen, G., and Svanvik, J. (1978). A comparison of glucagon, gastric inhibitory peptide, and secretin on gallbladder function, formation of bile, and pancreatic secretion in the cat. *Scand. J. Gastroenterol.* **13**, 919–925.

Jonderko, G., Jonderko, K., Konca, A., and Golab, T. (1989a). Effect of calcitonin on gallbladder volume between food intake and on its emptying after meals in humans. *Pol. Arch. Med. Wewn* **81**, 7–12.

Jonderko, K., Konca, A., Golab, T., and Jonderko, G. (1989b). Effect of calcitonin on gallbladder volume in man. *J. Gastroenterol. Hepatol.* **4**, 505–511.

Kashimura, J., Shimosegawa, T., Iguchi, K., Mochizuki, T., Yanaihara, N., Koizumi, M., and Toyota, T. (1993). The stimulatory effects and binding characteristics of PACAP27 in rat dispersed pancreatic acini. *Tohoku J. Exp. Med.* **171**, 243–254.

Kikuchi, Y., Koizumi, M., Shimosegawa, T., Kashimura, J., Suzuki, S., and Toyota, T. (1991). Islet amyloid polypeptide has no effect on amylase release from rat pancreatic acini stimulated by CCK-8, secretin, carbachol and VIP. *Tohoku J. Exp. Med.* **165**, 41–48.

Kline, L. W., Kaneko, T., Benishin, C. G., and Pang, P. K. (1991). Calcitonin gene-related peptide: An inhibitor of guinea pig gallbladder contraction. *Can. J. Physiol. Pharmacol.* **69**, 1149–1154.

Konturek, S. J., Bilski, J., and Cieszkowski, M. (1992). Role of cholecystokinin in the intestinal fat- and acid-induced inhibition of gastric secretion. *Regul. Pept.* **42**, 97–109.

Lam, W. F., Masclee, A. A., de Boer, S. Y., and Lamers, C. B. (1993). Hyperglycemia reduces gastric secretory and plasma pancreatic polypeptide responses to modified sham feeding in humans. *Digestion* **54**, 48–53.

Lam, W. F., Masclee, A. A., Muller, E. S., and Lamers, C. B. (1995). Effect of hyperglycemia on gastric acid secretion and gastrin release induced by intravenous amino acids. *Am. J. Clin. Nutr.* **61**, 1268–1272.

Lenz, H. J., and Brown, M. R. (1987). Intracerebroventricular administration of human calcitonin and human calcitonin gene-related peptide inhibits meal-stimulated gastric acid secretion in the dog. *Dig. Dis. Sci.* **32**, 409–416.

Lenz, H. J., Mortrud, M. T., Vale, W. W., Rivier, J. E., and Brown, M. R. (1984). Calcitonin gene-related peptide acts within the central nervous system to inhibit gastric acid secretion. *Regul. Pept.* **9**, 271–277.

Lenz, H. J., Mortrud, M. T., Rivier, J. E., and Brown, M. R. (1985). Central nervous system actions of calcitonin gene-related peptide on gastric acid secretion in the rat. *Gastroenterology* **88**, 539–544.

Lenz, H. J., Zimmerman, F. G., and Messmer, B. (1993). Regulation of canine gallbladder motility by brain peptides. *Gastroenterology* **104**, 1678–1685.

Lloyd, K. C., Amirmoazzami, S., Friedik, F., Heynio, A., Solomon, T. E., and Walsh, J. H. (1997). Candidate canine enterogastrones: Acid inhibition before and after vagotomy. *Am. J. Physiol.* **272**, G1236–G1242.

MacLellan, D. G., Upp, J. R., and Thompson, J. C. (1988). Influence of endogenous prostaglandins on secretin-mediated inhibition of gastric acid secretion in dogs. *Gastroenterology* **95**, 625–629.

Makhlouf, P. C., Zaki, M., Harrington, L., McCuen, R., and Schubert, M. L. (1996). Endogenous amylin stimulates somatostatin (SST) and inhibits gastrin secretion from the antrum of human, dog and rat stomach. *Gastroenterology* **110**, PA1096.

Manela, F. D., Ren, J. Y., Gao, J. S., McGuigan, J. E., and Harty, R. F. (1995). Calcitonin gene-related peptide modulates acid-mediated regulation of somatostatin and gastrin release from rat antrum. *Gastroenterology* **109**, 701–706.

Miyazato, M., Nakazato, M., Shiomi, K., Aburaya, J., Toshimori, H., Kangawa, K., Matsuo, H., and Matsukura, S. (1991). Identification and characterization of islet amyloid polypeptide in mammalian gastrointestinal tract. *Biochem. Biophys. Res. Commun.* **181**, 293–300.

Moore, J. G. (1980). The relationship of gastric acid secretion to plasma glucose in five men. *Scand. J. Gastroenterol.* **15**, 625–632.

Morley, J. E., Levine, A. S., and Silvis, S. E. (1981). Intraventricular calcitonin inhibits gastric acid secretion. *Science* **214**, 671–673.

Mulder, H., Lindh, A. C., Ekblad, E., Westermark, P., and Sundler, F. (1994). Islet amyloid polypeptide is expressed in endocrine cells of the gastric mucosa in the rat and mouse. *Gastroenterology* **107**, 712–719.

Mulder, H., Ekelund, M., Ekblad, E., and Sundler, F. (1997). Islet amyloid polypeptide in the gut and pancreas: Localization, ontogeny and gut motility effects. *Peptides* **18**, 771–783.

Mulholland, M. W., Garcia, R., Garcia, I., Taborsky, G. J., Jr., and Helton, S. (1989). Inhibition of pancreatic exocrine secretion in the rat by calcitonin gene-related peptide: Involvement of circulating somatostatin. *Endocrinology* **124**, 1849–1856.

Nakashima, Y., Appert, H. E., and Howard, J. M. (1977). The effects of calcitonin on pancreatic exocrine secretion in dogs. *Surg. Gynecol. Obstet.* **144**, 71–76.

Nealon, W. H., Beauchamp, R. D., Townsend, C. M., Jr., and Thompson, J. C. (1990). Additive interactions of calcitonin gene-related peptide and calcitonin on pancreatic exocrine function in conscious dogs. *Surgery* **107**, 434–441.

Nicholl, C. G., Bhatavdekar, J. M., Mak, J., Girgis, S. I., and Legon, S. (1992). Extrapancreatic expression of the rat islet amyloid polypeptide (amylin) gene. *J. Mol. Endocrinol.* **9**, 157–163.

Nishimura, M., Yokoyama, M., Taguchi, T., and Kitamura, Y. (1983). Spontaneous gastric erosions in NOD and KK-A gamma. *Lab. Anim. Sci.* **33**, 577–579.

O'Connor, T. P., and Diamond, J. (1999). Ontogeny of intestinal safety factors: Lactase capacities and lactose loads. *Am. J. Physiol.* **276**, R753–R765.

Ohtsuka, H., Iwanaga, T., Fujino, M. A., and Fujita, T. (1993). Amylin-containing cells in the gastro-entero-pancreatic (GeP) endocrine system of the rat and humans—an immunohistochemical study. *Acta Histochem. Cytochem.* **26**, 405–414.

Okimura, Y., Chihara, K., Abe, H., Kaji, H., Kita, T., Kashio, Y., and Fujita, T. (1986). Effect of intracerebroventricular administration of rat calcitonin gene-related peptide (CGRP), human calcitonin and [Asu1,7]-eel calcitonin on gastric acid secretion in rats. *Endocrinol. Jpn.* **33**, 273–277.

Okuma, Y., and Osumi, Y. (1986). Central cholinergic descending pathway to the dorsal motor nucleus of the vagus in regulation of gastric functions. *Jpn. J. Pharmacol.* **41**, 373–379.

Okuma, Y., Osumi, Y., Ishikawa, T., and Mitsuma, T. (1987). Enhancement of gastric acid output and mucosal blood flow by tripeptide thyrotropin releasing hormone microinjected into the dorsal motor nucleus of the vagus in rats. *Jpn. J. Pharmacol.* **43**, 173–178.

Owicki, J. C., Parce, J. W., Kercso, K. M., Sigal, G. B., Muir, V. C., Venter, J. C., Fraser, C. M., and McConnell, H. M. (1990). Continuous monitoring of receptor-mediated changes in the metabolic rates of living cells. *Proc. Natl. Acad. Sci. USA* **87**, 4007–4011.

Owyang, C. (1994). Negative feedback control of exocrine pancreatic secretion: Role of cholecystokinin and cholinergic pathway. *J. Nutr.* **124**, 1321S–1326S.

Pappas, T. N., Debas, H. T., Goto, Y., and Taylor, I. L. (1985). Peptide YY inhibits meal-stimulated pancreatic and gastric secretion. *Am. J. Physiol.* **248**, G118–G123.

Pappas, T. N., Debas, H. T., and Taylor, I. L. (1986). Enterogastrone-like effect of peptide YY is vagally mediated in the dog. *J. Clin. Invest.* **77**, 49–53.

Parce, J. W., Owicki, J. C., and Kercso, K. M. (1990). Biosensors for directly measuring cell affecting agents. *Ann. Biol. Clin. (Paris)* **48**, 639–641.

Parkes, D., Chen, K., Smith, P., and Young, A. (1999). Amylin does not suppress hypoglycemia-induced secretion of glucagon in rats. *Diabetes* **48**(suppl. 1), A425.

Paul, F. (1975). Intraindividually controlled studies in man on the inhibition of exocrine pancreas secretion using salmon calcitonin. *Verh. Dtsch. Ges. Inn. Med.* **81**, 1266–1268.

Pieber, T. R., Roitelman, J., Lee, Y., Luskey, K. L., and Stein, D. T. (1994). Direct plasma radioimmunoassay for rat amylin-(1–37): Concentrations with acquired and genetic obesity. *Am. J. Physiol.* **267**, E156–E164.

Pitchford, S., De Moor, K., and Glaeser, B. S. (1995). Nerve growth factor stimulates rapid metabolic responses in PC12 cells. *Am. J. Physiol.* **268**, C936–C943.

Pittner, R. A., and Fain, J. N. (1989). Exposure of cultured hepatocytes to cyclic AMP enhances the vasopressin-mediated stimulation of inositol phosphate production. *Biochem. J.* **257**, 455–460.

Piyachaturawat, P., Poprasit, J., and Glinsukon, T. (1991). Gastric mucosal secretions and lesions by different doses of streptozotocin in rats. *Toxicol. Lett.* **55**, 21–29.

Portincasa, P., Baldassarre, G., Palmieri, G., and Palasciano, G. (1989). In vitro effect of calcitonin on guinea pig gallbladder. *Boll. Soc. Ital. Biol. Sper.* **65**, 869–875.

Rasmussen, T. N., Harling, H., Rehfeld, J. F., and Holst, J. J. (1997). Calcitonin gene-related peptide (CGRP), a potent regulator of biliary flow. *Neurogastroenterol. Motil.* **9**, 215–220.

Ratner, R., Levetan, C., Schoenfeld, S., Organ, K., and Kolterman, O. (1998). Pramlintide therapy in the treatment of insulin-requiring type 2 diabetes: Results of a 1-year placebo-controlled trial. *Diabetes* **47**, A88.

Raufman, J. P., Malhotra, R., and Singh, L. (1991). PACAP-38, a novel peptide from ovine hypothalamus, is a potent modulator of amylase release from dispersed acini from rat pancreas. *Regul. Pept.* **36**, 121–129.

Rhee, J. C., Chang, T. M., Lee, K. Y., Jo, Y. H., and Chey, W. Y. (1991). Mechanism of oleic acid-induced inhibition on gastric acid secretion in rats. *Am. J. Physiol.* **260**, G564–G570.

Rodriguez, M. J., Ortiz, M., Vazquez, A., Lluch, M., and Ponz, F. (1982). Influence of temperature of the perfusion solution on the kinetics of intestinal absorption of glucose in rats. *Rev. Esp. Fisiol.* **38**, 345–348.

Rogers, R. C., McTigue, D. M., and Hermann, G. E. (1996). Vagal control of digestion: Modulation by central neural and peripheral endocrine factors. *Neurosci. Biobehav. Rev.* **20**, 57–66.

Rosenstock, J., Whitehouse, F., Schoenfeld, S., Dean, E., Blonde, L., and Kolterman, O. (1998). Effect of pramlintide on metabolic control and safety profile in people with type 1 diabetes. *Diabetes* **47**, A88.

Sakaguchi, T., and Sato, Y. (1987). D-glucose anomers in the nucleus of the tractus solitarius can reduce gastric acid secretion of rats. *Exp. Neurol.* **95**, 525–529.

Schjoldager, B. T., Mortensen, P. E., Christiansen, J., Orskov, C., and Holst, J. J. (1989). GLP-1 (glucagon-like peptide 1). and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig. Dis. Sci.* **34**, 703–708.

Schmidt, W. E., Seebeck, J., Hocker, M., Schwarzhoff, R., Schafer, H., Fornefeld, H., Morys-Wortmann, C., Folsch, U. R., and Creutzfeldt, W. (1993). PACAP and VIP stimulate enzyme secretion in rat pancreatic acini via interaction with VIP/PACAP-2 receptors: Additive augmentation of CCK/carbachol-induced enzyme release. *Pancreas* **8**, 476–487.

Sexton, P. M., Paxinos, G., Kenney, M. A., Wookey, P. J., and Beaumont, K. (1994). In vitro autoradiographic localization of amylin binding sites in rat brain. *Neuroscience* **62**, 553–567.

Shay, A. S., Kamarov, S. S., Fels, D., Meranze, M., Gruenstein, M., and Siplet, H. (1945). A simple method for uniform production of gastric ulceration in the rat. *Gastroenterology* **5**, 43–46.

Spannagel, A. W., Green, G. M., Guan, D., Liddle, R. A., Faull, K., and Reeve, J. R., Jr. (1996). Purification and characterization of a luminal cholecystokinin-releasing factor from rat intestinal secretion. *Proc. Natl. Acad. Sci. USA* **93**, 4415–4420.

Stacher, G., Bauer, P., Starker, H., and Schulze, D. (1976). Inhibitory effect of an intravenous glucose load on basal and insulin-stimulated gastric acid secretion in man. *Int. J. Clin. Pharmacol. Biopharm.* **13**, 107–112.

Tache, Y. (1992). Inhibition of gastric acid secretion and ulcers by calcitonin gene-related peptide. *Ann. N Y Acad. Sci.* **657**, 240–247.

Tache, Y., Gunion, M., Lauffenberger, M., and Goto, Y. (1984). Inhibition of gastric acid secretion by intracerebral injection of calcitonin gene related peptide in rats. *Life Sci.* **35**, 871–878.

Tache, Y., Stephens, R. L., Jr., and Ishikawa, T. (1989). Central nervous system action of TRH to influence gastrointestinal function and ulceration. *Ann. N Y Acad. Sci.* **553**, 269–285.

Tache, Y., Raybould, H., and Wei, J. Y. (1991). Central and peripheral actions of calcitonin gene-related peptide on gastric secretory and motor function. *Adv. Exp. Med. Biol.* **298**, 183–198.

Takeuchi, K., Takehara, K., Tajima, K., Kato, S., and Hirata, T. (1997). Impaired healing of gastric lesions in streptozotocin-induced diabetic rats: Effect of basic fibroblast growth factor. *J. Pharmacol. Exp. Ther.* **281**, 200–207.

Toshimori, H., Narita, R., Nakazato, M., Asai, J., Mitsukawa, T., Kangawa, K., Matsuo, H., and Matsukura, S. (1990). Islet amyloid polypeptide (IAPP) in the gastrointestinal tract and pancreas of man and rat. *Cell Tissue Res.* **262**, 401–406.

Umeda, Y., and Okada, T. (1987). Inhibition of gastric acid secretion by human calcitonin gene-related peptide with picomolar potency in guinea-pig parietal cell preparations. *Biochem. Biophys. Res. Commun.* **146**, 430–436.

Vine, W., Blase, E., Koda, J., and Young, A. (1998). Plasma amylin concentrations in fasted and fed rats quantified by a monoclonal immunoenzymometric assay. *Horm. Metab. Res.* **30**, 581–585.

Warzecha, Z., Dembinski, A., Ceranowicz, P., Konturek, P. C., Stachura, J., Konturek, S. J., and Niemiec, J. (1997). Protective effect of calcitonin gene-related peptide against caerulein-induced pancreatitis in rats. *J. Physiol. Pharmacol.* **48**, 775–787.

Weber, E., and Ehrlein, H. J. (1998). Relationships between gastric emptying and intestinal absorption of nutrients and energy in mini pigs. *Dig. Dis. Sci.* **43**, 1141–1153.

Whitehouse, F., Ratner, R., Rosenstock, J., Schoenfeld, S., and Kolterman, O. (1998). Pramlintide showed positive effects on body weight in type 1 and type 2 diabetes. *Diabetes* **47**, A9.

Young, A. A., and Gedulin, B. (2000). Effect of amylin on intestinal glucose transport. *Diabetologia* **43**, A179.

Young, A. A., Carlo, P., Rink, T. J., and Wang, M.-W. (1992). 8–37hCGRP, an amylin receptor antagonist, enhances the insulin response and perturbs the glucose response to infused arginine in anesthetized rats. *Mol. Cell Endocrinol.* **84**, R1–R5.

Young, A. A., Gedulin, B., Vine, W., Percy, A., and Rink, T. J. (1995). Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin. *Diabetologia* **38**, 642–648.

Young, A. A., Gedulin, B. R., and Rink, T. J. (1996a). Dose-responses for the slowing of gastric emptying in a rodent model by glucagon-like peptide (7–36)NH2, amylin, cholecystokinin, and other possible regulators of nutrient uptake. *Metabolism* **45**, 1–3.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996b). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Young, A., Gedulin, B., Srivastava, V., Jodka, C., and Nikoulina, S. (2002). Peptide YY[3–36] inhibits gastric emptying via a neuroendocrine pathway that includes the a*rea postrema*. *Diabetes* **51**, A405.

Young, A. A., Jodka, C., Pittner, R., Parkes, D., and Gedulin, B. R. (2005). Dose-response for inhibition by amylin of cholecystokinin-stimulated secretion of amylase and lipase in rats. *Regul. Pept.* In press.

Yuan, C. S., and Barber, W. D. (1993). Area postrema: Gastric vagal input from proximal stomach and interactions with nucleus tractus solitarius in cat. *Brain Res. Bull.* **30**, 119–125.

Zaki, M., Koduru, S., Harrington, L., McCuen, R., Vuyyuru, L., and Schubert, M. L. (1996). Endogenous amylin stimulates somatostatin (SST) and thus inhibits histamine and acid secretion in the fundus of the stomach. *Dig. Dis. Week* A-2.

Zanelli, J. M., Stracca-Gasser, M., Gaines-Das, R. E., and Guidobono, F. (1992). The short term effect of peripherally administered brain-gut peptides on gastric acid secretion in rats. *Agents Actions* **35**, 122–129.

Zdon, M. J., Adrian, T. E., and Modlin, I. M. (1988). Gastric somatostatin release: Evidence for direct mediation by calcitonin gene-related peptide and vasoactive intestinal peptide. *J. Surg. Res.* **44**, 680–686.

Zhang, S. X., and Huang, C. G. (1993). Stimulatory effect of vasoactive intestinal peptide microinjected into dorsal vagal complex on gastric acid secretion in rats. *Sheng Li Hsueh Pao* **45**, 568–574.

Andrew Young

Inhibition of Glucagon Secretion

I. Summary

This chapter describes a physiological and profound effect of amylin to inhibit meal-related glucagon secretion. Glucagon is processed from a large precursor, proglucagon, in a tissue-specific manner in pancreatic α-cells. In addition to amino acid nutrient stimuli, glucagon is also secreted in response to stressful stimuli, such as hypoglycemia and hypovolemia. Glucagon primarily acts on liver to initiate glycogenolysis and gluconeogenesis, resulting in a rapid increase in endogenous production of glucose. With longer stimulation, glucagon action at the liver results in a glucose-sparing activation of free fatty acid oxidation and production of ketones.

During hypoglycemia, glucagon secretion is clearly a protective feedback, defending the organism against damaging effects of low glucose in brain and nerves (neuroglycopenia). Amino acid-stimulated glucagon secretion during meals has a different purpose: amino acids stimulate insulin

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secretion, which mobilizes amino acid transporters and effects their storage in peripheral tissues. At the same time, insulin obligatorily recruits GLUT4 glucose transporters in muscle and fat. The hypoglycemic potential of such GLUT4 mobilization is averted only by the simultaneous liberation of endogenous glucose in response to feedforward (anticipatory) glucagon secretion.

The effect of amylin and its agonists to inhibit amino acid-stimulated glucagon secretion is both potent (EC_{50} = 18 pM) and profound (~70% inhibition). This glucagonostatic action appears to be extrinsic to the pancreatic islet, occurring in intact animals and in patients, but not in isolated islets or isolated perfused pancreas preparations. On the other hand, the effect of hypoglycemia to stimulate glucagon secretion, which is intrinsic to the islet and occurs in isolated preparations, is not affected by amylin or its agonists.

The physiological interpretation of these actions fits with the general concept, illustrated in Fig. 1, that amylin and insulin secreted in response to meals shut down endogenous production as a source of glucose, in favor of that derived from the meal. Amylin and insulin secreted in response to nutrients already absorbed act as a feedback switch for glucose sourcing. The insulinotropic (incretin) gut peptides, GLP-1 and GIP, secreted in response to yet-to-be-absorbed intraluminal nutrients, amplify β-cell secretion and thereby activate the glucose sourcing switch in a feedforward manner.

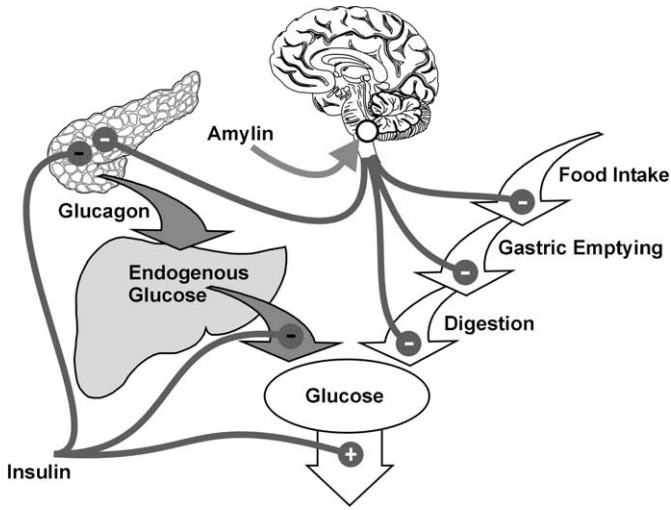

FIGURE 1 Role of amylin, with insulin, in switching the glucose source after meals from endogenous production (dark arrows) to meal-derived sources (white arrows). This action of amylin is extrapancreatic (shown here as likely CNS-mediated).

Hypoglycemia-stimulated glucagon secretion and nutrient (amino acid)-stimulated glucagon secretion are two clearly different processes, differently affected by amylin. The balance of glucose fluxes is disturbed in diabetic states, partly as a result of inappropriate glucagon secretion. Although glucose production due to glucagon secreted in response to hypoglycemia is normal or even reduced in diabetic patients, the secretion of glucagon (and production of endogenous glucose) in response to protein meals is typically exaggerated. Absence of appropriate β-cell suppression of α-cell secretion has been invoked as a mechanism that explains exaggerated glucagon responses, especially prevalent in patients with deficient β-cell secretion (type 1 diabetes and insulinopenic type 2 diabetes). A proposed benefit of insulin replacement therapy is the reduction of absolute or relative hyperglucagonemia. High glucagon is said to be necessary for ketosis in severe forms of diabetes. A further benefit of reversing hyperglucagonemia is reduction of the excessive endogenous glucose production that contributes to fasting and postprandial hyperglycemia in diabetes.

The idea that amylin is a part of the β-cell drive that normally limits glucagon secretion after meals fits with the observation that glucagon secretion is exaggerated in amylin-deficient states (diabetes characterized by β-cell failure). This proposal is further supported by the observation that postprandial glucagon suppression is restored following amylin replacement therapy in such states. These observations argue for a therapeutic case for amylin replacement in patients in whom excess glucagon action contributes to fasting and postprandial hyperglycemia and ketosis. The selectivity of amylin's glucagonostatic effect (wherein it is restricted to meal-related glucagon secretion, while preserving glucagon secretion and glucagon action during hypoglycemia) may confer additional benefits; the patient population amenable to amylin replacement therapy is likely to also be receiving insulin replacement therapy, and is thereby susceptible to insulin-induced hypoglycemia.

Most explorations of the biology of amylin have used the endogenous hormone in the cognate species (typically rat amylin in rat studies). Clinical studies have typically employed the amylinomimetic agent pramlintide. Studies of amylinomimetic effects on glucagon secretion include effects of rat amylin in anesthetized non-diabetic rats (Jodka *et al.*, 2000; Parkes *et al.*, 1999; Young *et al.*, 1995), effects of rat amylin in isolated perfused rat pancreas (Silvestre *et al.*, 1999), effects of pramlintide in anesthetized non-diabetic rats (Gedulin *et al.*, 1997b,c,d, 1998), effects of pramlintide in patients with type1 diabetes (Fineman *et al.*, 1997a,b,c,d, 1998a; Holst, 1997; Nyholm *et al.*, 1996, 1997a,b,c; Orskov *et al.*, 1999; Thompson and Kolterman, 1997), and effects in patients with type 2 diabetes (Fineman *et al.*, 1998b). In addition, effects of amylin antagonists have been observed in isolated preparations (Silvestre *et al.*, 1996), and effects of antagonists or neutralizing antibody have been determined in whole-animal preparations (Gedulin *et al.*, 1997a,e,f).

II. Glucagon Secretion in Insulinopenic Diabetes

Insulin-dependent diabetes mellitus (IDDM) is characterized by relative or absolute hyperglucagonemia (Müller *et al.*, 1970; Unger *et al.*, 1970) and exaggerated glucagon secretion in response to amino acid (Raskin *et al.*, 1976; Unger *et al.*, 1970) or protein stimuli (Kawamori *et al.*, 1985; Müller *et al.*, 1970). Further, it appears that the presence of glucagon is essential in the pathogenesis of the full syndrome that results from complete insulin deficiency and that elevated glucagon concentrations complicate the course of the disease (Dobbs *et al.*, 1975), contributing to marked endogenous hyperglycemia (Raskin and Unger, 1978) and hyperketonemia (Unger, 1978), which are present if insulin deficiency is associated with glucagon excess, but not if both glucagon and insulin are absent (Unger, 1985). Glucagon suppression could be a potentially useful adjunct to conventional antihyperglycemic treatment of diabetes (Unger, 1978).

It is now well established that at least four key influences regulate the secretion of pancreatic islet hormones: plasma levels of vital nutrients such as glucose and amino acids, the autonomic nervous system, circulating hormones such as the incretins, and islet hormones themselves (Ashcroft and Ashcroft, 1992). Much work has established that β-cell secretion is promoted by glucagon, while the β-cell products insulin (Argoud *et al.*, 1987) and amylin (Dégano *et al.*, 1993) reportedly reduce insulin secretion.

Insulin partly inhibits pancreatic α-cell secretion of glucagon (Raskin *et al.*, 1975), a so-called glucagonostatic effect. It has been proposed that the exaggerated glucagon secretion in IDDM may be attributable to the loss of a restraining influence of insulin on pancreatic α-cells (Samols *et al.*, 1986; Unger and Foster, 1992). Because pancreatic α-cells remain sensitive to the restraining influence of insulin in IDDM patients (Raskin *et al.*, 1978), the suppression of glucagon secretion has been proposed as one of the benefits of insulin therapy.

A. Arginine-Stimulated Secretion in Anesthetized Rats

In view of the possibility that, like other islet hormones, amylin might influence the secretion of the others, its effect on glucagon secretion was studied *in vivo*. In this work, (Gedulin *et al.*, 1997g; Young *et al.*, 1995), the effect of amylin on arginine-stimulated secretion of glucagon was examined in anesthetized rats. Because amylin administration can acutely change plasma glucose concentration in rats (Young *et al.*, 1991) and can acutely inhibit insulin secretion (Dégano *et al.*, 1993), both of which can affect glucagon secretion, the influence of those potential confounders was standardized using the hyperinsulinemic euglycemic clamp technique (DeFronzo *et al.*, 1979) during amylin infusion at different rates.

Anesthetized male Sprague Dawley rats were cannulated via the femoral artery and vein for sampling and infusions, respectively, during a hyperinsulinemic euglycemic clamp procedure (glucose 6 mM). Infusions of rat amylin (0, 3.6, 12, 36, or 120 pmol/kg/min) began 60 min before a 10 min infusion of 2 mmol L-arginine (delivered so as to avoid hypotensive stimulation of glucagon secretion; Lindsey *et al.,* 1975).

The data obtained are plotted in Fig. 2. Plasma glucose concentrations and insulin concentrations, which themselves can alter glucagon release (Maruyama *et al.,* 1984), and mean arterial pressure did not differ between treatment groups for the 60 min following arginine infusion. L-arginine provoked a 160 pM increase in plasma glucagon concentration within 20 min of administration in the absence of infused amylin. Continuously infused amylin reduced integrated glucagon secretion by 47–67% at the three highest amylin infusion rates ($P < 0.05$–0.01).

Steady-state plasma amylin concentrations obtained during and following different amylin infusion rates were calculable from other kinetic studies (Young *et al.,* 1996), and could be derived from infusion rate using the expression $[\text{amylin}] = 10^{(\log \text{inf rate}) \times 1.18 + 1.024}$, where [amylin] was measured in pM and infusion rate in pmol/kg/min. The plasma amylin concentrations thereby obtained were used to construct a concentration response that yielded an EC_{50} of 18 pM $\pm$ 0.28 log units for glucagon suppression, as shown in Fig. 3. This concentration was within the range of plasma amylin values reported to circulate in rats (Pieber *et al.,* 1994), and indicated that glucagon suppression was likely a physiological effect of endogenous amylin in this species.

III. Effects of Amylin on Glucagon Release from Isolated Preparations

It was postulated that amylin secreted from the β-cell-rich islet medulla into the local islet portal circulation might be carried to α-cells on its passage to the islet cortex, and act there directly to inhibit glucagon secretion. When the microanatomy of islets of Langerhans was considered (Redecker *et al.,* 1992; Weir and Bonner-Weir, 1990), it was anticipated that amylin secreted into the local portal circulation would be of high concentration as it flowed to the α-cell-rich cortex (Bonner-Weir and Orci, 1982). For example, in a semiquantitative histochemical study, insulin concentrations within the islet were estimated to be 100–200 times higher than concentrations found in plasma (Bendayan, 1993).

A potential direct effect of amylin on glucagon secretion in islets has been explored in several studies of the isolated perfused pancreas of the rat. But in apparent contradiction to the powerful glucagonostatic effects of amylin and pramlintide observed in intact rats, earlier studies of glucagon secretion in the

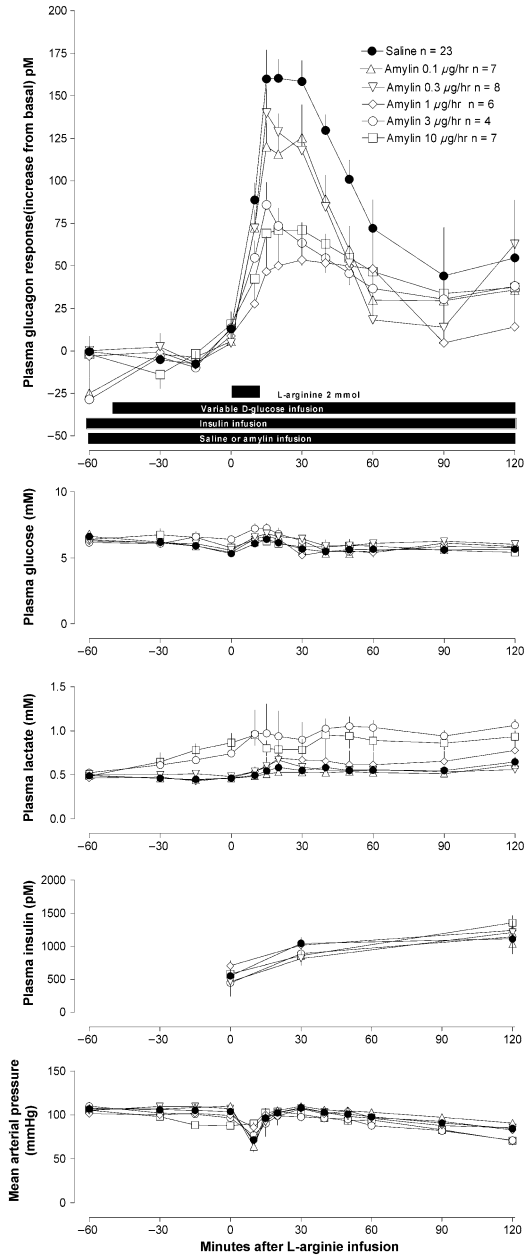

FIGURE 2 Dose dependence of effects of rat amylin on arginine-stimulated glucagon secretion in anesthetized rats. Data from Gedulin *et al.* (1997g) and Young *et al.* (1995).

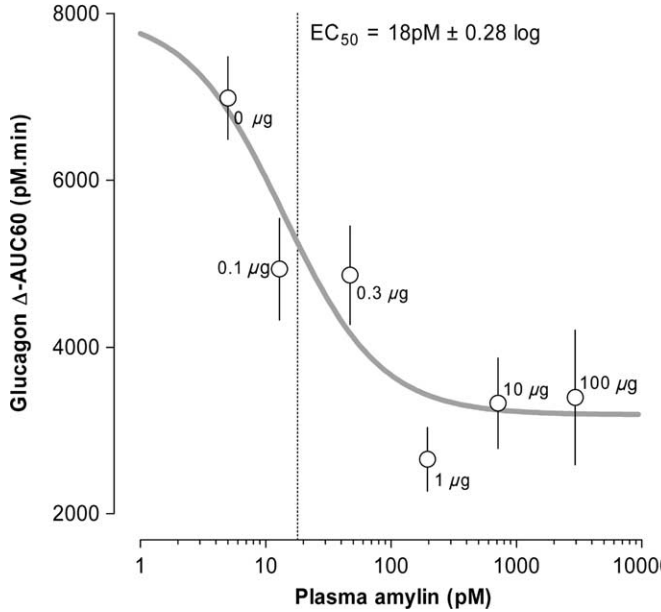

FIGURE 3 Concentration-response for glucagonostatic effect of rat amylin in anesthetized rats. Data from Gedulin *et al.* (1997g) and Young *et al.* (1995).

isolated perfused pancreas indicated no effect of rat amylin (up to 750 nM) on arginine-stimulated glucagon secretion (Inoue *et al.*, 1993; Silvestre *et al.*, 1990). A later study by the same authors (Dégano *et al.*, 1993) suggested that the peptide used in the original study may have had reduced biological activity. Because many preparations of amylin peptide commercially available at the time of that study were impure (Cobb *et al.*, 1992; Cody *et al.*, 1991) or had reduced, variable, or undetermined biological activity (Lehman-deGaeta *et al.*, 1991), isolated perfused pancreas experiments were repeated using material established by Amylin Pharmaceuticals Inc. as biologically active in isolated soleus muscle. The same result was obtained (Silvestre *et al.*, 1999), as described next, indicating that amylin did not inhibit stimulated glucagon secretion in the isolated pancreas and that a glucagonostatic effect was therefore not intrinsic to the isolated pancreas.

A. Isolated Perfused Rat Pancreas

Pancreata from male Wistar donor rats were obtained under pentobarbital anesthesia and were dissected and perfused *in situ* in a modification (Silvestre *et al.*, 1986) of the procedure of Leclercq-Meyer *et al.*

(Leclercq-Meyer *et al.*, 1976). The arterial side of a nonrecycled system was perfused with a Krebs-Henseleit buffer to which was added glucose (3.2 mM, 5.5 mM, or 11 mM), rat amylin (1 nM), 5 mM L-arginine (to stimulate glucagon secretion), vasoactive intestinal peptide (VIP) (1 nM), or carbachol (50 μM). Glucagon responses to L-arginine, carbachol, and VIP shown in Fig. 4 indicate, first, that the perfused pancreas preparation was functional. Addition of rat amylin did not significantly modify the glucagon responses to L-arginine, carbachol, or VIP. In these three experiments, amylin was also without effect on glucagon release during the 10 min (unstimulated) perfusion period preceding the infusion of secretagogues.

B. Response to Glycopenia

Pancreata initially perfused at a glucose concentration of 11 mM were abruptly exposed to 3.2 mM glucose (Silvestre *et al.*, 2001), as shown in Fig. 5. In the absence of amylin, the reduction of infusate glucose concentration resulted in a progressive increase in glucagon output from 129 $\pm$ 31 to 578 $\pm$ 99 pg/min at $t = 30$ min ($P < 0.05$). Restoration of an 11 mM glucose concentration promptly decreased glucagon release to basal values. Co-infusion of amylin from $t = 0$ until $t = 30$ min had no effect on the 25 min integrated glucagon response ($P = 0.8$) and had no apparent effect on the glucagon secretory pattern.

The effect of amylin on the secretion of glucagon was studied in the reverse experimental design, in which periods of low glucose concentration (3.2 mM) bracketed a period of euglycemia (7 mM). The inhibitory effect of 7 mM glucose concentration on glucagon output was not significantly modified by amylin infusion (15 min integrated decline; $P = 0.7$), as shown in Fig. 6.

C. Isolated Islets

In a further test of potential direct effects of amylin (Silvestre *et al.*, 2001), pancreatic islets of Langerhans were isolated from whole pancreas using a method of collagenase digestion originally described by Lacy and Kostianovsky (Lacy and Kostianovsky, 1967), and modified by Lakey *et al.* (Lakey *et al.*, 1996). Cleaned, minced pancreas digested with collagenase-P plus DNAse was applied to a Ficoll gradient to isolate the islets. Hand-picked islets could be stored in culture for 3–4 days until experimentation. Separate treatments consisted of addition of the following: glucose (3 mM) (control); glucose (3 mM) + L-arginine (10 mM); glucose (3 mM) + L-arginine

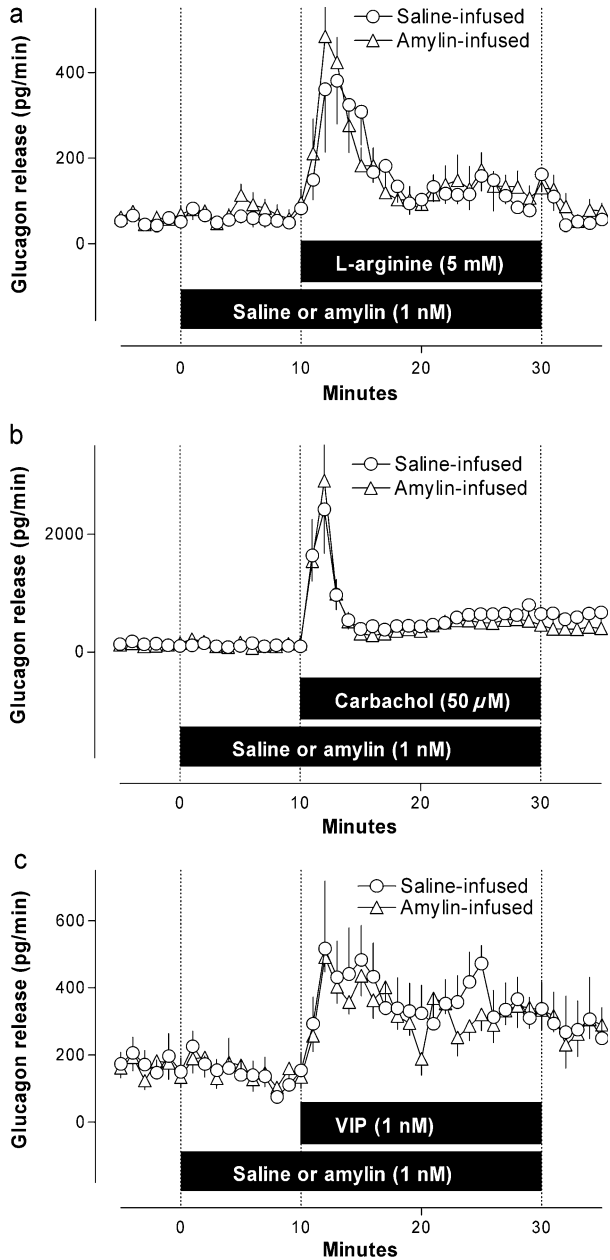

FIGURE 4 Effect of rat amylin on (a) arginine-induced, (b) carbachol-induced, and (c) VIP-induced glucagon secretion from isolated perfused pancreas. Data from Silvestre *et al.* (2001).

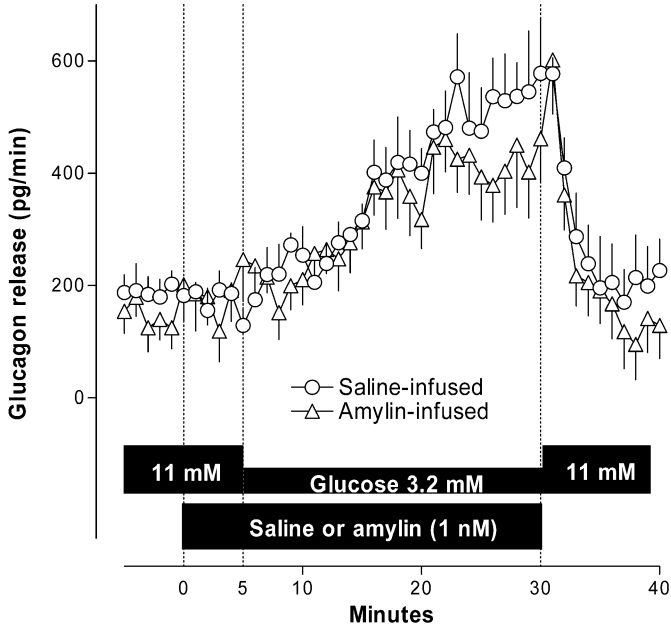

FIGURE 5 Effect of rat amylin on glucagon release from isolated rat pancreas stimulated by a fall in perfusate glucose concentration. Data from Silvestre *et al.* (2001).

(10 mM) + somatostatin (100 nM); glucose (3 mM) + L-arginine (10 mM) + rat amylin (100 nM).

Addition of arginine resulted in a 15.5-fold increase in the rate of glucagon secretion over that observed in the presence of 3 mM glucose (designated basal; $P < 0.02$), as shown in Fig. 7. Addition of somatostatin to islets exposed to arginine reduced this 15.5-fold stimulation of glucagon secretion by $55 \pm 21\%$. In contrast, addition of rat amylin to buffer containing arginine had no glucagonostatic effect ($102 \pm 46\%$ of islets treated with arginine alone).

IV. Effects of Amylin in Whole-Animal Preparations

A. Hypoglycemia-Stimulated Glucagon Secretion

Anesthetized fasted male Sprague Dawley rats were cannulated via artery and vein. Either rat amylin (50 pmol/kg/min) or saline was infused intravenously, and 30 min later insulin was added at 5 mU/min. Samples were taken sequentially for glucose and glucagon assay. In rats pre-infused with saline only for 60 min, insulin reduced plasma glucose from 5.72 ± 0.22 to 2.11 ± 0.11 mM ($P < 0.001$), as shown in Fig. 8. In rats pre-infused with

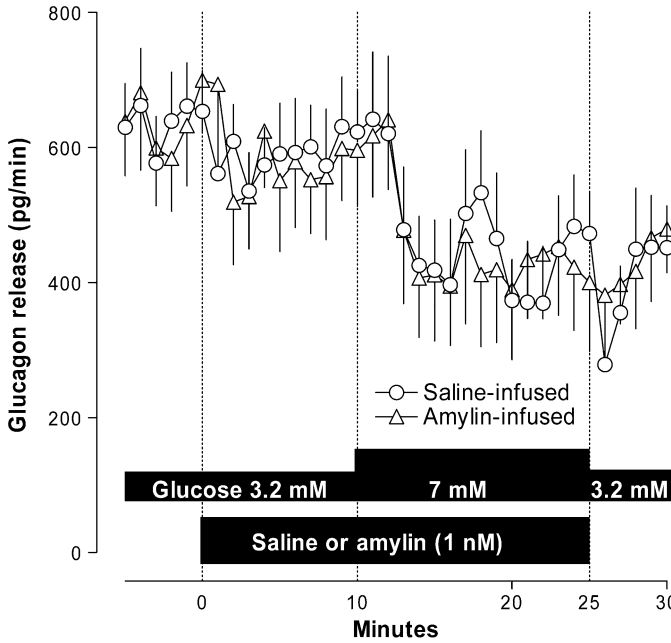

FIGURE 6 Effect of rat amylin on euglycemic suppression of glucagon secretion in isolated pancreas in which glucagon secretion was stimulated by low glucose in perfusate. Data from Silvestre *et al.* (2001).

amylin, plasma glucose concentration was initially higher (6.39 ± 0.17 mM), consistent with the glycemic effect of amylin in this species (Young *et al.*, 1991). Insulin reduced glucose to 2.39 ± 0.17 mM ($P < 0.001$), and it remained between 1.6 and 2.2 mM for the subsequent 60 min in both groups. The 9.5-fold increase in glucagon concentration observed during hypoglycemia in the amylin-treated rats (217 ± 18 to 2070 ± 273 pg/ml) was not different from the 8.2-fold increase observed in saline-treated rats (262 ± 35 to 2141 ± 348 pg/ml). Glucagon AUC (2 hr) was not different between groups ($P = 0.91$).

Amylin infused at a high rate is reported to increase plasma glucose in rats (Young *et al.*, 1991), an effect that might confound the interpretation of the influence of amylin per se on glucagon secretion. To accommodate the influence of glucose, the glucagon response was analyzed as a function of current plasma glucose concentration. The nonlinear relationship between plasma glucagon and glucose concentrations was similar whether or not amylin was being infused. A LOWESS (trend-following) curve was fitted to the glucagon:glucose scatterplot to approximate the center of distribution. In amylin-infused rats, the proportion of all data points above the curve was as great as, or greater than, the proportion in saline-infused rats, indicating

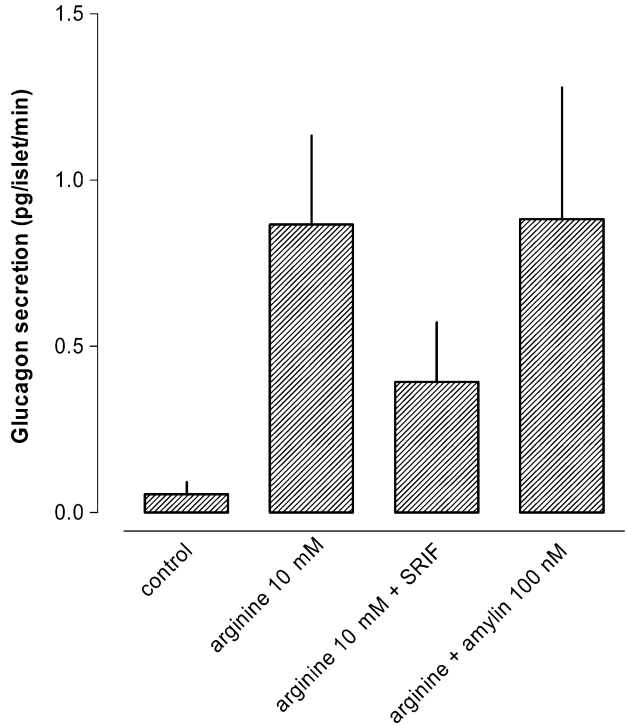

FIGURE 7 Effect of 100 nM rat amylin on glucagon secretion stimulated by L-arginine in isolated islets. Data from Silvestre *et al.* (2001).

that amylin did not diminish hypoglycemia-stimulated glucagon secretion (Fig. 9).

B. Selective Effect on Arginine-Stimulated versus Hypoglycemia-Stimulated Glucagon Secretion

To verify that amylin indeed exhibited glucagon suppression following nutrient stimuli but not during hypoglycemia, it was clearly necessary to demonstrate both phenomena in the same experiment. Animals were anesthetized and prepared as described previously. Thirty minutes after surgery, saline or rat amylin (50 pmol/kg/min) was continuously infused intravenously until the end of the experiment. Insulin was infused concurrently at 2 mU/min, and a variable glucose infusion was used to clamp plasma glucose at 5.67 ± 0.06 mM from 1 hr before until 2 hr after a

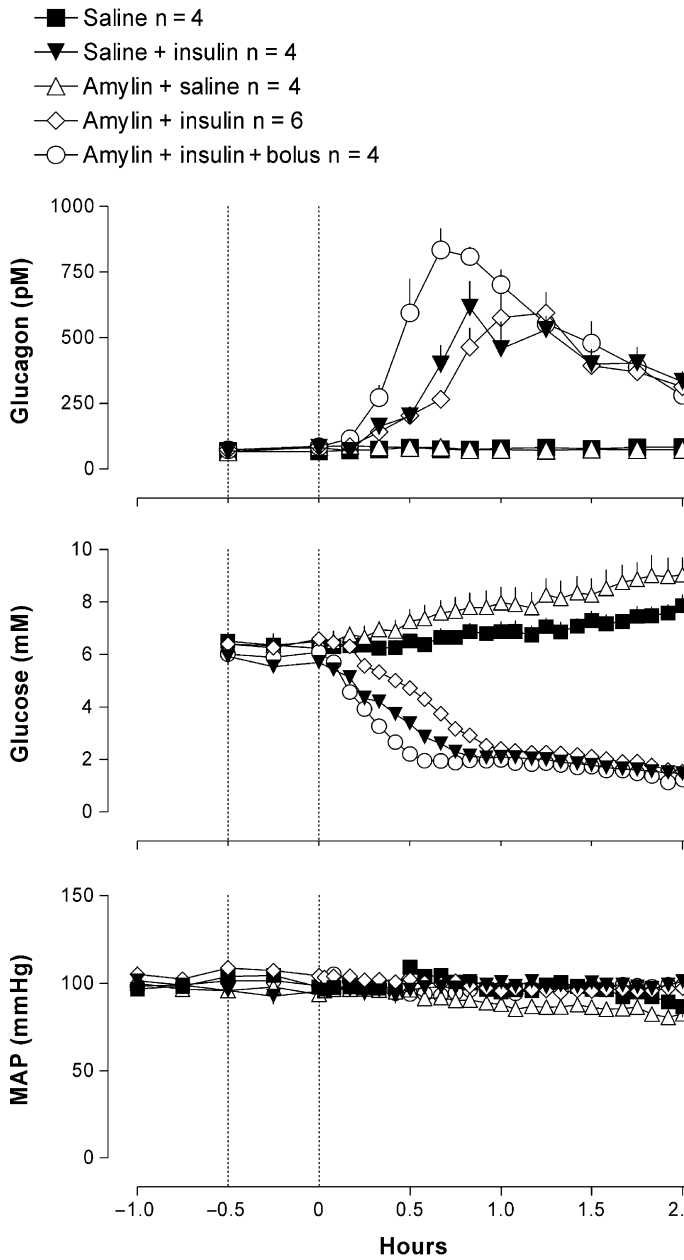

FIGURE 8 Effects of infusion of rat amylin on glucagon secretion in anesthetized rats infused with saline or hypoglycemic doses of insulin. Data from Silvestre *et al.* (2001).

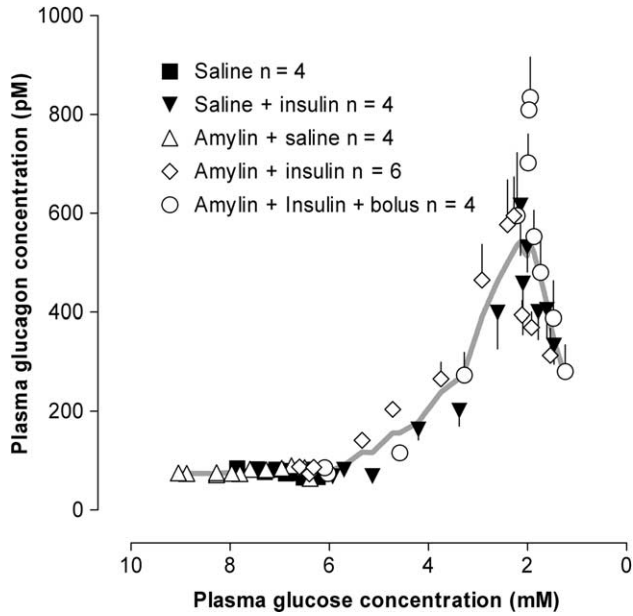

FIGURE 9 Effect of co-infusion of rat amylin on glucose–glucagon relationship in insulin-treated anesthetized rats. Data from Silvestre *et al.* (2001).

2 mmol L-arginine challenge (delivered over 10 min, as detailed previously). After 2 hr, the variable glucose infusion was stopped, allowing plasma glucose to fall and remain low. Hypoglycemic profiles during this portion of the experiment were matched by those of amylin-treated rats receiving an additional 90 mU insulin bolus when glucose infusion was stopped. Results are shown in Fig. 10.

In amylin-infused animals, steady-state plasma amylin concentration was 589 pM ± 29% (CV), a concentration previously shown to maximally inhibit arginine-induced glucagon secretion. Mean arterial pressure remained between 93 and 106 mmHg throughout the experiment and decreased slightly during administration of L-arginine. The 90 min glucagon response to the L-arginine challenge was reduced by 45% in the amylin-infused rats ($P < 0.05$), affirming a glucagonostatic effect in this context.

Within 20 min of cessation of the variable glucose infusion, insulin reduced plasma glucose from 5.67 mM to 2.44 mM (amylin-treated rats) and to 2.39 mM (saline-treated rats). Glucose remained between 1.6 and 2.2 mM over the next 100 min. In response to this hypoglycemic challenge, the 12.4-fold increase in plasma glucagon concentration in amylin-treated rats (205 ± 43 to 2544 ± 264 pg/ml) was indistinguishable from the 11.2-fold increase observed in controls (247 ± 49 to 2761 ± 382 pg/ml; glucagon AUC[120–240], $P = 0.44$). That is, in this animal model, amylin infusion

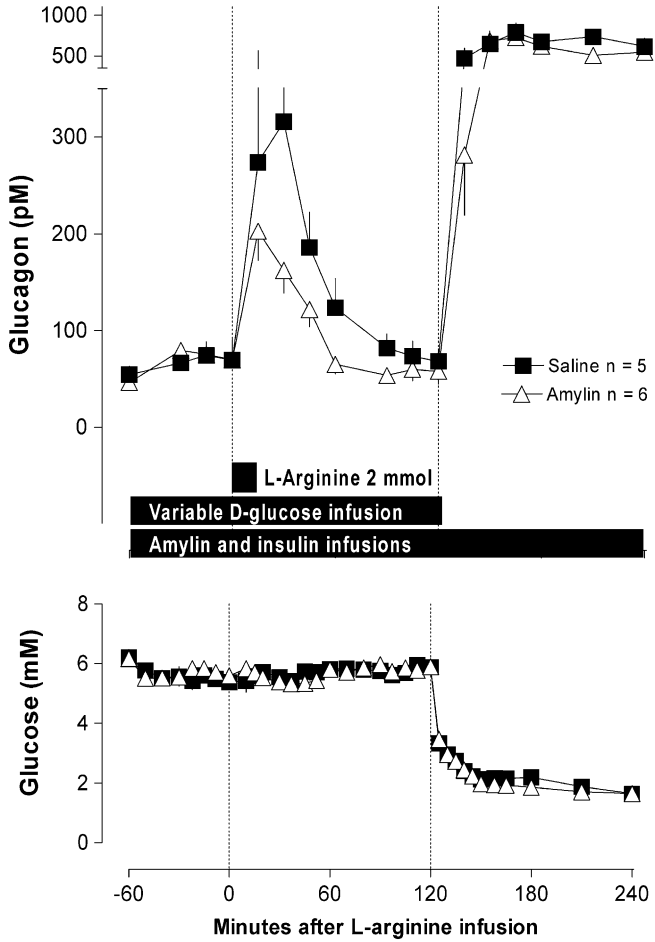

FIGURE 10 Differential effects of infused rat amylin on arginine-stimulated versus hypoglycemia-stimulated glucagon secretion in intact anesthetized rats. Data from Silvestre *et al.* (2001).

selectively inhibited arginine-stimulated glucagon secretion, consistent with a previous report (Gedulin *et al.*, 1997g), but did not affect hypoglycemia-stimulated glucagon secretion.

Selective inhibition of nutrient-stimulated glucagon secretion is likely to be of therapeutic interest, since it is selectively nutrient-stimulated secretion that is abnormally elevated in insulin-deficient diabetes. The preceding experiments provided a clue as to how selective hypersecretion and selective inhibition might occur. It appeared that amylinergic suppression of nutrient-stimulated glucagon secretion was extrinsic to the pancreas, being present only in intact animals, and was therefore likely to be autonomically

mediated. Glucagon secretion in response to glycopenia can, however, be observed in isolated preparations, is intrinsic to the pancreas, and is not obligatorily dependent upon intact autonomic innervation (explaining why, in isolated preparations, it is unaffected by amylin's autonomic inhibitory drive).

V. Pharmacology of Glucagonostatic Effect

Amylin's suppression of amino acid-induced glucagon secretion was similar to that reported for salmon calcitonin (Sgambato *et al.*, 1981; Starke *et al.*, 1981). In *in vivo* experiments in rats, CGRP infusions also inhibited arginine-stimulated glucagon secretion (Pettersson and Ahren, 1988). Those results, combined with the similar effect of amylin, are consistent with this action being mediated via a classic amylin pharmacology (Beaumont *et al.*, 1993). The combination of results is not accommodated solely via a CGRPergic pathway, for example, since CGRP receptors do not appreciably respond to salmon calcitonin (Beaumont *et al.*, 1993). The pharmacology of suppression of stimulated glucagon secretion was further tested using the selective amylin receptor antagonist AC187 (Gedulin *et al.*, 1997e). Infusion of AC187 in anesthetized glucose-clamped rats increased plasma glucagon concentrations from 82 $\pm$ 9 pM to 160 $\pm$ 30 pM ($P < 0.03$), a level higher than observed in saline-infused rats (103 $\pm$ 9 pM; $P < 0.02$). This result suggested a tonic endogenous activation of an amylinergic pathway that could be blocked pharmacologically. Observations that plasma glucagon levels were 61% higher in animals that had received a specific neutralizing anti-amylin antibody than in animals administered non-specific antibody suggested that the endogenous activator was amylin (Gedulin *et al.*, 1997e). That is, these results supported a physiological glucagonostatic role of amylin in rats.

VI. Effect of Pramlintide in Anesthetized Rats

The effects of pramlintide on arginine-stimulated glucagon secretion were reported at several regional meetings (Gedulin *et al.*, 1997b,c,d, 1998) and in a technical report (Gedulin, unpublished). In experiments similar to those described previously for rat amylin, male Sprague Dawley rats were surgically prepared and treated in a hyperinsulinemic euglycemic clamp, with arginine infused as a glucagon secretagogoue. Animals were infused from 30 min before until 120 min after L-arginine with pramlintide (0 [saline], 0.1, 1, or 10 μg/hr). A concentration response was determinable from infusion rate:concentration relationships obtained in separate parallel

experiments (Young *et al.*, 1996). In those experiments, a relationship was developed: [pramlintide] = $10^{(1.15 \times \text{infusion rate})+2.35}$, where [pramlintide] was measured in pM, and infusion rate in μg/hr.

Similarly to amylin, pramlintide infusion could inhibit the plasma glucagon response to arginine (ΔAUC60) by up to 56 $\pm$ 5.3% ($P < 0.01$ versus saline controls) with an EC_{50} of 30.4 pM $\pm$ 0.38 log units. Such a concentration was similar to that attained in humans with potentially therapeutic doses of pramlintide.

Arterial plasma glucose and insulin concentrations, and arterial pressure, for the 60 min after intravenous infusion of L-arginine and during euglycemic clamps were not different between treatment groups, indicating constancy of those factors that could influence glucagon secretion. The glucose infusion rates required to maintain euglycemia did not differ between groups treated with pramlintide or saline ($P = 0.43$), indicating no acute effect on insulin sensitivity.

VII. Clinical Studies

In several clinical studies in patients with type 1 diabetes, postprandial secretion of glucagon was elevated, and was inhibited by administration of pramlintide (Fineman *et al.*, 1997a, 1998a,b; Levetan *et al.*, 2003; Nyholm *et al.*, 1997a, 1999; Thompson *et al.*, 1997). Pramlintide also inhibited postprandial glucagon secretion in patients with type 2 diabetes (Fineman *et al.*, 1998a,b). In a crossover study in type 1 diabetic patients, pramlintide inhibited glucagon secretion during normoglycemia, but not during insulin-induced hypoglycemia (Nyholm *et al.*, 1996). Concomitant pramlintide administration did not affect the glycogenic response to a glucagon challenge in patients with type 1 diabetes (Orskov *et al.*, 1997).

Thus, in most respects, the selective actions of amylin on glucagon secretion identified in animals were also seen with pramlintide in humans. Amylinergic inhibition of nutrient-stimulated glucagon secretion could prove useful in those aspects of deranged metabolic control that are attributable to excess glucagon action in diabetes that is characterized by deficiency of insulin and amylin.

References

Argoud, G. M., Schade, D. S., and Eaton, R. P. (1987). Insulin suppresses its own secretion *in vivo*. *Diabetes* **36**, 959–962.

Ashcroft, F. M., and Ashcroft, S. J. H. (1992). Mechanism of insulin secretion. *In* "Insulin: Molecular Biology to Pathology" (F. M. Ashcroft, and S. J. H. Ashcroft, Eds.), pp. 97–150. Oxford University Press, New York.

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Bendayan, M. (1993). Pathway of insulin in pancreatic tissue on its release by the B-cell. *Am. J. Physiol.* **264**, G187–G194.

Bonner-Weir, S., and Orci, L. (1982). New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* **31**, 883–889.

Cobb, J. E., Kassel, D. B., Sugg, E. E., Burkhart, W., Geddie, N., Stele, A., and Anderegg, R. J. (1992). Mercury in a commercial preparation of rat amylin. *Pept. Res.* **5**, 161–164.

Cody, W. L., Giordani, A. B., Werness, S., Reily, M. D., Bristol, J. A., Zhu, G., and Dudley, D. T. (1991). Analysis of rat amylin amide from commercial sources: Identification of a mercury complex. *Bioorg. Med. Chem. Lett.* **1**, 415–420.

DeFronzo, R. A., Tobin, J. D., and Andres, R. (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. *Am. J. Physiol.* **237**, E214–E223.

Dégano, P., Silvestre, R. A., Salas, M., Peiró, E., and Marco, J. (1993). Amylin inhibits glucose-induced insulin secretion in a dose-dependent manner. Study in the perfused rat pancreas. *Regul. Pept.* **43**, 91–96.

Dobbs, R., Sakurai, H., Sasaki, H., Faloona, G., Valverde, I., Baetens, D., Orci, L., and Unger, R. (1975). Glucagon: Role in the hyperglycemia of diabetes mellitus. *Science* **187**, 544–547.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1997a). Glucagon secretion in patients with type I diabetes was inhibited by the human amylin analogue, pramlintide. *Diabet. Med.* **14**, S29.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1997b). The human amylin analogue pramlintide inhibited glucagon secretion in type I diabetic subjects. *79th Annual Meeting of the Endocrine Society, Program and Abstracts*, p. 472.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1997c). The human amylin analogue pramlintide inhibited glucagon secretion in type I diabetic subjects. *Diabetes* **40**, 30A.

Fineman, M. S., Kolterman, O. G., Thompson, R. G., and Koda, J. E. (1997d). The human amylin analogue pramlintide inhibited glucagon secretion in type I diabetic subjects. *Diabetologia* **40**, A355.

Fineman, M., Koda, J., and Kolterman, O. (1998a). Subcutaneous administration of a human amylin analogue suppresses postprandial plasma glucagon concentrations in type I diabetic patients. *Diabetes* **47**, A89.

Fineman, M., Organ, K., and Kolterman, O. (1998b). The human amylin analogue pramlintide suppressed glucagon secretion in patients with type 2 diabetes. *Diabetologia* **41**, A167.

Gedulin, B., Jodka, C., Percy, A., and Young, A. (1997a). Neutralizing antibody and the antagonist AC187 may inhibit glucagon secretion in rats. *Diabetes* **40**, 238A.

Gedulin, B., Jodka, C., and Young, A. (1997b). Pramlintide inhibits arginine-induced glucagon secretion in rats. *Diabet. Med.* **14**, S26.

Gedulin, B., Jodka, C., and Young, A. (1997c). Pramlintide inhibits arginine-induced glucagon secretion in rats. *Diabet. Metab.* **23**, P051.

Gedulin, B., Jodka, C., and Young, A. (1997d). Pramlintide inhibits arginine-induced glucagon secretion in rats. *Diabetes und Stoffwechsel* **6**, 54.

Gedulin, B., Percy, A., Jodka, C., and Young, A. (1997e). Endogenous amylin inhibits glucagon secretion, as demonstrated by studies using neutralizing antibody and the antagonist AC187. *Diabet. Med.* **14**, S18.

Gedulin, B., Percy, A., Jodka, C., and Young, A. (1997f). Studies using neutralizing antibody and the antagonist AC187 reveal that endogenous amylin inhibits glucagon secretion. *Diabetologia* **40**(suppl. 1), A300.

Gedulin, B. R., Rink, T. J., and Young, A. A. (1997g). Dose-response for glucagonostatic effect of amylin in rats. *Metabolism* **46**, 67–70.

Gedulin, B., Jodka, C., Lawler, R., and Young, A. (1998). Concentration-response for pramlintide inhibition of arginine-induced glucagon secretion in rats. *Programs and Abstracts: 80th Annual Meeting of the Endocrine Society*, p. 276.

Holst, J. J. (1997). Enteroglucagon. *Annu Rev. Physiol.* **59**, 257–271.

Inoue, K., Hiramatsu, S., Hisatomi, A., Umeda, F., and Nawata, H. (1993). Effects of amylin on the release of insulin and glucagon from the perfused rat pancreas. *Horm. Metab. Res.* **25**, 135–137.

Jodka, C., Parkes, D., and Young, A. A. (2000). Amylin inhibits arginine-stimulated but not hypoglycemia-stimulated glucagon secretion in anesthetized rats. *Diabet. Res. Clin. Pract.* **50**, S392.

Kawamori, R., Shichiri, M., Kikuchi, M., Yamasaki, Y., and Abe, H. (1985). The mechanism of exaggerated glucagon response to arginine in diabetes mellitus. *Diabet. Res. Clin. Pract.* **1**, 131–137.

Lacy, P. E., and Kostianovsky, M. (1967). Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* **16**, 35–39.

Lakey, J. R., Warnock, G. L., Ao, Z., and Rajotte, R. V. (1996). Bulk cryopreservation of isolated islets of Langerhans. *Cell Transplant* **5**, 395–404.

Leclercq-Meyer, V., Marchand, J., Leclercq, R., and Malaisse, W. J. (1976). Glucagon and insulin release by the *in vitro* perfused rat pancreas. *Diabet. Metab.* **2**, 57–65.

Lehman-deGaeta, L. S., Willis, A. C., Young, A. A., Foot, E. A., Albrecht, E., Leighton, B., Rink, T. J., and Cooper, G. J. S. (1991). Variability of chemically synthesized human amylin. *Diabetes* **40**, 236A.

Levetan, C., Want, L. L., Weyer, C., Strobel, S. A., Crean, J., Wang, Y., Maggs, D. G., Kolterman, O. G., Chandran, M., Mudaliar, S. R., and Henry, R. R. (2003). Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglycerides excursions among patients with type 1 diabetes intensively treated with insulin pumps. *Diabet. Care* **26**, 1–8.

Lindsey, C. A., Faloona, G. R., and Unger, R. H. (1975). Plasma glucagon levels during rapid exsanguination with and without adrenergic blockade. *Diabetes* **24**, 313–316.

Maruyama, H., Hisatomi, A., Orci, L., Grodsky, G. M., and Unger, R. H. (1984). Insulin within islets is a physiologic glucagon release inhibitor. *J. Clin. Invest.* **74**, 2296–2299.

Müller, W. A., Faloona, G. R., Aguilar-Parada, E., and Unger, R. H. (1970). Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. *N. Engl. J. Med.* **283**, 109–115.

Nyholm, B., Moller, N., Gravholt, C. H., Orskov, L., Mengel, A., Bryan, G., Moyses, C., Alberti, K. G., and Schmitz, O. (1996). Acute effects of the human amylin analog AC137 on basal and insulin-stimulated euglycemic and hypoglycemic fuel metabolism in patients with insulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* **81**, 1083–1089.

Nyholm, B., Orskov, L., Hove, K., Gravholt, C., Moller, N., Alberti, N., and Schmitz, O. (1997a). The amylin analogue pramlintide decreases post-prandial plasma glucose and glucagon in IDDM. *Diabetes* **46**.

Nyholm, B., Orskov, L., Hove, K. Y., Gravholt, C. H., Moller, N., Alberti, K. G. M. M., and Schmitz, O. (1997b). The amylin analogue pramlintide decreases postprandial plasma glucose and glucagon in IDDM patients. *Diabetologia* **40**, A46.

Nyholm, B., Orskov, L., and Schmitz, O. (1997c). The amylin analogue pramlintide decreased post-prandial plasma glucose and glucagon in patients with type I diabetes. *Diabet. Med.* **14**, S29.

Nyholm, B., Orskov, L., Hove, K., Gravholt, C., Moller, N., Alberti, K., Moyses, C. K. O., and Schmitz, O. (1999). The amylin analog pramlintide improves glycemic control and reduces postprandial glucagon concentrations in patients with type 1 diabetes mellitus. *Metabolism* **48**, 935–941.

Orskov, L., Nyholm, B., Hove, K. Y., Gravholt, C. H., Moller, O., Kolterman, O., Alberti, K. G. M. M. A., and Schmitz, O. (1997). Effects of the amylin analogue pramlintide on the glucose response to a glucagon challenge in IDDM. *Diabetes* **46**, 155A.

Orskov, L., Nyholm, B., Yde Hove, K., Gravholt, C. H., Moller, N., and Schmitz, O. (1999). Effects of the amylin analogue pramlintide on hepatic glucagon responses and intermediary metabolism in Type 1 diabetic subjects. *Diabet. Med.* **16**, 867–874.

Parkes, D., Chen, K., Smith, P., and Young, A. (1999). Amylin does not suppress hypoglycemia-induced secretion of glucagon in rats. *Diabetes* **48**(suppl. 1), A425.

Pettersson, M., and Ahren, B. (1988). Insulin and glucagon secretion in rats: Effects of calcitonin gene-related peptide. *Regul. Pept.* **23**, 37–50.

Pieber, T. R., Roitelman, J., Lee, Y., Luskey, K. L., and Stein, D. T. (1994). Direct plasma radioimmunoassay for rat amylin- (1–37): Concentrations with acquired and genetic obesity. *Am. J. Physiol.* **267**, E156–E164.

Raskin, P., and Unger, R. H. (1978). Glucagon and diabetes. *Med. Clin. North Am.* **62**, 713–722.

Raskin, P., Fujita, Y., and Unger, R. H. (1975). Effect of insulin-glucose infusions on plasma glucagon levels in fasting diabetics and nondiabetics. *J. Clin. Invest.* **56**, 1132–1138.

Raskin, P., Aydin, I., and Unger, R. H. (1976). Effect of insulin on the exaggerated glucagon response to arginine stimulation in diabetes mellitus. *Diabetes* **25**, 227–229.

Raskin, P., Aydin, I., Yamamoto, T., and Unger, R. H. (1978). Abnormal alpha cell function in human diabetes: The response to oral protein. *Am. J. Med.* **64**, 988–997.

Redecker, P., Seipelt, A., Jorns, A., Bargsten, G., and Grube, D. (1992). The microanatomy of canine islets of Langerhans: Implications for intra-islet regulation. *Anat. Embryol. (Berl.)* **185**, 131–141.

Samols, E., Bonner-Weir, S., and Weir, G. C. (1986). Intra-islet insulin-glucagon-somatostatin relationships. *Clin. Endocrinol. Metab.* **15**, 33–58.

Sgambato, S., Passariello, N., Giugliano, D., Torella, R., and D'Onofrio, F. (1981). Effect of calcitonin on plasma glucose, C-peptide, glucagon and growth hormone responses to arginine in insulin-dependent diabetic subjects. *Acta Diabetol. Lat.* **18**, 235–241.

Silvestre, R. A., Miralles, P., Moreno, P., Villanueva, M. L., and Marco, J. (1986). Somatostatin, insulin and glucagon secretion by the perfused pancreas from the cysteamine-treated rat. *Biochem. Biophys. Res. Commun.* **134**, 1291–1297.

Silvestre, R. A., Peiró, E., Dégano, P., Miralles, P., and Marco, J. (1990). Inhibitory effect of rat amylin on the insulin responses to glucose and arginine in the perfused rat pancreas. *Regul. Pept.* **31**, 23–31.

Silvestre, R. A., Salas, M., Rodriguez-Gallardo, J., Garcia-Hermida, O., Fontela, T., and Marco, J. (1996). Effect of (8–32). salmon calcitonin, an amylin antagonist, on insulin, glucagon and somatostatin release: Study in the perfused pancreas of the rat. *Br. J. Pharmacol.* **117**, 347–350.

Silvestre, R. A., Rodríguez-Gallardo, J., Gutiérrez, E., García, P., Egido, E. M., and Marco, J. (1999). Failure of amylin to directly affect glucagon release. Study in the perfused rat pancreas. *Diabetologia* **42**, A146.

Silvestre, R. A., Rodriguez-Gallardo, J., Jodka, C., Parkes, D. G., Pittner, R. A., Young, A. A., and Marco, J. (2001). Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas. *Am. J. Physiol.* **280**, E443–E449.

Starke, A., Keck, E., Berger, M., and Zimmermann, H. (1981). Effects of calcium and calcitonin on circulating levels of glucagon and glucose in diabetes mellitus. *Diabetologia* **20**, 547–552.

Thompson, R., and Kolterman, O. (1997). Review of clinical data with pramlintide, a human amylin analogue. *79th Annual Meeting of the Endocrine Society, Program and Abstracts*, p. 473.

Thompson, R., Kolterman, O., Peterson, J., and Pearson, L. (1997). Pramlintide reduced 24-hour glucose and serum fructosamine concentrations in people with type I diabetes mellitus. *Can. J. Diabet. Care* **21**, 26.

Unger, R. H. (1978). Role of glucagon in the pathogenesis of diabetes: The status of the controversy. *Metabolism* **27**, 1691–1709.

Unger, R. H. (1985). Glucagon physiology and pathophysiology in the light of new advances. *Diabetologia* **28**, 574–578.

Unger, R. H., and Foster, D. W. (1992). Diabetes mellitus. *In* "Williams Textbook of Endocrinology" (J. D. Wilson, and D. W. Foster, Eds.), 8th ed., pp. 1273–1275. W.B. Saunders, Philadelphia.

Unger, R. H., Aguilar-Parada, E., Muller, W. A., and Eisentraut, A. M. (1970). Studies of pancreatic alpha cell function in normal and diabetic subjects. *J. Clin. Invest.* **49**, 837–848.

Weir, G. C., and Bonner-Weir, S. (1990). Islets of Langerhans—the puzzle of intraislet interactions and their relevance to diabetes. *J. Clin. Invest.* **85**, 983–987.

Young, A. A., Wang, M. W., and Cooper, G. J. S. (1991). Amylin injection causes elevated plasma lactate and glucose in the rat. *FEBS Lett.* **291**, 101–104.

Young, A. A., Jodka, C. M., Green, D. E., and Gedulin, B. R. (1995). Inhibition of arginine-induced glucagon secretion by amylin in rats. *Diabetes* **44**, 238A.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Andrew Young

Inhibition of Insulin Secretion

I. Summary

Reports of the effects of amylin and amylin agonists on insulin secretion have varied widely. Some confusion can be attributed to the use of human amylin, which has been shown to readily fall out of solution resulting in low estimates of bioactivity. Some confusion can be resolved by assessing the probability that this had happened. The view taken here, supported by authors using reliable and well-characterized ligands (representing the preponderance of recent studies), is that exogenously administered amylin agonists inhibit insulin secretion, at least partly via activation of an amylin-like receptor linked to G_i-mediated inhibition of cAMP in islets. There may additionally be autonomic extrapancreatic effects of amylin on insulin secretion that derive from its action at the area postrema. Studies with amylin receptor antagonists, including human studies, indicate that endogenously secreted amylin may physiologically inhibit β-cell secretion

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(insulin and amylin) via feedback inhibition that is characteristic of many other hormones. Part of this inhibition may be local (paracrine), as indicated by the amylin sensitivity of isolated preparations and the fact that the concentration of secreted products in the islet interstitium can be over 100-fold higher than in the circulation (Bendayan, 1993).

II. Background

Initial reports of the effect of amylin on insulin secretion conflict with more recent reports. This may be a good illustration of how scientific knowledge evolves.

Prior to 1992, most (15/24, 63%) reports (Ahren and Pettersson, 1990; Ar'Rajab and Ahren, 1991; Bretherton-Watt *et al.,* 1990; Broderick and Gold, 1991; Broderick *et al.,* 1991; Fehmann *et al.,* 1990; Ghatei *et al.,* 1990; Gilbey *et al.,* 1989; Gold *et al.,* 1990; Nagamatsu *et al.,* 1990a,b; O'Brien *et al.,* 1990; Pettersson and Ahren, 1990; Tedstone *et al.,* 1989, 1990) concluded that amylin had no impact on insulin secretion. A minority reported an insulinostatic effect (Dégano *et al.,* 1991; Johnson *et al.,* 1990; Kogire *et al.,* 1991; Marco *et al.,* 1990; Murakami *et al.,* 1990; Ohsawa *et al.,* 1989; Peiró *et al.,* 1991; Silvestre *et al.,* 1990a,b). After 1992, when most studies used rat amylin instead of human amylin (Rodriguez-Gallardo *et al.,* 1995; Young *et al.,* 1992a), 45/49 (92%) of reports described an insulinostatic effect (Ahren *et al.,* 1998; Bennet *et al.,* 1994; Bloom, 1994; Bretherton-Watt *et al.,* 1992a,b; Chuang *et al.,* 1992; Dégano *et al.,* 1992, 1993; Fürnsinn *et al.,* 1992, 1994; Gebre-Medhin *et al.,* 1998; Gedulin *et al.,* 1992, 1993; Göke *et al.,* 1993, 1993; Inoue *et al.,* 1993; Kulkarni *et al.,* 1996; Leaming *et al.,* 1995; Lewis *et al.,* 1988; Marco and Silvestre, 1997; O'Harte *et al.,* 1998; Rodriguez-Gallardo *et al.,* 1995; Salas *et al.,* 1994, 1995; Sandler and Stridsberg, 1994; Silvestre *et al.,* 1992, 1993a,b, 1994, 1996, 1997; Smith and Bloom, 1995; Stridsberg *et al.,* 1993; Suzuki *et al.,* 1992; Wagoner *et al.,* 1992, 1993; Wang *et al.,* 1993, 1997; Young and Gedulin, unpublished; Young *et al.,* 1992a, 1993, 1994, 1995), with four reporting no effect (Barakat *et al.,* 1994; Nagamatsu *et al.,* 1992; Panagiotidis *et al.,* 1992; Wang *et al.,* 1997).

The reasons for the highly significant switch in preponderance of conclusions ($P < 0.0001$, Fischer's exact test) are not fully clear, but they are likely to include initial use of human amylin. The adverse physicochemical properties of human amylin resulted in commercial batches with highly variable purity (as low as 5%) and biological activity (as low as 1% of native amylin) (Lehman-deGaeta *et al.,* 1991). Compared to rat amylin, human amylin produced very inconsistent results (Rodriguez-Gallardo *et al.,* 1995). Reasons for a heterogeneous literature on amylin's pancreatic effects may also include nonmethodological phenomena, such as biases

in the processes by which scientific findings are audited, peer-reviewed, and accepted for publication. Of the nine reports that initially described an insulinostatic effect, five came from the laboratory of Marco *et al.* in Madrid, whose group has published 17 communications that all report an insulinostatic action. Amylin Pharmaceuticals, Inc. and corporate collaborators have published 10 reports that described an insulinostatic action. Other groups with access to commercial batches of material have observed insulinostatic effects only sporadically. For example, Steven Bloom's lab produced three studies that did not observe an insulinostatic effect and seven that did. And the five publications from Per Westermark's group comprise three against and two for an insulinostatic effect.

Effects of amylin and amylin agonists have been studied in isolated β-cells and β-cell-like cell lines (14 reports), in isolated pancreatic islets (14 reports), in isolated perfused pancreas (20 reports), *in vivo* in animals (18 reports), and in humans (five reports). Most recent studies have used rat amylin. Some further insights may be obtained from the literature on the effects of salmon calcitonin, which is an amylin agonist.

III. Effects of Amylin on Insulin Secretion

A. Isolated β-Cells and β-Cell-like Lines

The first indication that amylin might directly inhibit secretion from its cells of origin came from the discovery that calcitonin gene-related peptide (CGRP), an amylin agonist, inhibited insulin secretion from dissociated rat β-cells, as measured in a hemolytic plaque assay (Lewis *et al.*, 1988). Some authors, applying high concentrations of amyloidogenic (fibril-forming) amylin species, such as human amylin, have proposed a cytotoxic effect on isolated β-cells and neurons (Lorenzo and Yankner, 1994; Lorenzo *et al.*, 1994; May *et al.*, 1993), but not with rat amylin or non-amyloidogenic molecular species (May *et al.*, 1993). Some have proposed that the cytotoxic effect of human amylin may adversely affect insulin secretion in metabolic disease (Lorenzo *et al.*, 1994).

In dissociated β-cells from mice, Wagoner and others at Glaxo showed that rodent amylin directly inhibited glucose-stimulated electrical activity, although this occurred at ambient concentrations of amylin that exceeded those measured in plasma (Wagoner *et al.*, 1993). Importantly, they also showed that this effect of amylin could be blocked with the amylin receptor antagonist AC253 (Wagoner and Dukes, unpublished), indicating that the inhibitory effect of amylin was likely to be receptor mediated, rather than a non-specific "cytotoxic" effect that would require mechanical (disruptive) contact of fibrils with the cell surface (Lorenzo *et al.*, 1994). A receptor-mediated action was supported by observations that minor changes in

TABLE I

Conclusion	*Pre-1992*	*1992–present*
Inhibition of insulin secretion	Dégano *et al.*, 1991; Johnson *et al.*, 1990; Kogire *et al.*, 1991; Marco *et al.*, 1990; Murakami *et al.*, 1990; Ohsawa *et al.*, 1989; Peiró *et al.*, 1991; Silvestre *et al.*, 1990a,b	Ahren *et al.*, 1998; Bennet *et al.*, 1993, 1994; Bloom, 1994; Bretherton-Watt *et al.*, 1992a,b; Chuang *et al.*, 1992; Dégano *et al.*, 1992, 1993; Fürnsinn *et al.*, 1992, 1994; Gebre-Medhin *et al.*, 1998; Gedulin *et al.*, 1992, 1993; Göke *et al.*, 1993, 1993; Inoue *et al.*, 1993; Kulkarni *et al.*, 1996; Leaming *et al.*, 1995; Lewis *et al.*, 1988; Marco and Silvestre, 1997; O'Harte *et al.*, 1998; Rodriguez-Gallardo *et al.*, 1995; Salas *et al.*, 1994, 1995; Sandler and Stridsberg, 1994; Silvestre *et al.*, 1992, 1993a,b, 1994, 1996, 1997; Smith and Bloom, 1995; Stridsberg *et al.*, 1993; Suzuki *et al.*, 1992; Wagoner *et al.*, 1992, 1993; Wang *et al.*, 1993, 1997; Young and Gedulin, unpublished; Young *et al.*, 1992a, 1993, 1994, 1995
No effect on insulin secretion	Ahren and Pettersson, 1990; Ar'Rajab and Ahren, 1991; Bretherton-Watt *et al.*, 1990; Broderick and Gold, 1991; Broderick *et al.*, 1991; Fehmann *et al.*, 1990; Ghatei *et al.*, 1990; Gilbey *et al.*, 1989; Gold *et al.*, 1990; Nagamatsu *et al.*, 1990a,b; O'Brien *et al.*, 1990; Pettersson and Ahren, 1990; Tedstone *et al.*, 1989, 1990	Barakat *et al.*, 1994; Nagamatsu *et al.*, 1992; Panagiotidis *et al.*, 1992; Wang *et al.*, 1997

the ligand disrupted insulinostatic activity. Stimulated insulin secretion in glucose-sensitive BRIN-BD11 cells was inhibited by amylin, but not if amylin was glycated at the N-terminal lysine (O'Harte *et al.*, 1998).

Other evidence for a direct hormonal effect on insulin secretion comes from studies of cell lines (principally rat, hamster, and mouse insulinoma lines; RIN, HIT, and β TC3) whose behavior is held to approximate that of primary β-cells. Initial studies in RIN m5F cells (Murakami *et al.*, 1990) showed a pattern of inhibition by amylin that suggested an effect on adenylate cyclase. These effects of amylin were dose dependent and occurred whether insulin secretion was stimulated by glyceraldehyde, isoproterenol,

arginine, or forskolin (Suzuki *et al.,* 1992). The observation that amylin inhibited GLP-1-stimulated cAMP production in Rin m5F cells (Göke *et al.,* 1993a,b) also supported an effect on the adenylate cyclase system. But in other studies in Rin m5F cells, amylin and CGRP were stimulatory, not inhibitory (Barakat *et al.,* 1994), and in HIT (Broderick and Gold, 1991; Broderick *et al.,* 1991) and βTC3 cells (Nagamatsu *et al.,* 1990a, 1992) no effect on insulin secretion was observed. A potential problem of β-cell lines is that they may not contain the full complement of modulators of secretion (including peptide hormone receptors) present in primary β-cells.

Insulin biosynthesis was dose-dependently inhibited by amylin, and stimulated by glucose, in RIN cells (Chuang *et al.,* 1992).

B. Isolated Pancreatic Islets

Reported effects of amylin agonists on insulin production by isolated pancreatic islets are heterogeneous. Some discrepancies may be explicable. In reports in which amylin inhibited nutrient-stimulated insulin secretion (Ohsawa *et al.,* 1989; Wang *et al.,* 1993), this occurred with amylin concentrations around 1 μM (Ar'Rajab and Ahren, 1991; Nagamatsu *et al.,* 1992) (Fig. 1). When no effect on isolated islets was observed (Broderick and Gold, 1991; Broderick *et al.,* 1991), amylin concentrations in the media were often below this range. Some authors, discounting an insulinostatic effect of amylin (Broderick and Gold, 1991; Broderick *et al.,* 1991; Tedstone *et al.,* 1989, 1990) used amylin acid (deamidated peptide), which had been separately shown to have low biological activity (Roberts *et al.,* 1989).

High nominal concentrations of amylin necessary to inhibit insulin secretion from isolated islets had led many authors (Ar'Rajab and Ahren, 1991; Broderick and Gold, 1991; Broderick *et al.,* 1991; Nagamatsu *et al.,* 1990a,b; Tedstone *et al.,* 1989, 1990) to conclude that the effect, if present, was unlikely to be physiologically relevant. The first study to examine the effect of amylin receptor antagonists on insulin secretion *in vivo* (Young *et al.,* 1992a) showed an augmentation of nutrient-stimulated insulin secretion, consistent with the removal of an inhibitory effect. These findings indicated that the conclusions of physiological irrelevance may have been premature. Amylin antagonists administered alone were subsequently shown to enhance insulin secretion in the same isolated islet preparations in which only elevated amylin concentrations (~1 μM) had previously shown insulin inhibitory effect (Wang *et al.,* 1993). Augmentation (disinhibition) of insulin secretion was subsequently also observed with administration of amylin receptor antagonists in isolated perfused pancreas preparations (Salas *et al.,* 1994; Silvestre *et al.,* 1996), in intact rats (Bennet *et al.,* 1994; Gedulin *et al.,* 1993; Young and Gedulin, unpublished; Young *et al.,* 1993, 1994), and in diabetic humans (Leaming *et al.,* 1995). All authors of those

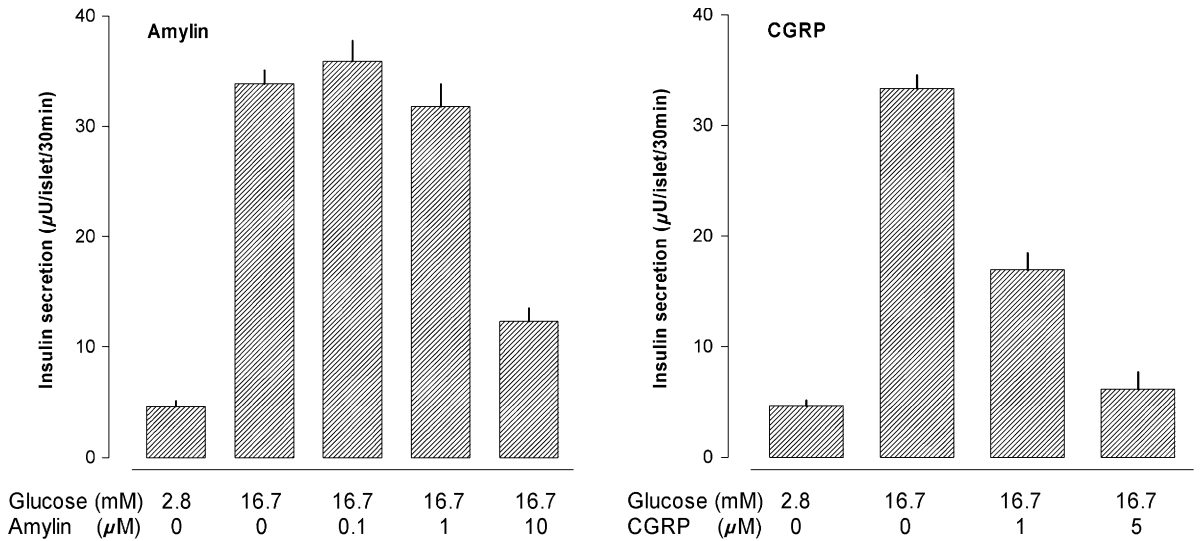

FIGURE 1 Effect of amylin (left panel) or CGRP (right panel) on glucose-stimulated insulin release from isolated rat islets. Data from Ohsawa *et al.* (1989).

reports concluded that endogenously secreted amylin was a physiological regulator of insulin secretion.

C. Isolated Perfused Pancreas Preparations

Most of the work in this field using the isolated perfused pancreas preparation has come from the laboratory of Marco (16 out of 20 reports: Dégano *et al.*, 1991, 1992, 1993; Marco *et al.*, 1990; Peiró *et al.*, 1991; Rodriguez-Gallardo *et al.*, 1995; Salas *et al.*, 1994, 1995; Silvestre *et al.*, 1990a,b, 1992, 1993a,b, 1994, 1996, 1997). However, Fehmann *et al.* were the first to publish on inhibition by amylin of insulin secretion from perfused pancreas (Fehmann *et al.*, 1990), followed by Marco *et al.* (Marco *et al.*, 1990; Silvestre *et al.*, 1990b). Fehmann *et al.* described an effect with only 10 pM rat amylin in the perfusate, a potency that few would have credited at the time. Later that same year, another group showed statistically significant inhibition of insulin response with an amylin concentration of 5 pM in the perfusate (O'Brien *et al.*, 1990), but since the effect disappeared with higher concentrations, they interpreted it as artifact. But in a careful dose–response study in the perfused pancreas, Dégano *et al.* (Dégano *et al.*, 1993) investigated the dose dependency of amylin inhibition of glucose-induced insulin release in the isolated perfused pancreas from non-fasted rats. In the first experiment, rat amylin was infused as a priming dose followed by 20 min infusions containing 7.5, 75, 750, 7500, and 75,000 pM amylin. Insulin release was stimulated by increasing glucose in the perfusate from 5.5 to 9 mM. Glucose-stimulated insulin secretion was reduced by up to 70% with perfusate amylin concentrations of 75 pM and above (the EC_{50} was calculable as ~40 pM) (Fig. 2). In a second experiment, restoration of glucose-stimulated insulin secretion after washout with an amylin-free perfusate precluded a durable toxic effect of amylin on the β cell.

Effects with amyloidogenic human amylin were observed in some conditions, but at high concentrations (Kogire *et al.*, 1991). The physicochemical properties of human amylin ensure that little remains in solution during pancreas perfusion, and it is not recommended in studies of amylin agonists (Rodriguez-Gallardo *et al.*, 1995). On the other hand, the robustness of data obtained from isolated perfused pancreas using rat amylin has enabled studies of mode of action. Inhibition of insulin secretion was not explained by changes in secretion of somatostatin, an insulinostatic agent (Peiró *et al.*, 1991), or glucagon, an insulinotropic agent (Silvestre *et al.*, 1990a). Observations in Rin m5F cells that amylin inhibited GLP-1-stimulated cAMP production in Rin m5F cells (Göke *et al.*, 1993a,b) supported an effect on the adenylate cyclase system.

In the perfused rat pancreas model, Silvestre *et al.* (Silvestre *et al.*, 1994), using several insulin secretogogues that stimulate secretion via the adenyl cyclase/cAMP system, also concluded that the inhibitory effect of amylin

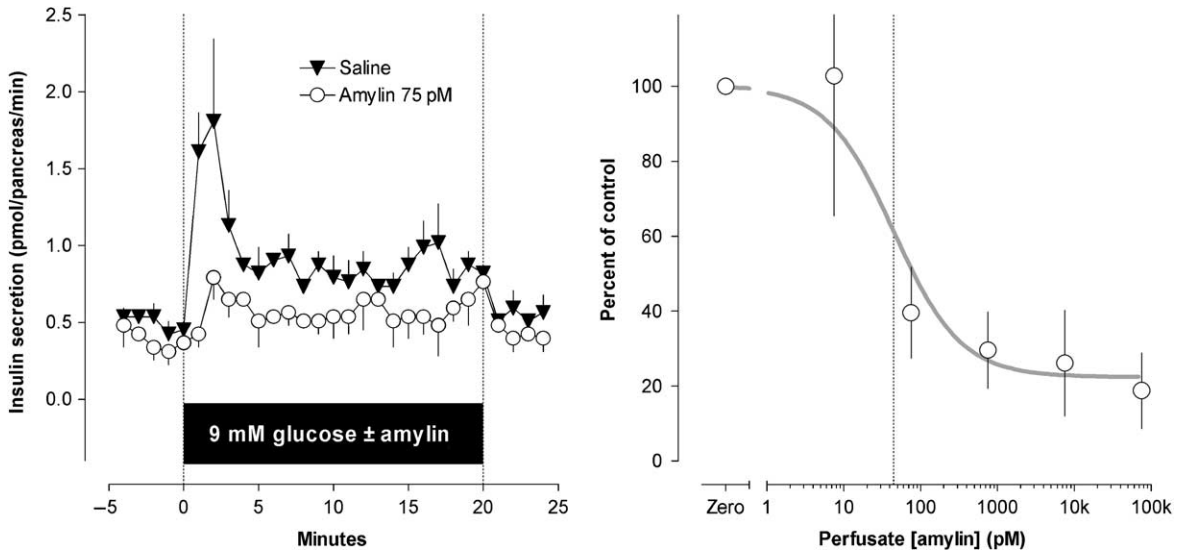

FIGURE 2 Effect of amylin on glucose-stimulated insulin secretion from perfused rat pancreas. Time course (left panel); concentration response (right panel); $EC_{50} = 45$ pM. Data derived from Dégano *et al.* (1993).

on insulin secretion may be via interference with this system. Following perfusion of either saline or amylin (75 pM) for 5 min, glucagon (10 nM), gastric inhibitory peptide (GIP, 1 nM), forskolin (1 μM), or isobutylmethyl xanthine (IBMX, 75 μM) was infused for up to 20 min, with or without amylin; insulin levels were measured before dosing and for up to 25 min afterward. Amylin inhibited the insulin response to glucagon, GIP, and IBMX by approximately 70, 90, and 75%, respectively, and also inhibited the early phase response for secretion stimulated by forskolin (~74%). Pertussis toxin interferes with signaling via G_i protein. The loss of amylin's inhibitory activity following treatment with pertussis toxin (Silvestre *et al.*, 1993a, 1994) implicates G_i in amylin's receptor-mediated action at β-cells to reduce cAMP signaling.

D. Whole Animals

When amylin was administered to intact animals, most reports described an inhibition of nutrient-stimulated insulin secretion (rats: Fürnsinn *et al.*, 1994; Gedulin *et al.*, 1992; Young and Gedulin, unpublished; cats: Johnson *et al.*, 1990). The amylin agonist salmon calcitonin had a similar action (Young *et al.*, 1995). However, no effect was observed in five reports. One of these used human amylin from a commercial batch shown to have low purity and biological activity (Lehman-deGaeta *et al.*, 1991). Aggregation of human amylin results in dissolution of as little as 1% of added material (Young *et al.*, 1992b). Other reports (Tedstone *et al.*, 1989, 1990) used not only the aggregable human sequence, but also the non-amidated form, shown to have at least 100-fold lower biological activity (Roberts *et al.*, 1989). A further report in which no difference in insulin or glucose profiles was observed over 9 days (Gold *et al.*, 1990) used non-amidated human amylin pressed into pellets with stearate and implanted subcutaneously. In these latter cases, it is doubtful if significant immunoreactive (or biologically active) material circulated. Plasma concentrations were not reported. A group previously working with human amylin (Ghatei *et al.*, 1990) reported an absence of effect with amidated and non-amidated amylins (species not described) on insulin secretion in rabbits. Nonetheless, there was one study for which no ready explanation of lack of activity was apparent. Rat amylin (amidated) was infused continuously at a high rate (~1 μg/kg/min) into rats without observable effects on either glucose or insulin profiles (Ar'Rajab and Ahren, 1991).

The effect of amylin on insulin secretion in non-fasted conscious rats in a hyperglycemic clamp model was reported by Fürnsinn *et al.* (Fürnsinn *et al.*, 1994). Rats were infused with saline or with amylin (8.5 or 85 pmol/min for 2 hr; 85 pmol/min for 24 hr), which was predicted to result in supraphysiological plasma concentrations of 0.5 and 7.2 nM, respectively (Young *et al.*, 1996). Glucose-stimulated increments in plasma insulin concentration

were reduced by 31 and 53%, respectively, following 2 hr infusions of 8.5 and 85 pmol/min of amylin, but no insulinostatic effect was observed following 24 hr of high-dose infusion (Fürnsinn *et al.*, 1994). In another study in rats, in which amylin was infused at 50 and 250 μg/hr, insulin responses to a 5-mmol/kg intravenous glucose bolus were reduced by 20 and 37%, respectively (Gedulin *et al.*, 1992). At an infusion rate of 5 μg/hr, rat amylin inhibited the 60-min insulin response to infused glucose by 40% (Young *et al.*, 1995). Salmon calcitonin, an amylin agonist, infused at 5.5 μg/hr also inhibited insulin secretion in the same model (Young *et al.*, 1995).

The insulinostatic effects of amylin (25 μg/hr infusion) were compared in 18 hr fasted and fed rats (Young and Gedulin, unpublished). Secretory responses were characterized by the slope of the regression of insulin versus prevailing plasma glucose. The slope, or "insulinogenic index," thus obtained, was 2.2-fold higher in fed than in fasted rats. The reduction in slope with amylin was greater (55%) in fasted rats, in which prevailing amylin was low, than in fed rats (12% reduction).

IV. Pharmacology of Insulinostatic Effect

A. Amylin Antagonists

Plasma amylin concentrations were predicted to have remained above the physiological range in all of the *in vivo* studies of amylin's insulinostatic effect, leaving the physiological relevance of this effect unresolved. Such issues can be amended using receptor antagonists, when they are available, to block the effects of endogenous hormone.

The insulinostatic actions of endogenous amylin were first probed by Young *et al.* (Young *et al.*, 1992a) using CGRP[8–37], previously shown (Wang *et al.*, 1991) to block the effects of exogenous amylin. Insulin secretion in response to infused L-arginine (2 mmol) was examined with and without CGRP[8–37] in fed rats. In CGRP[8–37]-treated animals, plasma insulin concentration was 2- to 3-fold higher than in saline-infused controls, consistent with blockade of an insulin inhibitory effect of endogenous amylin. Because CGRP[8–37] does not exclusively block amylin receptors, but also blocks CGRP receptors, the experiment was repeated using AC66 (salmon calcitonin[8–32]), which blocks amylin and calcitonin, but not CGRP, receptors. A similar augmentation of arginine-stimulated insulin secretion was observed (Young *et al.*, 1992a), indicating that the insulinostatic effect was mediated via amylin-like receptors rather than CGRP-like receptors.

Augmentation (disinhibition) of insulin secretion has subsequently been observed with administration of various amylin receptor antagonists in

isolated perfused pancreas preparations (Salas *et al.*, 1994; Silvestre *et al.*, 1996), in rats (Bennet *et al.*, 1994; Young *et al.*, 1994), and in humans (Leaming *et al.*, 1995). In perfused pancreas, addition of 10 μM AC66 increased glucose-stimulated insulin secretion 2.5-fold (Salas *et al.*, 1994) without changing glucagon or somatostatin secretion (Silvestre *et al.*, 1996). In rats infused with amylin[8–37] (an amylin receptor antagonist), the insulin response to an arginine challenge was 2-fold higher than in saline-infused controls (Bennet *et al.*, 1994). A 1.8-fold increase in glucose-stimulated insulin secretion was observed in rats pre-infused with the amylin antagonist AC187 (Young *et al.*, 1994). Several researchers have thus concluded that endogenous amylin tonically inhibits β-cell secretion.

In a clinical study (Leaming *et al.*, 1995; Mather *et al.*, 2002), the amylin antagonist AC253 caused a 44% increase in insulin response during a hyperglycemic clamp in obese (hyperamylinemic) subjects (Leaming *et al.*, 1995), but had no effect on insulin secretion in a comparatively amylin-deficient weight-matched type 2 diabetic cohort (Fig. 3). That result is somewhat similar to observations in fasted and fed rats (Young and Gedulin, unpublished), where AC187 significantly augmented insulin secretion in fed (amylin replete) rats (+24%, $P < 0.002$), but not in fasted (amylin-depleted) rats (+5%, ns). That is, amylin blockers best act in amylin-replete situations (e.g., fed state, non-diabetic state) but not in relatively amylin-depleted situations (e.g., fasted state, diabetic state).

B. Amylin Agonists

Bretherton-Watt *et al.* infused synthetic human amylin into seven normal volunteers at a rate of 50 pmol/kg/min prior to, and in association with, a 0.5 g/kg intravenous glucose challenge (Bretherton-Watt *et al.*, 1990) but observed no difference in insulin response with or without amylin. In an abstract, they reported that infusion at 150 pmol/kg/min also had no effect on insulin secretion (Gilbey *et al.*, 1989), but subsequently noted in a full publication (Bretherton-Watt *et al.*, 1990) that 86 $\pm$ 2% of the human amylin peptide was lost onto the infusion set tubing. Although plasma immunoreactivity reached 1330 $\pm$ 90 pM, orders of magnitude above physiological concentrations, there was no indication that the immunoreactive material was biologically active. For example, there was no difference in plasma glucagon profiles, and there were no reports of nausea, which would have been expected at such concentrations. In a separate report (Bretherton-Watt *et al.*, 1992b), the same authors investigated the effects of 25 and 50 μg/hr infusions of human amylin on glucose-stimulated insulin secretion in six normal individuals. Peptide losses to tubing were lower (29, 26%) than in the previous study, and plasma amylin concentrations were higher. The highest infusion rate caused nausea, so it is presumed that biologically

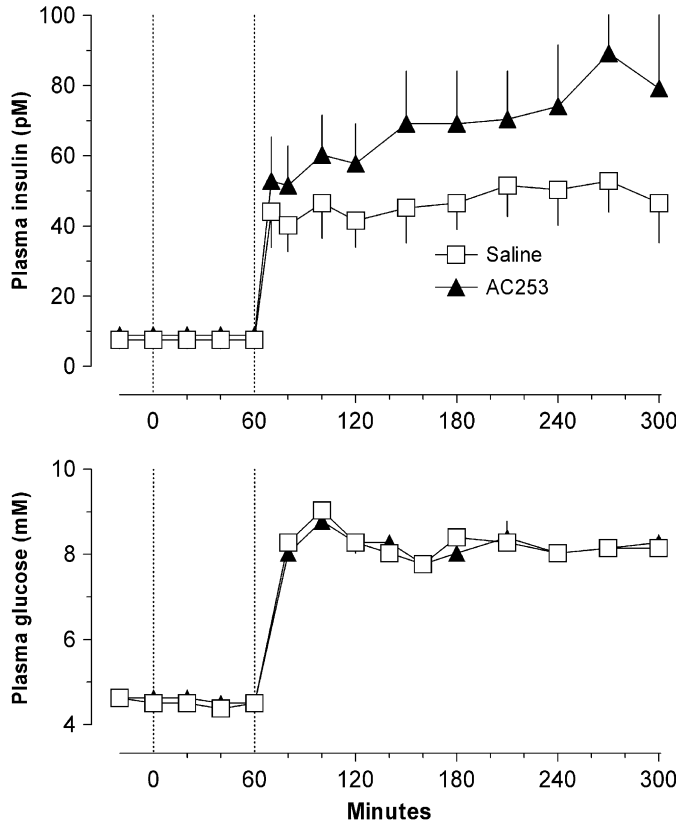

FIGURE 3 Effect of the amylin antagonist AC253 on glucose-stimulated insulin response in obese, glucose-tolerant humans. Data from Mather *et al.* (2002).

active material was present. At the high dose, human amylin inhibited insulin AUC by 35.7% ($P < 0.01$).

Stridsberg *et al.* described a patient with an insulinoma secreting an amylin-like substance (Stridsberg *et al.*, 1992). The molecular nature of the 15,000–25,000 pM circulating immunoreactivity was not known other than that it had a molecular weight of ~6300 Da; it may have therefore been a pro-peptide (Stridsberg *et al.*, 1993). Plasma diluted to 10% from this patient inhibited glucose-stimulated insulin release from cultured islets by 27% compared to plasma from normal subjects. This subject had normal insulin sensitivity, as measured by a euglycemic clamp, but failed to increase plasma insulin concentration during an intravenous glucose challenge (Stridsberg *et al.*, 1993).

Salmon calcitonin acutely inhibited nutrient-stimulated insulin secretion in humans (Cantalamessa *et al.*, 1978; Lunetta *et al.*, 1981; Petralito *et al.*,

1979). Salmon calcitonin inhibited insulin secreted in response to a mixed meal by 70%, although part of this reduction may have been secondary to a simultaneously observed slowing of gastric emptying (and reduction in nutrient assimilation) (Jonderko *et al.,* 1990). This was shown to be independent of effects on gastric emptying in rats, in which salmon calcitonin blunted the insulin response to an intravenous glucose challenge (Young *et al.,* 1995). Responsivity to salmon calcitonin established this response as being independent of CGRP receptors (at which salmon calcitonin does not act).

The literature on the insulinostatic effect of CGRP is variable. In human studies, CGRP did not affect arginine-stimulated insulin secretion (Ahren, 1990; Beglinger *et al.,* 1988; Edwards and Bloom, 1994). CGRP did inhibit insulin secretion stimulated by arginine in rats (Pettersson and Ahren, 1988) and insulin secreted in response to various stimuli in mice (Pettersson *et al.,* 1987). CGRP had no effect on insulin release from neonatal rat islets but inhibited the response in islets from adult rats (Ishizuka *et al.,* 1988), while adrenomedullin, an agonist for CGRPergic but not calcitoninergic or amylinergic responses (Vine *et al.,* 1996), did not inhibit insulin secretion from islets (Mulder *et al.,* 1996). It is possible that CGRP may act at calcitonin or amylin receptors (at which it is a weak agonist) to inhibit insulin secretion. An absence of effect of adrenomedullin (which acts at CGRP and adrenomedullin but not amylin or calcitonin receptors) thus fits with this interpretation.

In healthy humans, in addition to salmon calcitonin, mammalian calcitonins (porcine and/or human) also inhibit arginine-stimulated insulin secretion (Sgambato *et al.,* 1986; Stevenson *et al.,* 1985). This deviates somewhat from a purely amylinergic pattern, in which mammalian calcitonins are much less potent than teleost (eel and salmon) calcitonins. It is possible that calcitonin receptors mediate feedback inhibition of insulin secretion, for which amylin is a cognate ligand.

V. Localization of Effects on Insulin Secretion

Studies performed in isolated β-cells (Chuang *et al.,* 1992; Murakami *et al.,* 1990; Suzuki *et al.,* 1992; Wagoner *et al.,* 1993), isolated islets (Ohsawa *et al.,* 1989; Wang *et al.,* 1993), and the isolated perfused pancreas (Dégano *et al.,* 1991, 1992, 1993; Fehmann *et al.,* 1990; Marco *et al.,* 1990; Peiró *et al.,* 1991; Rodriguez-Gallardo *et al.,* 1995; Salas *et al.,* 1994, 1995; Silvestre *et al.,* 1990a,b, 1992, 1993a,b, 1994, 1996, 1997) clearly support a direct insulinostatic effect of amylin. However, the possibility also exists that amylin modulation of islet secretion, including that of insulin, also involved extrapancreatic mechanisms. It has been shown, for example, that the inhibition of nutrient-stimulated glucagon secretion by amylin

involves a pathway extrinsic to the pancreas (Silvestre *et al.*, 2001). A series of experiments conducted by Edwards *et al.* in calves (Adrian *et al.*, 1983; Bloom and Edwards, 1981, 1984, 1985a,b; Bloom *et al.*, 1984) pointed strongly to a central contribution, via the vagus, in the control of insulin secretion. Consistent with those findings, a preliminary communication (Young *et al.*, 2004) indicated that the area postrema in rats may be a source of nutrient-related and hormone-modulated autonomic control of insulin secretion. Since most neurons therein are amylin sensitive (Riediger *et al.*, 1999, 2002), it is possible that some portion of amylin's insulinostatic action (and that of other β-cell products) may be autonomically mediated via receptors in the area postrema.

References

Adrian, T. E., Bloom, S. R., and Edwards, A. V. (1983). Neuroendocrine responses to stimulation of the vagus nerves in bursts in conscious calves. *J. Physiol. (Lond.)* **344**, 25–35.

Ahren, B. (1990). Effects of galanin and calcitonin gene-related peptide on insulin and glucagon secretion in man. *Acta Endocrinol. (Copenh.)* **123**, 591–597.

Ahren, B., and Pettersson, M. (1990). Calcitonin gene-related peptide (CGRP) and amylin and the endocrine pancreas. *Int. J. Pancreatol.* **6**, 1–15.

Ahren, B., Oosterwijk, C., Lips, C. J., and Hoppener, J. W. (1998). Transgenic overexpression of human islet amyloid polypeptide inhibits insulin secretion and glucose elimination after gastric glucose gavage in mice. *Diabetologia* **41**, 1374–1380.

Ar'Rajab, A., and Ahren, B. (1991). Effects of amidated rat islet amyloid polypeptide on glucose-stimulated insulin secretion *in vivo* and *in vitro* in rats. *Eur. J. Pharmacol.* **192**, 443–445.

Barakat, A., Skoglund, G., Boissard, C., Rosselin, G., and Marie, J. C. (1994). Calcitonin gene-related peptide and islet amyloid polypeptide stimulate insulin secretion in RINm5F cells through a common receptor coupled to a generation of cAMP. *Biosci. Rep.* **14**, 1–13.

Beglinger, C., Koehler, E., Born, W., Fischer, J. A., Keller, U., Hanssen, L. E., and Gyr, K. (1988). Effect of calcitonin and calcitonin gene-related peptide on pancreatic functions in man. *Gut* **29**, 243–248.

Bendayan, M. (1993). Pathway of insulin in pancreatic tissue on its release by the B-cell. *Am. J. Physiol.* **264**, G187–G194.

Bennet, W. M., Beis, C. S., Ghatei, M. A., Byfield, P. G. H., and Bloom, S. R. (1994). Amylin tonally regulates arginine-stimulated insulin secretion in rats. *Diabetologia* **37**, 436–438.

Bloom, S. R. (1994). Paracrine regulation of regulatory peptides. *Biomed. Res.* **15**, 1–4.

Bloom, S. R., and Edwards, A. V. (1981). The role of the parasympathetic system in the control of insulin release in the conscious calf. *J. Physiol. (Lond.)* **314**, 37–46.

Bloom, S. R., and Edwards, A. V. (1984). Characteristics of the neuroendocrine responses to stimulation of the splanchnic nerves in bursts in the conscious calf. *J. Physiol. (Lond.)* **346**, 533–545.

Bloom, S. R., and Edwards, A. V. (1985). Effects of certain metabolites on pancreatic endocrine responses to stimulation of the vagus nerves in conscious calves. *J. Physiol. (Lond.)* **362**, 303–310.

Bloom, S. R., and Edwards, A. V. (1985). The role of the sympathetic system in the control of insulin release in response to hyperglycaemia in conscious calves. *J. Physiol. (Lond.)* **362**, 311–317.

Bloom, S. R., Edwards, A. V., and Ghatei, M. A. (1984). Neuroendocrine responses to stimulation of the splanchnic nerves in bursts in the conscious adrenalectomized calf. *J. Physiol. (Lond.)* **346**, 519–531.

Bretherton-Watt, D., Gilbey, S. G., Ghatei, M. A., Beacham, J., and Bloom, S. R. (1990). Failure to establish islet amyloid polypeptide (amylin) as a circulating beta cell inhibiting hormone in man. *Diabetologia* **33**, 115–117.

Bretherton-Watt, D., Ghatei, M. A., Jamal, H., Gilbey, S. G., Jones, P. M., and Bloom, S. R. (1992a). The physiology of calcitonin gene-related peptide in the islet compared with that of islet amyloid polypeptide (amylin). *Ann. N Y Acad. Sci.* **657**, 299–312.

Bretherton-Watt, D., Gilbey, S. G., Ghatei, M. A., Beacham, J., Macrae, A. D., and Bloom, S. R. (1992b). Very high concentrations of islet amyloid polypeptide are necessary to alter the insulin response to intravenous glucose in man. *J. Clin. Endocrinol. Metab.* **74**, 1032–1035.

Broderick, C. L., Brooke, G. S., DiMarchi, R. D., and Gold, G. (1991). Human and rat amylin have no effects on insulin secretion in isolated rat pancreatic islets. *Biochem. Biophys. Res. Commun.* **177**, 932–938.

Broderick, C. L., and Gold, G. (1991). Human and rat amylin have no effect on insulin secretion in isolated rat islets or in HIT cells. *Diabetes* **40**, 233A.

Cantalamessa, L., Cantania, A., Reschini, E., and Peracchi, M. (1978). Inhibitory effect of calcitonin on growth hormone and insulin secretion in man. *Metabolism* **27**, 987–992.

Chuang, L. M., Wu, H. P., Jou, T. S., Tai, T. Y., and Lin, B. J. I. (1992). Inhibitory effect of islet amyloid polypeptide on glucose-induced proinsulin biosynthesis in rat insulinoma cells. *Pancreas* **7**, 472–476.

Dégano, P., Peiro, E., Silvestre, R. A., Coma, I., Salas, M., and Marco, J. (1991). Insulin responses of the amylin-infused rat pancreas to glucose vasoactive intestinal polypeptide and arginine. *Diabetologia* **34**, A42.

Dégano, P., Salas, M., Peiro, E., Silvestre, R. A., and Marco, J. (1992). On the mechanism of the inhibitory effect of amylin on insulin secretion: Study in the perfused rat pancreas. *Diabetologia* **35**, A115.

Dégano, P., Silvestre, R. A., Salas, M., Peiró, E., and Marco, J. (1993). Amylin inhibits glucose-induced insulin secretion in a dose-dependent manner. Study in the perfused rat pancreas. *Regul. Pept.* **43**, 91–96.

Edwards, A. V., and Bloom, S. R. (1994). Pancreatic endocrine responses to substance P and calcitonin gene-related peptide in conscious calves. *Am. J. Physiol.* **30**, E847–E852.

Fehmann, H. C., Weber, V., Göke, R., Göke, B., Eissele, R., and Arnold, R. (1990). Islet amyloid polypeptide (IAPP; amylin) influences the endocrine but not the exocrine rat pancreas. *Biochem. Biophys. Res. Commun.* **167**, 1102–1108.

Fürnsinn, C., Leuvenink, H., Roden, M., Nowotny, P., and Waldhäusl, W. (1992). Inhibition of glucose induced insulin secretion by amylin in rats *in vivo*. *Diabetologia* **35**, A29.

Fürnsinn, C., Leuvenink, H., Roden, M., Nowotny, P., Schneider, B., Rohac, M., Pieber, T., Clodi, M., and Waldhausl, W. (1994). Islet amyloid polypeptide inhibits insulin secretion in conscious rats. *Am. J. Physiol.* **267**, E300–E305.

Gebre-Medhin, S., Mulder, H., Pekny, M., Westermark, G., Tornell, J., Westermark, P., Sundler, F., Ahren, B., and Betsholtz, C. (1998). Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). *Biochem. Biophys. Res. Commun.* **250**, 271–277.

Gedulin, B., Rink, T. J., Larson, E., and Young, A. A. (1992). Effects of amylin infusion on plasma glucose and insulin during i.v. glucose tolerance test in anesthetized rats.

Gedulin, B., Larson, E., Provost, S., and Koda, J. (1993). The selective amylin antagonist, AC187, enhances the insulin response during intravenous glucose tolerance tests in anesthetized rats. *Diabetes* **42**(suppl. 1), 229A.

Ghatei, M. A., Datta, H. K., Zaidi, M., Bretherton-Watt, D., Wimalawansa, S. J., Mac Intyre, I., and Bloom, S. R. (1990). Amylin and amylin-amide lack an acute effect on blood glucose and insulin. *J. Endocrinol.* **124**, R9–R11.

Gilbey, S. G., Bretherton-Watt, D., Beacham, J., Ghatei, M. A., and Bloom, S. R. (1989). High dose amylin in man: Unexpected failure to affect intravenous glucose tolerance. *BDA Diab. Med.* **6**, 5A.

Göke, R., McGregor, G. P., and Göke, B. (1993a). Amylin alters biological effects of GLP-1 in the ß-cell. *Digestion* **54**, 355–356.

Göke, R., McGregor, G. P., and Göke, B. (1993b). Amylin alters the biological action of the incretin hormone GLP-1 (7–36)amide. *Life Sci.* **53**, 1367–1372.

Gold, G., Dimarchi, R. D., Broderick, C. L., Bue, J. M., Brooke, G. S., and Yen, T. T. (1990). Human amylin has no effect on either rate of insulin release or concentration of glucose in rodents. *Diabetes* **39**, 142A.

Inoue, K., Hiramatsu, S., Hisatomi, A., Umeda, F., and Nawata, H. (1993). Effects of amylin on the release of insulin and glucagon from the perfused rat pancreas. *Horm. Metab. Res.* **25**, 135–137.

Ishizuka, J., Singh, P., Greeley, G. H., Jr., Townsend, C. M., Jr., Cooper, C. W., Tatemoto, K., and Thompson, J. C. (1988). A comparison of the insulinotropic and the insulin-inhibitory actions of gut peptides on newborn and adult rat islet cells. *Pancreas* **3**, 77–82.

Johnson, K. H., O'Brien, T. D., Jordan, K., Betsholtz, C., and Westermark, P. (1990). The putative hormone islet amyloid polypeptide (IAPP) induces impaired glucose tolerance in cats. *Biochem. Biophys. Res. Commun.* **167**, 507–513.

Jonderko, K., Jonderko, G., and Golab, T. (1990). Effect of calcitonin on gastric emptying and on serum insulin and gastrin concentrations after ingestion of a mixed solid-liquid meal in humans. *J. Clin. Gastroenterol.* **12**, 22–28.

Kogire, M., Ishizuka, J., Thompson, J. C., and Greeley, G. H. (1991). Inhibitory action of islet amyloid polypeptide and calcitonin gene-related peptide on release of insulin from the isolated perfused rat pancreas. *Pancreas* **6**, 459–463.

Kulkarni, R. N., Smith, D. M., Ghatei, M. A., Jones, P. M., and Bloom, S. R. (1996). Investigation of the effects of antisense oligodeoxynucleotides to islet amyloid polypeptide mRNA on insulin release, content and expression. *J. Endocrinol.* **151**, 341–348.

Leaming, R., Johnson, A., Hook, G., Hanley, R., and Baron, A. (1995). Amylin modulates insulin secretion in humans. Studies with an amylin antagonist. *Diabetologia* **38**, A113.

Lehman-deGaeta, L. S., Willis, A. C., Young, A. A., Foot, E. A., Albrecht, E., Leighton, B., Rink, T. J., and Cooper, G. J. S. (1991). Variability of chemically synthesized human amylin. *Diabetes* **40**, 236A.

Lewis, C. E., Clark, A., Ashcroft, S. J., Cooper, G. J. S., and Morris, J. F. (1988). Calcitonin gene-related peptide and somatostatin inhibit insulin release from individual rat B cells. *Mol. Cell Endocrinol.* **57**, 41–49.

Lorenzo, A., and Yankner, B. A. (1994). Beta-amyloid neurotoxicity requires fibril formation and is inhibited by Congo red. *Proc. Natl. Acad. Sci. USA* **91**, 12243–12247.

Lorenzo, A., Razzaboni, B., Weir, G. C., and Yankner, B. A. (1994). Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* **368**, 756–760.

Lunetta, M., Infantone, E., Spanti, D., and Mughini, L. (1981). Effects of synthetic salmon calcitonin administration on gastrin, immunoreactive insulin and growth hormone release after protein meal in uremic patients. *J. Endocrinol. Invest.* **4**, 185–188.

Marco, J., and Silvestre, R. (1997). Effect of amylin on islet cell secretion. *Exp. Clin. Endocrinol. Diabet.* **105**, 68.

Marco, J., Peiro, E., Dégano, P., Miralles, P., and Silvestre, R. E. (1990). Amylin inhibits insulin secretion in the perfused rat pancreas. *Diabetes* **39**, 136A.

Mather, K. J., Paradisi, G., Leaming, R., Hook, G., Steinberg, H. O., Fineberg, N., Hanley, R., and Baron, A. D. (2002). Role of amylin in insulin secretion and action in humans: Antagonist studies across the spectrum of insulin sensitivity. *Diabet. Metab. Res. Rev.* **18**, 118–126.

May, P. C., Boggs, L. N., and Fuson, K. S. (1993). Neurotoxicity of human amylin in rat primary hippocampal cultures—similarity to Alzheimer's disease amyloid-beta neurotoxicity. *J. Neurochem.* **61**, 2330–2333.

Mulder, H., Ahren, B., Karlsson, S., and Sundler, F. (1996). Adrenomedullin: Localization in the gastrointestinal tract and effects on insulin secretion. *Regul. Pept.* **62**, 107–112.

Murakami, M., Suzuki, S., Sato, Y., Shintami, S., Abe, S., Suzuki, K., Ishuzuka, J., and Toyota, T. (1990). Effects of amylin on insulin secretion from RIN m5F cells. *Diabetes* **39**, 266A.

Nagamatsu, S., Carroll, R., and Steiner, D. F. (1990). IAPP effects on pancreatic beta cell function in normal rat islets and BTC3 cell line. *Diabetes* **39**, 67A.

Nagamatsu, S., Carroll, R. J., Grodsky, G. M., and Steiner, D. F. (1990). Lack of islet amyloid polypeptide regulation of insulin biosynthesis or secretion in normal rat islets. *Diabetes* **39**, 871–874.

Nagamatsu, S., Nishi, M., and Steiner, D. F. (1992). Effects of islet amyloid polypeptide (IAPP) on insulin biosynthesis or secretion in rat islets and mouse beta TC3 cells. Biosynthesis of IAPP in mouse beta TC3 cells. *Diabet. Res. Clin. Pract.* **15**, 49–55.

O'Brien, T. D., Westermark, P., and Johnson, K. H. (1990). Islet amyloid polypeptide (IAPP) does not inhibit glucose-stimulated insulin secretion from isolated perfused rat pancreas. *Biochem. Biophys. Res. Commun.* **170**, 1223–1228.

O'Harte, F. P. M., Abdel Wahab, Y. H. A., Conlon, J. M., and Flatt, P. R. (1998). Glycated IAPP shows a reduced inhibitory action on insulin secretion. *Biochem. Soc. Trans.* **26**, S6.

Ohsawa, H., Kanatsuka, A., Yamaguchi, T., Makino, H., and Yoshida, S. (1989). Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets. *Biochem. Biophys. Res. Commun.* **160**, 961–967.

Panagiotidis, G., Salehi, A. A., Westermark, P., and Lundquist, I. (1992). Homologous islet amyloid polypeptide: Effects on plasma levels of glucagon, insulin and glucose in the mouse. *Diabet. Res. Clin. Pract.* **18**, 167–171.

Peiró, E., Dégano, P., Silvestre, R. A., and Marco, J. (1991). Inhibition of insulin release by amylin is not mediated by changes in somatostatin output. *Life Sci.* **49**, 761–765.

Petralito, A., Lunetta, M., Liuzzo, A., Fiore, C. E., and Heynen, G. (1979). Effects of salmon calcitonin on blood glucose and insulin levels under basal conditions after intravenous glucose load. *J. Endocrinol. Invest.* **2**, 209–211.

Pettersson, M., and Ahren, B. (1988). Insulin and glucagon secretion in rats: Effects of calcitonin gene-related peptide. *Regul. Pept.* **23**, 37–50.

Pettersson, M., and Ahren, B. (1990). Failure of islet amyloid polypeptide to inhibit basal and glucose-stimulated insulin secretion in model experiments in mice and rats. *Acta Physiol. Scand.* **138**, 389–394.

Pettersson, M., Lundquist, I., and Ahren, B. (1987). Neuropeptide Y and calcitonin gene-related peptide: Effects on glucagon and insulin secretion in the mouse. *Endocr. Res.* **13**, 407–417.

Riediger, T., Rauch, M., Jurat, G., and Schmid, H. A. (1999). Central nervous targets for pancreatic amylin. *Pflugers Arch.* **437**, R142.

Riediger, T., Schmid, H. A., Lutz, T. A., and Simon, E. (2002). Amylin and glucose co-activate area postrema neurons of the rat. *Neurosci. Lett.* **328**, 121–124.

Roberts, A., Leighton, B., Todd, J. A., Cockburn, D., Schofield, P. N., Sutton, R., Holt, S., Boyd, Y., Day, A. J., Foot, E. A., Willis, A. C., Reid, K. B. M., and Cooper, G. J. S. (1989).

Molecular and functional characterization of amylin, a peptide associated with type 2 diabetes mellitus. *Proc. Natl. Acad. Sci. USA* **86**, 9662–9666.

Rodriguez-Gallardo, J., Silvestre, R. A., Salas, M., and Marco, J. (1995). Rat amylin versus human amylin: Effects on insulin secretion in the perfused rat pancreas. *Med. Sci. Res.* **23**, 569–570.

Salas, M., Silvestre, R. A., Gutiérrez, E., Fontels, T., Garcia-Hermida, O., and Marco, J. (1994). Potentiation of the insulin response to glucose by an amylin antagonist (8–32 salmon calcitonin). *Diabetologia* **37**, A116.

Salas, M., Silvestre, R. A., Garcia-Hermida, O., Fontela, T., Rodriguez-Gallardo, J., and Marco, J. (1995). Inhibitory effect of amylin (islet amyloid polypeptide) on insulin response to non-glucose stimuli: Study in perfused rat pancreas. *Diabet. Metab.* **21**, 269–273.

Sandler, S., and Stridsberg, M. (1994). Chronic exposure of cultured rat pancreatic islets to elevated concentrations of islet amyloid polypeptide (IAPP) causes a decrease in islet DNA content and medium insulin accumulation. *Regul. Pept.* **53**, 103–109.

Sgambato, S., Passariello, N., Paolisso, G., Marano, A., Buoninconti, R., and Tesauro, P. (1986). Effect of human calcitonin (hCT) on glucose- and arginine-stimulated insulin secretion. *Acta Diabetol. Lat.* **23**, 13–22.

Silvestre, R. A., Peiro, E., Dégano, P., Iglesias, A., Miralles, P., and Marco, J. (1990a). Rat amylin inhibits insulin release without affecting glucagon and somatostatin output in the rat pancreas. *Diabetologia* **33**, A40.

Silvestre, R. A., Peiró, E., Dégano, P., Miralles, P., and Marco, J. (1990b). Inhibitory effect of rat amylin on the insulin responses to glucose and arginine in the perfused rat pancreas. *Regul. Pept.* **31**, 23–31.

Silvestre, R. A., Dégano, P., Salas, M., Peiro, E., and Marco, J. (1992). Calcitonin gene-related peptide (CGRP) and amylin inhibition of insulin release is reversed by the 8–37 CGRP fragment. *Diabetologia* **35**, A29.

Silvestre, R. A., Salas, M., Dégaño, P., Fontela, T., García-Hermida, O., and Marco, J. (1993a). Prevention of the inhibitory effect of amylin on GIP-induced insulin release by Pertussis toxin pretreatment. *Diabetologia* **36**, A136.

Silvestre, R. A., Salas, M., Degano, P., Peiro, E., and Marco, J. (1993b). Reversal of the inhibitory effects of calcitonin gene-related peptide (CGRP) and amylin on insulin secretion by the 8–37 fragment of human CGRP. *Biochem. Pharmacol.* **45**, 2343–2347.

Silvestre, R. A., Salas, M., Garcia-Hermida, O., Fontela, T., Degano, P., and Marco, J. (1994). Amylin (islet amyloid polypeptide) inhibition of insulin release in the perfused rat pancreas: Implication of the adenylate cyclase/cAMP system. *Regul. Pept.* **50**, 193–199.

Silvestre, R. A., Salas, M., Rodriguez-Gallardo, J., Garcia-Hermida, O., Fontela, T., and Marco, J. (1996). Effect of (8–32). salmon calcitonin, an amylin antagonist, on insulin, glucagon and somatostatin release: Study in the perfused pancreas of the rat. *Br. J. Pharmacol.* **117**, 347–350.

Silvestre, R. A., Rodríguez-Gallardo, J., Gutiérrez, E., and Marco, J. (1997). Influence of glucose concentration on the inhibitory effect of amylin on insulin secretion: Study in the perfused rat pancreas. *Regul. Pept.* **68**, 31–35.

Silvestre, R. A., Rodriguez-Gallardo, J., Jodka, C., Parkes, D. G., Pittner, R. A., Young, A. A., and Marco, J. (2001). Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas. *Am. J. Physiol.* **280**, E443–E449.

Smith, D. M., and Bloom, S. R. (1995). Paracrine/autocrine control of the islet and the amylin family. *Biochem. Soc. Trans.* **23**, 336–340.

Stevenson, J. C., Adrian, T. E., Christofides, N. D., and Bloom, S. R. (1985). Effect of calcitonin on gastrointestinal regulatory peptides in man. *Clin. Endocrinol. (Oxf.)* **22**, 655–660.

Stridsberg, M., Wilander, E., Öberg, K., Lundqvist, G., and Eriksson, B. (1992). Islet amyloid polypeptide-producing pancreatic islet cell tumor. *Scand. J. Gastroenterol.* **27**, 381–387.

Stridsberg, M., Berne, C., Sandler, S., Wilander, E., and Oberg, K. (1993). Inhibition of insulin secretion, but normal peripheral insulin sensitivity, in a patient with a malignant endocrine pancreatic tumour producing high amounts of an islet amyloid polypeptide-like molecule. *Diabetologia* **36**, 843–849.

Suzuki, S., Murakami, M., Abe, S., Satoh, Y., Shintani, S., Ishizuka, J., Suzuki, K., Thompson, J. C., and Toyota, T. (1992). The effects of amylin on insulin secretion from Rin m5F cells and glycogen synthesis and lipogenesis in rat primary cultured hepatocytes. *Diabet. Res. Clin. Pract.* **15**, 77–84.

Tedstone, A. E., Nezzer, T., Hughes, S. J., Clark, A., and Matthews, D. R. (1989). The effects of islet amyloid peptide and calcitonin gene-related peptide on insulin secretion in anaesthetised rats and from isolated rat islets. *BDA Diabet. Med.* **6**, A38.

Tedstone, A. E., Nezzer, T., Hughes, S. J., Clark, A., and Matthews, D. R. (1990). The effect of islet amyloid polypeptide (amylin) and calcitonin gene-related peptide on glucose removal in the anaesthetized rat and on insulin secretion from rat pancreatic islets *in vitro*. *Biosci. Rep.* **10**, 339–345.

Vine, W., Beaumont, K., Gedulin, B., Pittner, R., Moore, C. X., Rink, T. J., and Young, A. A. (1996). Comparison of the *in vitro* and *in vivo* pharmacology of adrenomedullin, calcitonin gene-related peptide and amylin in rats. *Eur. J. Pharmacol.* **314**, 115–121.

Wagoner, P. K., Dukes, I. D., and Worley, J. F., III (1992). Amylin inhibition of glucose-induced changes in electrical activity, intracellular calcium and insulin release in mouse beta-cells. *Diabetes* **41**, 103A.

Wagoner, P. K., Chen, C., Worley, J. F., Dukes, I. D., and Oxford, G. S. (1993). Amylin modulates ß-cell glucose sensing via effects on stimulus-secretion coupling. *Proc. Natl. Acad. Sci. USA* **90**, 9145–9149.

Wang, F., Westermark, G., Gasslander, T., and Permert, J. (1997). Effect of islet amyloid polypeptide on somatostatin inhibition of insulin secretion from isolated rat pancreatic islets. *Regul. Pept.* **72**, 61–67.

Wang, M. W., Young, A. A., Rink, T. J., and Cooper, G. J. S. (1991). 8–37h-CGRP antagonizes actions of amylin on carbohydrate metabolism *in vitro* and *in vivo*. *FEBS Lett.* **291**, 195–198.

Wang, Z.-L., Bennet, W. M., Ghatei, M. A., Byfield, P. G. H., Smith, D. M., and Bloom, S. R. (1993). Influence of islet amyloid polypeptide and the 8–37 fragment of islet amyloid polypeptide on insulin release from perifused rat islets. *Diabetes* **42**, 330–335.

Young, A. A., Carlo, P., Rink, T. J., and Wang, M.-W. (1992a). 8–37hCGRP, an amylin receptor antagonist, enhances the insulin response and perturbs the glucose response to infused arginine in anesthetized rats. *Mol. Cell Endocrinol.* **84**, R1–R5.

Young, A. A., Gedulin, B., Wolfe-Lopez, D., Greene, H. E., Rink, T. J., and Cooper, G. J. S. (1992b). Amylin and insulin in rat soleus muscle: Dose responses for cosecreted noncompetitive antagonists. *Am. J. Physiol.* **263**, E274–E281.

Young, A. A., Gedulin, B., Larson, E., and Rink, T. J. (1993). Evidence from studies using a specific blocker for a metabolic effect of endogenous amylin *in vivo*. *Diabetologia* **36** (suppl. 1), A136.

Young, A. A., Gedulin, B., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., Larson, E., and Rink, T. J. (1994). Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat—Evidence for a metabolic role of endogenous amylin. *FEBS Lett.* **343**, 237–241.

Young, A. A., Wang, M. W., Gedulin, B., Rink, T. J., Pittner, R., and Beaumont, K. (1995). Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. *Metabolism* **44**, 1581–1589.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Young, A. A., Jodka, C., Hoyt, J., and Gedulin, B. (2004). Involvement of the *area postrema* in nutrient-stimulated insulin secretion in rats. *Diabetes* **53**(suppl. 1), A324–A325(abstract 1344–P).

Andrew Young

Effects on Plasma Glucose and Lactate

I. Summary

Injection of amylin or amylin agonists, including human and rat amylin, pramlintide, salmon calcitonin, and calcitonin gene-related peptide (CGRP), increases the plasma levels of lactate and glucose in non-diabetic fasting rats and mice. This response can be useful in identifying and defining amylin agonists (amylinomimetic agents) (Cooper *et al.*) and has been investigated in several studies. Increases in plasma glucose and lactate are not present in all species. In humans, for example, increases in lactate are observed at high pramlintide doses but not at doses that would be used to therapeutically regulate plasma glucose. In species where it occurs, the increase in plasma lactate with amylin is comparable to that observed with exercise or adrenergic agents, and it is distinguishable from the very high levels observed during lactic acidosis (as may occur with biguanides). In contrast to lactic acidosis,

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the plasma lactate with amylin is derived from skeletal muscle rather than liver.

Increases in plasma lactate and glucose in some species may initially appear inconsistent with a glucose-lowering effect of amylin agonists. But glycemic effects are due to actions in skeletal muscle and are present only in some species, whereas glucose-lowering actions are attributable to effects in gastrointestinal systems and are present in all species studied to date. And while glycemic effects are most pronounced in the fasted state, glucose-lowering effects are most pronounced in the postprandial state. Since they were discovered first, effects of higher doses of amylin on plasma glucose, especially in the fasted state, are described first and are related to concomitant changes in plasma lactate. These effects are prominent in rodents but are barely discernible in humans. Effects of lower doses of pramlintide to suppress plasma glucose profiles in the postprandial period are also observable in normal and diabetic rats, however, and are covered here as well.

The relationship between plasma lactate and glucose concentrations can be confusing. Via some mechanisms, changes in plasma glucose can drive changes in lactate, while via different mechanisms, changes in lactate can drive changes in glucose concentration. The recursive loop created by these separate links, and for which its discoverers received the Nobel prize, is the Cori cycle (Cori, 1931). This cycle of substrate fluxes, simplified as plasma glucose → muscle glycogen → plasma lactate → liver glycogen → plasma glucose, is important in the redistribution of carbohydrate fuels in some species (Cori and Cori, 1929) and is discussed here in relation to the role of amylin.

II. Plasma Lactate Concentration

A. Profiles

The glycemic and lactemic effects of rat amylin were first noted in rats (Young *et al*., 1991b). Intravenous or subcutaneous injections of 100 μg rat amylin to fasted rats caused a rapid increase in plasma lactate followed by a slower increase in plasma glucose. When amylin alone was injected intravenously, plasma lactate concentration increased 3-fold, and was maximal by 30 min. Glucose increased around 2-fold and was maximal between 1 and 2 hr after injection. A similar pattern was observed during infusion of somatostatin to prevent secretion of islet hormones, implying that insulin and glucagon, for example, were not essential for the response. There were no changes in circulating catecholamines that would implicate them in the response. The pattern following subcutaneous amylin dosing was similar to that observed after intravenous dosing, but was slightly delayed. When sodium lactate was infused to simulate the amylin-generated profile, increases in plasma glucose were comparable to those noted after amylin

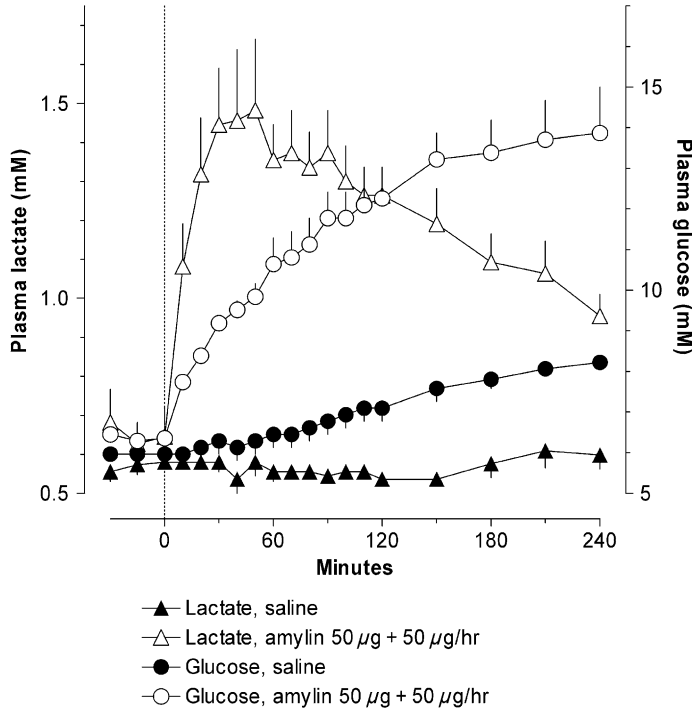

FIGURE 1 Changes in plasma glucose and lactate concentrations after a primed continuous intravenous infusion of rat amylin (50 μg + 50 μg/hr) that aimed to invoke a step change in plasma amylin concentration. The more rapid increase in plasma lactate suggested that it drove the slower increase in plasma glucose. Data from Young *et al.* (1993).

injection, suggesting that the lactate rise might drive the glycemic response (Young *et al.*, 1991b) (Fig. 1).

In each of several reports, plasma lactate began to increase within 15 min of intravenous amylin injection and peaked within 30 min, having risen ~2- to 3-fold (typically changing from ~0.5 mM to ~1.5 mM) (Wang *et al.*, 1991b,c; Young *et al.*, 1991a,b,c,d, 1992, 1993a,b). The character of the change in plasma lactate concentration (magnitude and time course) was similar to that observed 60 years earlier with epinephrine in fed rats (Cori *et al.*, 1930b). Maximal lactate responses were not attained within the amylin dose range examined in fasting rats, so ED_{50} could not be formally derived (Wang *et al.*, 1991b; Young *et al.*, 1993b).

B. Dependence upon Fasted/Fed State

The lactate response depended upon whether the rat was fasted or fed. When a 100 μg intravenous dose of rat amylin was administered to fed rats, the integrated increase in plasma lactate was ~50% greater than in

comparably treated fasted rats (Young *et al.*, 1993). The amplified lactate profile in fed rats was also present if a constant load of sodium lactate (1.75 mmol/rat, mimicking that evoked by 100 μg amylin) was administered (Young *et al.*, 1993). This result suggested that differences in lactate clearance, rather than differences in lactate appearance, were responsible for the observed differences in plasma lactate profile. For example, the increased clearance of plasma lactate due to activated gluconeogenesis in the fasted state could account for the lesser rise in plasma lactate concentration.

This interpretation was supported by experiments in which glucagon (100 μg i.v.) was co-administered with amylin (100 μg i.v.) in fasted rats. Under such conditions, the plasma lactate response to amylin was less than without glucagon (Young *et al.*, 1993). These observations were consistent with gluconeogenesis being a determinant of lactate clearance. That is, when gluconeogenesis was activated by fasting or by glucagon administration, for example, the effect of lactate addition to plasma (whether invoked by amylin administration or by direct lactate infusion) was less than when gluconeogenesis is less stimulated, as in the fed state, or when glucagon was not administered (Fig. 2).

An increase in plasma lactate concentration following amylin administration was also apparent in mice. Intravenous bolus injection of 50 μg rat amylin into fasted BALB/c mice resulted in an increase in plasma lactate from 2 to 4 mM within 15 min of injection (Wang *et al.*, 1992). The ED_{50} for this response was 1.1 μg/mouse (17 nmol/kg). In contrast to rats, plasma lactate responses in fed mice were somewhat similar to those in fasted mice (Wang *et al.*, 1992).

The plasma lactate profiles evoked by intravenous doses of human amylin, rat amylin, and pramlintide were similar for each peptide in fasted anesthetized rats (Young *et al.*, 1996), and were similar to a previously described effect of rat amylin (Young *et al.*, 1993). Although a maximally effective dose for lactate could not be derived (Young *et al.*, 1996), effects on plasma lactate were significant with both rat amylin and pramlintide at doses $\geq$ 10 μg/rat.

C. Pharmacology

In fasted anesthetized rats, rat amylin or pramlintide was continuously infused intravenously. Plasma lactate elevations were significant with infusion rates of 1 μg/hr for each peptide, estimated to result in plasma concentrations of 214 and 219 pM, respectively (Young *et al.*, 1996). Salmon calcitonin, an amylin agonist, evoked a similar increase in plasma lactate concentration when administered intravenously in fasted rats (Rink *et al.*, 1993; Young *et al.*, 1995). In dose–response comparisons, salmon calcitonin was slightly more potent than rat amylin (Young *et al.*, 1995), while CGRP appeared somewhat less potent (Young *et al.*, 1993). The observed order of

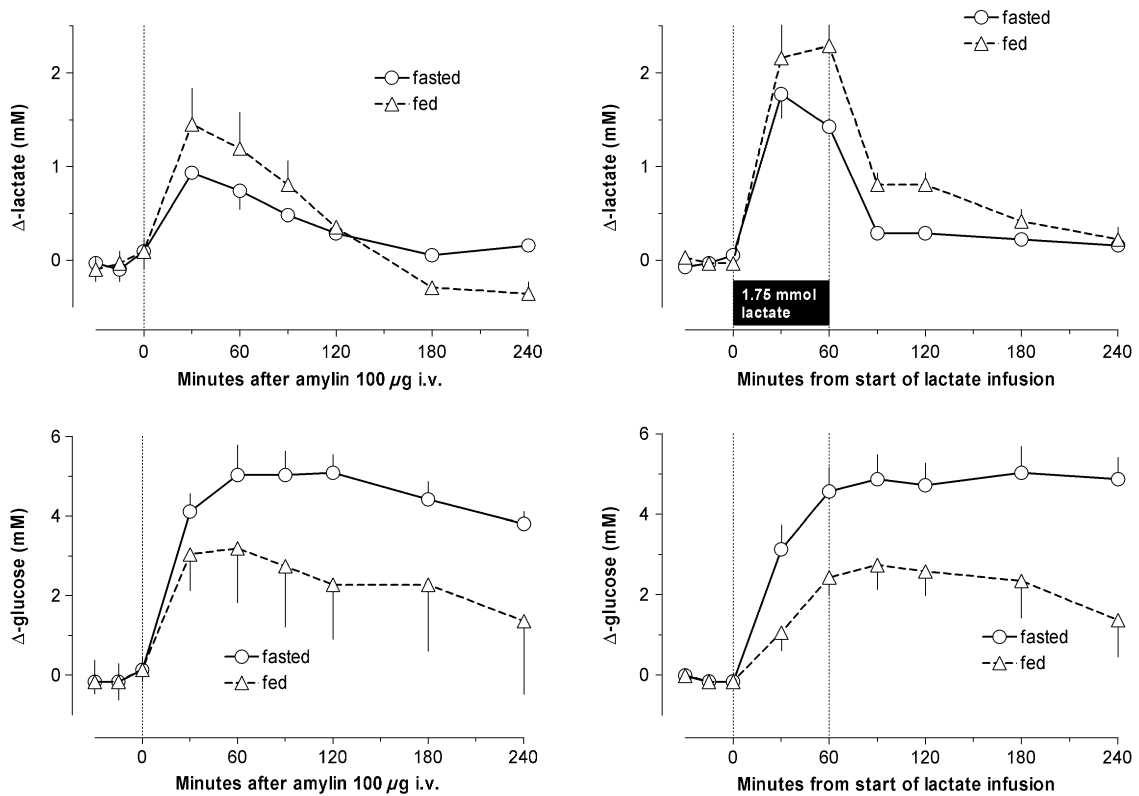

FIGURE 2 Relationships between change in plasma lactate and change in plasma glucose in fasted and fed rats. The increment in plasma lactate (upper panels) was smaller, and the increment in plasma glucose (lower panels) was greater, in fasted compared to fed rats, regardless of whether changes in lactate were invoked by amylin administration (left panels) or by direct lactate infusion (right panels). Data from Young *et al.* (1993).

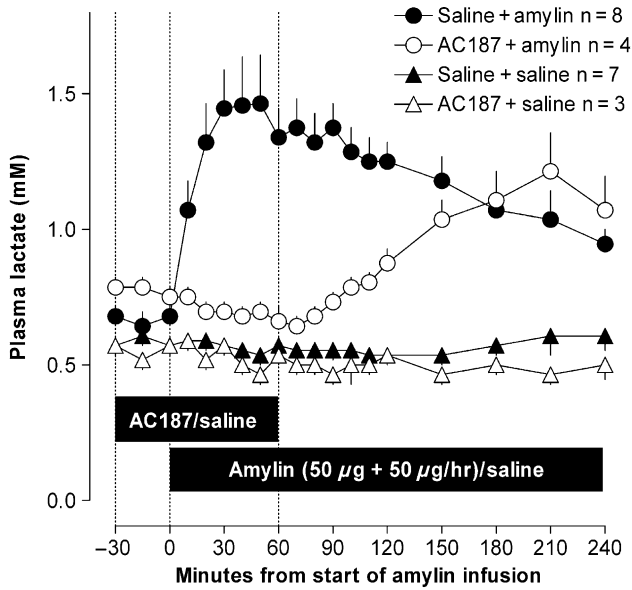

FIGURE 3 Blockade of amylin-induced lactate response with the amylin antagonist AC187 in anesthetized rats. Data from Young *et al.* (1994).

potency in the same rat model, salmon calcitonin > amylin > CGRP, was consistent with a classical amylin pharmacology (Beaumont *et al.*, 1993). This conclusion was also supported by observations that the selective amylin receptor antagonist AC187 blocked increases in plasma lactate evoked by administration of exogenous amylin in rats (Young *et al.*, 1994). The truncated peptide CGRP[8–37], which similarly blocks amylin effects when present at high concentrations (Wang *et al.*, 1991c), also blocked amylin-stimulated lactate responses when delivered at a high infusion rate (0.5 mg + 5 mg/hr) (Wang *et al.*, 1991c) (Fig. 3).

D. Role of Endogenous Amylin

It was uncertain from dose–response studies using exogenous peptide whether changes in lactate could result from physiological changes in plasma amylin concentration. In this regard, studies using AC187 to block the effects of endogenous amylin were informative. In anesthetized rats, release of endogenous amylin (and insulin) was stimulated by intravenous glucose with or without co-administration of AC187. Because AC187 augmented insulin release (discussed in the previous chapter of this volume, "Inhibition of Insulin Secretion"), another control was required wherein additional insulin was administered to match the (augmented) profile observed in

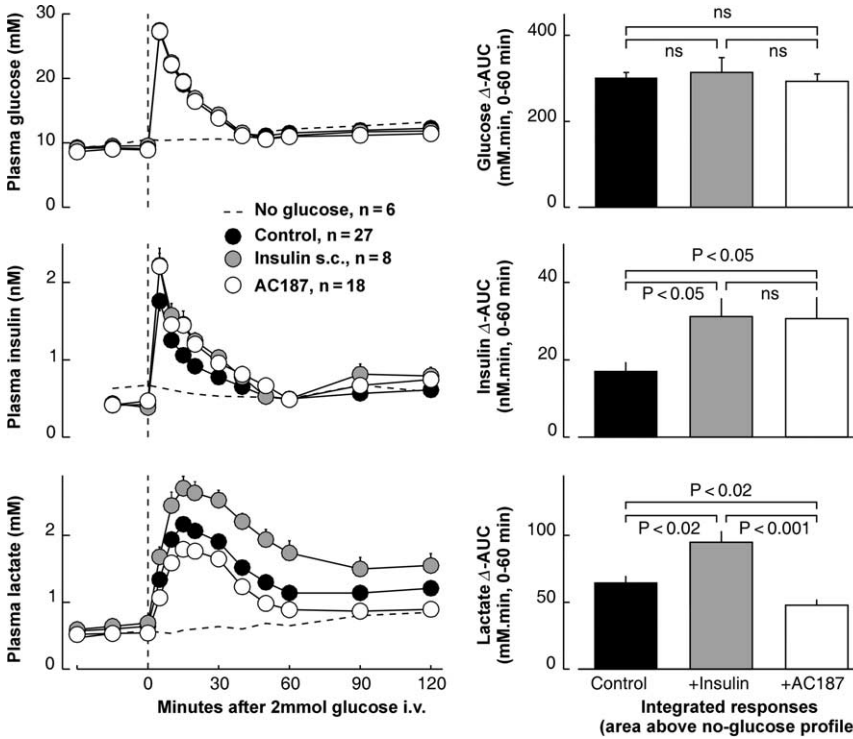

FIGURE 4 Glucose, insulin, and lactate profiles in glucose-challenged anesthetized rats infused with saline, the amylin antagonist AC187, or insulin (to match the increased insulin secretion resulting from AC187). Despite matched glucose and insulin profiles, animals treated with the amylin antagonist showed a reduced lactate response. These results suggested that up to half of the lactate surge observed after a glucose challenge could be mediated via an amylinergic mechanism, as described later. Data from Young *et al.* (1994).

AC187-treated rats. With glucose and insulin profiles thus matched, the lactate surge observed after the glucose challenge was halved in the presence of AC187 (Young *et al.*, 1994). This result indicated that a sizeable component of the lactate response in that preparation was mediated via amylin-sensitive pathways (Fig. 4).

In a similar experimental design in fasted anesthetized rats, a 2:1 molar excess of neutralizing monoclonal anti-amylin antibody (F025-27) suppressed by 94% the lactate surge following an intravenous glucose challenge (Vine *et al.*, 1995b).

In an *in situ* perfused rat hindlimb preparation, which is mainly represented by muscle, lactate production was calculated as the product of arteriovenous differences and flow (measured with electromagnetic flow probes). To avoid accumulation of lactate, perfusion of hepatic and systemic circulations were maintained by retaining an essentially intact animal, and

instead catheterizing a minor (ilio-lumbar) vein along with the carotid artery to measure arteriovenous differences following subcutaneous injection of amylin (100 μg) or saline. Exogenous amylin (100 μg s.c.) increased hindlimb lactate production from 2.6 to 4 μmol/min (Vine *et al.*, 1995c).

E. Source of Lactate

In an animal preparation in which pancreatic perfusion and secretion were still intact, an intravenous challenge of 2 mmol D-glucose increased hindlimb lactate production from 2.3 to 3 μmol/min. This increase was abrogated by prior intravenous infusion of AC187 (0.5 mg + 1 mg/hr for 2 hr) (Vine *et al.*, 1995a,c) and suggested that it was therefore mediated via amylin-sensitive tissues. The incremental production of lactate from the hindlimb (muscle) after a glucose challenge was similar in magnitude to that evoked by exogenous amylin in other studies. That result was consistent with the surge in hindlimb lactate production being at least partly attributable to endogenous amylin.

In summary, amylin administration was associated with a rapid increase in plasma lactate in rodents. The magnitude of the response appeared to be related to the prevailing rate of lactate clearance, by gluconeogenesis, for example. Studies using AC187, and the potency of exogenous amylin to increase plasma lactate, suggest that it may be a physiological effect in rats. Endogenous amylin might account for some component of the lactemic response typically observed with a glucose challenge. Amylin-sensitive tissues implicated in the response are likely to include skeletal muscle.

III. Plasma Glucose Concentration

A. Profiles

In both fasted and fed BALB/c mice, 50 μg intravenous bolus doses of rat (= mouse) amylin increased plasma glucose by ~50% (from ~8 mM to ~12 mM) with an ED_{50} of 11.8 μg (155 nmol/kg) (Wang *et al.*, 1992). Intravenous amylin injections also increased plasma glucose concentration in rats (Young *et al.*, 1990, 1991b,d). In fasted anesthetized rats, the ED_{50} for glucose elevation was 16 μg (12 nmol/kg) (Wang *et al.*, 1991b; Young *et al.*, 1993), while in another study, the glycemic response was still submaximal at doses as high as 1 mg, precluding derivation of an ED_{50} (Young *et al.*, 1996). Glucose elevations occurred when lactate elevations were also detected. Glycemic responses were similar after administration of human amylin, rat amylin, or pramlintide in fasted rats (Young *et al.*, 1996). Effects were significant with doses $\geq$10 μg. With continuous intravenous amylin

infusion, glycemic responses were significant for rat amylin and pramlintide at infusion rates of 1 μg/hr (Young *et al*., 1996).

B. Pharmacology

In a dose–response study, salmon calcitonin increased plasma glucose in fasted anesthetized rats with a potency slightly greater than that of rat amylin (ED_{50} 4.4 versus 10.8 μg) (Young *et al*., 1995), while the glucose elevating potency of CGRP in the same model was slightly less than that of rat amylin (Young *et al*., 1993). The order of potency for glucose elevation was salmon calcitonin > amylin > CGRP, consistent with an amylin pharmacology (Beaumont *et al*., 1993). This order contrasted with that for effects on blood pressure, where the pattern of CGRP ≫ amylin ≫ salmon calcitonin was instead consistent with a CGRPergic pharmacology (Young *et al*., 1993, 1995).

C. Relationship to Lactate and Glucagon

Effects of amylin and amylin agonists on plasma glucose were invariably associated with a lactate response, even though they were pharmacologically dissociable from other effects, such as blood pressure lowering. The effects of different antagonists to block lactemic responses to exogenous amylin also prevented glycemic responses (Wang *et al*., 1991c). This association provided clues to the mechanism underlying amylin-induced glucose elevation in rats.

Glycemic responses to amylin were different in character from those mediated by glucagon. In fasted rats, in which liver glycogen is depleted, glucagon (100 μg) evoked a lesser glucose increment than did the same dose of rat amylin (Δ-glucose 2.9 mM versus 4.3 mM) (Young *et al*., 1991a). Glucagon also showed a 5-fold lower *in vivo* potency (Wang *et al*., 1991a). In contrast, glucagon evoked a greater glycemic response than amylin in fed rats (Young *et al*., 1993). In all instances, increases in plasma glucose with amylin were associated with increases in plasma lactate, while glucagon increased plasma glucose, but not plasma lactate concentration (Fig. 5).

The glycemic response to rat amylin was enhanced when gluconeogenic efficiency would be expected to be high, such as in fasting or following glucagon administration. The glycemic response to an amylin injection was greater in fasted than in fed rats (Young *et al*., 1993), as was the glycemic response to infusions of sodium lactate that mimicked the lactemic effect of amylin (Young *et al*., 1993). Under multiple scenarios, the magnitude of lactemic and glycemic responses were inversely related.

Co-administration of glucagon and amylin resulted in a glycemic synergy, exceeding the sum of individual glycemic effects (Young *et al*., 1993).

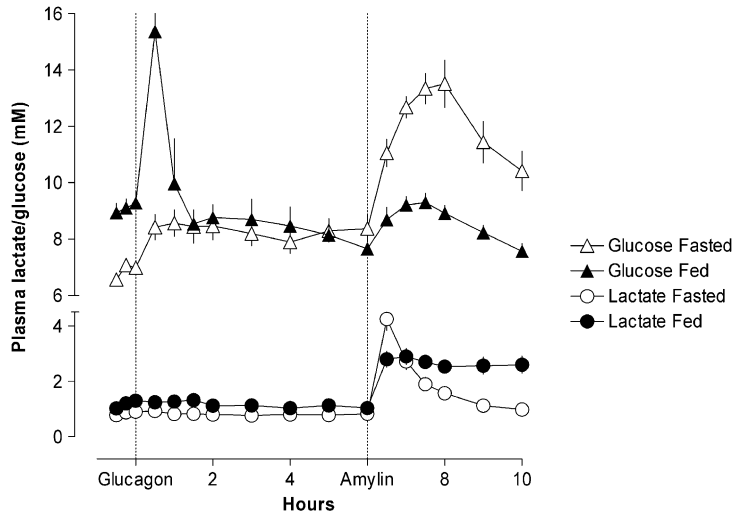

FIGURE 5 Contrasting effects of glucagon and amylin when delivered as intravenous bolus injections into fasted (open symbols) and fed rats (filled symbols). Glucagon, delivered at $t = 0$, increased glucose (triangles) the most in fed animals, without significantly changing plasma lactate concentrations (circles). In contrast, amylin injected at 6 hr increased glucose the most in fasted rats, in association with a rapid decay in plasma lactate, while in fed rats the decay in lactate was slower and glycemic increments were less.

A similar synergy in glycemic effect was observed when salmon calcitonin and glucagon were co-administered (Young *et al.*, 1995).

IV. Timing of Changes

In fasted anesthetized rats, the time courses of changes in plasma amylin concentration, lactate concentration, glucose concentration, and blood pressure were compared following an intravenous bolus injection of 100 μg rat amylin (Young *et al.*, 1993). An increase in plasma lactate was the most rapid metabolic event, followed by a slower increase in plasma glucose. Other reports have showed a similar sequence (Young *et al.*, 1991b, 1993). The same succession was observed when amylin was administered by primed/continuous intravenous infusion (Young *et al.*, 1993) or when salmon calcitonin was administered (Young *et al.*, 1995). The plasma concentration profiles, wherein the lactate surge preceded that of glucose, were similar to those originally reported with epinephrine (Cori *et al.*, 1930a) and suggested a similar mechanism of action. These profiles were unlike the slower increases in plasma lactate that follow insulin administration or glucose infusions wherein the lactate lags behind the increase in glucose flux.

V. Mechanisms Linking Changes in Glucose and Lactate

The resemblance between epinephrine- and amylin-associated glucose/lactate profiles suggested a similar mechanism, wherein the glycemic excursions were due to gluconeogenesis and were driven principally by changes in the availability of lactate, the dominant gluconeogenic precursor. Results of experiments in which lactate was infused to mimic amylin-mediated changes in plasma lactate were consistent with this interpretation. Effects of amylin in skeletal muscle, described in the following chapter, include glycogen breakdown and lactate production and mirror the effects of catecholamines. It thus appeared that in rats amylin acted to increase activity of the Cori cycle (Rink *et al*., 1991; Young, 1993, 1994; Young *et al*., 1991c).

A renewed appreciation of the metabolic importance of the Cori cycle followed the demonstration that in many species liver glycogen is derived mainly from circulating lactate (an indirect pathway), instead of directly from dietary glucose (Newgard *et al*., 1983, 1984). This result fitted with effects described by Cori and Cori, in which muscle glucogenolysis led to increases in plasma lactate and thence to synthesis of liver glycogen (Cori and Cori, 1929).

VI. The Hyperlactemic Clamp

The need to test the effects of amylin on lactate flux (Cori cycle activity) led to the development of the hyperlactemic clamp. The hyperlactemic clamp procedure is analogous to the glucose clamp procedure developed by Reuben Andres (DeFronzo *et al*., 1979) and to the voltage clamp on which the glucose clamp was modeled. In the hyperlactemic clamp procedure, plasma lactate was measured frequently and kept constant by adjusting infusion rate of a sodium lactate solution (Gedulin *et al*., 1993, 1994; Rink and Gedulin, 1993; Rink *et al*., 1994). The rate at which lactate needed to be infused to maintain isolactemia (e.g., 4 mM) depended upon both the rate at which lactate was being endogenously released into the plasma, and the rate at which it was being consumed from the plasma. When plasma lactate was raised 10-fold, from ~0.4 mM to 4 mM, it became by far the dominant gluconeogenic substrate, such that the contribution from glycerol and amino acids could practically be ignored.

Under circumstances in which endogenous lactate production was presumably steady (and low), the infusion rate of exogenous lactate could be used to directly assess gluconeogenesis. Conversely, if the rate of lactate consumption (e.g., due to gluconeogenesis) was constant, for example, by maintaining hyperlactemia, then changes in infusion rate required for isolactemia would mirror endogenous release. That is, increases in endogenous lactate production would decrease the lactate infusion required to maintain

isolactemia. This is similar to the reduction in glucose infusion required to maintain euglycemia when a meal is ingested, or when glucagon is administered.

When plasma lactate was clamped by variable lactate infusion at 6 mM, an intravenous bolus of rat amylin (100 μg) into fasted rats resulted in a near-total elimination in the need for exogenous lactate infusion to maintain isolactemia (Young, 1992). This effect lasted 30–40 min and indicated that

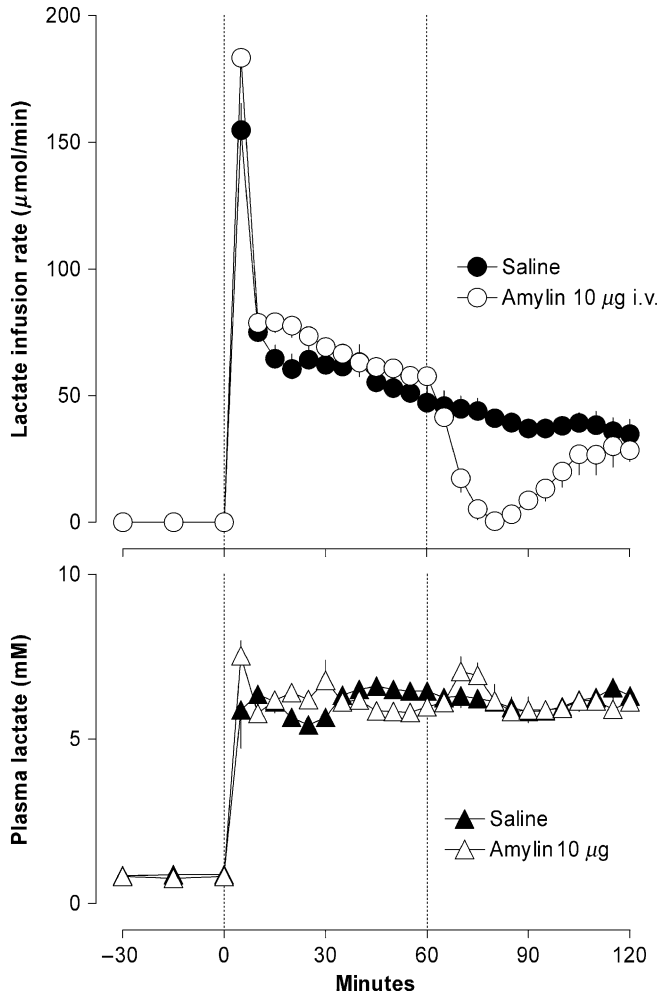

FIGURE 6 Hyperlactemic clamp quantifying the lactate flux invoked by amylin. Beginning at $t = 0$, lactate was infused at a variable rate (upper panel) to maintain a plasma concentration of 6 mM (lower panel). The reduction in infusion rate required to maintain isolactemia following a 10 μg intravenous injection of amylin at 60 min was a measure of lactate release invoked by amylin. Data from Young (1992).

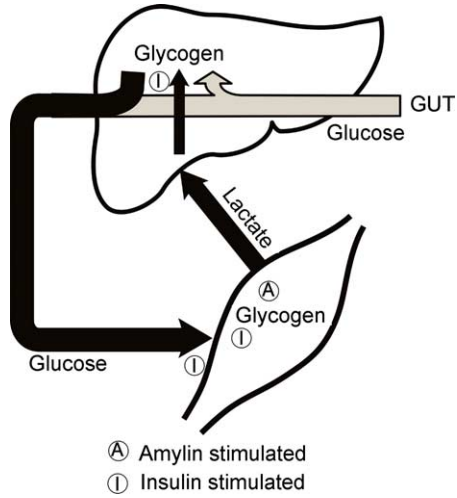

FIGURE 7 Conjoint effects of insulin and amylin on Cori cycle activity in rats. Insulin promotes glucose uptake of meal-derived glucosyls into muscle glycogen. Amylin promotes transfer of glucosyls from muscle to liver.

amylin at that dose had liberated lactate into the plasma at a rate of ~18 mg/kg/min. This result supported the conclusion that amylin increased Cori cycle activity in the rat (Young, 1993, 1994).

Conversely, the administration of glucagon during the isolactemic clamp resulted in a large and immediate increase in lactate infusion required to maintain isolactemia (Gedulin *et al.*, unpublished) (Figs. 6 and 7).

VII. Postprandial Glucose

The effects of amylin and amylinomimetics on plasma glucose depend upon the test system in which they are used. Differences are due to independent activation of different mechanisms in skeletal muscle (for lactemic and glycemic actions, as above), and in gastrointestinal systems (for glucose-smoothing actions). Paradoxically, since they were the first-described actions of human amylin, effects on skeletal muscle carbohydrate metabolism observed in rats appear not to be a major feature of the human response to amylinomimetics. In humans, glucose-smoothing features seem to predominate. Under appropriate conditions, the latter effects of amylinomimetics can be demonstrated in rats.

The effect of pramlintide on plasma glucose concentration was determined in conscious rats following an oral glucose challenge. Conscious rats were injected s.c with 1 μg pramlintide 5 min prior to gavage with 1 ml 50% D-glucose. The rate of increase in plasma glucose after pramlintide injection

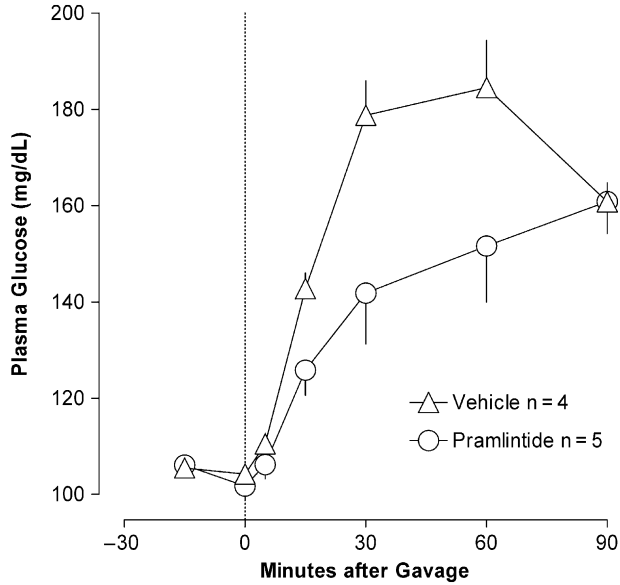

FIGURE 8 Plasma glucose profiles after an oral glucose challenge in rats injected with pramlintide or vehicle 5 min prior to gavage. Data from Young *et al.* (1996).

was slower than after saline pre-injection (Young *et al.*, 1996). This effect resembled that obtained with amylin during an oral nutrient challenge in the dog (Brown *et al.*, 1994) and with pramlintide in humans (Kolterman *et al.*, 1995) (Fig. 8).

In summary, in the context of a meal, amylin administration resulted in a suppression of rise in plasma glucose concentration in rats, dogs, and humans. In the absence of a meal, amylin administration was associated with an increase in plasma glucose in rodents, but not in humans. The elevation in plasma glucose following amylin administration to rats was a consequence of increased availability of lactate as a gluconeogenic substrate. The magnitude of glucose elevation was also dependent upon the efficiency of gluconeogenesis. There was a synergy of both glucose-elevating effects when an amylinomimetic was combined with glucagon.

References

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Brown, K., Menius, A., Sandefer, E., Edwards, J., and James, M. (1994). The effects of amylin on changes in plasma glucose and gastric emptying following an oral glucose load in conscious dogs. *Diabetes* **43**(suppl. 1), 172A.

Cooper, G. J. S., Young, A., and Rink, T. J., inventors. Identifying compounds which affect amylin activity—using an *in vivo* biological model in which amylin increases levels of lactate and glucose. U.S. patent 4,521,430.

Cori, C. F. (1931). Mammalian carbohydrate metabolism. *Physiol. Rev.* **11**, 143–275.

Cori, C. F., and Cori, G. T. (1929). Glycogen formation in the liver from d- and l-lactic acid. *J. Biol. Chem.* **81**, 389–403.

Cori, C. F., Cori, G. T., and Buchwald, K. W. (1930a). The mechanisms of epinephrine action. VI. Changes in blood sugar, lactic acid, and blood pressure during continuous intravenous injection of epinephrine. *Am. J. Physiol.* **93**, 273–283.

Cori, C. F., Cori, G. T., and Buchwald, K. W. (1930b). The mechanism of epinephrine action. V. Changes in liver glycogen and blood lactic acid after injection of epinephrine and insulin. *J. Biol. Chem.* **86**, 375–388.

DeFronzo, R. A., Tobin, J. D., and Andres, R. (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. *Am. J. Physiol.* **237**, E214–E223.

Gedulin, B., Larson, E., Young, A., and Rink, T. (1993). Combined hyperlactemic and hyperglycemic clamps in anesthetized rats with acute pancreatectomy or somatostatin infusion to study hepatic effects of amylin. Conference Proceedings: American Diabetes Association Southern California Research Symosium. Irvine, California.

Gedulin, B., Larson, E., Young, A. A., and Rink, T. J. (1994). Combined hyperlactemic and hyperglycemic clamps in anesthetized rats with acute pancreatectomy or somatostatin infusion; effects of amylin on endogenous lactate and glucose production. *Program and Abstracts, the Endocrine Society, 76th Annual Meeting*, p. 373.

Kolterman, O. G., Gottlieb, A., Moyses, C., and Colburn, W. (1995). Reduction of postprandial hyperglycemia in subjects with IDDM by intravenous infusion of AC137, a human amylin analogue. *Diabet. Care* **18**, 1179–1182.

Newgard, C. B., Hirsch, L. J., Foster, D. W., and McGarry, J. D. (1983). Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. A direct or an indirect pathway? *J. Biol. Chem.* **258**, 8046–8052.

Newgard, C. B., Moore, S. V., Foster, D. W., and McGarry, J. D. (1984). Efficient hepatic glycogen synthesis in refeeding rats requires continued carbon flow through the gluconeogenic pathway. *J. Biol. Chem.* **259**, 6958–6963.

Rink, T., and Gedulin, B. (1993). Lactate clamp: A new technique for studying carbohydrate metabolism *in vivo*. *Diabetes* **42**(suppl. 1), 245A.

Rink, T. J., Young, A. A., Wang, M.-W., Gedulin, B. R., and Cooper, G. J. S. (1991). Amylin hormone has complementary actions to insulin in control of Cori cycle activity. *Diabetes* **40**, 21A.

Rink, T. J., Beaumont, K., and Young, A. A. (1993). Salmon calcitonin potently mimics amylin actions on isolated soleus muscle and in increasing plasma lactate and glucose in rats. *Br. J. Pharmacol.* **108**, 318P.

Rink, T. J., Larson, E., Young, A. A., and Gedulin, B. (1994). Effects of amylin on lactate metabolism in the rat: Studies using hyperactemic/hyperglycemic clamps. *Diabetologia* **37**, A117.

Vine, W., Gedulin, B., Smith, P., Rink, T., and Young, A. (1995a). Blockade of endogenous amylin decreases release of lactate from muscle in rats. *Diabetologia* **38**, 155.

Vine, W., Percy, A., Gedulin, B., Moore, C., Smith, P., Crocker, L., Koda, J., and Young, A. (1995b). Evidence for metabolic action of endogenous amylin from effects of a neutralizing monoclonal antibody in rats. *Diabetes* **44**, 251A.

Vine, W., Smith, P., Lachappell, R., Rink, T. J., and Young, A. A. (1995c). Lactate production from the rat hindlimb is increased after glucose administration and is suppressed by a selective amylin antagonist: Evidence for action of endogenous amylin in skeletal muscle. *Biochem. Biophys. Res. Commun.* **216**, 554–559.

Wang, M. W., Carlo, P., Rink, T. J., and Young, A. A. (1991a). Amylin is more potent and more effective than glucagon in raising plasma glucose concentration in fasted, anesthetized rats. *Biochem. Biophys. Res. Commun.* **181**, 1288–1293.

Wang, M.-W., Cooper, G. J. S., and Young, A. A. (1991b). Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic effects of amylin in the rat. *Diabetes* **40**, 241A.

Wang, M. W., Young, A. A., Rink, T. J., and Cooper, G. J. S. (1991c). 8–37h-CGRP antagonizes actions of amylin on carbohydrate metabolism *in vitro* and *in vivo*. *FEBS Lett.* **291**, 195–198.

Wang, M. W., Carlo, P., Fineman, M., Rink, T. J., and Young, A. A. (1992). Induction of acute hyperglycemia, hyperlactemia and hypocalcemia in fed and fasted BALB/c mice by intravenous amylin injection. *Endocr Res.* **18**, 321–332.

Young, A. A. (1992). The role of the B-cell hormone, amylin, on endogenous lactate and glucose production in fuel homeostasis. *In* "Exercise: The Physiological Challenge." (P. M. Hill, Ed.). Conference Publishing, Auckland, New Zealand.

Young, A. A. (1993). Amylin and its effects on the cori cycle. *In* "Taking Control in Diabetes." Synergy Medical Education," Surrey, UK. 15–21.

Young, A. A. (1994). Amylin regulation of fuel metabolism. *J. Cell Biochem.* **55**, 12–18.

Young, D. A., Deems, R. O., Deacon, R. W., McIntosh, R. H., and Foley, J. E. (1990). Effects of amylin on glucose metabolism and glycogenolysis *in vivo* and *in vitro*. *Am. J. Physiol.* **259**, E457–E461.

Young, A. A., Wang, M.-W., and Cooper, G. J. S. (1991). Amylin, but not glucagon, acts to produce hyperglycemia and elevated blood lactate and hepatic glucose production in the fasted rat. *Diabetes* **40**, 21A.

Young, A. A., Wang, M. W., and Cooper, G. J. S. (1991). Amylin injection causes elevated plasma lactate and glucose in the rat. *FEBS Lett.* **291**, 101–104.

Young, A. A., Wang, M.-W., Cooper, G. J. S., and Mott, D. M. (1991). Amylin and insulin exert complementary control over Cori cycle activity. *J. Cell Biochem.* **15**(part B), 68.

Young, A. A., Wang, M.-W., Rink, T. J., and Cooper, G. J. S. (1991). Effects of intravenous injections of amylin in the fasted, anaesthetized rat. *J. Physiol.* **438**, 250P.

Young, A. A., Cooper, G. J. S., Rink, T. J., and Wang, M.-W. (1992). Amylin, a newly discovered pancreatic beta cell hormone stimulates glycogenolysis in skeletal muscle and evokes hyperlactemia and hyperglycemia in anesthetized fasted rats. *Hypertension (Dallas)* **19**, I131.

Young, A. A., Cooper, G. J. S., Carlo, P., Rink, T. J., and Wang, M. W. (1993a). Response to intravenous injections of amylin and glucagon in fasted, fed, and hypoglycemic rats. *Am. J. Physiol.* **264**, E943–E950.

Young, A. A., Rink, T. J., and Wang, M. W. (1993b). Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-I (CGRP-alpha). in the fasted, anaesthetized rat. *Life Sci.* **52**, 1717–1726.

Young, A. A., Gedulin, B., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., Larson, E., and Rink, T. J. (1994). Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat—evidence for a metabolic role of endogenous amylin. *FEBS Lett.* **343**, 237–241.

Young, A. A., Wang, M. W., Gedulin, B., Rink, T. J., Pittner, R., and Beaumont, K. (1995). Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. *Metabolism* **44**, 1581–1589.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Andrew Young

Effects in Skeletal Muscle

I. Summary

The first biological action of amylin to be described was the inhibition of insulin-stimulated incorporation of radiolabeled glucose into glycogen in the isolated soleus muscle of the rat. This antagonism of insulin action in muscle was non-competitive, occurring with equal potency and efficacy at all insulin concentrations.

Amylin inhibited activation of glycogen synthase, partially accounting for the inhibition of radiolabeled glucose incorporation. However, this did not account for a low rate of labeling at higher amylin concentrations, wherein the radioglycogen accumulation was even less than in incubations where insulin was absent.

The principal action of amylin accounting for reduction of insulin-stimulated accumulation of glycogen was activation of glycogen phosphorylase

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via a cyclic AMP-, protein kinase C-dependent signaling pathway to cause glycogenolysis (glycogen breakdown). At physiological concentrations, amylin activated glycogen phosphorylase at its ED_{50}, but because glycogen phosphorylase is present in such high activity, the resulting flux out of glycogen was estimated to be similar to insulin-mediated flux of glucosyl moieties into glycogen. Thus, in the rat, endogenous amylin secreted in response to meals appeared to mobilize carbon from skeletal muscle.

Amylin-induced glycogenolysis resulted in intramuscular accumulation of glucose-6-phosphate and release of lactate from tissue beds that included muscle. When muscle glycogen was pre-labeled with tritium in the three position, amylin could be shown to evoke the release of free glucose. This is made possible by glucosyl moieties cleaved at the branch points in glycogen being released as free glucose, rather than being phosphorylated, as occurs with the bulk of the glycogen glucosyls. Free glucose is free to exit cells via facilitated transport, down a concentration gradient that might exist under such circumstances. When measured by a sensitive technique utilizing efflux of labeled glucose, amylin was reported to not affect muscle glucose transport. In most of the above respects, amylin behaved similarly to catecholamines in skeletal muscle.

The pharmacology of amylin's effects on muscle glycogen metabolism was consistent with a classic amylin pharmacology in whole animals and in isolated soleus muscle. In one cell line, the pharmacology was CGRPergic.

Amylin, like insulin, stimulated Na^+/K^+ ATPase activity and enhanced muscle contractility *in vitro*.

II. Glycogen Metabolism

A. Formation, Breakdown, and Content

Insulin promotes glycogen synthesis in skeletal muscle, and this can be assessed by following the incorporation of radiolabeled glucose into ethanol-extractable glycogen in isolated rat soleus muscle (Crettaz *et al.,* 1980; Cuendet *et al.,* 1976; Le Marchand-Brustel and Freychet, 1980; Le Marchand-Brustel *et al.,* 1978). Soleus muscle is used because it is very insulin sensitive, and its shape is advantageous for lengthwise division (stripping) and, hence, maintenance *in vitro*. The first biological action of native and synthetic human amylin to be discovered was an inhibition of radioglucose incorporation into glycogen (Leighton and Cooper, 1988; Leighton *et al.,* 1988). Many reports since then have confirmed this effect on soleus muscle glycogen with rat and human amylin, pramlintide (Young *et al.,* 1996), and other amylinomimetic agents (Beaumont *et al.,* 1994, 1995a; Castle *et al.,* 1998; Dimitriadis *et al.,* 1998a,b; Kim and Youn, 1996, 1997; Kreutter *et al.,* 1990, 1993, 1994, 1995; Lawrence and Zhang, 1994; Leighton *et al.,*

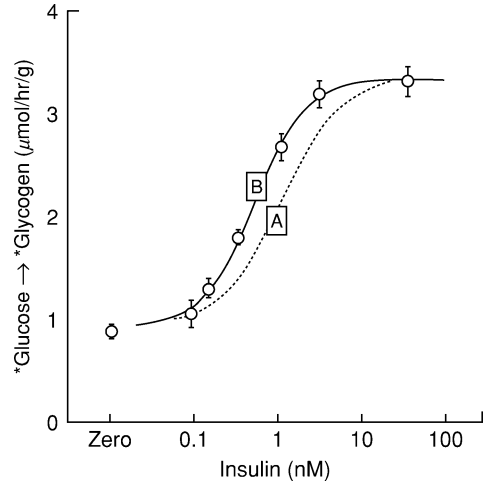

FIGURE 1 Insulin dose response for the incorporation of radiolabeled glucose into glycogen in isolated stripped soleus muscle from rats. From Young *et al.* (1992).

1990; Pittner *et al.*, 1994a,b, 1995a, 1996; Rink *et al.*, 1993; Weiel *et al.*, 1993; Young *et al.*, 1991, 1992a,b, 1993b) (Fig. 1).

Impairment of insulin-stimulated glucose storage (as glycogen in muscle) is a key feature (Lillioja *et al.*, 1986; Young *et al.*, 1988) and a predictor (Bogardus *et al.*, 1986) of insulin resistance in humans. This feature aggregates in families (Lillioja *et al.*, 1987). With the advent of the ability to directly track muscle glycogen with ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Jue *et al.*, 1989), it was subsequently confirmed that the storage defect in type 2 diabetes was indeed a reduced rate of muscle glycogen synthesis (Rothman *et al.*, 1991; Shulman *et al.*, 1990). ^{31}P- and ^{13}C-NMR showed the same result in obese insulin-resistant subjects (Petersen *et al.*, 1998) and in the families of patients with type 2 diabetes (Price *et al.*, 1996).

The observation that amylin opposed some aspects of insulin action in rat soleus muscle glycogen led to the proposal that it might be implicated in the pathogenesis of insulin resistance in humans (Cooper *et al.*, 1988, 1989; Leighton and Cooper, 1988, 1990). This hypothesis has not been supported by chronic use of amylinomimetics in humans or by acute use of amylin antagonists in humans (Leaming *et al.*, 1995).

B. Isolated Stripped Rat Soleus Muscle

There is little clear evidence of an effect of amylinomimetics on muscle glycogen metabolism in humans as evidenced by the cascade of events, described below, for rat muscle. However, the effect on glycogen metabolism

in skeletal muscle is such a robust feature of the amylin response in rats that the isolated stripped soleus muscle became the first bioassay for development of amylinomimetic agents, including pramlintide (Janes *et al.*, 1996). The method for studying glycogen metabolism in isolated rat soleus muscle has been described in detail by Young *et al.* (Young *et al.*, 1992b). Briefly, the m. soleus was removed from a rat killed after a 4 hr fasting period and split into four equal strips. These were incubated in flasks containing 10 ml of Krebs-Ringer bicarbonate buffer containing recombinant human insulin (7.1 nM). The continuously gassed flasks were placed in a shaking water bath, and peptides under investigation were added at appropriate dilutions. Following a 30 min pre-incubation period, 0.5 μCi of U-^{14}C- glucose was added to each flask and incubation continued for 60 min; the muscle pieces were then removed, weighed, and frozen. Following digestion with potassium hydroxide and precipitation of glycogen with cold ethanol, ^{14}C-glycogen content was determined in a scintillation counter. The rate of glucose incorporation into glycogen (expressed as μmol/g muscle/hr) was obtained from the specific activity of ^{14}C-glucose in the incubation medium and the activity of the glycogen from the muscle (Fig. 2).

The soleus muscle assay indicated that the potency for pramlintide was similar to that of rat amylin and was slightly (but significantly) greater than that of human amylin ($P < 0.01$) (Young *et al.*, 1996). Respective EC_{50} values for these three compounds were 3.0 nM, 4.97 nM, and 7.63 nM, respectively, for reversal of insulin-stimulated radioglucose incorporation into glycogen. Part of the slightly greater apparent potency of pramlintide

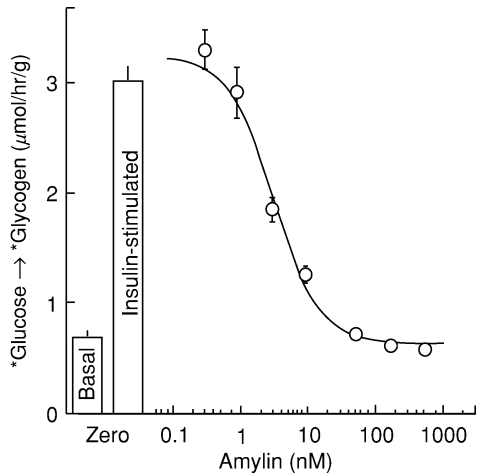

FIGURE 2 Amylin dose response for the reversal of insulin-stimulated incorporation of radiolabeled glucose into isolated rat soleus muscle. Basal is without added hormones. "Insulin-stimulated" is with human insulin added at a final concentration of 7.1 nM. From Young *et al.* (1992).

over human amylin may have been attributable to aggregation of the latter *in vitro*. Depending upon method of assessing peptide concentrations present in the incubation, aggregation has been shown to lead to an artifactual rightward shift of the dose–response curve for human amylin in this assay (Young *et al.*, 1992).

C. Other Muscles

Soleus muscle in rodents is composed mainly of type 1 (slow-twitch oxidative) muscle. Amylin (and calcitonin gene-related peptide [CGRP]) have been reported to affect muscle glycogen metabolism in extensor digitorum longus, a muscle composed mainly of type 2 (fast-twitch glycolytic) fibers (Leighton *et al.*, 1989). Similar actions of these peptides were also reported in diaphragm (Foot *et al.*, 1990; Leighton *et al.*, 1990). An apparent trophic effect of amylin in primary cardiac myocytes was less potent than that of CGRP and appeared to be mediated via CGRP receptors (Bell *et al.*, 1995).

D. Pharmacology

Following the identification of the high affinity of salmon calcitonin and the low affinity of mammalian calcitonins to amylin binding sites in nucleus accumbens, these ligands were tested in isolated soleus muscle. It was found that the order of potency of these ligands for ^{125}I-amylin displacement from nucleus accumbens (salmon calcitonin > rat amylin > rat calcitonin) was matched by the order of potency in inhibiting radioglucose incorporation in glycogen (EC_{50}s of 0.39, 3.1, and 74 nM, respectively, in one series of experiments, Beaumont *et al.*, 1993; 0.4, 8.4, and 376 nM in another, Young *et al.*, 1995) (Fig. 3).

Affinity at amylin binding sites in nucleus accumbens was compared with potency in blocking amylin action in isolated soleus muscle for three truncated peptide antagonists of varying affinities at other receptors. CGRP8–37], originally described as a CGRP antagonist (Maggi *et al.*, 1991), salmon calcitonin[8–32] (AC66), which does not appreciably interact at CGRP receptors, and ac-[Asn^{30}],[Tyr^{32}]sCT$^{8–32}$ (AC187), which is selective for amylin versus CGRP or calcitonin receptors (Young *et al.*, 1994), were progressively more potent in displacement of ^{125}I-amylin from nucleus accumbens (IC_{50}s of 13, 1.9, and 0.48 nM, respectively). As with amylinomimetic peptides, the order of potency for the blockade by these antagonists of amylinergic effects on soleus muscle glycogen (AC187 > AC66 > CGRP$^{8–37}$) was the same as the order of binding affinities at nucleus accumbens membranes (Beaumont *et al.*, 1995a). The pharmacology of nucleus accumbens binding matched the pharmacology of inhibition of muscle glycogen radiolabeling. As a further example, adrenomedullin, which is an agonist at CGRP

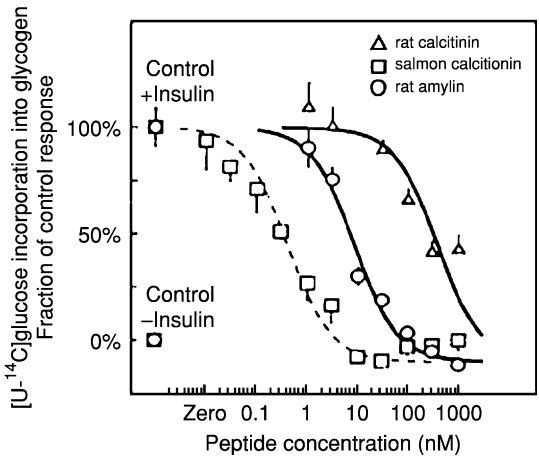

FIGURE 3 Dose responses for reversal of incorporation of radiolabeled glucose for salmon calcitonin, amylin, and rat calcitonin. The order of potency, salmon calcitonin > amylin > rat calcitonin, is consistent with an amylin-like pharmacology (Young *et al.*, 1995).

receptors and is potently hypotensive (Kitamura *et al.*, 1993), had a low affinity to nucleus accumbens and did not affect soleus muscle glycogen metabolism or exhibit other amylinergic actions such as inhibition of gastric emptying (Vine *et al.*, 1996). In contrast to the amylin-like pharmacology observed for glycogen metabolism in soleus muscle, two cell lines commonly used as a model of muscle, L6 myocytes and C2C12 cells, did not exhibit amylin-like pharmacology, but instead had a pharmacology consistent with that of CGRP receptors; CGRP was orders of magnitude more potent than amylin, and salmon calcitonin was without effect (Pittner *et al.*, 1996). Others working with L6 cells also found their pharmacology to be CGRP-like (Coppock *et al.*, 1996; Zhu *et al.*, 1991), but some of these authors erred in presuming that L6 cells reflected muscle pharmacology *in vivo* and mistakenly concluded that the cellular effects and physiological actions of amylin were therefore mediated through receptors for CGRP (Zhu *et al.*, 1991). Indeed, CGRP receptors appeared not to be involved in the regulation of muscle glycogen metabolism by either amylin or CGRP (Beaumont *et al.*, 1995b).

E. Interactions with Insulin in Isolated Skeletal Muscle

The functional relationship between the actions of insulin and rat amylin in isolated rat soleus muscle was elucidated in a study in which radioglucose incorporation into glycogen was determined over a range of insulin/amylin combinations to create an insulin/amylin response surface. It was revealed that amylin reduced the maximally achievable magnitude, but not the potency (EC_{50} of 0.78–1.52 nM), of insulin-stimulated radioglucose incorporation in this series of experiments. Conversely, prevailing insulin

concentration set the initial magnitude of effect that amylin dose-dependently reversed with a potency (EC_{50} of 8.2–12.1 nM) that was independent of the prevailing insulin concentration (Young *et al.,* 1992). That is, amylin and insulin appeared to have independent effects, via separate signaling systems, on a common measure. Consistent with the behavior of non-competitive antagonists, amylin did not affect transduction of the insulin signal or vice versa. A prediction following from this observation was that an amylin: insulin mixture (as is in reality secreted from β-cells) would result in a bell-shaped dose–response curve. Using increasing strengths of a 14% amylin: insulin molar mixture, the presence of a bell-shaped concentration response for both rat and human amylin was confirmed experimentally (Young *et al.,* 1992) (Fig. 4), and it illustrated that an amylin effect could not be countered

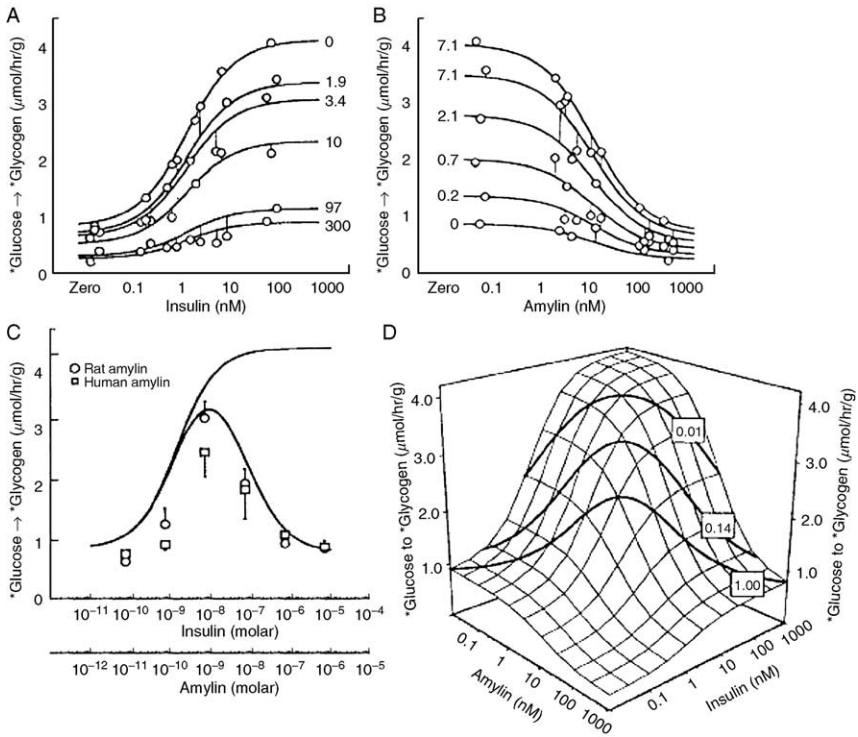

FIGURE 4 (A) Insulin concentration responses at different fixed amylin concentrations, and (B) amylin concentration responses at different fixed insulin concentrations for radiolabeled glucose incorporation into rat soleus muscle. The maximal magnitude of response to insulin or amylin, but not the apparent potency of insulin or amylin, was affected by the presence of amylin or insulin, respectively. (C) Bell-shaped concentration response when soleus muscle was incubated in different concentrations of an amylin:insulin mixture in a 14% molar ratio. (D) complete response surface describing tested concentrations of amylin and insulin. Mixtures in a 1, 14, and 100% molar ratio are shown as transects on the surface. From Young *et al.* (1992).

simply by increasing insulin action. This result argued for independence of mechanisms on skeletal muscle glycogen.

III. Muscle Glycogen Synthase and Glycogen Content

Glycogen synthase enzyme in muscle is insulin sensitive and is rate determining for the incorporation of glucose into glycogen (Larner *et al.*, 1979; Roach and Larner, 1976). Early interpretations of the effect of amylin to inhibit the insulin-stimulated incorporation of radiolabeled glucose into glycogen included a proposed inhibition of glycogen synthase activity. Inhibition of glycogen synthase activity of 43–60% has been reported in isolated soleus muscle incubated with high (>100 nM) concentrations of amylin (Deems *et al.*, 1991b; Lawrence and Zhang, 1994) and in perfused hindlimb (Castle *et al.*, 1998).

Examination of muscle glycogen content after exposure to amylin indicated that, more than just a slowing of the rate of glycogen synthesis, there was a net decrease in total glycogen content (Castle *et al.*, 1998; Deems *et al.*, 1991b; Kreutter *et al.*, 1989, 1995; Lawrence and Zhang, 1994; Pittner *et al.*, 1994a,b, 1995a; Young *et al.*, 1990). It could be inferred that in circumstances such as these in the presence of amylin, where glycogen accumulation rate was negative but rates of glycogen labeling with U-^{14}C-glucose were positive (albeit reduced), the rate of labeling was a marker of, but could not quantitatively track, net glycogen synthesis in muscle (Young *et al.*, 1992).

IV. Glycogen Phosphorylase

Another glycogen-related process to consider was glycogenolysis, which occurs via an enzymatic pathway that is distinct from synthesis, the rate-limiting step of which is glycogen phosphorylase. Glycogen phosphorylase in skeletal muscle is activated by states associated with high energy demand (e.g., by intracellular Ca^{2+} following frequent contractions, hypoxia, or high AMP) or in response to catecholamines. The latter stimulation of glycogen phosphorylase is mediated via cyclic AMP. Total phosphorylase enzyme activity exceeds that of synthase by 12- to 85-fold (Benzo and Stearns, 1982; Le Marchand-Brustel and Freychet, 1980), so activity of this enzyme can be a major determinant of glycogen balance in muscle. The result of phosphorylase-mediated glycogenolysis is the sometimes "explosive" liberation of glucose-1-phosphate intracellularly, which rapidly converts to glucose-6-phosphate to enter glycolysis. In skeletal muscle, amylin activated glycogen phosphorylase (Deems *et al.*, 1991a,b; Lawrence and Zhang, 1994; Pittner *et al.*, 1994a,b, 1995a; Young *et al.*, 1991) (Fig. 5).

The predominant role of glycogenolysis as a source of amylin-induced carbon flux was demonstrated by a novel technique (Young *et al.*, 1993a,b)

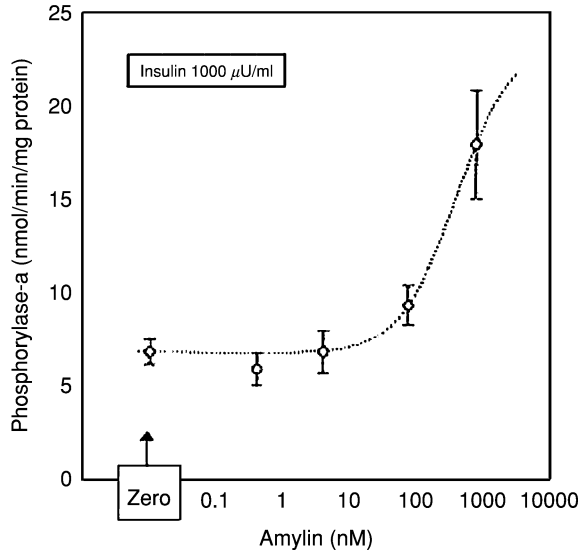

FIGURE 5 Amylin concentration response for the activation of glycogen phosphorylase in isolated stripped rat soleus muscle (Young *et al.*, 1991).

derived from one developed in human studies (Young *et al.*, 1988). The method tracked the fate of 3-^{3}H-glucose after it was preloaded into muscle glycogen. Tritium from this form of labeled glucose either remains with the glucose or, if the glucose is committed to the glycolytic pathway, is lost to water in the hexose → triose step of glycolysis. Tritiated water is a reaction product, and the rate of appearance of ^3H_2O can be used as a marker of glycolytic rate. Rats were fasted for 20 hr and then exercised for 1 hr to profoundly deplete muscle glycogen. Rats were then anesthetized and infused with insulin and 1g D-glucose containing 500 μCi 3-^{3}H-glucose to replete muscle with labeled glycogen. Labeled glucose was given a further 2 hr to clear from plasma before rats received one of three intravenous treatments: (1) 0.1 ml saline, (2) 100 μg rat amylin, or (3) 100 μg rat amylin preceded by 0.5 mg infusion of the antagonist CGRP[8–37]. Serial sampling for ^3H_2O activity revealed a 6.4-fold increase in tritiated water production within 30 min of injection of amylin compared with controls. This observation indicated that amylin had activated glycolysis, for example, following stimulation of glycogenolysis. This action was inhibited by CGRP[8–37] (Young *et al.*, 1993).

V. Cyclic Amp in Muscle

Initial reports by Stace *et al.* (Stace *et al.*, 1992) that amylin increased cyclic AMP in muscle were surrounded by speculation that it did not (Deems *et al.*, 1991b; Lawrence and Zhang, 1994). It was eventually resolved that

amylin and amylinomimetics did indeed stimulate cyclic AMP accumulation in muscle (Kruetter *et al.,* 1994; Moore and Rink, 1993; Pittner *et al.,* 1994a,b, 1995a, 1996). This activation was associated with the activation of glycogen phosphorylase with concentration responses that were comparable (Pittner *et al.,* 1995a). Amylin stimulation of soleus muscle also activated cAMP-dependent protein kinase A (PKA) (Weiel *et al.,* 1993), an element of the signaling pathway connecting cAMP to activation of phosphorylase and other intracellular events. While it is possible that other signaling cascades could be activated by amylin in muscle, so far there has been no indication of such.

VI. Intracellular Glucose-6-Phosphate in Muscle

Additionally, glucose-6-phosphate was elevated with amylin treatment *in vitro* (Young *et al.,* 1990), *in vivo* (Kim and Youn, 1996, 1997; Young *et al.,* 1990), and in the perfused hindlimb (Castle *et al.,* 1998). Intracellular flooding with glucose-6-phosphate is a predicted consequence of a glycogenolytic rate that exceeds glycolytic rate.

VII. Lactate Efflux from Muscle

Efflux of lactate from muscle was observed *in vitro* (Pittner *et al.,* 1994a, 1995a), *in vivo* (Vine *et al.,* 1995), and in the perfused hindlimb (Castle *et al.,* 1998), following amylin administration. In the rat, glucose administration is typically followed by an increase in plasma lactate, the origin of which has been much debated. In an experimental preparation in the anesthetized rat that examined substrate fluxes across the hindlimb (predominantly a muscle bed), amylin administration increased net lactate efflux from muscle into the circulation from 2.6 to 4.0 μmol/min (Vine *et al.,* 1995). Moreover, when the antagonist AC187 was perfused alone, the lactate efflux from hindlimb following a glucose challenge was reduced. Those findings were consistent with a contribution of endogenous amylin, secreted in response to a glucose challenge, having acted at skeletal muscle to release lactate (Vine *et al.,* 1995).

VIII. Glucose Efflux from Muscle

Essentially, muscle is not perceived as a glucose-producing organ, because it lacks the enzyme glucose-6-phosphatase to convert glucose-6-phosphate produced by glycogenolysis or gluconeogenesis into free glucose. Only glucose-producing organs such as liver and kidney (and β-cells) contain this

enzyme. But plasma glucose increased in frogs during recovery from exercise, and this new glucose was shown in hepatectomized frogs to originate in muscle (Fournier and Guderley, 1993). The likely explanation was glucosidic breakdown; during the hydrolysis of branched-chain molecular glycogen, approximately every 10th cleavage involved a branch point at α-1,6 linkages instead of at the α-1,4 linkages that constitute the majority of main chains. Hydrolysis of the α-1,6 glucosidic bonds yield free intracellular glucose that can then exit the muscle cell via glucose transporters. This possible pathway was not thought to be relevant in mammalian muscle because of the presence of hexokinase that scavenges free glucose in muscle and rapidly converts it to glucose-6-phosphate (Fournier and Guderley, 1993), thereby maintaining low intracellular glucose concentrations and the gradient down which glucose flows from the extracellular space. But high intracellular glucose-6-phosphate concentrations, such as those that are produced by amylin- and catecholamine-mediated glycogenolysis, inhibit hexokinase and could create the environment that would allow net glucose efflux from muscle.

To study this phenomenon in mammals, a technique described previously was developed to radiolabel the muscle glycogen pool with 3-^{3}H-glucose, a process that was enhanced by prior glycogen-depleting exercise and the addition of insulin (Young *et al.,* 1993). Tritium at the three position on glucose is retained on glucose during glycogen synthesis and breakdown, for example, and is only lost to water during glycolysis and is eventually eliminated. After a short time, infused 3-^{3}H-glucose is cleared from plasma and allows 3-^{3}H-glucose newly entering the plasma to be easily detected (Young *et al.,* 1993). When this technique was used in anesthetized rats administered rat amylin to induce glycogenolysis, 3-^{3}H-glucose appeared in plasma, consistent with the release of new free glucose from muscle (Young *et al.,* 1993). It is possible that glucosidic release of free glucose from muscle due to amylin and other glycogenolytic agents, such as catecholamines, could be significant in maintaining glucose balance in some circumstances.

IX. Potencies for Amylin Effects in Muscle

The EC_{50} for amylin activation of cyclic AMP was 0.48 nM. The EC_{50} for change in muscle glycogen content was 0.9 nM, for phosphorylase activation, 2.2 nM, and for the increase in muscle lactate production, 1.5 nM (Pittner *et al.,* 1995a). These potencies were not discernibly different from each other and were consistent with their linkage in a mechanistic cascade.

The cascade was consistent with amylin effects on muscle glycogen being primarily due to stimulation of a specific receptor coupled (via G_s) to adenylate cyclase, which resulted in formation of the active (*a*) form of glycogen phosphorylase, resultant glycogenolysis, reductions of muscle glycogen content,

and increases in muscle glucose-6-phosphate content, thereby increasing glycolysis to an extent that the oxidative capacity of the muscle cell was exceeded, spilling lactate from the cell. This cascade of events is equivalent to that previously described for catecholamines in muscle (Fig. 6).

EC_{50}s in this analysis are those observed *in vitro*, where diffusional barriers exist due to absence of a microcirculation. On the presumption that the diffusional barrier is equal for all ligands and for all responses in the soleus muscle preparation, the *in vitro* EC_{50} is useful for identifying relative potency and, thereby, the pharmacology of receptors involved and the relationship between responses. However, interstitial concentrations of ligand (that is, concentrations near cell surface receptors) in *in vitro* preparations are likely to be lower than in *in vivo,* where the microcirculation is intact. *In vitro* potencies therefore likely represent a lower bound of *in vivo*

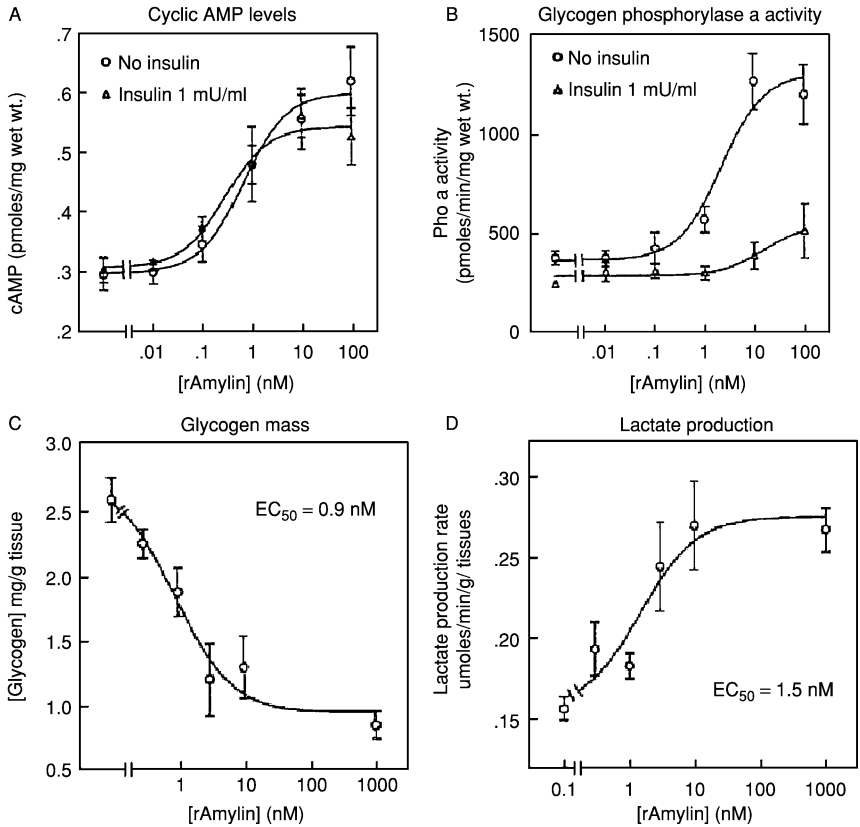

FIGURE 6 Amylin concentration-responses for (A) activation of cyclic AMP, (B) activation of glycogen phosphorylase, (C) depletion of muscle glycogen, and (D) muscle release of lactate in isolated stripped rat soleus muscle (Pittner *et al.*, 1995a).

potencies and may be less informative in identifying effects that prevail at physiological concentrations of ligand.

X. Transport of Glucose, 3-O-Methylglucose, and 2-Deoxyglucose

It is of some clinical importance whether or not amylin and amylinomimetic agents affect glucose transport in muscle, since they are used in individuals in whom insulin-mediated glucose transport via GLUT4 is often impaired. Glucose transport is commonly measured in cellular and whole body systems using labeled glucose analogs, especially 3-O-methylglucose (3OMG) and 2-deoxyglucose (2DOG).

An attribute of 3OMG is that it enters cells normally via glucose transporters, but is not further metabolized. Intracellular concentrations eventually come into equilibrium with those outside the cell, since the molecule can be transported in either direction. Until equilibration occurs, rates of intracellular accumulation of 3OMG can be used to assess glucose transport.

The limitation of the bidirectional flux of 3OMG can be overcome with 2DOG, a glucose analog that is not only transported, but also phosphorylated by hexokinase to 2-deoxyglucose-6-phosphate, but is not metabolized further. This feature essentially results in intracellular trapping of 2DOG at a rate that can reflect glucose transport. But 2DOG is toxic and cannot be meaningfully used except in acute non-clinical studies.

Both techniques are aptly applied in cell-based assays in which extracellular and intracellular compartments are easily studied. But interpretation of data can be difficult in isolated skeletal muscle, for example, where the diffusional barrier from bathing medium to cell membrane is significant, concentration gradients in the interstitium may be significant, the fractional volumes of interstitial and intracellular compartments are unknown, and assumed values can result in large errors. Assumptions in the case of 3OMG need to include that equilibrium is not being approached. In the case of 2DOG, it is assumed that transported analog is being phosphorylated and trapped.

Several studies have nonetheless looked at effects of amylin on levels of 3OMG and 2DOG in skeletal muscle. Where 3OMG accumulation in skeletal muscle has been measured, the effect of amylin has been to cause either no change (Young *et al.,* 1990) or a decrease (Castle *et al.,* 1998; Dimitriadis *et al.,* 1998a,b; Wallberg-Henrikson *et al.,* 1990; Zierath *et al.,* 1991, 1992). Muscles studied have included rat soleus (Dimitriadis *et al.,* 1998a,b; Young *et al.,* 1990), rat gastrocnemius (Young *et al.,* 1990), whole rat hindlimb (Castle *et al.,* 1998), rat epitrochlearis (Wallberg-Henrikson *et al.,* 1990), and human quadriceps muscle (Wallberg-Henrikson *et al.,*

1990; Zierath *et al.,* 1991, 1992). The concentration response in human muscle indicated an effect with amylin concentrations of 1 nM or greater, but not at 10 or 100 pM.

The results of these studies would appear to indicate, on balance, that amylin inhibited glucose transport in muscle, and some authors have concluded such as recently as 1998 (Dimitriadis *et al.,* 1998b). However, these conclusions fail to take into consideration the effect that net glucose efflux from muscle, described previously (Young *et al.,* 1993), would have on this measure. Glucose efflux into the extracellular space immediately adjacent to glucose transporters would tend to dilute 3OMG molecules with free glucose and reduce the probability that a 3OMG molecule would attach to a transporter. Similar issues may pertain to other glycogenolytic agents, such as catecholamines, for which 3OMG influx measurements would indicate an inhibition of glucose transport in muscle.

In an inspired accommodation of the limitations of 3OMG influx measurement in muscle, Clausen and Flatman developed a muscle assay in which 3OMG was allowed to accumulate until it was in equilibrium with the bathing medium (Clausen and Flatman, 1987). Then, by changing to a label-free medium, the rate of its appearance in the medium by reverse transport, related to number of transporters, could be followed with high sensitivity. In this assay, insulin increased rates of 3OMG efflux, but catecholamines had no effect (Clausen and Flatman, 1987). In a similar set of experiments in isolated soleus muscle, insulin increased the rate of 3OMG efflux (Pittner *et al.,* 1995b,c), and in agreement with Clausen and Flatman (Clausen and Flatman, 1987), epinephrine had no effect. Importantly, rat amylin at a concentration of 100 nM did not affect the rate of glucose efflux. That is, by this robust method, it appears that there is no direct effect of amylin on glucose transport in skeletal muscle.

In experiments looking at 2DOG in muscle, amylin and amylinomimetics appear to consistently reduce its accumulation. This is true in diaphragm (Hothersall and Muirhead, 1990; Hothersall *et al.,* 1990), L6 myocytes (Kreutter *et al.,* 1989, 1990), and soleus muscle (Kreutter *et al.,* 1990, 1994; Young *et al.,* 1990). It is observed with CGRP and with rat and human amylin. Some authors inferred that slowing of 2DOG accumulation was due to inhibition of hexokinase by glucose-6-phosphate, as it flooded cells following glycogenolysis (Young *et al.,* 1990). This explanation fits with the observation that amylin inhibition of 2DOG uptake is more a feature of glycogen-replete muscles from non-fasted rats than it is of glycogen-depleted muscles from fasted rats (Pittner *et al.,* 1995b,c), in which glycogenolysis would have a lesser effect on intracellular glucose-6--phosphate. Effects of amylin on 2DOG could be blocked with $sCT^{8–32}$ (AC66), an amylin antagonist that does not interact with CGRP receptors (Kreutter *et al.,* 1995). Effects of CGRP, possibly mediated via CGRP receptors, were not blocked with AC66 (Kreutter *et al.,* 1995).

XI. Na^+/K^+ Atpase in Muscle

Shifts in plasma K^+ concentration following amylin administration in rats appeared similar in character to those observed following administration in insulin and led to the prediction that amylin would activate Na^+/K^+ ATPase in muscle (Young *et al.*, 1996). A similar effect was observed with catecholamines (Clausen and Flatman, 1987) and with CGRP (Andersen and Clausen, 1993) in isolated skeletal muscle. A stimulation of Na^+/K^+ ATPase by amylin in muscle alluded to in 1996 (Clausen, 1996) was subsequently fully described (James *et al.*, 1999). Thus, in skeletal muscle, CGRP (Andersen and Clausen, 1993) and amylin (Clausen, 1996), like insulin and epinephrine (Clausen and Flatman, 1987), clearly activate Na^+/K^+-ATPase and could point to this being a CGRPergic effect. However, a similar effect of salmon calcitonin on skeletal muscle (Andersen and Clausen, 1993) is not consistent with this interpretation but is instead consistent with an amylin-like pharmacology. An association between reduced sodium pumping and a propensity to insulin resistance has been described (Mattiasson *et al.*, 1992). Endogenous digoxin-like and ouabain-like molecules that inhibit Na^+/K^+-ATPase are more prevalent in diabetic states and have been proposed to have a role in the pathogenesis of insulin resistance (Martinka *et al.*, 1997). In this context, activation of muscle Na^+/K^+-ATPase by amylin and amylinomimetics would, if anything, be expected to promote muscle insulin sensitivity rather than insulin resistance, perhaps explaining its lack of effect on the latter (Gilbey *et al.*, 1989; Wilding *et al.*, 1994).

References

Andersen, S. L., and Clausen, T. (1993). Calcitonin gene-related peptide stimulates active Na(+)-K+ transport in rat soleus muscle. *Am. J. Physiol.* **264**, C419–C429.

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Beaumont, K., Moore, C. X., Pittner, R., Prickett, K., Gaeta, L. S. L., Rink, T. J., and Young, A. A. (1994). Differential antagonism of amylin's metabolic and vascular actions with amylin receptor antagonists. *Can. J. Physiol. Pharmacol.* **72**, 31.

Beaumont, K., Moore, C. X., Pittner, R. A., Prickett, K. S., Gaeta, L. S. L., Rink, T. J., and Young, A. A. (1995a). Differential antagonism of amylin's metabolic and vascular actions with amylin receptor antagonists. *Can. J. Physiol. Pharmacol.* **73**, 1025–1029.

Beaumont, K., Pittner, R. A., Moore, C. X., Wolfe-Lopez, D., Prickett, K. S., Young, A. A., and Rink, T. J. (1995b). Regulation of muscle glycogen metabolism by CGRP and amylin: CGRP receptors not involved. *Br. J. Pharmacol.* **115**, 713–715.

Bell, D., Schluter, K. D., Zhou, X. J., McDermott, B. J., and Piper, H. M. (1995). Hypertrophic effects of calcitonin gene-related peptide (CGRP) and amylin on adult mammalian ventricular cardiomyocytes. *J. Mol. Cell Cardiol.* **27**, 2433–2443.

Benzo, C. A., and Stearns, S. B. (1982). Glycogen synthase and phosphorylase activities in skeletal muscle from genetically diabetic (db/db) mice. *Horm. Metab. Res.* **14**, 130–133.

Bogardus, C., Lillioja, S., Zawadski, J., Young, A., Abbott, W., Reaven, G., Knowler, W., Bennett, P., Mott, D., Howard, B., and Foley, J. (1986). Prospective study of metabolic changes during transition from normal to impaired glucose tolerance. *Clin. Res.* **34**, 681A.

Castle, A. L., Kuo, C. H., Han, D. H., and Ivy, J. L. (1998). Amylin-mediated inhibition of insulin-stimulated glucose transport in skeletal muscle. *Am. J. Physiol.* **275**, E531–E536.

Clausen, T. (1996). Long- and short-term regulation of the Na^+/K^+ pump in skeletal muscle. *News Physiol. Sci.* **11**, 24–30.

Clausen, T., and Flatman, J. A. (1987). Effects of insulin and epinephrine on Na^+-K^+ and glucose transport in soleus muscle. *Am. J. Physiol.* **252**, E492–E499.

Cooper, G. J. S., Leighton, B., Dimitriadis, G. D., Parry-Billings, M., Kowalchuk, J. M., Howland, K., Rothbard, J. B., Willis, A. C., and Reid, K. B. (1988). Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc. Natl. Acad. Sci. USA* **85**, 7763–7766.

Cooper, G. J. S., Roberts, A. N., Leighton, B., and Willis, A. C. (1989). Amylin and insulin resistance in the pathogenesis of type 2 insulin-dependent diabetes mellitus: Molecular correlates of biological activity. *Diabetologia* **32**, 477A.

Coppock, H. A., Owji, A. A., Bloom, S. R., and Smith, D. M. (1996). A rat skeletal muscle cell line (L6). expresses specific adrenomedullin binding sites but activates adenylate cyclase via calcitonin gene-related peptide receptors. *Biochem. J.* **318**, 241–245.

Crettaz, M., Prentki, M., Zaninetti, D., and Jeanrenaud, B. (1980). Insulin resistance in soleus muscle from obese Zucker rats: Involvement of several defective sites. *Biochem. J.* **186**, 525–534.

Cuendet, G. S., Loten, E. G., Jeanrenaud, B., and Renold, A. E. (1976). Decreased basal, noninsulin-stimulated glucose uptake and metabolism by skeletal soleus muscle isolated from obese-hyperglycemic (ob/ob). mice. *J. Clin. Invest.* **58**, 1078–1088.

Deems, R. O., Deacon, R., and Young, D. A. (1991a). Amylin activates glycogen phosphorylase via a cAMP-independent mechanism. *Diabetes* **40**, 20A.

Deems, R. O., Deacon, R. W., and Young, D. A. (1991b). Amylin activates glycogen phosphorylase and inactivates glycogen synthase via a cAMP-independent mechanism. *Biochem. Biophys. Res. Commun.* **174**, 716–720.

Dimitriadis, G., Crowne, E., Clark, A., and Dunger, D. (1998a). Effects of islet amyloid polypeptide on IGF-1 induced glucose disposal in skeletal muscle. *Diabetologia* **41**, A166.

Dimitriadis, G., Crowne, E., Clark, A., and Dunger, D. B. (1998b). Islet amyloid polypeptide decreases the effects of insulin-like growth factor-I on glucose transport and glycogen synthesis in skeletal muscle. *Int. J. Biochem. Cell Biol.* **30**, 1039–1046.

Foot, E. A., Da Costa, C., and Leighton, B. (1990). Amylin and CGRP decrease insulin-mediated glycogenesis in rat diaphragm *in vivo*. *Diabetologia* **33**, A112.

Fournier, P. A., and Guderley, H. (1993). Glucosidic pathways of glycogen breakdown and glucose production by muscle from postexercised frogs. *Am. J. Physiol.* **265**, R1141–R1147.

Gilbey, S. G., Bretherton-Watt, D., Beacham, J., Ghatei, M. A., and Bloom, S. R. (1989). High dose amylin in man: Unexpected failure to affect intravenous glucose tolerance. *BDA Diabet. Med.* **6**, 5A.

Hothersall, J. S., and Muirhead, R. P. (1990). Effect of amylin and calcitonin gene-related peptide on insulin-stimulated glucose and calcium transport in the diaphragm. *Biochem. Soc. Trans.* **18**, 1241–1243.

Hothersall, J. S., Muirhead, R. P., and Wimalawansa, S. (1990). The effect of amylin and calcitonin gene-related peptide on insulin-stimulated glucose transport in the diaphragm. *Biochem. Biophys. Res. Commun.* **169**, 451–454.

James, J. H., Wagner, K. R., King, J. K., Leffler, R. E., Upputuri, R. K., Balasubramaniam, A., Friend, L. A., Shelly, D. A., Paul, R. J., and Fischer, J. E. (1999). Stimulation of both

aerobic glycolysis and Na (+)-K (+)-ATPase activity in skeletal muscle by epinephrine or amylin. *Am. J. Physiol.* **277**, E176–E186.

Janes, S., Gaeta, L., Beaumont, K., Beeley, N., and Rink, T. (1996). The selection of pramlintide for clinical evaluation. *Diabetes* **45**, 235A.

Jue, T., Rothman, D. L., Shulman, G. I., Tavitian, B. A., De Fronzo, R. A., and Shulman, R. G. (1989). Direct observation of glycogen synthesis in human muscle with 13C NMR. *Proc. Natl. Acad. Sci. USA* **86**, 4489–4491.

Kim, J. K., and Youn, J. H. (1996). Suppression of glucose metabolism during euglycemic hyperinsulinemic clamps causes insulin resistance in skeletal muscle in rats. *Diabetes* **45**, 166A.

Kim, J. K., and Youn, J. H. (1997). Prolonged suppression of glucose metabolism causes insulin resistance in rat skeletal muscle. *Am. J. Physiol.* **35**, E288–E296.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., and Eto, T. (1993). Adrenomedullin: A novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.* **192**, 553–560.

Kreutter, D., Orena, S. J., and Andrews, K. M. (1989). Suppression of insulin-stimulated glucose transport in L6 myocytes by calcitonin gene-related peptide. *Biochem. Biophys. Res. Commun.* **164**, 461–467.

Kreutter, D., Orena, S. J., and Andrews, G. C. (1990). Induction of insulin resistance by amylin in isolated soleus muscle and cultured myocytes. *Diabetes* **39**, 121A.

Kreutter, D. K., Torchia, A. J., Schmidberger, F. J., and Stevenson, R. W. (1993). Failure of amylin to suppress insulin action due to desensitization by chronic amylin infusion. *Diabetes* **42**, 36A.

Kruetter, D., Brodeur, A. M., Torchia, A. J., Stevenson, R. W., and Groton, C. T. (1994). Homologous desensitization to amylin; evidence for distinct amylin and CGRP receptors. *Diabetes* **43**, 533.

Kreutter, D. K., Brodeur, A. M., Torchia, A. J., Orena, S. J., and Stevenson, R. W. (1995). Inhibition of insulin action in rat soleus muscle by amylin is mediated by a sCT (8–32)-sensitive receptor. *Diabetes* **44**, 244A.

Larner, J., Roach, P. J., Huang, L. C., Brooker, G., Murad, F., and Hazen, R. (1979). Hormonal control of glycogen metabolism. *Adv. Exp. Med. Biol.* **111**, 103–123.

Lawrence, J. C., and Zhang, J. N. (1994). Control of glycogen synthase and phosphorylase by amylin in rat skeletal muscle—hormonal effects on the phosphorylation of phosphorylase and on the distribution of phosphate in the synthase subunit. *J. Biol. Chem.* **269**, 11595–11600.

Leaming, R., Johnson, A., Hook, G., Hanley, R., and Baron, A. (1995). Amylin modulates insulin secretion in humans: Studies with an amylin antagonist. *Diabetologia* **38**, A113.

Leighton, B., and Cooper, G. J. S. (1988). Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle *in vitro*. *Nature* **335**, 632–635.

Leighton, B., and Cooper, G. J. S. (1990). The role of amylin in the insulin resistance of non-insulin-dependent diabetes mellitus. *Trend Biochem. Sci.* **15**, 295–299.

Leighton, B., Cooper, G. J. S., Willis, A. C., and Rothbard, J. B. (1988). Amylin inhibits glucose utilisation in the soleus muscle of the rat *in vitro*. *Diabetologia* **31**, 513A.

Leighton, B., Foot, E. A., and Cooper, G. J. S. (1989). The effects of calcitonin gene-related peptide and amylin on glycogen metabolism in muscle are dependent on skeletal muscle fibre type. *BDA Diabet. Med.* **6**, A4.

Leighton, B., Da Costa, C., and Foot, E. (1990). Effect of synthetic human amylin on glycogen synthesis in skeletal muscle *in vivo*. *Biochem. Soc. Trans.* **18**, 980.

Le Marchand-Brustel, Y., and Freychet, P. (1980). Alteration of glycogen synthase activation by insulin in soleus muscles of obese mice. *FEBS Lett.* **120**, 205–208.

Le Marchand-Brustel, Y., Jeanrenaud, B., and Freychet, P. (1978). Insulin binding and effects in isolated soleus muscle of lean and obese mice. *Am. J. Physiol.* **234**, E348–E358.

Lillioja, S., Mott, D. M., Zawadzki, J. K., Young, A. A., Abbott, W. G., and Bogardus, C. (1986). Glucose storage is a major determinant of *in vivo* "insulin resistance" in subjects with normal glucose tolerance. *J. Clin. Endocrin. Metab.* **62**, 922–927.

Lillioja, S., Mott, D. M., Zawadzki, J. K., Young, A. A., Abbott, W. G., Knowler, W. C., Bennett, P. H., Moll, P., and Bogardus, C. (1987). *In vivo* insulin action is familial characteristic in nondiabetic Pima Indians. *Diabetes* **36**, 1329–1335.

Maggi, C. A., Chiba, T., and Giuliani, S. (1991). Human alpha-calcitonin gene-related peptide-(8–37). as an antagonist of exogenous and endogenous calcitonin gene-related peptide. *Eur. J. Pharmacol.* **192**, 85–88.

Martinka, E., Galajada, P., Ochodnicky, M., Lichardus, B., Straka, S., and Mokan, M. (1997). Endogenous digoxin-like immunoactivity and diabetes mellitus: Facts and hypotheses. *Med. Hypotheses* **49**, 271–275.

Mattiasson, I., Berntorp, K., and Lindgarde, F. (1992). Insulin resistance and $Na^+/K(+)$-ATPase in hypertensive women: A difference in mechanism depending on the level of glucose tolerance. *Clin. Sci. (Colch.)* **82**, 105–111.

Moore, C., and Rink, T. J. (1993). Amylin activates adenylyl cyclase in rat soleus muscle. *Diabetes* **42**, 257A.

Petersen, K. F., Hendler, R., Price, T., Perseghin, G., Rothman, D. L., Held, N., Amatruda, J. M., and Shulman, G. I. (1998). C-13/P-31 NMR studies on the mechanism of insulin resistance in obesity. *Diabetes* **47**, 381–386.

Pittner, R. A., Wolfe-Lopez, D., Lupien, J., Beaumont, K., Young, A. A., and Rink, T. J. (1994a). Amylin-evoked dose-dependent increase in cAMP, activation of glycogen phosphorylase, lactate release and reduction in glycogen content in rat skeletal muscle. *Can. J. Physiol. Pharmacol.* **72**, 225.

Pittner, R. A., Wolfe-Lopez, D., Lupien, J., Beaumont, K., Young, A. A., and Rink, T. J. (1994b). Amylin increases cyclic AMP levels and stimulates glycogenolysis in rat skeletal muscle. *Diabetologia* **37**, A117.

Pittner, R., Beaumont, K., Young, A., and Rink, T. (1995a). Dose-dependent elevation of cyclic AMP, activation of glycogen phosphorylase, and release of lactate by amylin in rat skeletal muscle. *Biochim. Biophys. Acta* **1267**, 75–82.

Pittner, R. A., Lopez, D., Young, A. A., and Rink, T. (1995b). *In* Similar effects of amylin and epinephrine on glucose transport in isolated rat soleus muscle Program and Abstracts, Endocrine Society 77th Annual Meeting, p. 379.

Pittner, R. A., Wolfe-Lopez, D., Young, A. A., and Rink, T. J. (1995c). Amylin and epinephrine have no direct effect on glucose transport in isolated rat soleus muscle. *FEBS Lett.* **365**, 98–100.

Pittner, R. A., Wolfe-Lopez, D., Young, A. A., and Beaumont, K. (1996). Different pharmacological characteristics in L (6). and C2C12 muscle cells and intact rat skeletal muscle for amylin, CGRP and calcitonin. *Br. J. Pharmacol.* **117**, 847–852.

Price, T. B., Perseghin, G., Duleba, A., Chan, W., Chase, J., Rothman, D. L., Shulman, R. G., and Shulman, G. I. (1996). NMR studies of muscle glycogen synthesis in insulin-resistant offspring of parents with non-insulin-dependent diabetes mellitus immediately after glycogen-depleting exercise. *Proc. Natl. Acad. Sci. USA* **93**, 5329–5334.

Rink, T. J., Beaumont, K., and Young, A. A. (1993). Salmon calcitonin potently mimics amylin actions on isolated soleus muscle and in increasing plasma lactate and glucose in rats. *Br. J. Pharmacol.* **108**, 318P.

Roach, P. J., and Larner, J. (1976). Rabbit skeletal muscle glycogen synthase: II. Enzyme phosphorylation state and effector concentrations as interacting control parameters. *J. Biol. Chem.* **251**, 1920–1925.

Rothman, D. L., Shulman, R. G., and Shulman, G. I. (1991). NMR studies of muscle glycogen synthesis in normal and non-insulin-dependent diabetic subjects. *Biochem. Soc. Trans.* **19**, 992–994.

Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., De Fronzo, R. A., and Shulman, R. G. (1990). Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N. Engl. J. Med.* **322**, 223–228.

Stace, P. B., Fatania, H. R., Jackson, A., Kerbey, A. L., and Randle, P. J. (1992). Cyclic AMP and free fatty acids in the longer-term regulation of pyruvate dehydrogenase kinase in rat soleus muscle. *Biochim. Biophys. Acta* **1135**, 201–206.

Vine, W., Smith, P., Lachappell, R., Rink, T. J., and Young, A. A. (1995). Lactate production from the rat hindlimb is increased after glucose administration and is suppressed by a selective amylin antagonist: Evidence for action of endogenous amylin in skeletal muscle. *Biochem. Biophys. Res. Commun.* **216**, 554–559.

Vine, W., Beaumont, K., Gedulin, B., Pittner, R., Moore, C. X., Rink, T. J., and Young, A. A. (1996). Comparison of the *in vitro* and *in vivo* pharmacology of adrenomedullin, calcitonin gene-related peptide and amylin in rats. *Eur. J. Pharmacol.* **314**, 115–121.

Wallberg-Henrikson, H., Zierath, J. R., Andreasson, K., Galuska, D., Engstrom, U., Bestholtz, C., and Westermark, P. (1990). Islet amyloid polypeptide inhibits insulin-stimulated glucose transport in skeletal muscle independent of changes in glycogen levels. *Diabetologia* **33**, A40.

Weiel, J., Hull-Ryde, E., Irsula, O., and Frangakis, C. (1993). Activation of PKa by amylin in skeletal muscle. *Diabetes* **42**, 129A.

Wilding, J. P. H., Khandan-Nia, N., Bennet, W. M., Gilbey, S. G., Beacham, J., Ghatei, M. A., and Bloom, S. R. (1994). Lack of acute effect of amylin (islet associated polypeptide) on insulin sensitivity during hyperinsulinaemic euglycaemic clamp in humans. *Diabetologia* **37**, 166–169.

Young, A. A., Bogardus, C., Wolfe-Lopez, D., and Mott, D. M. (1988). Muscle glycogen synthesis and disposition of infused glucose in humans with reduced rates of insulin-mediated carbohydrate storage. *Diabetes* **37**, 303–308.

Young, D. A., Deems, R. O., Deacon, R. W., McIntosh, R. H., and Foley, J. E. (1990). Effects of amylin on glucose metabolism and glycogenolysis *in vivo* and *in vitro*. *Am. J. Physiol.* **259**, E457–E461.

Young, A. A., Mott, D. M., Stone, K., and Cooper, G. J. S. (1991). Amylin activates glycogen phosphorylase in the isolated soleus muscle of the rat. *FEBS Lett.* **281**, 149–151.

Young, A. A., Crocker, L., Clarke, H., Wolf-Lopez, D., and Rink, T. (1992a). Streptozotocin treatment of fatty zucker rats reverses insulin resistance in soleus muscle. *Diabetes* **41**, 107A.

Young, A. A., Gedulin, B., Wolfe-Lopez, D., Greene, H. E., Rink, T. J., and Cooper, G. J. S. (1992b). Amylin and insulin in rat soleus muscle: Dose responses for cosecreted noncompetitive antagonists. *Am. J. Physiol.* **263**, E274–E281.

Young, A., Carlo, P., Wolfe-Lopez, D., and Wang, M.-W. (1993a). Novel *in vivo* technique for continuous observation of muscle glycogenolysis. *Diabetes* **42**, 241A.

Young, A. A., Carlo, P., Smith, P., Wolfe-Lopez, D., Pittner, R., Wang, M. W., and Rink, T. (1993b). Evidence for release of free glucose from muscle during amylin-induced glycogenolysis in rats. *FEBS Lett.* **334**, 317–321.

Young, A. A., Gedulin, B., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., Larson, E., and Rink, T. J. (1994). Selective amylin antagonist suppresses rise in plasma lactate after intravenous glucose in the rat—evidence for a metabolic role of endogenous amylin. *FEBS Lett.* **343**, 237–241.

Young, A. A., Wang, M. W., Gedulin, B., Rink, T. J., Pittner, R., and Beaumont, K. (1995). Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. *Metabolism* **44**, 1581–1589.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Zhu, G. C., Dudley, D. T., and Saltiel, A. R. (1991). Amylin increases cyclic AMP formation in L6 myocytes through calcitonin gene-related peptide receptors. *Biochem. Biophys. Res. Commun.* **177**, 771–776.

Zierath, J. R., Galuska, D., Engström, U., Betsholtz, C., Westermark, P., and Wallberg-Henriksson, H. (1991). Islet amyloid polypeptide inhibits insulin and phorbol ester stimulated glucose transport in rat and human skeletal muscle. *Diabetes* **40**, 23A.

Zierath, J. R., Galuska, D., Engstrom, A., Johnson, K. H., Betsholtz, C., Westermark, P., and Wallberg-Henriksson, H. (1992). Human islet amyloid polypeptide at pharmacological levels inhibits insulin and phorbol ester-stimulated glucose transport in in vitro incubated human muscle strips. *Diabetologia* **35**, 26–31.

Andrew Young

Effects in Liver

I. Summary

Amylin and calcitonin gene-related peptide (CGRP) were each shown to stimulate endogenous glucose production *in vivo* in rats. Neither peptide had any effect on any of several measures of intermediary carbohydrate metabolism in isolated hepatocytes or isolated perfused liver in rats.

The possibility exists that augmentation of endogenous glucose production was secondary to release of lactate from muscle into the plasma, thereby stimulating gluconeogenesis by increasing the availability of substrate. Results from hyperlactemic clamp preparations, which allowed for direct measurement of such an effect, suggested that there were additional mechanisms that accounted for amylin stimulation of endogenous glucose production in rats.

There is no evidence that amylin increases endogenous glucose production in humans.

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II. Effects of Amylinomimetic Agents on Endogenous Glucose Production

Endogenous production of glucose, derived either from breakdown of glycogen or from gluconeogenesis, is considered to occur primarily in liver. Rate of appearance in the plasma of glucose from all sources (R_a) can be measured by a tracer dilution technique (Wolfe, 1984). If a radiolabeled glucose tracer is constantly infused, its steady state activity in the plasma depends upon the rate of addition of nonlabeled glucose (R_a); if R_a is high, the glucose specific activity is low. During glucose clamping procedures, the rate of infusion of exogenous glucose is subtracted from R_a to obtain the rate of endogenous glucose production.

Endogenous glucose production has been measured during administration of amylin or amylinomimetic agents in 10 reports. Of these, nine explored endogenous glucose production during amylin infusion (Betts *et al.*, 1991; Choi *et al.*, 1991; Frontoni *et al.*, 1991; Holder *et al.*, 1991; Koopmans *et al.*, 1990, 1991; Molina *et al.*, 1990; Young *et al.*, 1990, 1991c) and three measured it during infusion of CGRP (Choi *et al.*, 1991; Molina *et al.*, 1989, 1990). In all reports, the amylinomimetic agent increased endogenous glucose production or blunted insulin suppression of endogenous glucose production. These findings appear to robustly support the finding that amylinomimetic agents can increase endogenous glucose production (interpreted as hepatic glucose production). This effect was present at circulating amylin concentrations of 220 pM (Koopmans *et al.*, 1991).

Following 4 weeks of subcutaneous injections of pramlintide (30 μg q.i.d.) in humans, there was no effect compared to placebo on endogenous glucose production or on the glycemic response to a glucagon challenge (Orskov *et al.*, 1999). In rats made diabetic with streptozotocin (STZ), daily amylin injections restored the depleted liver glycogen characteristic of that model (Young *et al.*, 1991a).

III. Direct Effects of Amylin in Hepatocytes

There was no evidence of a direct effect on indices related to glucose production in six studies of amylin effect in isolated hepatocytes (Fillers *et al.*, 1991; Morishita *et al.*, 1992; Pittner, 1997; Pittner *et al.*, 1996; Stephens and Hermeling, 1991; Stephens *et al.*, 1991).

In one particularly comprehensive study (Pittner, 1997), the effects of amylin and CGRP were compared to those of insulin and glucagon in primary monolayer cultures of rat hepatocytes on the following parameters: (1) cAMP production, (2) glycogen phosphorylase and synthase activities, (3) glycogen synthesis from both glucose and lactate, (4) glycogen

mass, (5) gluconeogenesis from lactate, (6) induction of the gluconeogenic enzyme tyrosine aminotransferase, and (7) chronic changes in lactate flux. Neither amylin nor CGRP (1 pM–100 nM) had a significant effect on any of these parameters, whereas significant effects of both insulin and glucagon were demonstrated. Neither amylin nor CGRP appeared to affect insulin or glucagon action (Pittner, 1997; Pittner *et al.*, 1996).

Effects of amylin and CGRP on several processes involved in carbohydrate metabolism were investigated in rat hepatocytes, non-parenchymal cells (Kupffer, Ito, and endothelial cells), and alveolar macrophages. There was no significant evidence of specific amylin binding sites in hepatocytes, and in contrast to the 25-fold increase in cyclic AMP induced by glucagon (10 nM), there was no cyclic AMP stimulation by amylin (100 nM), and no effect on glycogen phosphorylase activity, glucose output, lactate uptake, glycogen synthesis, glycogen mass, or tyrosine aminotransferase activity. These results were consistent with the notion that amylin did not exert a direct effect on hepatocytes (Pittner, 1997). Minor activation of cyclic AMP was observed only in non-parenchymal cells, consistent with other reports (Stephens and Hermeling, 1991; Stephens *et al.*, 1991).

Further support for the absence of a direct effect of amylin in hepatocytes was its failure to modulate insulin stimulation of glucokinase gene expression (Nouspikel *et al.*, 1992).

IV. Effects of Amylin in Isolated Perfused Liver

There are five reports in which the effects of amylin on glucose production from the isolated perfused liver were described (Nishimura *et al.*, 1991, 1992; Roden *et al.*, 1990, 1991, 1992). In none of them was an effect of amylin noted, even though the preparations responded appropriately to insulin, glucagon, and gluconeogenic substrates. These reports also were consistent with the notion that amylin did not exert a direct effect in liver.

V. Cori Cycle-Independent Effects on Endogenous Glucose Production

The absence of amylin effect in each of six studies in hepatocytes, and in each of five studies in the isolated perfused liver, contrasted with the ability of amylinomimetic agents to increase endogenous glucose production measured by tracer dilution in each of 10 studies in whole animals. There was the possibility that the hepatic response to amylin was different in isolated and intact preparations, as had been shown for other amylineric responses, including those of the endocrine pancreas. But it had also been shown from studies in which lactate infusions mimicked the plasma lactate

profiles obtained with amylin (Young *et al.*, 1991b, 1993b) that amylin might augment hepatic glucose output indirectly through Cori cycling of muscle-derived lactate. To understand whether there were effects of amylin on liver glucose production *in vivo* distinct from its promotion of gluconeogenic substrate supply, the latter needed to be kept constant. While this could be achieved in an isolated perfused preparation, the effects of amylin (or any hormone) on endogenous glucose production had not previously been explored *in vivo* under conditions in which the substrate drive had been maintained constant.

To study this question, the hyperlactemic clamp (described previously in relation to the measurement of lactate flux) was developed (Gedulin *et al.*, 1994; Rink and Gedulin, 1993; Rink *et al.*, 1994). In one manifestation of the hyperlactemic clamp, plasma lactate concentration was maintained by variable lactate infusion at 4 mM, approximately 10-fold higher than basal plasma lactate concentration. At this level it was expected to dominate substrate drive for gluconeogenesis. Plasma glucose was also maintained constant at 12 mM, thereby equalizing the lactate → glucose gradient up which gluconeogenesis was being driven, and facilitating the measurement of endogenous glucose production by the tritiated tracer dilution technique. The effects of endogenous pancreatic hormones were minimized in some experiments by somatostatin infusions. However, this peptide tends to produce hypotension in rats, so in other experiments secretion of endogenous pancreatic hormones was eliminated by subtotal acute pancreatectomy. In this elaborately controlled *in vivo* preparation, amylin increased endogenous glucose production by 35.4% (somatostatin experiments, $P < 0.05$) or by 51% (pancreatectomy experiments, $P < 0.05$) (Gedulin *et al.*, 1994; Rink *et al.*, 1994).

That is, amylin had an effect of increasing endogenous glucose production (R_a), independent of its effect of increasing gluconeogenic substrate supply, but this observation was manifest only in intact animals and not in isolated liver preparations or hepatocytes. The possibility that substrate-independent effects of amylin on R_a observed in intact animals may have been mediated via the autonomic nervous system is presently untested. A possible site of action is the kidney, a recognized gluconeogenic tissue that is amylin responsive. Another possibility to be considered and quantified is the glucosidic release of free glucose from muscle (Young *et al.*, 1993a) following amylin-induced glycogenolysis, as described in Chapter 11 of this volume.

In the one study in which it was measured, there was no indication that amylinomimetics increased endogenous glucose production in humans. In one crossover study in patients with type 1 diabetes, 4 weeks therapy with pramlintide ending the night prior to measurement resulted in no difference in isotopically measured endogenous glucose production (Orskov *et al.*, 1997a,b, 1999).

References

Betts, J. J., Lupien, J. R., and Horton, E. S. (1991). Amylin induces *in vivo* insulin resistance in rats. *Diabetes* **40**, 35A.

Choi, S. B., Frontoni, S., and Rossetti, L. (1991). Mechanism by which calcitonin gene-related peptide antagonizes insulin action *in vivo*. *Am. J. Physiol.* **260**, E321–E325.

Fillers, W. S., Cohen, D. K., and Bell, P. A. (1991). Amylin sensitivity in isolated hepatocytes. *Diabetes* **40**, 21A.

Frontoni, S., Choi, S. B., Banduch, D., and Rossetti, L. (1991). *In vivo* insulin resistance induced by amylin primarily through inhibition of insulin-stimulated glycogen synthesis in skeletal muscle. *Diabetes* **40**, 568–573.

Gedulin, B., Larson, E., Young, A. A., and Rink, T. J. (1994). Combined hyperlactemic and hyperglycemic clamps in anesthetized rats with acute pancreatectomy or somatostatin infusion; effects of amylin on endogenous lactate and glucose production. *Program and Abstracts, the Endocrine Society, 76th Annual Meeting*, 373.

Holder, J. C., Cawthorne, M. A., Cooper, G. J. S., Coyle, P. J., Rink, T. J., and Smith, S. A. (1991). Acute effects of amylin on hepatic and peripheral insulin action in the rat. *Diabetologia* **34**, A104.

Koopmans, S. J., vanMansfeld, A. D. M., Jansz, H. S., Krans, H. M. J., Radder, J. K., Frolich, M., deBoer, S. F., Kreutter, D. K., Andrews, G. C., and Maassen, J. A. (1990). Pancreatic amylinamide induces *in vivo* hepatic and peripheral insulin resistance in the rat. *Diabetes* **39**, 101A.

Koopmans, S. J., vanMansfeld, A. D. M., Jansz, H. S., Krans, H. M. J., Radder, J. K., Frölich, M., deBoer, S. F., Kreutter, D. K., Andrews, G. C., and Maassen, J. A. (1991). Amylin-induced in vivo insulin resistance in conscious rats: The liver is more sensitive to amylin than peripheral tissues. *Diabetologia* **34**, 218–224.

Molina, J. M., Cooper, G. J. S., and Olefsky, J. M. (1989). Calcitonin gene-related peptide causes insulin resistance *in vivo*. *Clin. Res.* **37**, 456A.

Molina, J. M., Cooper, G. J. S., Leighton, B., and Olefsky, J. M. (1990). Induction of insulin resistance *in vivo* by amylin and calcitonin gene-related peptide. *Diabetes* **39**, 260–265.

Morishita, T., Yamaguchi, A., Yamatani, T., Nakamura, A., Arima, N., Yamashita, Y., Nakata, H., Fujita, T., and Chiba, T. (1992). Effects of islet amyloid polypeptide (amylin) and calcitonin gene-related peptide (CGRP) on glucose metabolism in the rat. *Diabet. Res. Clin. Pract.* **15**, 63–69.

Nishimura, S., Sanke, T., Machida, K., Bessho, H., Nakai, K., Negoro, T., Kondo, M., and Nanjo, K. (1991). Effects of islet amyloid polypeptide (IAPP/amylin) on glucose output in perfused rat liver. *Diabetes* **40**, 242A.

Nishimura, S., Sanke, T., Machida, K., Bessho, H., Hanabusa, T., Nakai, K., and Nanjo, K. (1992). Lack of effect of islet amyloid polypeptide on hepatic glucose output in the *in situ* perfused rat liver. *Metabolism* **41**, 431–434.

Nouspikel, T., Gjinovci, A., Li, S. L., and Iynedjian, P. B. (1992). Unimpaired effect of insulin on glucokinase gene expression in hepatocytes challenged with amylin. *FEBS Lett.* **301**, 115–118.

Orskov, L., Nyholm, B., Hove, K., Gravholt, C., Moller, N., Kolterman, O., Alberti, K., and Schmitz, O. (1997). Effects of the amylin analogue pramlintide on the glucose response to a glucagon challenge in IDDM. *Diabetologia* **40**, A355.

Orskov, L., Nyholm, B., Hove, K. Y., Gravholt, C. H., Moller, O., Kolterman, O., Alberti, K. G. M. M., and Schmitz, O. (1997). Effects of the amylin analogue pramlintide on the glucose response to a glucagon challenge in IDDM. *Diabetes* **46**, 155A.

Orskov, L., Nyholm, B., Hove, K. Y., Gravholt, C. H., Moller, N., and Schmitz, O. (1999). Effects of the amylin analogue pramlintide on hepatic glucagon responses and intermediary metabolism in type 1 diabetic subjects. *Diabet. Med.* **16**, 867–874.

Pittner, R. A. (1997). Lack of effect of calcitonin gene-related peptide and amylin on major markers of glucose metabolism in hepatocytes. *Eur. J. Pharmacol.* **325**, 189–197.

Pittner, R., Wolfe-Lopez, D., Beaumont, K., and Rink, T. (1996). Lack of effect of amylin on major markers of glycogenolysis and gluconeogenesis on isolated rat hepatocytes. *Diabetes* **45**, 235A.

Rink, T., and Gedulin, B. (1993). Lactate clamp: A new technique for studying carbohydrate metabolism *in vivo*. *Diabetes* **42**(suppl. 1), 245A.

Rink, T. J., Larson, E., Young, A. A., and Gedulin, B. (1994). Effects of amylin on lactate metabolism in the rat: Studies using hyperactemic/hyperglycemic clamps. *Diabetologia* **37**, A117.

Roden, M., Kothbauer, I., Vierhapper, H., and Waldhäusl, W. (1990). Failure of amylin to affect insulin sensitivity in the isolated perfused rat liver. *Diabetologia* **33**, A39.

Roden, M., Fürnsinn, C., Vierhapper, H., and Waldhäusl, W. (1991). Amylin does not cause hepatic insulin-resistance in the perfused rat liver. *Diabetes* **40**, 36A.

Roden, M., Liener, K., Furnsinn, C., Nowotny, P., Hollenstein, U., Vierhapper, H., and Waldhausl, W. (1992). Effects of islet amyloid polypeptide on hepatic insulin resistance and glucose production in the isolated perfused rat liver. *Diabetologia* **35**, 116–120.

Stephens, T. W., and Hermeling, R. N. (1991). Liver amylin/CGRP receptors are present only in non-parenchymal cells and do not directly regulate liver glucose metabolism in the rat. *Diabetes* **40**, 300A.

Stephens, T. W., Heath, W. F., and Hermeling, R. N. (1991). Presence of liver CGRP/amylin receptors in only nonparenchymal cells and absence of direct regulation of rat liver glucose metabolism by CGRP/amylin. *Diabetes* **40**, 395–400.

Wolfe, R. R. (1984). "Tracers in Metabolic Research: Radioisotope and Stable Isotope/Mass Spectometry Methods." A. R. Liss, New York.

Young, A. A., Crocker, L. B., Wolfe-Lopez, D., and Cooper, G. J. S. (1991). Daily amylin replacement reverses hepatic glycogen depletion in insulin-treated streptozotocin diabetic rats. *FEBS Lett.* **287**, 203–205.

Young, A. A., Wang, M. W., and Cooper, G. J. S. (1991). Amylin injection causes elevated plasma lactate and glucose in the rat. *FEBS Lett.* **291**, 101–104.

Young, A. A., Wang, M.-W., Rink, T. J., and Cooper, G. J. S. (1991). Effects of intravenous injections of amylin in the fasted, anaesthetized rat. *J. Physiol.* **438**, 250P.

Young, A. A., Carlo, P., Smith, P., Wolfe-Lopez, D., Pittner, R., Wang, M. W., and Rink, T. (1993). Evidence for release of free glucose from muscle during amylin-induced glycogenolysis in rats. *FEBS Lett.* **334**, 317–321.

Young, A. A., Cooper, G. J. S., Carlo, P., Rink, T. J., and Wang, M. W. (1993). Response to intravenous injections of amylin and glucagon in fasted, fed, and hypoglycemic rats. *Am. J. Physiol.* **264**, E943–E950.

Young, D. A., Deems, R. O., Deacon, R. W., McIntosh, R. H., and Foley, J. E. (1990). Effects of amylin on glucose metabolism and glycogenolysis *in vivo* and *in vitro*. *Am. J. Physiol.* **259**, E457–E461.

Andrew Young

Effects in Fat

I. Summary

Biological actions in adipocytes can be mediated directly via circulating hormones or indirectly via sympathetic innervation of fat. Direct effects can be usefully assessed in preparations in which adipocytes have been dissociated from each other, and are therefore directly exposed to substrate and hormones.

Amylin appears to have no direct effects in isolated adipocytes.

II. Effects of Amylin in Isolated Adipocytes

Only four reports address the effects of amylinomimetic agents in isolated adipocytes. Cooper *et al.* studied the effects of synthetic human amylin in isolated adipocytes from rats (Cooper *et al.*, 1988). Adipocytes

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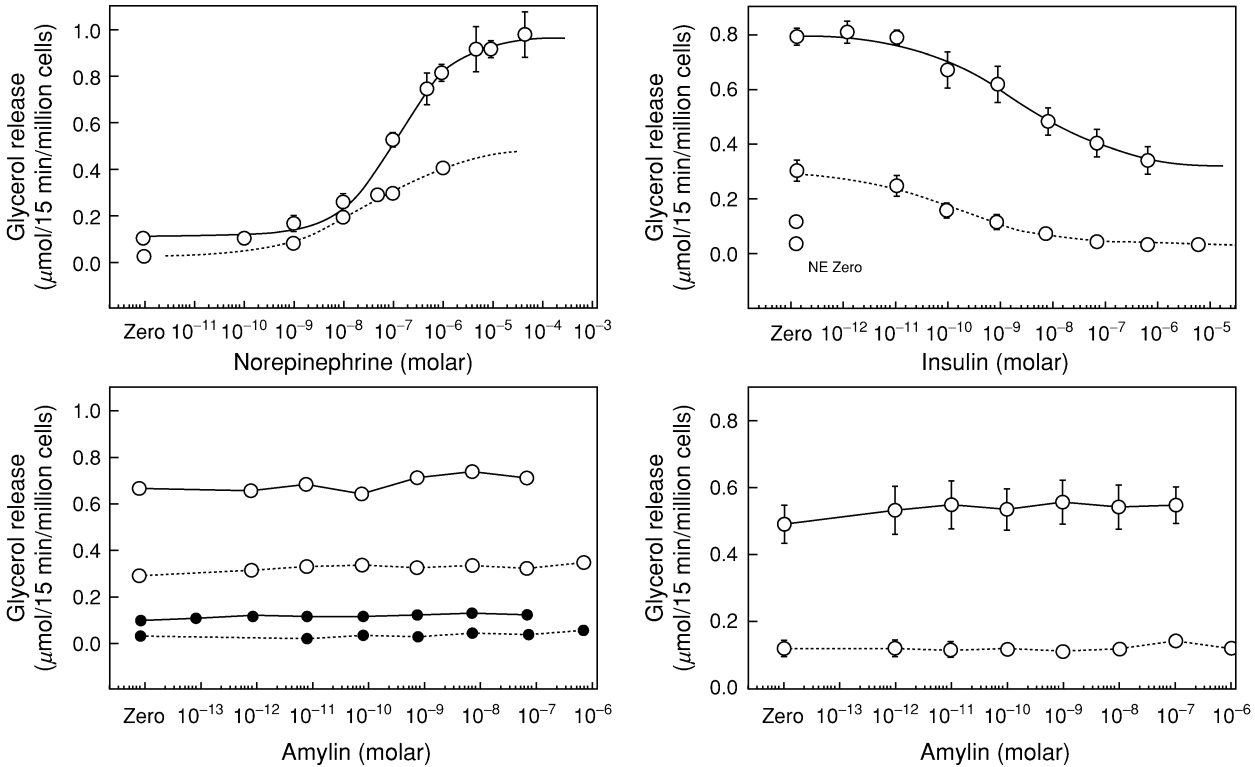

FIGURE 1 Left panel: Absence of effect of amylin at any dose to affect basal (closed circle) or epinephrine-stimulated (open circle) lipolysis in white adipocytes (solid line) or brown adipocytes (broken line). Right panel: Absence of effect of amylin at any dose to affect the antilipolytic effect of insulin in white adipocytes (solid line) or brown adipocytes (broken line) (Lupien and Young, 1993).

were prepared and dissociated by collagenase digestion as previously described (Green and Newsholme, 1979). They were incubated in medium containing U-^{14}C-glucose, ± insulin and ± human amylin (120 nM). Rates of glucose oxidation were assessed by the production of $^{14}CO_2$. Adipocytes showed a dose-dependent increase in the $^{14}CO_2$ production rate with addition of insulin, verifying that adipocytes were responsive, at least to that hormone. But addition of 120 nM human amylin had no effect. Effects of hormones on lipogenesis were assessed by rupturing adipocytes and separating aqueous and lipid fractions in an isopropanol/heptane/sulfuric acid step, and counting ^{14}C in the lipid fraction. Triacylglycerol production (lipogenesis) increased dose-dependently with addition of insulin. In contrast, amylin had no demonstrable effect on white adipocyte carbohydrate oxidation or insulin-stimulated incorporation of glucose into triacylglycerol.

In a similar preparation but using adipocytes obtained from lean, obese, and type 2 diabetic humans (Sinha *et al.*, 1991), Sinha *et al.* tested the effects of human amylin and calcitonin gene-related peptide (CGRP) on rates of uptake of labeled glucose in the absence and presence of insulin concentrations ranging from 10 pM to 100 nM. Neither human amylin (nominal concentrations of 100 pM, 10 nM, 100 nM) nor CGRP had an effect on basal or stimulated glucose transport. In addition, amylin had no effect on glucose oxidation in this system. A subsequent analysis of frozen incubation media revealed no extant amylin immunoreactivity, raising the question of what were the concentrations of active peptide during incubations.

In contrast, a recent report described expression of CGRP and adrenomedullin (but not amylin) in isolated human adipocytes in response to inflammation, and a dose-dependent lipolytic effect of CGRP and adrenomedullin (Linscheid *et al.*, 2005). An effect of adrenomedullin is more consistent with a CGRPergic than with an amylin-like pharmacology. There are no reports of effects of salmon calcitonin in isolated adipocytes.

Lupien and Young (Lupien and Young, 1993) studied amylin effects in white and brown adipocytes, using rat amylin to circumvent the limitations of human amylin in media solutions (Young *et al.*, 1992).

Amylin's effects on lipolysis in both white and brown adipocytes were determined in normal rats by measuring extracellular release of glycerol. Amylin (0.1 pM to 1 μM) did not alter basal or norepinephrine-stimulated (0.1 μM, 1 μM) lipolysis. Additionally when insulin (1 nM, 10 nM) was used in combination with norepinephrine, the antilipolytic effect of insulin was not altered by amylin. Thus, amylin did not affect basal or stimulated lipolysis in the presence or absence of insulin. Amylin had no effect in either white or brown isolated adipocytes (Fig. 1).

The absence of direct effect on adipocytes does not preclude an indirect effect. Autonomic activity, or inactivity (for example, from denervation; Shi *et al.*, 2005) has a major effect on cellularity and growth of fat depots.

References

Cooper, G. J. S., Leighton, B., Dimitriadis, G. D., Parry-Billings, M., Kowalchuk, J. M., Howland, K., Rothbard, J. B., Willis, A. C., and Reid, K. B. (1988). Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc. Natl. Acad. Sci. USA* **85**, 7763–7766.

Green, A., and Newsholme, E. A. (1979). Sensitivity of glucose uptake and lipolysis of white adipocytes of the rat to insulin and effects of some metabolites. *Biochem. J.* **180**, 365–370.

Linscheid, P., Seboek, D., Zulewski, H., Keller, U., and Muller, B. (2005). Autocrine/paracrine role of inflammation-mediated calcitonin gene-related peptide and adrenomedullin expression in human adipose tissue. *Endocrinology.* **146**, 2699–2708.

Lupien, J. R., and Young, A. A. (1993). No measurable effect of amylin on lipolysis in either white or brown isolated adipocytes from rats. *Diabet. Nutr. Metab.* **6**, 13–18.

Shi, H., Song, C. K., Giordano, A., Cinti, S., and Bartness, T. J. (2005). Sensory or sympathetic white adipose tissue denervation differentially affects depot growth and cellularity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R1028–R1037.

Sinha, M. K., Madigan, T., Cooper, G. J. S., and Caro, J. F. (1991). Effect of amylin and calcitonin gene-related peptide-1 (CGRP-1). on glucose transport in isolated adipocytes from lean, obese and NIDDM patients. *Diabetes* **40**(suppl. 1), 239A.

Young, A. A., Gedulin, B., Wolfe-Lopez, D., Greene, H. E., Rink, T. J., and Cooper, G. J. S. (1992). Amylin and insulin in rat soleus muscle: Dose responses for cosecreted noncompetitive antagonists. *Am. J. Physiol.* **263**, E274–E281.

Andrew Young

Cardiovascular Effects

I. Summary

Amylin can lower blood pressure in anesthetized animals (in which reflex bradycardia is absent), or evoke reflex bradycardia. This effect is likely in response to vasodilatation mediated via calcitonin gene-related peptide (CGRP) receptors, and only occurs at concentrations two to three orders of magnitude higher than physiological amylin concentrations.

There is suggestive, but not fully established, evidence for an amylin-like pharmacology with cardiotropic effects, consisting of inotropy (stimulation of contractility) and suppression of secretion of atrial natriuretic peptide (ANP).

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II. Effects of Amylin on Blood Pressure

The first demonstration of an effect of amylin on blood pressure and on vasodilator activity was in New Zealand White rabbits (Brain *et al.*, 1990). At 100-fold the dose of CGRP (10 nmol versus 100 pmol) amylin caused a similar fall in blood pressure (11 mm Hg). Vasoactivity was assessed by the rapidity with which a co-injected radiolabel disappeared from its subcutaneous injection site. Clearance of subcutaneously injected ^{133}Xe was dose-dependently increased by co-administration of either human amylin or CGRP. The potency of amylin was around 100-fold lower than that of CGRP (Fig. 1).

The first described effect of amylin on cardiovascular function in anesthetized rats was a reduction in blood pressure when rat amylin was injected intravenously as a 100 μg bolus (Young *et al.*, 1991). The 35 mm Hg fall in mean arterial pressure, which returned to baseline values over the next 30 min, was not associated with changes in plasma epinephrine or norepinephrine concentrations. Subcutaneous injections of the same amylin dose did not affect blood pressure, although glycemic and lactemic effects (described in Chapter 10) were present, indicating that they were not secondary to cardiovascular effects (Young *et al.*, 1991). Changes in plasma amylin concentration and changes in blood pressure followed very similar time courses, with an immediacy of effect that suggested a direct vasodilator effect (Young *et al.*, 1993). The clear association between blood pressure and simultaneously measured intravascular concentrations prompted a meta-analysis for the hypotensive concentration response for pramlintide

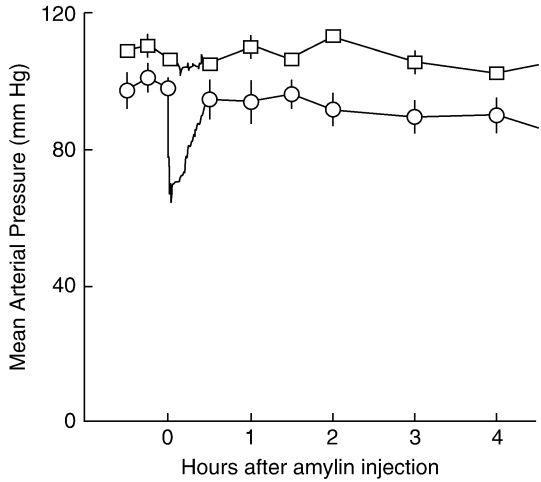

FIGURE 1 Acute effect of rat amylin injected as a 100 μg intravenous bolus on mean arterial pressure in anesthetized rats. From Young *et al.* (1991).

(Young *et al.*, 1996).The synthesis of 302 blood pressure measurements that could be paired with a concurrent plasma pramlintide measurement resulted in a robust relationship with an EC_{50} of 59.8 nM ± 0.09 log (Young *et al.*, 1996). This result concurred with the response obtained with steady plasma amylin concentrations resulting from continuous intravenous infusions (Young *et al.*, 1996). The EC_{50} for lowering of mean arterial pressure exceeded physiological concentrations of amylin, and therapeutic concentrations of the analog pramlintide, by approximately three orders of magnitude. The concentration response for this effect challenges any interpretation that it may be physiological, and prompts an examination that it may represent pharmacological action at receptors for allied ligands (Fig. 2).

Dose responses for the effects of intravenous rat amylin and CGRP on mean arterial pressure were compared in anesthetized rats (Wang *et al.*, 1991a; Young *et al.*, 1993). Both compounds lowered mean arterial pressure with greatest effect observed within 1–2 min, followed by a return to basal within 10–30 min. CGRP was ~44-fold more potent than amylin in producing hypotension, similar to the ~100-fold difference in vasodilatory potency observed in rabbit skin (Brain *et al.*, 1990). Different potencies and orders of potency for effects on blood pressure, glucose, lactate, and calcium (Wang *et al.*, 1991a; Young *et al.*, 1993) suggested that these effects were

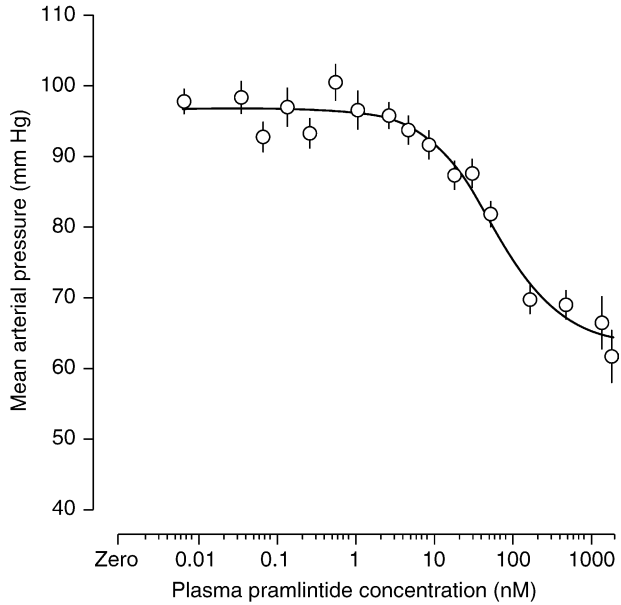

FIGURE 2 Relationship between plasma pramlintide concentration, delivered via subcutaneous, intravenous bolus or intravenous infusion, and simultaneously measured mean arterial pressure (Young *et al.*, 1996).

mediated via different receptors. For example, the ED_{50} for the hypotensive effect of amylin was 34 μg, but for the hypocalcemic effect it was 0.5 μg (Wang *et al.*, 1991a).

A small pressor response of low doses of rat amylin in anesthetized rats was reported in one study (Haynes *et al.*, 1997) but was not noted in others (Wang *et al.*, 1991a; Young *et al.*, 1993).

In conscious rats with arterial catheters (Gardiner *et al.*, 1991a), intravenous amylin infusion (2.5 nmol/kg/min) lowered mean arterial pressure by up to 21 $\pm$ 2 mm Hg ($P < 0.05$). This hypotensive effect was prevented by co-infusion of CGRPα[8–37] (Gardiner *et al.*, 1991a,c). In the same study, flow in renal, mesenteric, and hindquarter beds was assessed with Doppler flow probes during intravenous infusion of rat amylin at several rates. Heart rate increased by up to ~30%, and mean arterial pressure decreased by up to ~10% (Gardiner *et al.*, 1991b). From the tabulated data, ED_{50}s for these responses were assessed as being at least 0.63 and 0.74 nmol/kg/min, respectively. These infusion rates can be predicted from separately published pharmacokinetic data (Young *et al.*, 1996) to correspond to EC_{50}s of 197 and 211 nM, three to four orders of magnitude higher than physiological concentrations. Similarly, in anesthetized rats, decrements in mean arterial pressure during 3 hr continuous intravenous infusion allowed an amylin concentration response to be determined (Young *et al.*, 1996). The EC_{50} for the blood pressure effect of rat amylin was 25 nM $\pm$ 0.1 log, or three orders of magnitude above physiological concentrations (Fig. 3).

The hemodynamic effects of subcutaneous pramlintide have been evaluated in conscious rats fitted with a blood pressure transducer/transmitter (Data Sciences) in the abdominal aorta. Effects of subcutaneous doses of 0, 10, 100, or 1000 μg pramlintide, administered in a Latin square design, were evaluated for mean systolic, mean diastolic, mean arterial pressures, heart rate, and relative locomotor activity. Only the highest dose (1000 μg), estimated to result in plasma concentrations of ~30 nM, evoked a significant decrease in blood pressure, increase in heart rate, and increase in pulse pressure (systolic – diastolic), consistent with a reflexive response to vasodilation (Young *et al.*, 1996). Pramlintide continuously infused lowered mean arterial pressure with an ED_{50} of 167 nM $\pm$ 0.11 log (Young *et al.*, 1996).

It is significant, in view of a vasodilatory effect of amylin described previously, and in view of activation of the renin-angiotensin-aldosterone system described in Chapter 15, that chronic (52 week) dosing with pramlintide did not change blood pressure in humans at any dose (Young *et al.*, 1999).

In summary, amylin can lower blood pressure in anesthetized animals (in which reflex bradycardia is absent) or evoke reflex bradycardia in response to vasodilatation. This effect is likely mediated via CGRP receptors, and only occurs at concentrations two to three orders of magnitude higher than physiological amylin concentrations.

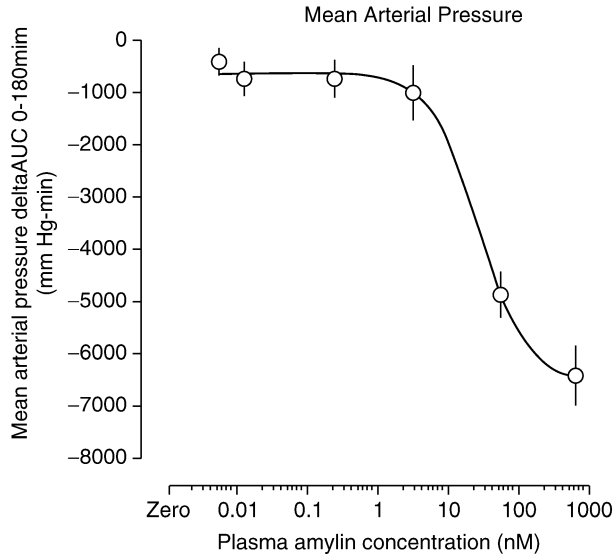

FIGURE 3 Relationship between steady state plasma amylin concentration attained by continuous intravenous infusion and blood pressure response. From Young *et al.* (1996).

III. Effects of Amylin in Specific Vascular Beds

A. Kidney

Amylin was ~400-fold less potent than CGRP in vasodilating isolated perfused rat kidneys. The selective amylin antagonist AC187 did not affect actions of either amylin or CGRP. Amylin displaced labeled CGRP from kidney binding sites ~80-fold less potently than did CGRP; in contrast, CGRP and amylin were equally potent in displacing labeled amylin from its kidney binding sites (Haynes *et al.*, 1994). These data are consistent with kidney vasodilator actions being mediated via CGRP receptors, rather than via amylin receptors.

In a similar study in perfused rat kidney, vascular tone was increased by addition of 3 μM norepinephrine. Potential vasodilator effects of rat CGRPα, CGRPβ, [Cys(ACM)$^{2-7}$]hCGRP (a putative CGRP2 agonist), rat amylin, and salmon calcitonin (sCT) were compared. The magnitude of effect of rat amylin was ~25% of that observed with rat CGRPα and CGRPβ. [Cys(ACM)$^{2-7}$]hCGRP (a CGRP2 agonist) and sCT (an amylin agonist with no action at CGRP receptors) were entirely without effect (Castellucci *et al.*, 1993). These results were consistent with renal vasodilatation occurring via CGRP rather than via amylin- or calcitonin-like receptors.

In a similar study in perfused rat kidney, vascular tone was increased by addition of 0.1 mM phenylephrine. Potential vasodilator effects of rat CGRPα, human CGRPα, and amylin were compared. The CGRPs were approximately equipotent in renal vasodilator activity, and were 12- to 26-fold more potent than amylin (Chin *et al.*, 1993, 1994). [Cys(ACM)$^{2-7}$] hCGRP (CGRP2 agonist) was without effect (Chin *et al.*, 1994).

In the dose–response study described earlier (Gardiner *et al.*, 1991b) in which the effects of infused rat amylin on hydraulic conductance in renal, mesenteric, and hindquarter beds were measured, conductance increased in renal and hindquarter vascular beds, with renal blood flow being the more sensitive (Gardiner *et al.*, 1991b). Hemodynamic changes were reversed by CGRP[8–37] (Gardiner *et al.*, 1991b).

B. Mesenteric Vascular Bed

In the preparation just described (Gardiner *et al.*, 1991b), there was no change in vasoactivity in the mesenteric bed. In a separate report, human amylin was vasodilatory in the mesenteric vascular bed of the rat, and was slightly less potent than human CGRP in relaxing vessels preconstricted with U46619, a thromboxane A2 mimic (Champion *et al.*, 1998). Both CGRP[8–37] and human amylin[8–37] blocked each of these responses.

C. Cutaneous Vascular Beds

Intravital microscopy was used to observe relaxation of 20–40 μm arterioles preconstricted with endothelin in the hamster cheek pouch. Vasodilator activities of human CGRPα and CGRPβ, rat CGRPα, and rat amylin were tested. The CGRPs were similar in potency and were 80- to 200-fold more potent than the effect of amylin (Hall and Brain, 1998). These authors reported that CGRP[8–37], considered a selective CGRP1 receptor antagonist, was more potent at blocking these responses than was human amylin [8–37] (Hall and Brain, 1998).

In anesthetized cats, pressure responses to intravenous bolus doses of adrenomedullin, CGRP, and amylin were measured. A balloon on a triple-lumen catheter guided via the jugular vein into the pulmonary artery of the left lower lung lobe enabled measurement of perfusion pressure at given flow rates and derivation of pulmonary vascular resistance. U46619 (Upjohn, Kalamazoo, MI) was added to raise intralobar arterial pressure to 35–40 mm Hg. Despite the absence of effect on systemic arterial pressure (Dewitt *et al.*, 1994), amylin was approximately equipotent with CGRP and adrenomedullin in this system, suggesting a preferential pulmonary vasodilator effect of amylin.

IV. Pharmacology of Vascular Effect

Amylin's hypotensive actions appear to be mediated by a pharmacological action at CGRP receptors, while its metabolic actions are mediated more potently by receptors with a distinct antagonist profile. High-affinity amylin binding sites present in rat nucleus accumbens bind ^{125}I-amylin with an affinity of 27 pM, have high affinity for sCT, and have moderately high affinity for CGRP. N-terminally truncated peptides tested for their ability to compete for ^{125}I-amylin binding were also compared for their respective abilities to antagonize the metabolic (soleus glycogen assay and hyperlactemia *in vivo*) and vascular actions of amylin. CGRP[8–37], sCT[8–32] (AC66), and ac-[Asn^{30},Tyr^{32}]sCT[8–32] (AC187) inhibited ^{125}I-amylin binding to rat nucleus accumbens with an order of potency of AC187 > sCT[8–32] > CGRP[8–37]. This order of potency matched that for inhibition of amylin's effects on isolated rat soleus muscle glycogen metabolism, and AC187 was more effective than either sCT[8–32] or CGRP [8–37] at reducing amylin-stimulated hyperlactemia in rats.

The order of potency just described for inhibition of amylin's metabolic actions differed from that for inhibition of ^{125}I-CGRP binding to SK-N-MC neuroblastoma cells (a CGRP receptor preparation), where CGRP[8–37] > AC187 > sCT[8–32]. This order of potency (rather than that described for soleus effects and nucleus accumbens binding) matched that for blocking hypotensive effects in rats. That is, amylin's hypotensive actions appear from antagonist studies to be mediated via CGRP receptors, in contrast to metabolic actions, which are mediated via a distinct pharmacology. The same (probably correct) conclusion has been drawn by others, who have (probably incorrectly) considered CGRP[8–37] selective for CGRP1 receptors (Castellucci *et al.*, 1993; Chin *et al.*, 1993, 1994; Gardiner *et al.*, 1991a,b,c; Haynes *et al.*, 1994). The (probably correct) conclusion that amylin receptors are not involved in hemodynamic responses is supported by the absence of vasoactive effect (Castellucci *et al.*, 1993) or hypotensive effect (Young *et al.*, 1995) of sCT, an amylin agonist (Young *et al.*, 1995) that binds to amylin (but not CGRP) receptors (Beaumont *et al.*, 1993). Conversely, adrenomedullin (Kitamura *et al.*, 1993) has CGRP-like hypotensive effects and is a more selective agonist at CGRP receptors (versus amylin or calcitonin receptors) than is CGRP, for example (Vine *et al.*, 1996). While adrenomedullin was near CGRP in its hypotensive potency, it was devoid of amylinergic or calcitoninergic action (inhibition of ^{14}C-glycogen formation in soleus muscle, hyperlactemia, hypocalcemia, and inhibition of gastric emptying). This result also supports the conclusion that it is CGRP-like receptors (rather than amylin- or calcitonin-like receptors) that underlie amylin's limited vasoactivity.

V. Direct Inotropic Effects

Bell and McDermott reported that amylin increased contractility of isolated ventricular cardiocytes (Bell and McDermott, 1995). Based upon effects in the presence of CGRP[8–37], they interpreted these effects to be mediated via $CGRP_1$ receptors and proposed that these effects were unlikely to be of physiological relevance but may have been of possible pathophysiological significance in hyperamylinemic states (Fig. 4).

A more recent paper described an effect of CGRP and of amylin to increase contractility in isolated cardiac tissue from pigs (Saetrum Opgaard *et al.*, 1999). Those authors also attributed the effect to an action via CGRP

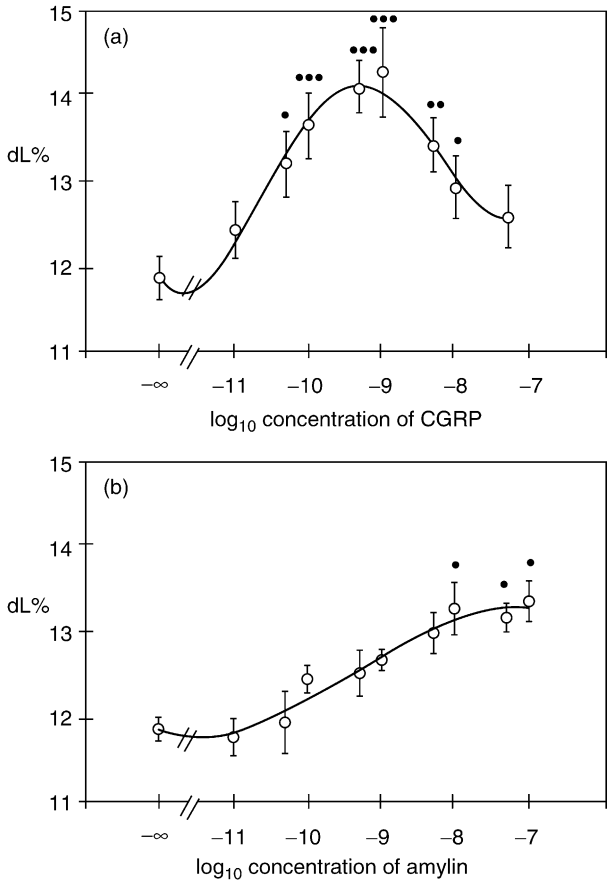

FIGURE 4 Concentration responses for effects of amylin and CGRP to augment electrically stimulated shortening of ventricular myocytes acutely isolated from rat heart. From Bell and McDermott (1995).

receptors, since it was blocked with CGRP[8–37]. CGRP can stimulate contractility in isolated human atrium (Franco-Cereceda *et al.*, 1987) and in guinea pig atrium (Franco-Cereceda and Lundberg, 1985). Amylin also produced a concentration-dependent inotropic effect in isolated left atrium of the guinea pig (Giuliani *et al.*, 1992), but it was 16–31 times less potent than α- and β-CGRP, respectively, and could be blocked with CGRP[8–37] at 1 μM. This pharmacology was somewhat different in urinary bladder strips, where amylin was ~100-fold less potent than the CGRPs, but the effects of ligands could not be blocked with CGRP[8–37] (Giuliani *et al.*, 1992). In another report of effects in whole rat hearts, both amylin and CGRP increased atrial contractility and suppressed the release of ANP, but CGRP was ~300-fold more potent than amylin (Piao *et al.*, 2004a).

A CGRPergic mechanism fitted with higher potencies for CGRP than for amylin, but did not explain, for example, an inotropic effect of calcitonins (Barabanova, 1976; Fiore *et al.*, 1978) that do not interact at CGRP receptors. On the other hand, not all studies observed an inotropic effect of sCT in isolated preparations (Chiba and Himori, 1977; Piao *et al.*, 2004b). One source of confusion may be the time domain over which inotropic responses are observed. Kaygisiz *et al.* (Kaygisiz *et al.*, 2003) observed positive inotropic effects of both CGRP and amylin in isolated rat hearts at low (1–100 nM) concentrations within 30 min. These effects subsequently decayed into a negative inotropic effect at 60 min and subsequent times. Effects were not seen with adrenomedullin, which argues against a CGRPergic pharmacology, since adrenomedullin behaves as a selective CGRP agonist (Vine *et al.*, 1996).

Conclusions based solely upon blockade with CGRP[8–37] can be erroneous, since CGRP[8–37] at appropriate doses can also block amylinergic effects (Wang *et al.*, 1991b). Blockade with an amylin-selective antagonist that is a poor blocker of CGRPergic activity, such as AC187, is more informative. AC187 blocked the inotropic effect of rat amylin in isolated perfused left atria of rats, and blocked the suppression of ANP (Piao *et al.*, 2004a).

The mechanism underlying increased contractility (inotropy) of amylinomimetic agents is unknown, but could involve Na^+/K^+-ATPase, for which there is evidence of an amylin effect. In the case of CGRP, one paper reported that the contractile effect includes a ouabain-sensitive component (Satoh *et al.*, 1986) (that is, involves Na^+/K^+-ATPase). The complicity of Na^+/K^+-ATPase in heart is clearer for catecholamines, which acutely stimulate Na^+/K^+-ATPase in myocytes (Desilets and Baumgarten, 1986; Dobretsov *et al.*, 1998). In skeletal muscle, CGRP (Andersen and Clausen, 1993) and amylin (Clausen, 1996), like insulin and epinephrine (Clausen and Flatman, 1987), activate Na^+/K^+-ATPase. Amylin activation of Na^+/K^+-ATPase in isolated soleus muscle in a high-potassium environment is associated with a restoration of contractility (Clausen, 1996). An effect of sCT to enhance

contractility in skeletal muscle (Andersen and Clausen, 1993) is not consistent with a CGRPergic pharmacology, but instead fits with an amylin-like pharmacology. It is conceivable that mechanisms present in skeletal muscle will also be present in cardiac muscle.

In summary, there is evidence in some isolated preparations for a stimulation of myocardial contractility and for an inhibition of secretion of ANP. Whether these effects are attributable to an amylin-like or to a CGRP-like pharmacology is yet to be fully established.

References

Andersen, S. L., and Clausen, T. (1993). Calcitonin gene-related peptide stimulates active Na(+)-K+ transport in rat soleus muscle. *Am. J. Physiol.* **264**, C419–C429.

Barabanova, V. V. (1976). Myocardial cell adenyl cyclase activity and the effect of thyrocalcitonin. *Fiziol. Zh SSSR Im I M Sechenova* **62**, 1355–1360.

Beaumont, K., Kenney, M. A., Young, A. A., and Rink, T. J. (1993). High affinity amylin binding sites in rat brain. *Mol. Pharmacol.* **44**, 493–497.

Bell, D., and McDermott, B. J. (1995). Activity of amylin at CGRP (1)-preferring receptors coupled to positive contractile response in rat ventricular cardiomyocytes. *Regul. Pept.* **60**, 125–133.

Brain, S. D., Wimalawansa, S., Mac Intyre, I., and Williams, T. J. (1990). The demonstration of vasodilator activity of pancreatic amylin amide in the rabbit. *Am. J. Pathol.* **136**, 487–490.

Castellucci, A., Maggi, C. A., and Evangelista, S. (1993). Calcitonin gene-related peptide (CGRP)1 receptor mediates vasodilation in the rat isolated and perfused kidney. *Life Sci.* **53**, PL153–PL158.

Champion, H., Bivalacqua, T., Pierce, R., Murphy, W., Coy, D., Hyman, A., McNamara, D., and Kadowitz, P. (1998). Amylin induces vasodilation by hCGRP-(8–37)/hAmylin-(8–37)-sensitive receptors in isolated resistance arteries from the mesenteric bed of the rat. *CGRP 1998: New Horizons in CGRP and Related Peptides Research* (abstract 15).

Chiba, S., and Himori, N. (1977). Effects of salmon calcitonin on SA nodal pacemaker activity and contractility in isolated, blood-perfused atrial and papillary muscle preparations of dogs. *Jpn. Heart J.* **18**, 214–220.

Chin, S. Y., Morton, I. K. M., and Hall, J. M. (1993). Vasodilator responses to CGRP in the rat isolated perfused kidney are mediated via CGRP1 receptors. *Br. J. Pharmacol.* **108**, 167P.

Chin, S. Y., Hall, J. M., Brain, S. D., and Morton, I. K. M. (1994). Vasodilator responses to calcitonin gene-related peptide (CGRP) and amylin in the rat isolated perfused kidney are mediated via CGRP (1). receptors. *J. Pharmacol. Exp. Ther.* **269**, 989–992.

Clausen, T. (1996). Long- and short-term regulation of the Na+/K+ pump in skeletal muscle. *News Physiol. Sci.* **11**, 24–30.

Clausen, T., and Flatman, J. A. (1987). Effects of insulin and epinephrine on Na+-K+ and glucose transport in soleus muscle. *Am. J. Physiol.* **252**, E492–E499.

Desilets, M., and Baumgarten, C. M. (1986). Isoproterenol directly stimulates the Na+-K+ pump in isolated cardiac myocytes. *Am. J. Physiol.* **251**, H218–H225.

Dewitt, B. J., Cheng, D. Y., Caminiti, G. N., Nossaman, B. D., Coy, D. H., Murphy, W. A., and Kadowitz, P. J. (1994). Comparison of responses to adrenomedullin and calcitonin gene-related peptide in the pulmonary vascular bed of the cat. *Eur. J. Pharmacol.* **257**, 303–306.

Dobretsov, M., Hastings, S. L., and Stimers, J. R. (1998). Na (+)-K+ pump cycle during beta-adrenergic stimulation of adult rat cardiac myocytes. *J. Physiol. (Lond.)* 507(Pt. 2), 527–539.

Fiore, C. E., Carnemolla, G., Grillo, S., Grimaldi, D. R., and Petralito, A. (1978). Inotropic effects of calcitonin in man. *Acta Cardiol.* **33**, 155–166.

Franco-Cereceda, A., and Lundberg, J. M. (1985). Calcitonin gene-related peptide (CGRP) and capsaicin-induced stimulation of heart contractile rate and force. *Naunyn Schmiedebergs Arch. Pharmacol.* **331**, 146–151.

Franco-Cereceda, A., Bengtsson, L., and Lundberg, J. M. (1987). Inotropic effects of calcitonin gene-related peptide, vasoactive intestinal polypeptide and somatostatin on the human right atrium *in vitro*. *Eur. J. Pharmacol.* **134**, 69–76.

Gardiner, S. M., Compton, A. M., Kemp, P. A., Bennett, T., Bose, C., Foulkes, R., and Hughes, B. (1991a). Antagonism of the haemodynamic actions of rat islet amyloid polypeptide by human α-calcitonin gene-related peptide [8–37] in conscious rats. *Br. J. Pharmacol.* **102**, 108P.

Gardiner, S. M., Compton, A. M., Kemp, P. A., Bennett, T., Bose, C., Foulkes, R., and Hughes, B. (1991b). Antagonistic effect of human α-calcitonin gene-related peptide [8–37] on regional hemodynamic actions of rat islet amyloid polypeptide in conscious Long Evans rats. *Diabetes* **40**, 948–951.

Gardiner, S. M., Compton, A. M., Kemp, P. A., Bennett, T., Bose, C., Foulkes, R., and Hughes, B. (1991c). Human alpha-cgrp 8–37 inhibits the haemodynamic effects of rat IAPP in conscious rats. *FASEB J.* **5**, A774.

Giuliani, S., Wimalawansa, S. J., and Maggi, C. A. (1992). Involvement of multiple receptors in the biological effects of calcitonin gene-related peptide and amylin in rat and guinea-pig preparations. *Br. J. Pharmacol.* **107**, 510–514.

Hall, J., and Brain, S. (1998). Amylin interacts with calcitonin gene-related peptide receptors in the microvasculature of the hamster cheek pouch *in vivo*. *Br. J. Pharmacol.* **107**, 510–514.

Haynes, J. M., Hodgson, W. C., and Cooper, M. E. (1997). Rat amylin mediates a pressor response in the anaesthetised rat: Implications for the association between hypertension and diabetes mellitus. *Diabetologia* **40**, 256–261.

Haynes, J. M., Wookey, P. J., Tikellis, C. M., Du, H. C., Sexton, P. M., and Cooper, M. E. (1994). Amylin and calcitonin gene related peptide in the rat kidney: Functional and autoradiographic studies. *Can. J. Physiol. Pharmacol.* **72**, 566.

Kaygisiz, Z., Erksap, N., Uyar, R., Kabadere, S., Kabadere, T. E., and Dernek, S. (2003). The effect of adrenomedullin, amylin fragment 8–37 and calcitonin gene-related peptide on contractile force, heart rate and coronary perfusion pressure in isolated rat hearts. *Acta Physiol. Hung.* **90**, 133–146.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., and Eto, T. (1993). Adrenomedullin: A novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.* **192**, 553–560.

Piao, F. L., Cao, C., Han, J. H., Kim, S. Z., Cho, K. W., and Kim, S. H. (2004a). Amylin-induced suppression of ANP secretion through receptors for CGRP1 and salmon calcitonin. *Regul. Pept.* **117**, 159–166.

Piao, F. L., Cao, C., Han, J. H., Kim, S. Z., and Kim, S. H. (2004b). Calcitonin gene-related peptide-induced suppression of atrial natriuretic peptide release through receptors for CGRP (1) but not for calcitonin and amylin. *Eur. J. Pharmacol.* **483**, 295–300.

Saetrum Opgaard, O., de Vries, R., Tom, B., Edvinsson, L., and Saxena, P. R. (1999). Positive inotropy of calcitonin gene-related peptide and amylin on porcine isolated myocardium. *Eur. J. Pharmacol.* **385**, 147–154.

Satoh, M., Oku, R., Maeda, A., Fujii, N., Otaka, A., Funakoshi, S., Yajima, H., and Takagi, H. (1986). Possible mechanisms of positive inotropic action of synthetic human calcitonin gene-related peptide in isolated rat atrium. *Peptides* **7**, 631–635.

Vine, W., Beaumont, K., Gedulin, B., Pittner, R., Moore, C. X., Rink, T. J., and Young, A. A. (1996). Comparison of the *in vitro* and *in vivo* pharmacology of adrenomedullin, calcitonin gene-related peptide and amylin in rats. *Eur. J. Pharmacol.* **314**, 115–121.

Wang, M.-W., Cooper, G. J. S., and Young, A. A. (1991a). Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic effects of amylin in the rat. *Diabetes* **40**, 241A.

Wang, M. W., Young, A. A., Rink, T. J., and Cooper, G. J. S. (1991b). 8–37h-CGRP antagonizes actions of amylin on carbohydrate metabolism *in vitro* and *in vivo*. *FEBS Lett.* **291**, 195–198.

Young, A. A., Wang, M. W., and Cooper, G. J. S. (1991). Amylin injection causes elevated plasma lactate and glucose in the rat. *FEBS Lett.* **291**, 101–104.

Young, A. A., Rink, T. J., and Wang, M. W. (1993). Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-I (CGRP-alpha). in the fasted, anaesthetized rat. *Life Sci.* **52**, 1717–1726.

Young, A. A., Wang, M. W., Gedulin, B., Rink, T. J., Pittner, R., and Beaumont, K. (1995). Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. *Metabolism* **44**, 1581–1589.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Young, A., Kolterman, O., and Hall, J. (1999). Amylin innocent in essential hypertension? *Diabetologia* **42**, 1029.

Andrew Young

Renal Effects

I. Summary

Amylin bound to kidney cortex in a distinctive pattern. Binding appeared specific in that it was displaceable with amylin antagonists. It was associated with activation of cyclic AMP (cAMP), and was thereby likely to represent receptor binding and activation. Amylin's principal effects at the kidney included a stimulation of plasma renin activity, reflected in aldosterone increases at quasi-physiological amylin concentrations. It was unclear whether this was a local or a systemic effect. Other renal effects in rats included a diuretic effect and a natriuretic effect. The latter was mainly driven by the diuresis, since urinary sodium concentration did not change.

Amylin had a transient effect to lower plasma potassium concentration. This effect was likely to be a consequence of activation of Na^+/K^+-ATPase, an action shared with insulin and catecholamines. Amylin lowered plasma

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calcium, particularly ionized calcium, likely due to an antiresorptive effect at osteoclasts.

Immunoreactive amylin was detected in the developing kidney. It appeared to have a trophic effect in kidney, and its absence resulted in renal dysgenesis.

Neurons in the subfornical organ (SFO), which has a role in fluid/electrolyte homeostasis, were potently activated by amylin. The dipsogenic and renal effects of amylin may be related to effects at the SFO.

II. Renovascular Effects

The first report of an amylin action at the kidney was renal vasodilation, described in Chapter 14 of this volume (Gardiner *et al.*, 1991). These actions were attributed to occur via calcitonin gene-related peptide 1 (CGRP1) receptors (Chin *et al.*, 1994; Hall and Brain, 1993) and were not blocked with the amylin receptor antagonist AC187 (Haynes *et al.*, 1994).

III. Amylin Binding in Kidney

The possibility of a direct effect of amylin at the kidney was raised when it was noted that it bound to kidney (Cooper *et al.*, 1992) in a pattern that appeared distinct from that of calcitonin or CGRP (Wookey *et al.*, 1996). The greater potency of the amylin antagonists AC66, AC413, and AC187 over hCGRP[8–37] in displacing labeled amylin indicated that the binding sites exhibited an amylin-like pharmacology (Haynes *et al.*, 1994; Wookey *et al.*, 1994a,b). Using labeled AC512 (an amylin antagonist that can bind to fixed tissue), Dilts *et al.* obtained a similar pattern of cortical, largely renotubular binding (Dilts *et al.*, 1995). Amylin binding at the kidney increased in the spontaneous hypertensive rat (SHR) and in surgically induced rat models of hypertension (Wookey *et al.*, 1997) but was not reduced by normalization of blood pressure with angiotensin converting enzyme inhibitors (Cao *et al.*, 1997), prompting an interpretation that amylin was somehow associated with blood pressure control. In the monkey, amylin also bound to the renal cortex (Cooper *et al.*, 1995a). In rats and monkeys, amylin bound to tubules (Chai *et al.*, 1998; Wookey *et al.*, 1996).

But since renal tubules are dense with proteases, it was unclear whether such binding was to receptors or to an enzymatic site. The observation that amylin could stimulate cAMP production in kidney slices indicated a receptor-mediated effect somewhere in the kidney (Sexton *et al.*, 1994; Wookey *et al.*, 1996). At cloned pig kidney calcitonin receptors (Lin *et al.*, 1991), amylin stimulated cAMP production as potently as did calcitonin, and since it circulates at greater concentrations than calcitonin, it may be a cognate ligand for such kidney receptors (Sexton *et al.*, 1994). In the monkey, amylin

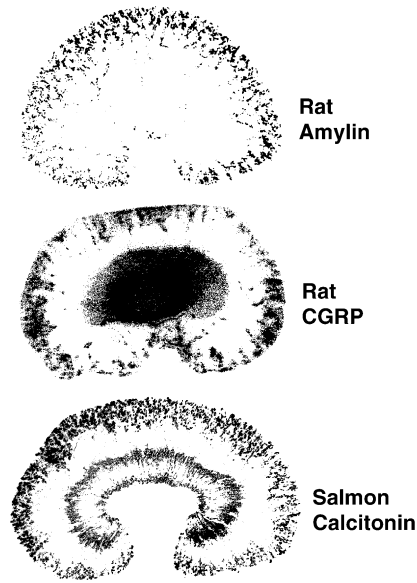

FIGURE 1 Binding of amylin, CGRP, and salmon calcitonin to rat kidney, showing distinctive cortical (glomerular) binding of ^{125}I-amylin. Images courtesy of Prof. Mark Cooper.

binding could be localized to the juxtaglomerular apparatus (Chai *et al.*, 1998; Sexton *et al.*, 1995), suggesting a possible effect in the renal renin-angiotensin system (Fig. 1).

IV. Effects on the Renin-Angiotensin-Aldosterone System

The first indication that amylin might affect the renin-angiotensin system was a doubling of plasma renin activity within 30 min of subcutaneous injection of 100 μg amylin in anesthetized rats (Young *et al.*, 1994c). Falls in blood pressure, which can themselves stimulate renin secretion, did not occur with this subcutaneous dose of rat amylin. The effect could be blocked with the amylin receptor blockers AC66, AC187 (Young *et al.*, 1994a), and AC625, pointing to an action mediated via an amylin-like receptor. The effect was not blocked with propranolol, indicating that it was independent of sympathetic activation (Young *et al.*, 1994c).

In human subjects, human amylin administered as primed/continuous infusions increased plasma renin activity by up to 97% (Cooper *et al.*, 1995b; McNally *et al.*, 1994) and, at some infusion rates, human amylin also increased aldosterone concentration by up to 62% (Nuttall *et al.*, 1995a,b; Young *et al.*, 1995). No change in blood pressure or plasma sodium concentration occurred at any dose (Young *et al.*, 1995).

A. Potency of Effect

Initial dose–response studies (Smith *et al.*, 1994; Young *et al.*, 1995) suggested that the effect could prevail at the elevated plasma amylin concentrations observed in insulin-resistant individuals. However, dose responses for effects on renin secretion were not performed in insulin-resistant animals (or humans). Since amylin resistance can be a concomitant feature of insulin resistance (as determined, for example, in effects on gastric emptying, described in Chapter 6), it should not be presumed that amylin concentrations that increase renin activity in insulin-sensitive individuals will do the same in insulin-resistant (hyperamylinemic) individuals.

B. Pharmacology of Effect

Increases in plasma renin activity with amylin in animals and humans are similar in magnitude and character to those reported for salmon calcitonin (Clementi *et al.*, 1986; Malatino *et al.*, 1987) and CGRP (both amylin agonists) (Braslis *et al.*, 1988; Gnaedinger *et al.*, 1989; Kurtz *et al.*, 1988; Palla *et al.*, 1995b). Concordance of effects of amylin, salmon calcitonin, and CGRP suggested an amylin-like pharmacology.

C. Hypothesis Linking Excess Amylin Action to Hypertension

The observation that amylin agonists could stimulate renin secretion led to the proposal that excess amylin action (for example, in hyperamylinemic insulin-resistant individuals) could contribute to obesity-related hypertension (Young *et al.*, 1994b). Others have since made similar speculations (Cooper, 1997; Cooper *et al.*, 1995b; Haynes *et al.*, 1997; Williams, 1994; Wookey and Cooper, 1998; Wookey *et al.*, 1996). This hypothesis was initially attractive for a number of reasons:

1. Hyperinsulinemia is robustly associated with essential (obesity-related) hypertension (Modan *et al.*, 1985; Welborn *et al.*, 1966), now recognized in the term syndrome X (Reaven, 1988). And although insulin had potentially hypertensive actions (DeFronzo, 1981; Landsberg, 1989), the data linking the metabolic defects of insulin resistance with hypertension were associative rather than causal, such that the precise nature of this relationship remained unexplained (Hall, 1993; Jarrett, 1991; Lefèbvre, 1993). Hyperinsulinemia per se is unlikely to be directly responsible for elevation of blood pressure (Hall, 1993; Jarrett, 1992; Lefèbvre, 1993); chronic infusions of insulin either systemically or intrarenally (Brands *et al.*, 1991; Briffeuil *et al.*, 1992; Hall *et al.*, 1990a,b, 1991a,b) failed

to elevate blood pressure in animal models. Patients with insulinoma, although hyperinsulinemic (and not hyperamylinemic; Nieuwenhuis *et al.,* 1992a,b), did not exhibit a propensity to be hypertensive (Pontiroli *et al.,* 1992; Sawicki *et al.,* 1992).

2. Agents that reduced β-cell secretion by improving insulin sensitivity, such as the thiazolidinediones (Kotchen, 1994; Yoshioka *et al.,* 1993) and metformin (Landin-Wilhelmsen, 1992; Morgan *et al.,* 1992), could also reduce blood pressure, as did other maneuvers that reduced β-cell secretion, including exercise (Reaven *et al.,* 1988) and somatostatin administration (Carretta *et al.,* 1989; Reaven *et al.,* 1989). Conversely, maneuvers that increased β-cell secretion, such as the administration of sulfonylurea drugs (Peuler *et al.,* 1993) or fructose feeding (Hwang *et al.,* 1987), were reported to increase blood pressure. None of these associations distinguished between effects that might be attributable to hypersecretion of insulin versus hypersecretion of amylin.

3. While the role of plasma renin in the pathogenesis of common forms of hypertension is still debated, partly because measured renin was thought not to vary with blood pressure (Meade *et al.,* 1983), many lines of evidence now implicate it. A study of normal weight, normotensive obese and hypertensive obese individuals (Licata *et al.,* 1994) found plasma renin activity to be a major covariant of blood pressure. In longitudinal studies, increases in arterial pressure associated with weight gain were also associated with increases in plasma renin activity (Hall *et al.,* 1993), while decreases in pressure associated with weight loss were associated with decreases in plasma renin activity (Tuck *et al.,* 1981). The effectiveness of renin inhibitors, angiotensin converting enzyme (ACE) inhibitors (Laragh *et al.,* 1977), and selective angiotensin II subtype 1 (AT1) receptor antagonists (Brunner *et al.,* 1993) in the treatment of obesity-related hypertension also pointed to a pathogenic role for the renin angiotensin system in this condition.

4. The only patient thus far described as having a tumor secreting an amylin-like substance was discovered during investigation for unexplained hypertension (Stridsberg *et al.,* 1992). Blood pressure returned to normal and metabolic state was ameliorated following treatment with streptozotocin. The patient died unexpectedly from a cerebral hemorrhage (Stridsberg *et al.,* 1993).

5. Renin-angiotensin elevations of the magnitude evoked with amylin can elevate blood pressure. While an 80-fold elevation of angiotensin II was required to acutely increase arterial pressure by 50 mm Hg in rats (Lever, 1993), lesser infusions could nonetheless lead to a slower pressor response (Dickinson and Lawrence, 1963; McCubbin *et al.,* 1965) that developed over 3–5 days (Brown *et al.,* 1981). In contrast to the 80-fold elevation required for an acute effect, it required only a 2- to 6-fold elevation

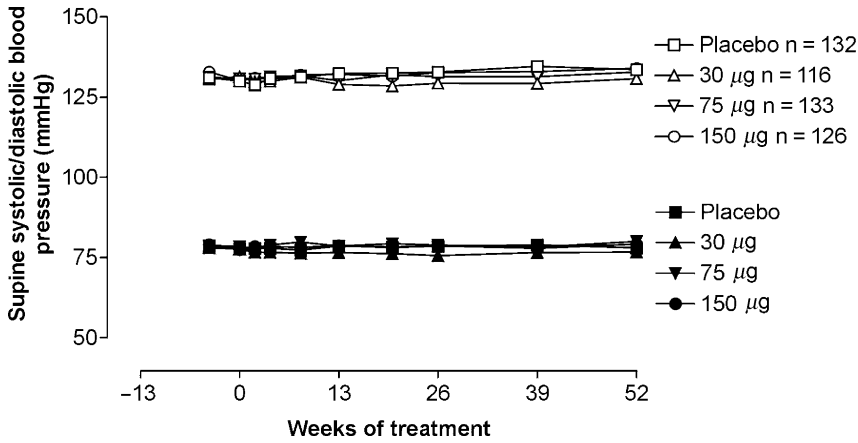

FIGURE 2 Absence of effect of 1 year of pramlintide administration on blood pressure in humans. From Young *et al.* (1999).

of angiotensin II to increase arterial pressure 50 mm Hg by the slow pressor effect (Lever, 1993).

The hypothesis wherein excess amylin action, via the renin-angiotensin-aldosterone axis, leads to elevations of blood pressure was explored in hyperamylinemic subjects with the amylin antagonist AC625 (Bryan *et al.*, 1995). While it blocked the effects of exogenous human amylin to stimulate renin secretion in humans, AC625 had no effect, when infused for 4 days, on blood pressure in hyperamylinemic subjects. Second, dogs made hyperinsulinemic, hyperamylinemic, hyperreninemic, and hypertensive by fat feeding showed no effect of 1 week continuous infusion of the potent amylin antagonist AC253. Finally, in a 1-year study in 507 insulin-treated type 2 diabetic patients (body mass 90.6 ± 18.2 kg; mean ± SD), some were treated three times daily with injections of pramlintide, at doses (30, 75, 150 μg three times daily) that resulted in plasma amylin activity equal to or greater than that in hypertensive individuals (up to 50 pM). There was no dose-related change in either systolic or diastolic blood pressures (Young *et al.*, 1999) (Fig. 2).

V. Effects on Kidney Fluid and Electrolyte Excretion

Effects of amylin infusions on renal function were determined in dose–response experiments in anesthetized rats with catheterized kidneys in which glomerular filtration rate and renal plasma flow were measured using infusions of ^{3}H-inulin and ^{14}C-p-aminohippuric acid (PAH), respectively. Urine flow and plasma and urinary sodium, potassium, and calcium were determined at 15 min intervals (Vine *et al.*, 1996, 1998).

Amylin at ~52 pM increased urine flow, and at ~193 pM, it also increased sodium excretion, glomerular filtration rate, and renal plasma flow. The EC_{50} for the diuretic effect was 64 pM ± 0.28 log. The natriuretic effect was largely determined by the diuresis, since urinary sodium concentration changed little (Vine *et al.*, 1998) (Fig. 3).

Renal calcium and potassium excretion were significantly elevated at plasma amylin concentrations of ~52 pM and ~193 pM, respectively. Higher concentrations of plasma amylin decreased plasma calcium and potassium and blunted urinary excretion of these electrolytes. A calciuretic effect was also described in dogs (Miles *et al.*, 1994), but the calciuresis was not sufficient to account for the lowering of plasma calcium, which was instead attributed to a calcitonin-like inhibition of bone resorption. Thus, in the rat (Vine *et al.*, 1998), diuresis and natriuresis appeared to be the most

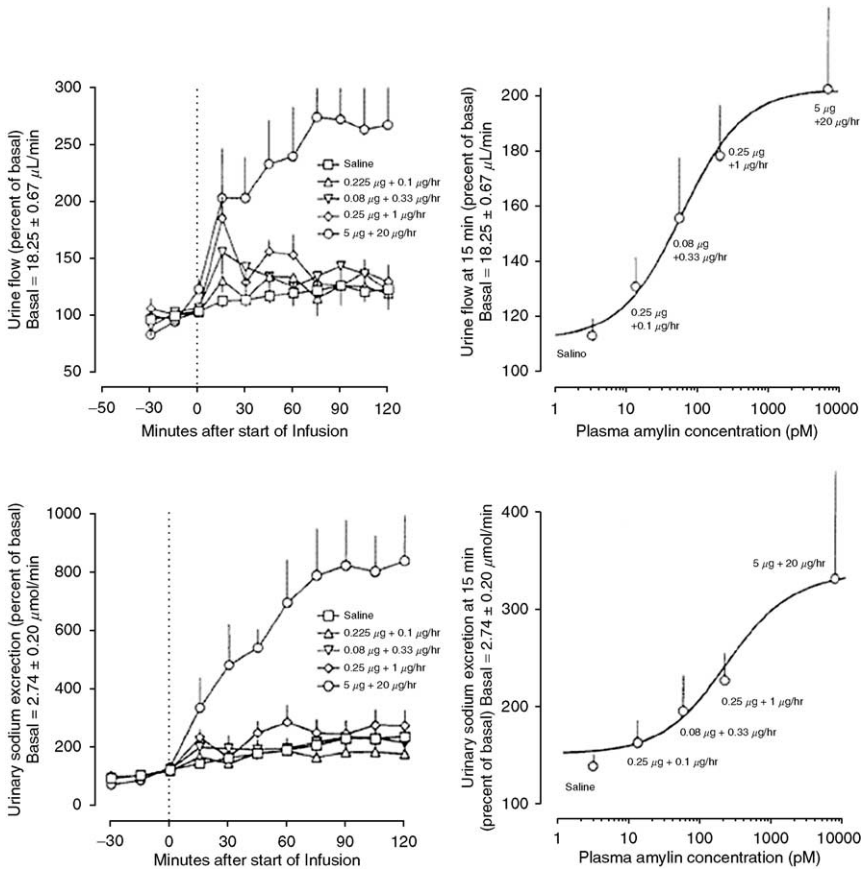

FIGURE 3 Concentration responses for diuretic and natriuretic effects of rat amylin in anesthetized rats. Redrawn from Vine *et al.* (1998).

amylin sensitive of the renal responses tested, being present at slightly above physiological concentrations. It was possible that such effects might have annulled any sodium retention resulting from activation of the renin-angiotensin system and thereby have accounted for no net effect on blood pressure in humans (Young *et al.*, 1999).

The effects of amylin on the kidney are similar to those described for calcitonins, especially salmon calcitonin, which exhibited a similar pattern of effects, including a potent natriuretic effect, a diuretic effect, and an anticalciuretic effect (Blakely *et al.*, 1997; Williams *et al.*, 1972). The anticalciuretic effect was apparent at physiological amylin concentrations in rats (Blakely *et al.*, 1997).

In summary, amylin bound to kidney cortex in a distinctive pattern. Binding appeared specific in that it was displaceable with amylin antagonists. It was associated with activation of cAMP, and was thereby likely to represent receptor binding and activation. Amylin's principal effects at the kidney included a stimulation of plasma renin activity, reflected in aldosterone increases at quasi-physiological amylin concentrations. It was unclear whether this was a local or systemic effect. Other renal effects in rats included a diuretic effect and a natriuretic effect. The latter was mainly driven by the diuresis, since urinary sodium concentration did not change.

VI. Effects on Plasma Electrolyte Concentrations

A. Sodium

Neither rat amylin (Vine *et al.*, 1998) nor pramlintide (Young *et al.*, 1996) had an effect on plasma sodium concentration in anesthetized rats.

B. Potassium

Plasma potassium measured in the same studies showed a transient decrease of about 0.4 mM within 1 hr of administration of amylin or pramlintide (Vine *et al.*, 1998; Young *et al.*, 1996). The character of this effect was similar to that observed with CGRP, insulin, and catecholamines, which activate Na^+/K^+-ATPase (Andersen and Clausen, 1993; Clausen and Flatman, 1987; Klimes *et al.*, 1984). Amylin (Clausen, 1996) and salmon calcitonin (Andersen and Clausen, 1993) were similarly shown to activate Na^+/K^+-ATPase. The correction of the hyperkalemia of diabetic patients in ketoacidotic crisis with insulin has been attributed to an insulin-mediated restoration of the ionic milieu, shifting accumulated extracellular potassium to the intracellular compartment. It is likely that the transient decrease in plasma potassium with amylin agonists represents a similar phenomenon (Vine *et al.*, 1998; Young *et al.*, 1996), since amylin agonists did not promote urinary potassium loss.

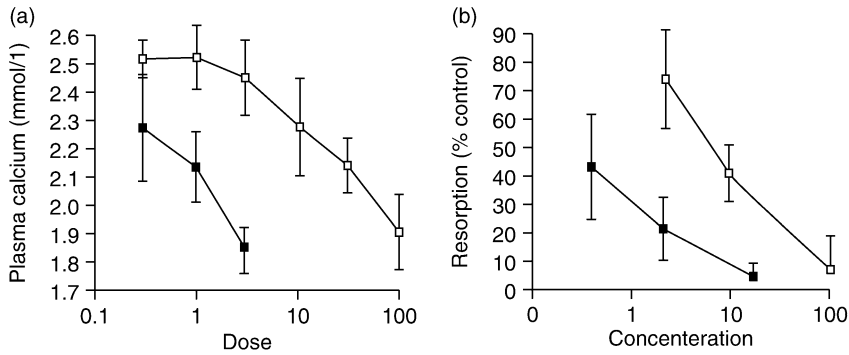

FIGURE 4 Comparison of effects of human amylin and human calcitonin on plasma calcium concentration in rats, and on resorptive activity of isolated rat osteoclasts *in vitro*. Redrawn from MacIntyre (1989).

C. Calcium

An effect of amylin on plasma calcium concentration was first noted in 1989 (Datta *et al.*, 1989b; MacIntyre, 1989), and several times since (Gilbey *et al.*, 1991; MacIntyre *et al.*, 1991; Young *et al.*, 1996) (Fig. 4).

In a comparison of the effects of intravenous rat amylin, human amylin, and pramlintide in rats, a reduction in total and ionized plasma calcium of similar magnitude with each compound was observed over a 2 hr period, with values for total calcium falling from a basal level of ~2.3 mM to ~1.8 mM (Young *et al.*, 1996). A similar result was obtained following dosage by the subcutaneous route. In an infusion study, the EC_{50}s for decrease in ionized calcium with rat amylin and pramlintide were 130 and 97 pM, respectively (Young *et al.*, 1996) (Fig. 5).

The potency of amylin's effects on plasma calcium is sufficient to have spawned speculation that it has a physiological role in skeletal maintenance (MacIntyre, 1989). MacIntyre (MacIntyre, 1989) proposed that the effect of calcitriol (1,25-dihydroxycholecalciferol; 1,25-dihydroxyvitamin D3; the most physiologically active metabolite of vitamin D) to stimulate β-cell secretion was consistent with a calcium regulatory role in which calcium retention by amylin augmented the effect of calcitriol to enhance calcium recuperation at renal tubules (Fig. 6).

D. Acid/Base Status

An intravenous bolus of 100 μg pramlintide resulted in no observable change in pH or pCO_2 in arterial blood of rats (Young *et al.*, 1996).

In summary, amylin had a transient effect to lower plasma potassium concentration. This effect was likely to be a consequence of activation of

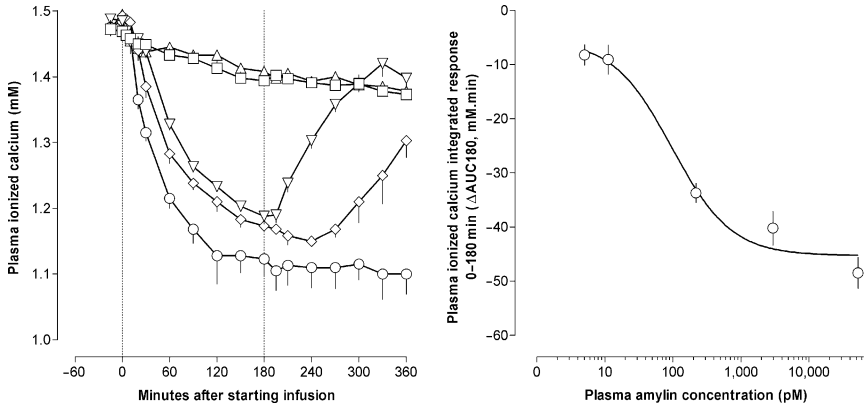

FIGURE 5 Concentration response for the effect of continuously infused rat amylin to lower plasma ionized calcium in rats. Redrawn from Young *et al.* (1996).

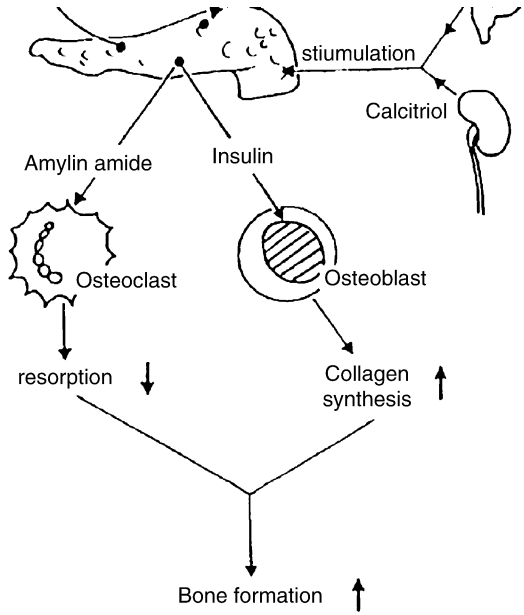

FIGURE 6 Proposed integration of bone-conserving roles of calcitriol and amylin (MacIntyre, 1989).

Na^+/K^+-ATPase, an action shared with insulin and catecholamines. Amylin lowered plasma calcium, particularly ionized calcium, likely due to an antiresorptive effect at osteoclasts.

VII. Effects in Isolated Kidney Preparations

Membranes from rat kidney cortex incubated with rat amylin increased cAMP production 3-fold. The amylin receptor antagonists AC187 and AC413, at tested doses, inhibited this effect, but hCGRP[8–37] did not, suggesting this effect was mediated via an amylin-like receptor (Wookey *et al.*, 1994, 1996). CGRP[8–37] was less potent than AC413 and AC66 (sCT[8–32]) in inhibiting amylin-stimulated cyclase activity (Wookey *et al.*, 1996) and was ~100-fold less potent in displacing 50 pM ^{125}I-rat amylin from kidney membranes (Wookey *et al.*, 1996).

In split-drop single-nephron micropuncture studies, and in contrast to the natriuretic effect in whole animal studies, systemically administered amylin promoted tubular sodium re-absorption by 28%, while AC187 reduced it 22% (Harris *et al.*, 1997). The mechanism was proposed to involve Na^+/H^+ exchange (Hiranyachattada *et al.*, 1995).

VIII. Effects on Kidney Development and Endothelial Integrity

In primary culture of rat proximal tubule cells from neonatal rat pups, amylin stimulated proliferation while the antagonists AC187, AC413, and AC512 blocked it (Harris *et al.*, 1997). Interestingly, amylin mRNA was transiently expressed between embryo day 17 and postnatal day 7. The location of these amylin gene transcripts was below the nephrogenic zone, associated with the primitive tubules of the developing nephrons. There was no evidence of expression in the normal adult kidney. In the developing kidney (metanephros), amylin peptide could also be detected by immunohistochemistry using a rabbit polyclonal anti-rat amylin antibody (Tikellis *et al.*, 1997). These studies suggested that amylin could act as a growth factor in kidney development. In support of this interpretation, kidney development was found to be disturbed in amylin gene knockout mice (Wookey *et al.*, 1999). In the cortices of the knockout mice, intertubular spaces were 4.8-fold greater than in controls ($P < 0.01$). These spaces were mostly lined with cells positive for the endothelial marker von Willebrand factor, thus representing a large expansion of the capillary volume. Such a picture was consistent with "tubular drop out" resulting from reduced expansion of developing proximal tubules, and was consistent with a role for amylin as a growth factor for the epithelial cells of the proximal tubules.

Circulating von Willebrand factor peptide, a marker from endothelial cells, distinguishes diabetic patients with nephropathy from those without it (Vischer *et al.*, 1998). Some have proposed that the absence of nephro-active agents from the pancreatic β-cell, recently proposed to include C-peptide (Wahren and Johansson, 1998), may aggravate the course of diabetic

nephropathy. It might similarly be possible that the absence of a trophic effect of amylin could be implicated in the nephropathy of insulinopenic diabetes, also characterized by lack of amylin.

Immunoreactive amylin was detected in the developing kidney. It appeared to have a trophic effect in kidney, and its absence resulted in renal dysgenesis.

IX. Effects on Subfornical Organ and Drinking Behavior

Fluid and electrolyte balance is controlled not only via renal excretion and effects on the renin-angiotensin-aldosterone system, but also by control of intake. Angiotensin, for example, not only evokes vasoconstriction and sodium retention in response to depletion of extracellular volume, but also stimulates thirst (Fitzsimons, 1998) via actions on the SFO, which it activates (McKinley *et al.,* 1992). The SFO is one of the specialized "sensory" areas of the brain, the circumventricular organs, that also include the organum vasculosum lateroterminalis (OVLT) and area postrema, where a leaky blood–brain barrier allows circulating peptide hormones to access neurons (Simon, 2000). Neurophysiological investigation of SFO neuronal activity showed that 61% of cells were stimulated by calcitonin and that almost all of these were angiotensin II sensitive (Schmid *et al.,* 1998). The effect of amylin and angiotensin II on spontaneous neuronal activity was examined using a rat SFO slice preparation. Superfusion with amylin and angiotensin II activated 72% and 69%, respectively, of the 32 SFO neurons tested for their reactivity to both peptides. The remaining neurons were insensitive; not a single neuron was inhibited. The specificity of the amylin-induced excitation was confirmed by co-application of an amylin antagonist (AC187) in a concentration 10-fold higher. AC187 totally blocked the excitatory effect (Rauch *et al.,* 1997). Amylin activation was not blocked with losartan, an angiotensin receptor antagonist, indicating that the activation by amylin was not secondarily mediated via an angiotensinergic mechanism (Riediger *et al.,* 1999b). The threshold concentration for amylin was below 10 nM and was thus similar to the threshold concentration observed with angiotensin II in this preparation. Concordance of amylin and angiotensin sensitivity in the SFO prompted *in vivo* studies of the effect of amylin on water intake, which are described more fully in Chapter 5.

In brief, subcutaneous injection of amylin and angiotensin II in water-sated, adult male rats caused drinking in 13/17 and 16/20 rats, respectively, whereas only 6 out of 33 control rats drank during the 2 hr period following the injection. The cumulative water intake of all rats receiving amylin or angiotensin II was increased (Rauch *et al.,* 1997; Riediger *et al.,* 1999b). These data provided the first direct evidence of a neural substrate for prandial

drinking, a phenomenon that had previously been regarded as a learned behavior (Rauch *et al.*, 1997).

In a pharmacological study looking at effects of amylin, CGRP, rat calcitonin, salmon calcitonin, AC187, and CGRP[8–37] on SFO neuronal activity, it was observed that (1) CGRP was a weaker agonist in the SFO than amylin, (2) salmon calcitonin excites SFO neurons, and (3) responses were blocked by AC187 but not by CGRP[8–37]. As described elsewhere, this pattern was inconsistent with activation via CGRP receptors, but was instead consistent with involvement of amylin-like (C3) and/or calcitonin-like (C1) receptors (Riediger *et al.*, 1999c).

The same authors have examined amylin action at the area postrema (described in a separate section), another circumventricular brain structure that serves as a multifunctional receptor organ and that, as an integrative structure, is also involved in the control of sodium and fluid intake (Simon, 2000). Nearly half of the neurons in this structure are amylin sensitive (Riediger *et al.*, 1999a). Amylin may stimulate water intake by acting on the SFO and inhibit food intake by acting on the area postrema (Simon, 2000).

In summary, neurons in the SFO, which has a role in fluid/electrolyte homeostasis, were potently activated by amylin. The dipsogenic and renal effects of amylin may be related to effects at the SFO.

References

Andersen, S. L., and Clausen, T. (1993). Calcitonin gene-related peptide stimulates active Na (+)-K+ transport in rat soleus muscle. *Am. J. Physiol.* **264**, C419–C429.

Blakely, P., Vaughn, D. A., and Fanestil, D. D. (1997). Amylin, calcitonin gene-related peptide, and adrenomedullin: Effects on thiazide receptor and calcium. *Am. J. Physiol.* **41**, F410–F415.

Brands, M. W., Mizelle, H. L., Gaillard, C. A., Hildebrandt, D. A., and Hall, J. E. (1991). The hemodynamic response to chronic hyperinsulinemia in conscious dogs. *Am. J. Hypertens.* **4**, 164–168.

Braslis, K. G., Fletcher, D. R., Shulkes, A., Scoggins, B. A., Tresham, J., and Hardy, K. J. (1988). The cardiovascular effects of human calcitonin gene-related peptide in conscious sheep. *J. Hypertens.* **6**, 881–887.

Briffeuil, P., Thu, T. H., and Kolanowski, J. (1992). Reappraisal of the role of insulin on sodium handling by the kidney—effect of intrarenal insulin infusion in the dog. *Eur. J. Clin. Invest.* **22**, 523–528.

Brown, A. J., Casals-Stenzel, J., Gofford, S., Lever, A. F., and Morton, J. J. (1981). Comparison of fast and slow pressor effects of angiotensin II in the conscious rat. *Am. J. Physiol.* **241**, H381–H388.

Brunner, H. R., Nussberger, J., Burnier, M., and Waeber, B. (1993). Angiotensin II antagonists. *Clin. Exp. Hypertens.* **15**, 1221–1238.

Bryan, G., Nuttall, A., and Moyses, C. (1995). First administration to man of the human amylin antagonist, AC625. *J. Invest. Med.* **43**, 296A.

Cao, Z. M., Wookey, P. J., Wu, L. L., Voskuil, M., vanGeenen, R. C. I., and Cooper, M. E. (1997). Renal amylin binding in normotensive and hypertensive rats: Effects of angiotensin converting enzyme inhibition with perindopril. *J. Hypertens.* **15**, 1245–1252.

Carretta, R., Fabris, B., Fischetti, F., Costantini, M., DeBiasi, F., Muiesan, S., Bardelli, M., Vran, F., and Campanacci, L. (1989). Reduction of blood pressure in obese hyperinsulinaemic hypertensive patients during somatostatin infusion. *J. Hypertens.* **7**(Suppl.), S196–S197.

Chai, S. Y., Christopoulos, G., Cooper, M. E., and Sexton, P. M. (1998). Characterization of binding sites for amylin, calcitonin, and CGRP in primate kidney. *Am. J. Physiol.* **274**, F51–F62.

Chin, S. Y., Hall, J. M., Brain, S. D., and Morton, I. K. M. (1994). Vasodilator responses to calcitonin gene-related peptide (CGRP) and amylin in the rat isolated perfused kidney are mediated via CGRP (1) receptors. *J. Pharmacol. Exp. Ther.* **269**, 989–992.

Clausen, T. (1996). Long- and short-term regulation of the Na+/K+ pump in skeletal muscle. *News Physiol. Sci.* **11**, 24–30.

Clausen, T., and Flatman, J. A. (1987). Effects of insulin and epinephrine on Na+-K+ and glucose transport in soleus muscle. *Am. J. Physiol.* **252**, E492–E499.

Clementi, G., Rapisarda, E., Fiore, C. E., Prato, A., Amico-Roxas, M., Millia, C., Bernardini, R., Maugeri, S., and Scapagnini, U. (1986). Effects of salmon calcitonin on plasma renin activity and systolic blood pressure in the rat. *Neurosci. Lett.* **66**, 351–355.

Cooper, M. E. (1997). Amylin, the kidney and hypertension. *Exp. Clin. Endocrinol. Diabet.* **105**, 67.

Cooper, M. E., Qin, H.-F., Panagiotopoulos, S., Cox, A., Bach, L. A., Sexton, P. M., and Jerums, G. (1992). Renal binding sites of rat islet amyloid polypeptide. *Proceedings: 9th International Congress of Endocrinology*, p. 10.03.065.

Cooper, M. E., Chai, S. Y., and Sexton, P. M. (1995a). Amylin binding to the primate kidney: Implications for the association of hypertension with insulin resistance. *Diabetologia* **38**, A131.

Cooper, M. E., McNally, P. G., Phillips, P. A., and Johnston, C. I. (1995b). Amylin stimulates plasma renin concentration in humans. *Hypertension* **26**, 460–464.

Datta, H. K., Zaidi, M., Wimalawansa, S. J., Ghatei, M. A., Beacham, J. L., Bloom, S. R., and MacIntyre, I. (1989b). *In vivo* and *in vitro* effects of amylin and amylin-amide on calcium metabolism in the rat and rabbit. *Biochem. Biophys. Res. Commun.* **162**, 876–881.

DeFronzo, R. A. (1981). The effect of insulin on renal sodium metabolism. A review with clinical implications. *Diabetologia* **21**, 165–171.

Dickinson, C. J., and Lawrence, J. R. (1963). A slowly developing pressor response to small concentrations of angiotensin. *Lancet* **i**, 1354–1356.

Dilts, R. P., Kenney, M., and Beaumont, K. (1995). Autoradiographic localization of binding sites for amylin and amylin antagonists in rat kidney. *J. Am. Soc. Nephrol.* **6**, 735.

Fitzsimons, J. T. (1998). Angiotensin, thirst, and sodium appetite. *Physiol. Rev.* **78**, 583–686.

Gardiner, S. M., Compton, A. M., Kemp, P. A., Bennett, T., Bose, C., Foulkes, R., and Hughes, B. (1991). Antagonistic effect of human α-calcitonin gene-related peptide [8–37] on regional hemodynamic actions of rat islet amyloid polypeptide in conscious Long Evans rats. *Diabetes* **40**, 948–951.

Gilbey, S., Ghatei, M. A., Bretherton-Watt, D., Jones, P. M., Beacham, I., Perera, T., Girgis, S., Bloom, S. R., and Zaidi, M. (1991). Amylin lowers serum calcium in Paget's bone disease: Further evidence for a role in calcium metabolism. *J. Bone Miner. Res.* **6**, S293.

Gnaedinger, M. P., Uehlinger, D. E., Weidmann, P., Sha, S. G., Muff, R., Born, W., Rascher, W., and Fischer, J. A. (1989). Distinct hemodynamic and renal effects of calcitonin gene-related peptide and calcitonin in men. *Am. J. Physiol.* **257**, E848–E854.

Hall, J. M., and Brain, S. D. (1993). Interaction of amylin with calcitonin gene-related peptide receptors in the microvasculature of the hamster cheek pouch *in vivo*. *Br. J. Pharmacol.* **126**, 280–284.

Hall, J. E. (1993). Hyperinsulinemia—a link between obesity and hypertension? *Kidney Int.* **43**, 1402–1417.

Hall, J. E., Brands, M. W., Kivlighn, S. D., Mizelle, H. L., Hildebrandt, D. A., and Gaillard, C. A. (1990a). Chronic hyperinsulinemia and blood pressure. Interaction with catecholamines? *Hypertension* **15**, 519–527.

Hall, J. E., Coleman, T. G., Mizelle, H. L., and Smith, M. J., Jr. (1990b). Chronic hyperinsulinemia and blood pressure regulation. *Am. J. Physiol.* **258**, F722–F731.

Hall, J. E., Brands, M. W., Dixon, W. N., Mizelle, H. L., and Hildebrandt, D. A. (1991a). Hyperinsulinemia does not elevate blood pressure in obese hypertensive dogs. *FASEB J.* **5**, A737.

Hall, J. E., Brands, M. W., Mizelle, H. L., Gaillard, C. A., and Hildebrandt, D. A. (1991b). Chronic intrarenal hyperinsulinemia does not cause hypertension. *Am. J. Physiol.* **260**, F663–F669.

Hall, J. E., Brands, M. W., Dixon, W. N., and Smith, M. J. (1993). Obesity-induced hypertension—renal function and systemic hemodynamics. *Hypertension* **22**, 292–299.

Harris, P. J., Cooper, M. E., Hiranyachattada, S., Berka, J. L., Kelly, D. J., Nobes, M., and Wookey, P. J. (1997). Amylin stimulates proximal tubular sodium transport and cell proliferation in the rat kidney. *Am. J. Physiol.* **41**, F13–F21.

Haynes, J. M., Wookey, P. J., Tikellis, C. M., Du, H. C., Sexton, P. M., and Cooper, M. E. (1994). Amylin and calcitonin gene related peptide in the rat kidney: Functional and autoradiographic studies. *Can. J. Physiol. Pharmacol.* **72**, 566.

Haynes, J. M., Hodgson, W. C., and Cooper, M. E. (1997). Rat amylin mediates a pressor response in the anaesthetised rat: Implications for the association between hypertension and diabetes mellitus. *Diabetologia* **40**, 256–261.

Hiranyachattada, S., Cooper, M. E., and Harris, P. J. (1995). Amylin stimulates rat renal proximal tubular fluid reabsorption via luminal Na+/H+ exchange. *Proc. Aust. Physiol. Pharmacol. Soc.* **26**, 42P.

Hwang, I. S., Ho, H., Hoffman, B. B., and Reaven, G. M. (1987). Fructose-induced insulin resistance and hypertension in rats. *Hypertension* **10**, 512–516.

Jarrett, R. J. (1991). Hyperinsulinemia and elevated blood pressure—cause, confounder, or coincidence? *Am. J. Epidemiol.* **134**, 327–328.

Jarrett, R. J. (1992). In defence of insulin—a critique of syndrome-X. *Lancet* **340**, 469–471.

Klimes, I., Howard, B. V., and Mott, D. M. (1984). Sodium-potassium pump in cultured fibroblasts from obese donors; no evidence for an inherent decrease of basal or insulin-stimulated activity. *Metabolism* **33**, 317–322.

Kotchen, T. A. (1994). Attenuation of experimental hypertension with agents that increase insulin sensitivity. *Drug. Dev. Res.* **32**, 100–103.

Kurtz, A., Muff, R., Born, W., Lundberg, J. M., Millberg, B. I., Gnadinger, M. P., Uehlinger, D. E., Weidmann, P., Hokfelt, T., and Fischer, J. A. (1988). Calcitonin gene-related peptide is a stimulator of renin secretion. *J. Clin. Invest.* **82**, 538–543.

Landin-Wilhelmsen, K. (1992). Metformin and blood pressure. *J. Clin. Pharm. Ther.* **17**, 75–79.

Landsberg, L. (1989). Obesity, metabolism, and hypertension. *Yale J. Biol. Med.* **62**, 511–519.

Laragh, J. H., Case, D. B., Wallace, J. M., and Keim, H. (1977). Blockade of renin or angiotensin for understanding human hypertension: A comparison of propranolol, saralasin and converting enzyme blockade. *Fed. Proc.* **36**, 1781–1787.

Lefèbvre, P. J. (1993). Syndrome-X. *Diabet. Nutr. Metab.* **6**, 61–65.

Lever, A. F. (1993). The fast and slowly developing pressor effect of angiotensin-II. *In* "The Renin-angiotensin System" (J. I. S. Robertson and M. G. Nicholls, Eds.), pp. 28.1–28.9. Gower Medical Publishing, London.

Licata, G., Scaglione, R., Ganguzza, A., Corrao, S., Donatelli, M., Parinello, G., Dichiara, M. A., Merlino, G., and Cecala, M. G. (1994). Central obesity and hypertension: Relationship between fasting serum insulin, plasma renin activity, and diastolic blood pressure in young obese subjects. *Am. J. Hypertens.* **7**, 314–320.

Lin, H. Y., Harris, T. L., Flannery, M. S., Aruffo, A., Kaji, E. H., Gorn, A., Kolakowski, L. F., Jr., Lodish, H. F., and Goldring, S. R. (1991). Expression cloning of an adenylate cyclase-coupled calcitonin receptor. *Science* **254**, 1022–1024.

MacIntyre, I. (1989). Amylinamide, bone conservation, and pancreatic beta cells. *Lancet* **2**, 1026–1027.

MacIntyre, I., Moonga, B. S., and Zaidi, M. (1991). Amylin a new calcium-regulating hormone from the pancreas. *J. Physiol. (Camb.)* **434**, 78P.

Malatino, L. S., Fiore, C. E., Foti, R., Guzzardi, F., and Tamburino, G. (1987). Acute effects of salmon calcitonin in man include stimulation of the renin-angiotensin-aldosterone system. *Miner Electrolyte Metab.* **13**, 316–322.

McCubbin, J. W., DeMoura, R. S., Page, I. H., and Olmsted, F. (1965). Arterial hypertension elicited by subpressor amounts of angiotensin. *Science* **149**, 1394–1395.

McKinley, M. J., Badoer, E., and Oldfield, B. J. (1992). Intravenous angiotensin II induces Fos-immunoreactivity in circumventricular organs of the lamina terminalis. *Brain Res.* **594**, 295–300.

McNally, P. G., Phillips, P. A., Johnston, C. I., Kolterman, O. G., and Cooper, M. E. (1994). Human amylin increases plasma renin in man: A possible link between hypertension and insulin resistance? *Diabetologia* **37**, A46.

Meade, T. W., Imeson, J. D., Gordon, D., and Peart, W. S. (1983). The epidemiology of plasma renin. *Clin. Sci.* **64**, 273–280.

Miles, P. D. G., Deftos, L. J., Moossa, A. R., and Olefsky, J. M. (1994). Islet amyloid polypeptide (amylin) increases the renal excretion of calcium in the conscious dog. *Calcif. Tissue Int.* **55**, 269–273.

Modan, M., Halkin, H., Almog, S., Lusky, A., Eshkol, A., Shefi, M., Shitrit, A., and Fuchs, Z. (1985). Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. *J. Clin. Invest.* **75**, 809–817.

Morgan, D. A., Ray, C. A., Balon, T. W., and Mark, A. L. (1992). Metformin increases insulin sensitivity and lowers arterial pressure in spontaneously hypertensive rats. *Hypertension* **20**, 421.

Nieuwenhuis, M. G., van Hulst, K. L., Hackeng, W. H. L., and Lips, C. J. M. (1992a). Islet amyloid polypeptide plasma concentrations in patients with insulinoma. *Diabetologia* **35**, A119.

Nieuwenhuis, M. G., van Mansfeld, A. D., van Unnik, J. A., Berends, M. J., and Lips, C. J. (1992b). No constant relationship between islet amyloid polypeptide (IAPP) and insulin expression in insulinomas. *Neth. J. Med.* **41**, 264–271.

Nuttall, A., Bryan, G. L., and Moyses, C. (1995a). Administration of human amylin increases plasma renin activity and plasma aldosterone in man. *Am. J. Hypertens.* **8**, 108A.

Nuttall, A., Bryan, G. L., and Moyses, C. (1995b). Intravenous human amylin increases plasma renin activity and plasma aldosterone in man. *Eur. Heart J.* **16**, 66.

Palla, R., Parrini, M., Panichi, V., Andreini, B., De Pietro, S., Migliori, M., Bianchi, A. M., Giovannini, L., Bertelli, A., Bertelli, A. A., *et al.* (1995b). Acute effects of calcitonin gene related peptide on renal haemodynamics and renin and angiotensin II secretion in patients with renal disease. *Int. J. Tissue React.* **17**, 43–49.

Peuler, J. D., Johnson, B. A. B., Phare, S. M., and Sowers, J. R. (1993). Sex-specific effects of an insulin secretagogue in stroke-prone hypertensive rats. *Hypertension* **22**, 214–220.

Pontiroli, A. E., Alberetto, M., and Pozza, G. (1992). Patients with insulinoma show insulin resistance in the absence of arterial hypertension. *Diabetologia* **35**, 294–295.

Rauch, M., Schmid, H. A., Koch, J., Riediger, T., and Simon, E. (1997). Blood-borne amylin stimulates water intake via central action on neurons of the subfornical organ. *Pflugers Arch.* **433**, 618.

Reaven, G. M. (1988). Role of insulin resistance in human disease. *Diabetes* **37**, 1595–1607.

Reaven, G. M., Ho, H., and Hoffman, B. B. (1988). Attenuation of fructose-induced hypertension in rats by exercise training. *Hypertension* **12**, 129–132.

Reaven, G. M., Ho, H., and Hoffmann, B. B. (1989). Somatostatin inhibition of fructose-induced hypertension. *Hypertension* **14**, 117–120.

Riediger, T., Rauch, M., Jurat, G., and Schmid, H. A. (1999a). Central nervous targets for pancreatic amylin. *Pflugers Arch.* **437**, R142.

Riediger, T., Rauch, M., and Schmid, H. A. (1999b). Actions of amylin on subfornical organ neurons and on drinking behavior in rats. *Am. J. Physiol.* **276**, R514–R521.

Riediger, T., Schmid, H. A., Young, A. A., and Simon, E. (1999c). Pharmacological characterisation of amylin-related peptides activating subfornical organ neurones. *Brain Res.* **837**, 161–168.

Sawicki, P. T., Heinemann, L., Starke, A., and Berger, M. (1992). Hyperinsulinaemia is not linked with blood pressure elevation in patients with insulinoma. *Diabetologia* **35**, 649–652.

Schmid, H. A., Rauch, M., and Koch, J. (1998). Effect of calcitonin on the activity of ANG II-responsive neurons in the rat subfornical organ. *Am. J. Physiol.* **43**, R1646–R1652.

Sexton, P. M., Houssami, S., Brady, C. L., Myers, D. E., and Findlay, D. M. (1994). Amylin is an agonist of the renal porcine calcitonin receptor. *Endocrinology* **134**, 2103–2107.

Sexton, P. M., Chai, S. Y., and Cooper, M. E. (1995). Amylin binds to the juxtaglomerular apparatus of the primate kidney: An explanation for the link between amylin and the renin-angiotensin system. *J. Am. Soc. Nephrol.* **6**(3), abstract 535.

Simon, E. (2000). Interface properties of circumventricular organs in salt and fluid balance. *News Physiol. Sci.* **15**, 61–67.

Smith, P., Carlo, P., Young, A. A., and Vine, W. (1994). Amylin potently increases plasma renin activity (PRA) in rats. *Program and Abstracts, the Endocrine Society 76th Annual Meeting*, p. 477.

Stridsberg, M., Wilander, E., Öberg, K., Lundqvist, G., and Eriksson, B. (1992). Islet amyloid polypeptide-producing pancreatic islet cell tumor. *Scand. J. Gastroenterol.* **27**, 381–387.

Stridsberg, M., Berne, C., Sandler, S., Wilander, E., and Oberg, K. (1993). Inhibition of insulin secretion, but normal peripheral insulin sensitivity, in a patient with a malignant endocrine pancreatic tumour producing high amounts of an islet amyloid polypeptide-like molecule. *Diabetologia* **36**, 843–849.

Tikellis, C., Wookey, P. J., Darby, I. A., and Cooper, M. E. (1997). Amylin acts as a growth factor during the development of the rat kidney. *Diabetologia* **40**, A148.

Tuck, M. L., Sowers, J., Dornfeld, L., Kledzik, G., and Maxwell, M. (1981). The effect of weight reduction on blood pressure, plasma renin activity, and plasma aldosterone levels in obese patients. *N. Engl. J. Med.* **304**, 930–933.

Vine, W., Smith, P., LaChappell, R., Percy, A., and Young, A. A. (1996). Effect of amylin on renal function in the rat. *Program and Abstracts, 10th International Congress of Endocrinology*, p. 419.

Vine, W., Smith, P., LaChappell, R., Blase, E., and Young, A. (1998). Effects of rat amylin on renal function in the rat. *Horm. Metab. Res.* **30**, 518–522.

Vischer, U. M., Emeis, J. J., Bilo, H. J., Stehouwer, C. D., Thomsen, C., Rasmussen, O., Hermansen, K., Wollheim, C. B., and Ingerslev, J. (1998). von Willebrand factor (vWf) as a plasma marker of endothelial activation in diabetes: Improved reliability with parallel determination of the vWf propeptide (vWf:AgII). *Thromb. Haemost.* **80**, 1002–1007.

Wahren, J., and Johansson, B. L. (1998). Ernst-Friedrich-Pfeiffer Memorial Lecture. New aspects of C-peptide physiology. *Horm. Metab. Res.* **30**, A2–A5.

Welborn, T. A., Breckenridge, A., Rubinstein, A. H., Dollery, C. T., and Fraser, T. R. (1966). Serum-insulin in essential hypertension and in peripheral vascular disease. *Lancet* **1**, 1336–1337.

Williams, B. (1994). Insulin resistance: The shape of things to come. *Lancet* **344**, 521–524.
Williams, C. C., Matthews, E. W., Moseley, J. M., and MacIntyre, I. (1972). The effects of synthetic human and salmon calcitonins on electrolyte excretion in the rat. *Clin. Sci.* **42**, 129–137.
Wookey, P. J., and Cooper, M. E. (1998). Amylin: Physiological roles in the kidney and a hypothesis for its role in hypertension. *Clin. Exp. Pharmacol. Physiol.* **25**, 653–660.
Wookey, P., Tikellis, C., Du, C., Sexton, P., Young, A. A., Gaeta, L. S. L., Prickett, K. S., Beaumont, K., and Cooper, M. E. (1994a). Identification, characterisation and localisation of amylin binding sites in rat kidney using specific amylin antagonists. *J. Hypertens.* **12**, S9.
Wookey, P. J., Berka, J., Rumble, J., Kellly, D., Du, H. C., and Cooper, M. E. (1994b). Amylin binding sites in rat kidney and stimulation of plasma renin activity: Characterization using antagonists. *Diabetologia* **37**, A116.
Wookey, P. J., Tikellis, C., Du, H. C., Qin, H. F., Sexton, P. M., and Cooper, M. E. (1996). Amylin binding in rat renal cortex, stimulation of adenylyl cyclase, and activation of plasma renin. *Am. J. Physiol.* **39**, F289–F294.
Wookey, P. J., Cao, Z., vanGeenen, R. C. I., Voskuil, M., Darby, I. A., Komers, R., and Cooper, M. E. (1997). Increased density of renal amylin binding sites in experimental hypertension. *Hypertension* **30**, 455–460.
Wookey, P., Xuereb, L., Alcorn, D., Parkes, D., Devine, E., Young, A., and Cooper, M. (1999). Renal pathology associated with the postnatal mouse: The amylin gene-deletion model. *J. Am. Assoc. Nephrol.* **10**, 413A.
Yoshioka, S., Nishino, H., Shiraki, T., Ikeda, K., Koike, H., Okuno, A., Wada, M., Fujiwara, T., and Horikoshi, H. (1993). Antihypertensive effects of CS-045 treatment in obese Zucker rats. *Metab. Clin. Exp.* **42**, 75–80.
Young, A. A., Gaeta, L. S. L., Prickett, K., Beaumont, K., Carlo, P., Smith, P., and Vine, W. (1994a). Inhibition of amylin-stimulated plasma renin activity by the selective amylin antagonist, AC187. *Program and Abstracts, the Endocrine Society, 76th Annual Meeting*, p. 478.
Young, A. A., Rink, T. J., Vine, W., and Gedulin, B. (1994b). Amylin and syndrome-X. *Drug Dev. Res.* **32**, 90–99.
Young, A. A., Vine, W., Carlo, P., Smith, P., Rink, T. J., Rumble, J., and Cooper, M. E. (1994c). Amylin stimulation of renin activity in rats: A possible link between insulin resistance and hypertension. *J. Hypertens.* **12**, S152.
Young, A., Nuttall, A., Moyses, C., Percy, A., Vine, W., and Rink, T. (1995). Amylin stimulates the renin-angiotensin-aldosterone axis in rats and man. *Diabetologia* **38**, A225.
Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.
Young, A., Kolterman, O., and Hall, J. (1999). Amylin innocent in essential hypertension? *Diabetologia* **42**, 1029.

Andrew Young

Effects on Bone

I. Summary

The actions of amylin on bone have been reviewed in several publications (MacIntyre, 1992a,b; MacIntyre *et al.,* 1991; Reid and Cornish, 1996; Tamura *et al.,* 1992a,b; Zaidi *et al.,* 1990a,c, 1993b). MacIntyre proposed that amylin or its derivatives or agonists would be useful for treating bone disorders, such as osteoporosis, Paget's disease, or bone loss resulting from malignancy, endocrine disorders, autoimmune arthritides, breakage and fracture, immobility and disease, or hypercalcaemia (MacIntyre, 1995).

II. Effects at Calcitonin Receptors

Human amylin was a full agonist at human T47D (calcitonin) receptors (Muff *et al.,* 1992), although it was 200-fold less potent than salmon

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calcitonin. In a similar T47D (human breast carcinoma) membrane preparation, human amylin, rat amylin, and pramlintide each bound to calcitonin receptors with affinities of 10.1, 2.7, and 5.1 nM, respectively. This affinity was 34- to 170-fold less than that of salmon calcitonin (Young *et al.*, 1996). Similarly, in another report on salmon calcitonin, human calcitonin, and human amylin binding in T47D membranes, human calcitonin was 15-fold less potent than salmon calcitonin (sCT), but 240-fold more potent than human amylin at displacing ^{125}I-sCT (Zimmermann *et al.*, 1997). Rat amylin was 300-fold less potent than rat calcitonin at stimulating cyclic AMP (cAMP) in rat C1a receptors (Beaumont *et al.*, 1994). And when transfected into an opossum cell line, human amylin was much less active than salmon calcitonin at the porcine calcitonin receptor (Muff *et al.*, 1994).

In contrast to these reports of greater potency of rat calcitonin over rat amylin at calcitonin receptors, in pig calcitonin receptors, salmon calcitonin, pig calcitonin, and rat calcitonin exhibited similar potency in stimulating cyclic AMP (Sexton *et al.*, 1994). Similarly, in another human breast carcinoma cell line, the MCF-7, human amylin and human calcitonin were similarly potent (EC_{50}s of 1.36 $\pm$ 0.22 versus 1.74 $\pm$ 0.41 nM, respectively) in increasing cAMP (Zimmermann *et al.*, 1997). In rabbit aortic endothelial cells transfected with the human calcitonin-2 receptor, human amylin was only 6.2-fold less potent than human calcitonin (53 $\pm$ 16 versus 8.5 $\pm$ 1.8 nM, respectively) in displacing bound ^{125}I-human calcitonin (Muff *et al.*, 1999) and was more potent than human calcitonin gene-related peptide α (CGRPα) or human adrenomedullin, which were 120-fold less potent than calcitonin. In cells co-transfected with receptor activity modifying proteins-1 and -3, affinity of human amylin was substantially increased (IC_{50} changed from 53 to 3.1 and 4.0 nM, respectively), and that for human calcitonin decreased so that it was 45 $\pm$ 2-fold and 126 $\pm$ 3-fold less potent than human amylin (Muff *et al.*, 1999). For stimulation of cAMP in these cells, human calcitonin and amylin were effective agonists, increasing cAMP production 8- to 14-fold, with EC_{50}s of $\sim$0.1 nM and 1 nM, irrespective of the presence or absence of receptor activity modifiying proteins (RAMP-1, -2, or -3).

In isolated osteoclasts, Zaidi *et al.* (Alam *et al.*, 1991; Zaidi *et al.*, 1991) found evidence for separate signaling pathways preferentially activated via amylin and calcitonin, respectively (Alam *et al.*, 1993a,b).

Since (in some receptor systems) amylins have potencies only slightly lower than mammalian calcitonins, these findings raise the possibility that circulating amylin (generally in higher concentration than circulating calcitonin) could, via activity at calcitonin receptors, mediate actions on bone. Alternatively, if some actions are mediated via receptors for which amylin may have greater potency than calcitonins, this would also raise the possibility of an effect of amylin on bone, as proposed in several publications (MacIntyre, 1989, 1992a,b; Reid and Cornish, 1996; Tamura *et al.*, 1992a,b; Zaidi *et al.*, 1990a,c, 1993b).

III. Effects on Calcium Concentrations

Several studies, including some described in Chapter 15 of this volume, have described a calcium-lowering effect of amylinomimetic agents, including human amylin (Datta *et al.*, 1989; MacIntyre, 1989; Zaidi *et al.*, 1990a,d), rat amylin (Young *et al.*, 1993, 1996), and pramlintide (Young *et al.*, 1996). It is interesting that deamidated amylin is also hypocalcemic (Tedstone *et al.*, 1989) despite its lack of metabolic actions. It is unknown whether this represents a separate signaling pathway or whether this represents a selective activation of calcitonin versus amylin receptors, for example.

In a comparison with the International Reference Preparation of human calcitonin, human amylin was as effective as human calcitonin in lowering plasma calcium in Wistar rats, but was ~40-fold less potent (Zaidi *et al.*, 1990a). Human amylin thus exhibited 2.5–5 MRC units/mg. It was nonetheless the most potent calcium-lowering peptide after calcitonin (Zaidi *et al.*, 1993b). In some respects, this potency was surprising, since the sequence identity to calcitonins was generally <13%. In contrast, CGRP, 30% identical to calcitonins, was 1000 times less potent (Zaidi *et al.*, 1990a) (Fig. 1).

Human amylin infused at 150 pmol/kg/min into five patients with Paget's disease evoked a fall in serum calcium and phosphate that was similar to that produced by 50 pmol/kg/min human calcitonin (Gilbey *et al.*, 1991b). A similar result was obtained in eight patients with Paget's disease in which intravenous bolus doses of 6 μg human calcitonin and 600 μg human amylin were compared; the absolute magnitude of hypocalcemic

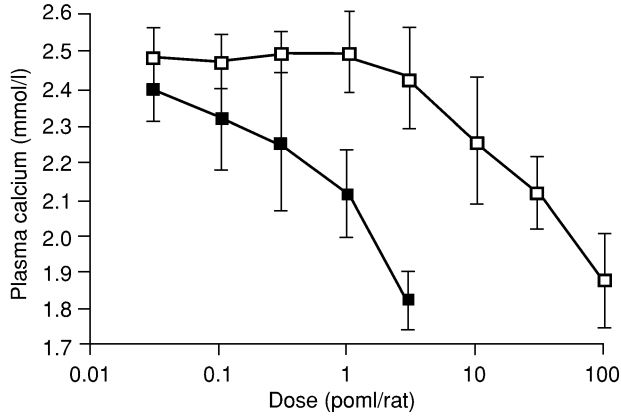

FIGURE 1 Comparison of calcium-lowering activities of human amylin and the International Reference Preparation of human calcitonin in Wistar rats. At 2.5–5 MRC units/mg, human amylin was the most potent peptide to be identified other than calcitonin (Zaidi *et al.*, 1990a).

effect was similar, but the response was longer in duration with amylin (Wimalawansa *et al.*, 1992) (Fig. 2).

Effects on plasma calcium concentration have been studied in dose–response studies in rats for rat amylin (Young *et al.*, 1993, 1996) and for pramlintide (Young *et al.*, 1996). Following bolus intravenous injections, dose responses for changes in total plasma calcium 120 min later were similar for rat amylin and CGRP, occurring with doses of 10 μg and greater (Young *et al.*, 1993). The hypocalcemic response to calcitonins and amylinomimetics takes ~3 hr to fully develop and represents largely a fall in ionized calcium (about half of total calcium present). Changes in ionized calcium measured with an ion-selective electrode were followed during 3 hr continuous infusions of rat amylin or pramlintide at different rates in anesthetized rats, enabling derivation of an EC_{50} for the hypocalcemic effects of these peptides. The EC_{50} for the hypocalcemic effect of rat amylin

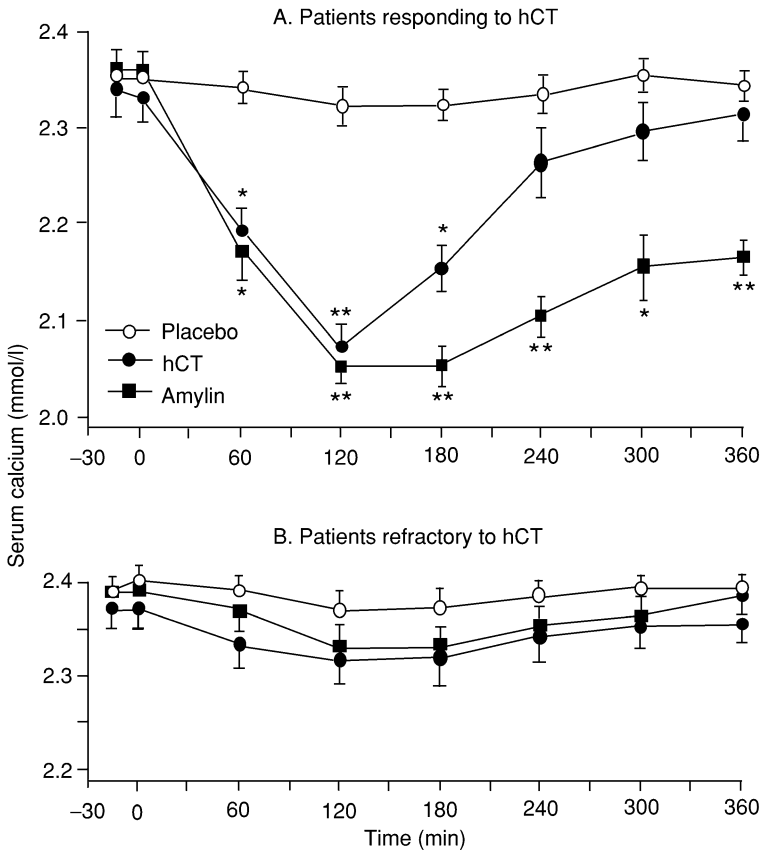

FIGURE 2 Effects of human amylin or human calcitonin injections on serum calcium concentrations in patients with Paget's disease of bone (Wimalawansa *et al.*, 1992).

obtained in this way was 97 pM ± 0.25 log; that for pramlintide was similar, at 130 pM ± 0.28 log. These concentrations are not dissimilar to those achievable with antidiabetic doses of pramlintide in humans.

The hypocalcemic effects of amylin appeared to be at least partly explicable by a direct effect on osteoclasts to inhibit bone resorption (Alam *et al.*, 1993a). The calciuretic effect of amylin was not sufficient to account for the fall in plasma calcium observed (Miles *et al.*, 1994).

IV. Effects on Osteoclasts

In addition to inducing profound hypocalcemia in rats and rabbits, human amylin was reported to abolish bone resorption by isolated osteoclasts *in vitro* (MacIntyre, 1989; Zaidi *et al.*, 1990a), a result that has been confirmed in other reports (Alam *et al.*, 1991, 1993a,b; Miyaura *et al.*, 1992; Moonga *et al.*, 1993; Zaidi *et al.*, 1991, 1993a) (Fig. 3).

In a comparison with the International Reference Preparation of human calcitonin in which primary rat osteoclasts were cultured on devitalized human bone and resorptive pit area measured by electron microscopy, human amylin was 33-fold less potent than the standard (Zaidi *et al.*, 1990a). In another osteoclast-bone resorption assessment that quantified in a direct comparison the area of resorption per bone slice, the following potency differences for antiresorptive effects were reported: amylin > βCGRP (10:1);

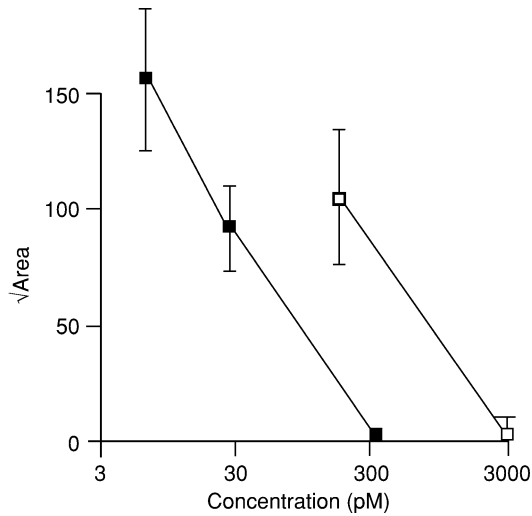

FIGURE 3 Comparison of antiresorptive activities of human amylin and the International Reference Preparation of human calcitonin in primary rat osteoclasts cultured on devitalized human bone. Pit area (root transformed) was measured by electron microscopy. Human amylin was 33-fold less potent than the standard (Zaidi *et al.*, 1990a).

sCT >> amylin (800:1), and human calcitonin > amylin (12:1) (Alam *et al.*, 1993a). The potency of deamidated amylin (amylin acid) was less than that of amidated amylin and approached that of βCGRP (Alam *et al.*, 1993a). As with the hypocalcemic potencies, the rank order of potency for anti-resorptive effects, calcitonin ≥ amylin > CGRP, was surprising in view of the sequence dissimilarity between calcitonins and amylin (~13% identity) versus a greater similarity between calcitonins and CGRPs (Zaidi *et al.*, 1993b). Others reported 60-fold lower potency of human amylin and CGRP versus human calcitonin in mouse

Osteoclasts are thought to be derived from cells of the monocyte macrophage lineage. Both human amylin and CGRP inhibited 1α,25-dihydroxyvitamin D3-induced bone resorption in a culture system containing osteoclast-like multinucleated cells, but at 60-fold lower potency than human amylin (Tamura *et al.*, 1992a).

Alveolar macrophages responded to CGRP and to amylin at 100-fold higher doses, but not to calcitonin with increases in cAMP and with a pharmacology that was CGRP-like (Owan and Ibaraki, 1994). But despite being ~100-fold less potent at CGRP receptors, the hypocalcemic potency of amylin was greater than that of CGRP (Datta *et al.*, 1989; Zaidi *et al.*, 1988, 1990a). Thus, effects at osteoclasts cannot be explained solely by action at CGRP receptors.

Zaidi and others quantifying osteoclast morphology discerned two separate behaviors that appeared to be mediated via separate signaling systems (Zaidi *et al.*, 1992, 1994): the "quiescent" Q-effect, characterized by a reduction in cell motility (Alam *et al.*, 1991, 1993a; Zaidi *et al.*, 1990b, 1991), and the "retraction" R-effect, wherein osteoclasts withdraw pseudopodia (Alam *et al.*, 1991). The Q-effect appears to correlate well with inhibition of bone resorption, is coupled to adenylyl cyclase via a G_s-like cholera toxin-sensitive G protein, and appears to be driven by application of amylin, CGRP, and calcitonins. This receptor pathway has been termed the "amylin site" (Zaidi *et al.*, 1991). The R-effect, in contrast, appears to be much more calcitonin selective, is mediated via a pertussis toxin-sensitive G protein, and is associated with changes in cytosolic $[Ca^{2+}]$ (Alam *et al.*, 1991; Zaidi *et al.*, 1993b).

Gilbey *et al.* described production of a substance with amylin-like immunoreactivity from osteoblasts, which they proposed could have a direct paracrine effect on osteoclasts within the same bone matrix (Gilbey *et al.*, 1991b). Cox *et al.*, using polyclonal antibody raised against full-length human amylin, found similar evidence for amylin-like immunoreactivity at resorptive sites of teeth subject to lateral strain in cats (Cox *et al.*, 1991), and suggested that amylin might be involved in bone remodeling.

In a transgenic model, amylin-null homozygotes and heterozygotes showed osteopenia due to excessive resorption, without apparent effect on markers of bone formation (Dacquin *et al.*, 2004). Amylin's *in vitro* effects

included inhibiting fusion of mononucleated osteoclast precursors into multinucleated osteoclasts via a signalling system that implicated ERK1/2 and an as-yet-uncharacterized receptor (Dacquin *et al.*, 2004).

V. Effects on Osteoblasts

In mouse primary osteoblasts, and in one osteoblast-like cell line, the KS-4 (but not all), amylin was a weak agonist in stimulating cAMP production (Miyaura *et al.*, 1992). Human calcitonin was without effect (potency CGRP > amylin >> calcitonin). Thus, in contrast to calcitonin, amylin appeared to be involved in both osteoclast and osteoblast functions. In rats made diabetic with streptozotocin (but not in non-diabetic controls) rat amylin delivered as a daily subcutaneous injection of 1 nmol/kg increased endosteal osteoblast number by ~45% ($P < 0.05$), partly restoring the deficit in osteoblast number relative to that observed in non-diabetic control rats (Romero *et al.*, 1993). In osteoblasts isolated from fetal rat calvaria, incubation with rat amylin increased cell number by up to 60%, with significant effects observed at amylin concentrations of 10 pM (Cornish *et al.*, 1994). When injected locally over the hemicalvaria of adult mice, amylin similarly increased osteoblast surface area, osteoblast number, thymidine incorporation, osteoid area, and mineralized bone area, while simultaneously decreasing local indices of resorption (Cornish *et al.*, 1994, 1995).

Cornish *et al.* reported that while both full-length rat amylin and the amylin[1–8] subpeptide stimulated osteoblasts, in contrast, inhibition of bone resorption in neonatal mouse calvariae occurred only with the intact amylin molecule. Their interpretation was that the dissociation of the actions of amylin suggested actions through two separate receptors, one on the osteoclast (possibly the calcitonin receptor) and a second on the osteoblast (Cornish *et al.*, 1998). Adrenomedullin produced a dose-dependent increase in cell number and ^{3}H-thymidine incorporation in cultures of fetal rat osteoblasts. This effect was also seen with adrenomedullin[15–52], [22–52], and [27–52], but adrenomedullin[40–52] was inactive. These effects were lost in the presence of amylin blockers, suggesting that they were mediated by an amylin-like receptor. Adrenomedullin also increased ^{3}H-thymidine and phenylalanine incorporation into cultured neonatal mouse calvaria but, unlike amylin, did not reduce bone resorption in this model. When injected daily for 5 days over the calvariae of adult mice, it increased indexes of bone formation 2- to 3-fold ($P < 0.0001$) and increased mineralized bone area by 14% ($P = 0.004$). It was concluded that adrenomedullin regulates osteoblast function and that it increases bone mass *in vivo* via an amylin-like receptor (Cornish *et al.*, 1997).

In a comparison of rat amylin and CGRP on stimulation of thymidine and phenylalanine incorporation in cultured fetal rat osteoblasts, amylin

was effective on these indices at concentrations 100-fold lower, and its maximal effects were about twice as great as those of CGRP. The ED_{50}s for the effects of amylin and CGRP on cell number were ~1 pM and 100 pM, respectively. From that result, and from preferential blockade with certain antagonists, it was concluded that amylin and CGRP probably acted through a common receptor to stimulate osteoblast growth, and that this receptor has a higher affinity for amylin than for CGRP (Cornish *et al.,* 1999b). Other groups also reported a proliferative effect on osteoblast lines (Villa *et al.,* 2000, 2003). Amylin, unlike CGRP, did not stimulate cAMP in primary human osteoblasts (Villa *et al.,* 2000) but appeared to activate the protein kinase C (PKC) signaling pathway (Villa *et al.,* 2003). A third group failed to see effects of amylin or CGRP on a human osteosarcoma cell line, SaOS-2, that did respond to calcitonin (Farley *et al.,* 2000).

When maximally stimulating concentrations of amylin, adrenomedullin, or insulin-like growth factor-1 (IGF-1) were combined, no added proliferative effect was observed in cultured osteoblasts, whereas additivity was achieved when either amylin or adrenomedullin was combined with maximal concentrations of TGFβ or EGF. The interpretation was that amylin, adrenomedullin, and IGF-1 share similar pathways of action in osteoblasts (Cornish *et al.,* 1999a).

VI. Effects in Models of Diabetic Osteopenia

In a study using streptozotocin induction of diabetes in 10-week-old rats as an animal model, analysis of osteocalcin and bone histomorphometry showed a low-turnover osteopenia in the diabetic animals. Amylin administered as a 1 nmol/kg daily subcutaneous dose resulted in a significant increase in bone volume in the normal rats, group B ($P < 0.05$), but was unable to significantly alter this parameter in the diabetic animals (Jacobs *et al.,* 1992; Romero *et al.,* 1995). In a shorter study, the same authors found an effect of amylin replacement on osteoblast number in streptozotocin diabetic rats (Romero *et al.,* 1993). The group of Barlet *et al.* examined several bone parameters in streptozotocin diabetic rats treated with daily subcutaneous injections of insulin, rat amylin (45 μg/kg), both, or neither (Horcajada-Molteni *et al.,* 2001). In contrast to untreated diabetic rats, those receiving amylin had normal femoral bone strength and normal metaphyseal, diaphyseal, and total bone mineral densities. In these experimental conditions, amylin appeared at least as effective as insulin in inhibiting diabetes-induced osteopenia, and appeared to increase bone density both by inhibiting resorption and by increasing osteoblastic activity.

As part of a 1 year clinical study in type 1 diabetic patients receiving pramlintide (30 or 60 μg q.i.d., or placebo), biochemical markers of bone resorption and formation were measured. In the subset of postmenopausal

women, a pattern of reduction on bone turnover was observed, best exemplified by a 20.2% reduction in bone alkaline phosphatase compared to the subset treated with pramlintide for 52 weeks (Bone *et al.*, 1999).

VII. Effects in Models of Osteoporosis

The ovarectomized rat is frequently used as an animal model of postmenopausal osteoporosis. In one study using this model, amylin delivered as a daily subcutaneous injection of 3, 30, or 300 μg/kg for 2 months had no discernable effect on histomorphometric parameters. However, calcitonin included as a positive control in this study also did not affect bone histomorphometry (Goodman *et al.*, 1997). In a similar study on ovarectomized rats administered rat amylin in daily 30μg/kg subcutaneous doses, distal metaphyseal (cancellous bone) and total femoral bone densities were higher in ovarectomized rats treated with amylin than in ovarectomized controls. The highest plasma osteocalcin concentration (indicative of new bone formation) was measured in amylin-treated ovarectomized rats. Simultaneously, urinary deoxypyridinoline excretion (indicative of bone resorption) was lower in amylin-treated than in control ovarectomized rats. These results were consistent with amylin treatment in ovarectomized rats having inhibited trabecular bone loss both by inhibiting resorption and by stimulating osteoblastic activity (Horcajada-Molteni *et al.*, 2000).

VIII. Effects on Bone in Humans

In addition to the postmenopausal diabetic patients described previously (Bone *et al.*, 1999), amylinomimetics have been administered in two studies to patients with Paget's disease of bone (Gilbey *et al.*, 1991a,b; Wimalawansa *et al.*, 1992), in which a sustained hypocalcemic effect was observed.

References

Alam, A. S., Moonga, B. S., Bevis, P. J., Huang, C. L., and Zaidi, M. (1991). Selective antagonism of calcitonin-induced osteoclastic quiescence (Q effect) by human calcitonin gene-related peptide-(Val8Phe37). *Biochem. Biophys. Res. Commun.* **179**, 134–139.

Alam, A. S. M., Moonga, B. S., Bevis, P. J. R., Huang, C. L. H., and Zaidi, M. (1993a). Amylin inhibits bone resorption by a direct effect on the motility of rat osteoclasts. *Exp. Physiol.* **78**, 183–196.

Alam, A. S. M. T., Bax, C. M. R., Shankar, V. S., Bax, B. E., Bevis, P. J. R., Huang, C. L. H., Moonga, B. S., Pazianas, M., and Zaidi, M. (1993b). Further studies on the mode of action of calcitonin on isolated rat osteoclasts—pharmacological evidence for a 2nd site mediating intracellular Ca2+ mobilization and cell retraction. *J. Endocrinol.* **136**, 7–15.

Beaumont, K., Pittner, R. A., Herich, J., Albrandt, K., and Moore, C. X. (1994). Coupling of two cloned rat calcitonin receptor isoforms to adenylyl cyclase and phospholipase C. *Program and Abstracts, Endocrine Society 76th Annual Meeting, June 15–18, Anaheim, CA*, 424.

Bone, H. G., Hurley, M. A., Goldstein, H., Fineman, M. S., and Kolterman, O. G. (1999). Effects of administration of pramlintide for 12 months on bone metabolism markers in people with type 1 diabetes. *Program and Abstracts, Endocrine Society 81st Annual Meeting, June 12–15, San Diego, CA*, 447.

Cornish, J., Callon, K. E., Cooper, G. J. S., and Reid, I. (1994). Amylin stimulates osteoblasts *in vitro* and *in vivo*. *J. Bone Miner. Res.* **9**, S299.

Cornish, J., Callon, K. E., Cooper, G. J. S., and Reid, I. R. (1995). Amylin stimulates osteoblast proliferation and increases mineralized bone volume in adult mice. *Biochem. Biophys. Res. Commun.* **207**, 133–139.

Cornish, J., Callon, K. E., Coy, D. H., Jiang, N. Y., Xiao, L. Q., Cooper, G. J. S., and Reid, I. R. (1997). Adrenomedullin is a potent stimulator of osteoblastic activity *in vitro* and *in vivo*. *Am. J. Physiol.* **36**, E1113–E1120.

Cornish, J., Callon, K. E., Lin, C. Q. X., Xiao, C. L., Mulvey, T. B., Coy, D. H., Cooper, G. J. S., and Reid, I. R. (1998). Dissociation of the effects of amylin on osteoblast proliferation and bone resorption. *Am. J. Physiol.* **37**, E827–E833.

Cornish, J., Callon, K. E., Grey, A. B., Balchin, L. M., Cooper, G. J. S., and Reid, I. R. (1999a). The proliferative effects of amylin and adrenomedullin on osteoblasts—an important role for the IGF-1 receptor. *Program and Abstracts: American Society of Bone and Mineral Research*, SA118.

Cornish, J., Callon, K. E., Lin, C. Q., Xiao, C. L., Gamble, G. D., Cooper, G. J., and Reid, I. R. (1999b). Comparison of the effects of calcitonin gene-related peptide and amylin on osteoblasts. *J. Bone Miner. Res.* **14**, 1302–1309.

Cox, M., Shanfeld, J., and Davidovitch, Z. (1991). Amylin amide peptide localized at bone resorption sites *in vivo*. *J. Dent. Res.* **70**, 578.

Dacquin, R., Davey, R. A., Laplace, C., Levasseur, R., Morris, H. A., Goldring, S. R., Gebre-Medhin, S., Galson, D. L., Zajac, J. D., and Karsenty, G. (2004). Amylin inhibits bone resorption while the calcitonin receptor controls bone formation *in vivo*. *J. Cell Biol.* **164**, 509–514.

Datta, H. K., Zaidi, M., Wimalawansa, S. J., Ghatei, M. A., Beacham, J. L., Bloom, S. R., and MacIntyre, I. (1989). *In vivo* and *in vitro* effects of amylin and amylin-amide on calcium metabolism in the rat and rabbit. *Biochem. Biophys. Res. Commun.* **162**, 876–881.

Farley, J., Dimai, H. P., Stilt-Coffing, B., Farley, P., Pham, T., and Mohan, S. (2000). Calcitonin increases the concentration of insulin-like growth factors in serum-free cultures of human osteoblast-line cells. *Calcif. Tissue Int.* **67**, 247–254.

Gilbey, S., Ghatei, M. A., Bretherton-Watt, D., Jones, P. M., Beacham, I., Perera, T., Girgis, S., Bloom, S. R., and Zaidi, M. (1991a). Amylin lowers serum calcium in Paget's bone disease: Further evidence for a role in calcium metabolism. *J. Bone Miner. Res.* **6**, S293.

Gilbey, S. G., Ghatei, M. A., Bretherton-Watt, D., Zaidi, M., Jones, P. M., Perera, T., Beacham, J., Girgis, S., and Bloom, S. R. (1991b). Islet amyloid polypeptide: Production by an osteoblast cell line and possible role as a paracrine regulator of osteoclast function in man. *Clin. Sci. (Colch.)* **81**, 803–808.

Goodman, G. R., Dissanayake, I. R., Gorodetsky, E., Zhou, H., Ma, Y. F., Jee, W. S. S., Santos, I. S. J., and Epstein, S. (1997). The effect of calcitonin and incremental doses of amylin on biochemical and histomorphometric parameters on the ovarectomized rats. *Program and Abstracts: American Society of Bone and Mineral Research*, T549.

Horcajada-Molteni, M. N., Davicco, M. J., Lebecque, P., Coxam, V., Young, A. A., and Barlet, J. P. (2000). Amylin inhibits ovariectomy-induced bone loss in rats. *J. Endocrinol.* **165**, 663–668.

Horcajada-Molteni, M. N., Chanteranne, B., Lebecque, P., Davicco, M. J., Coxam, V., Young, A. A., and Barlet, J.-P. (2001). Amylin and bone metabolism in streptozotocin-induced diabetic rats. *J. Bone Miner. Res.* **16**, 958–965.

Jacobs, T. W., Takazawa, M., Liu, C. C., Berlin, J. A., Katz, I. A., Stein, B., Joff, E. E., Cooper, G. J. S., and Epstein, S. (1992). Amylin stimulates bone cell turnover *in vivo* in normal and diabetic rats. *J. Bone Miner. Res.* **7**, S226.

MacIntyre, I. (1989). Amylinamide, bone conservation, and pancreatic beta cells. *Lancet* **2**, 1026–1027.

MacIntyre, I. (1992a). The calcitonin family of peptides. *Ann. N Y Acad. Sci.* **657**, 117–118.

MacIntyre, I. (1992b). The calcitonin peptide family: Relationship and mode of action. *Bone Miner.* **16**, 160–161.

MacIntyre, I. inventor, Amylin Pharmaceuticals Inc. (1995). Treatment of bone disorders. U.S. patent 5,405,831.

MacIntyre, I., Moonga, B. S., and Zaidi, M. (1991). Amylin a new calcium-regulating hormone from the pancreas. *J. Physiol. (Camb.)* **434**, 78P.

Miles, P. D. G., Deftos, L. J., Moossa, A. R., and Olefsky, J. M. (1994). Islet amyloid polypeptide (amylin) increases the renal excretion of calcium in the conscious dog. *Calcif. Tissue Int.* **55**, 269–273.

Miyaura, C., Tamura, T., Owan, I., Akatsu, T., and Suda, T. (1992). The mechanism of action of amylin in osteoclasts and osteoblasts. *Bone Miner.* **17**, I48.

Moonga, B. S., Pazianas, M., Alam, A. S. M. T., Shankar, V. S., Huang, C. L. H., and Zaidi, M. (1993). Stimulation of a Gs-like G-protein in the osteoclast inhibits bone resorption but enhances tartrate-resistant acid phosphatase secretion. *Biochem. Biophys. Res. Commun.* **190**, 496–501.

Muff, R., Stangl, D., Born, W., and Fischer, J. A. (1992). Comparison of a calcitonin gene-related peptide receptor in a human neuroblastoma cell line (SK-N-MC) and a calcitonin receptor in a human breast carcinoma cell line (T47D). *Ann. NY Acad. Sci.* **657**, 106–116.

Muff, R., Kaufmann, M., Born, W., and Fischer, J. A. (1994). Calcitonin inhibits phosphate uptake in opossum kidney cells stably transfected with a porcine calcitonin receptor. *Endocrinology* **134**, 1593–1596.

Muff, R., Bühlmann, N., Fischer, J. A., and Born, W. (1999). An amylin receptor is revealed following co-transfection of a calcitonin receptor with receptor activity modifying proteins-1 or -3. *Endocrinology* **140**, 2924–2927.

Owan, I., and Ibaraki, K. (1994). The role of calcitonin gene-related peptide (CGRP) in macrophages—the presence of functional receptors and effects on proliferation and differentiation into osteoclast-like cells. *Bone Miner.* **24**, 151–164.

Reid, I. R., and Cornish, J. (1996). Amylin and CGRP. *In* "Principles of Bone Biology" (J. P. Bilezikian, L. G. Raisz, and G. A. Rodan, Eds.), pp. 495–505. Academic Press, San Diego, CA.

Romero, D., Bryer, H. P., Rucinski, B., Cvetkovic, M., Liu, C. C., and Epstein, S. (1993). Amylin stimulates osteoblast number in the diabetic rat independent of serum IGF1. *J. Bone Miner. Res.* **8**, S369.

Romero, D. F., Bryer, H. P., Rucinski, B., Isserow, J. A., Buchinsky, F. J., Cvetkovic, M., Liu, C. C., and Epstein, S. (1995). Amylin increases bone volume but cannot ameliorate diabetic osteopenia. *Calcif. Tissue Int.* **56**, 54–61.

Sexton, P. M., Houssami, S., Brady, C. L., Myers, D. E., and Findlay, D. M. (1994). Amylin is an agonist of the renal porcine calcitonin receptor. *Endocrinology* **134**, 2103–2107.

Tamura, T., Miyaura, C., Owan, I., and Suda, T. (1992a). Mechanism of action of amylin in bone. *J. Cell Physiol.* **153**, 6–14.

Tamura, T., Owan, I., Miyaura, C., Akatsu, T., and Suda, T. (1992b). The mechanism of action of amylin on osteoclasts and osteoblasts. *Calcif. Tissue Int.* **50**, A42.

Tedstone, A. E., Nezzer, T., Hughes, S. J., Clark, A., and Matthews, D. R. (1989). The effects of islet amyloid peptide and calcitonin gene-related peptide on insulin secretion in anaesthetised rats and from isolated rat islets. *BDA Diab. Med.* **6**, A38.

Villa, I., Melzi, R., Pagani, F., Ravasi, F., Rubinacci, A., and Guidobono, F. (2000). Effects of calcitonin gene-related peptide and amylin on human osteoblast-like cells proliferation. *Eur. J. Pharmacol.* **409**, 273–278.

Villa, I., Dal Fiume, C., Maestroni, A., Rubinacci, A., Ravasi, F., and Guidobono, F. (2003). Human osteoblast-like cell proliferation induced by calcitonin-related peptides involves PKC activity. *Am. J. Physiol. Endocrinol. Metab.* **284**, E627–E633.

Wimalawansa, S. J., Gunasekera, R. D., and Datta, H. K. (1992). Hypocalcemic actions of amylin amide in humans. *J. Bone Miner. Res.* **7**, 1113–1116.

Young, A. A., Rink, T. J., and Wang, M. W. (1993). Dose response characteristics for the hyperglycemic, hyperlactemic, hypotensive and hypocalcemic actions of amylin and calcitonin gene-related peptide-I (CGRP-alpha). in the fasted, anaesthetized rat. *Life Sci.* **52**, 1717–1726.

Young, A. A., Vine, W., Gedulin, B. R., Pittner, R., Janes, S., Gaeta, L. S. L., Percy, A., Moore, C. X., Koda, J. E., Rink, T. J., and Beaumont, K. (1996). Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev. Res.* **37**, 231–248.

Zaidi, M., Chambers, T. J., Bevis, P. J., Beacham, J. L., Gaines Das, R. E., and MacIntyre, I. (1988). Effects of peptides from the calcitonin genes on bone and bone cells. *Q. J. Exp. Physiol.* **73**, 471–485.

Zaidi, M., Datta, H. K., Bevis, P. J. R., Wimalawansa, S. J., and Macintyre, I. (1990a). Amylin-amide: A new bone-conserving peptide from the pancreas. *Exp. Physiol.* **75**, 529–536.

Zaidi, M., Datta, H. K., Moonga, B. S., and MacIntyre, I. (1990b). Evidence that the action of calcitonin on rat osteoclasts is mediated by two G proteins acting via separate post-receptor pathways. *J. Endocrinol.* **126**, 473–481.

Zaidi, M., Moonga, B. S., Bevis, P. J. R., Bascal, Z. A., and Breimer, L. H. (1990c). The calcitonin gene peptides: Biology and clinical relevance. *Crit. Rev. Clin. Lab. Sci.* **28**, 109–174.

Zaidi, M., Moonga, B. S., Ghatei, M. A., Gilbey, S., Wimalawansa, S. J., Bloom, S. R., MacIntyre, I., and Datta, H. K. (1990d). Amylin: A new bone-conserving hormone from the pancreas: *In vivo* and *in vitro* studies on potency and mode of action. *Bone Miner. Res.* **5**, S76.

Zaidi, M., Alam, A. S. M. T., Soncini, R., Avaldi, F., and Moonga, B. S. (1991). Calcitonin acts upon two receptor subtypes on the osteoclast: The 'amylin-site' and the 'calcitonin-site'. *J. Bone Miner. Res.* **6**, S198.

Zaidi, M., Alam, A. S., Shankar, V. S., Bax, B. E., Moonga, B. S., Bevis, P. J., Pazianas, M., and Huang, C. L. (1992). A quantitative description of components of *in vitro* morphometric change in the rat osteoclast model: Relationships with cellular function. *Eur. Biophys. J.* **21**, 349–355.

Zaidi, M., Pazianas, M., Shankar, V. S., Bax, B. E., Bax, C. M., Bevis, P. J., Stevens, C., Huang, C. L., Blake, D. R., Moonga, B. S., *et al.* (1993a). Osteoclast function and its control. *Exp. Physiol.* **78**, 721–739.

Zaidi, M., Shankar, V. S., Huang, C. L. H., Pazianas, M., and Bloom, S. R. (1993b). Amylin in bone conservation—current evidence and hypothetical considerations. *Trends Endocrinol. Metab.* **4**, 255–259.

Zaidi, M., Bax, B. E., Shankar, V. S., Moonga, B. S., Simon, B., Alam, A. S. M. T., Das, R. E. G., Pazianas, M., and Huang, C. L. H. (1994). Dimensional analysis of osteoclastic bone resorption and the measurement of biologically active calcitonin. *Exp. Physiol.* **79**, 387–399.

Zimmermann, U., Fluehmann, B., Born, W., Fischer, J. A., and Muff, R. (1997). Coexistence of novel amylin-binding sites with calcitonin receptors in human breast carcinoma MCF-7 cells. *J. Endocrinol.* **155**, 423–431.

Andrew Young

Central Nervous System and Other Effects

I. Summary

Amylin enhanced the uptake of certain amino acids, crossed the blood–brain barrier, and increased body temperature. The physiological significance of these responses is currently unclear.

An effect of peripherally injected amylin to enhance weakly trained memory fitted with similar effects of other gastrointestinal peptide hormones.

Centrally administered amylin reduced locomotor and exploratory behavior.

Amylin administered alone was analgesic when administered peripherally, via a non-opiate pathway. When administered in combination with opiates, there was an opiate-sparing synergy.

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II. Effects on Amino Acid Transport

Relative to control injections, rat amylin injected intrahypothalamically into rats at doses of 2 μg increased brain content of L-tyrosine and L-tryptophan, especially when these amino acids were pre-injected intraperitoneally to obviate substrate supply as a rate-limiting step for brain uptake (Chance *et al.,* 1992). It was not known whether this represented a specific stimulation of a transport process, or whether it was a consequence of amylin activation of certain brain systems, such as the dopaminergic system, whose metabolism was also found to increase in corpus striatum with amylin administration (Balasubramaniam *et al.,* 1991; Chance *et al.,* 1992).

III. Amylin Transport across the Blood–Brain Barrier

Banks *et al.* (Banks *et al.,* 1995) studied the uptake of iodinated rat amylin in the brains of ICR mice at brain regions outside of the circumventricular organs, where amylin was known to bind, and at which the diffusional barrier is reduced. Following an intravenous bolus injection 15 min earlier, they frequently sampled carotid arterial blood to estimate brain vascular exposure to the label and used a kinetic model to derive a transport constant and determined that (1) material that entered the brain was intact, (2) amylin diffusion was similar to that of other peptides of similar molecular weight that enter the brain by a nonsaturable process, but (3) uptake was decreased by 60% in the presence of aluminum given intraperitoneally as the chloride, more indicative of a facilitated transport. Uptake of amylin was ~5-fold more rapid than that of morphine determined in this same test system (Banks *et al.,* 1995). It is unclear, however, whether there is any physiological significance attached to amylin that might cross the blood–brain barrier, as opposed, for example, to amylin signals generated at the circumventricular organs or within the brain by amylin-secreting neurons.

IV. Effects on Body Temperature

Effects on body temperature of injection of 1 μg rat amylin in 1 μl artificial cerebrospinal fluid (CSF) into the paraventricular hypothalamus of nine conscious rats was compared to similar injections of CSF alone, the [1–23] fragment of amylin, or amylin following pretreatment with indomethacin to block prostaglandin synthesis (Chance *et al.,* 1991). The relative hyperthermia of $>1^{\circ}$C evoked with amylin was present with or without indomethacin, indicating that this response was not mediated via prostaglandins.

In a study using hooded rats (Bouali *et al.,* 1995), a dose-dependent hyperthermia was observed with amylin doses between 1.25 and 20 μg, with

the maximal hyperthermic effect being a pyrexia of ~1.5°C 30–60 min after intracerebroventricular administration. The dose at which pyrexia was first observed was lower for calcitonin gene-related peptide (CGRP) than for rat amylin, suggesting that the response may have been mediated via CGRP receptors (Bouali *et al.*, 1995). But a similar response has also been described with salmon calcitonin, which interacts at amylin but not CGRP receptors (Sellami and de Beaurepaire, 1993). For salmon calcitonin, the brain structures implicated in the response included the dorsomedial nucleus of the hypothalamus, the centromedial nucleus of the thalamus, and the preoptic area, an area of thermoregulatory importance (Sellami and de Beaurepaire, 1993). Increases in body temperature occurred in spite of reports of decreased locomotor function following central amylin administration, suggesting the effect was not related to increases in activity, for example.

V. Effects on Memory

Gastrointestinal peptides, in addition to their roles in fuel homeostasis, also commonly modulate learning and memory (Morley *et al.*, 1992). "The relationship between hormones that regulate food intake (cholecystokinin, bombesin, gastrin-releasing peptide, pancreastatin, amylin) and those which are involved in memory processing continues to be described. It appears that the relationship between feeding and memory processing is not just fortuitous, but may have evolved to increase the likelihood of future successful foraging activity" (Flood and Morley, 1992).

An example is cholecystokinin (CCK), which in addition to inhibiting food intake is considered to be part of the mechanism by which feeding enhances memory. The effect of CCK appears to be mediated via the amygdala (Morley *et al.*, 1995), a brain structure in which amylin-containing neurons have been identified (Dilts *et al.*, 1995). Arginine vasopressin, oxytocin, angiotensin II, insulin, growth factors, serotonin, melanin concentrating hormone, histamine, bombesin, gastrin-releasing peptide, glucagon-like peptide-1, CCK, dopamine, corticotropin-releasing factor (Gulpinar and Yegen, 2004), and ghrelin (Carlini *et al.*, 2002) increased learning and memory.

Morley *et al.* reported an effect of rat amylin to enhance memory in rats and mice (Edwards and Morley, 1992; Flood and Morley, 1992; Morley and Flood, 1994; Morley *et al.*, 1992, 1995).

Amylin increased retention only when administered peripherally, and only in association with "weak" training (Flood and Morley, 1992). CD-1 mice were trained to choose one arm of a T-maze under conditions of weak (55 dB buzzer, 0.3 mA footshock, 30 s intertrial interval, four trials) and strong (65 dB buzzer, 0.35 mA footshock, 45 s intertrial interval, five trials) conditions of retention. Amylin delivered by intraperitoneal injection in doses of 5–100 μg/kg (but not by intracerebroventricular injection) immediately after weak

training reduced the number of trials required to reacquire the original T-maze arm choice 1 week later. This result was interpreted as having promoted retention, but it was differential, depending upon the training stimulus. Amylin actually impaired retention 1 week after strong conditioning (Flood and Morley, 1992). A similar differential pattern was observed with other compounds capable of improving memory.

Rate of loss of behaviors (extinction) upon cessation of conditioning stimuli was dose-dependently faster if rat amylin was immediately given intracerebroventricularly in CFY rats implanted with cannulae (Kovacs and Telegdy, 1996).

VI. Effects on Locomotor Activity, Grooming, and Stereotypy

Rat amylin (0, 2.5, 5, or 10 μg) was injected via implanted cannulae into the lateral ventricles of rats that were then scored for 4 min for locomotor activity (number of cage sections explored with forelegs), grooming (washing, licking, scratching), and stereotypic behavior (sniffing) (Clementi *et al.*, 1996).

Amylin dose-dependently reduced locomotor activity and antagonized the hyperactivity induced by amphetamine, but had no effect on the other behaviors (Clementi *et al.*, 1996). Ambulatory activity, measured as the total number (out of 36) of floor units entered in an open field test, was decreased by intracerebroventricular rat amylin in CFY rats (Kovacs and Telegdy, 1996), but the number of rearings (standing on hind legs) and groomings was increased (Kovacs and Telegdy, 1996). Locomotor activity determined in a photocell-activated apparatus was also dose-dependently inhibited by injection of 2.5 to 20 μg doses of rat amylin via lateral ventricular cannulae in hooded rats (Bouali *et al.*, 1995). A similar decrease in locomotor and exploratory behaviors was observed when rat amylin was injected into the nucleus accumbens shell (Baldo and Kelley, 1999, 2001) (Fig. 1).

VII. Effects on Pain

Potential effects of intracerebroventricular amylin on latency of tail withdrawal from 49°C water were tested in hooded rats at doses up to 80 μg. No effect was observed (Bouali *et al.*, 1995).

In view of the lack of central antinociceptive activity, it was therefore surprising that peripheral (subcutaneous and intraperitoneal) doses of rat amylin were potently and dose-dependently analgesic in a mouse model of visceral pain (Young, 1997). Amylin administered to Swiss Webster mice inhibited writhing induced by intraperitoneal injection of dilute (2%) acetic

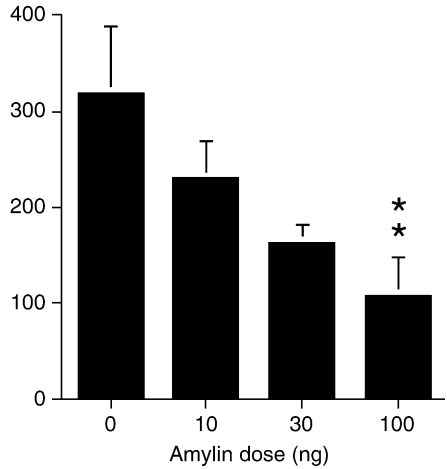

FIGURE 1 Dose response for effects of amylin infusions directed to the core of the nucleus accumbens on locomotor activity in the subsequent 30 min (Baldo and Kelley, 2001).

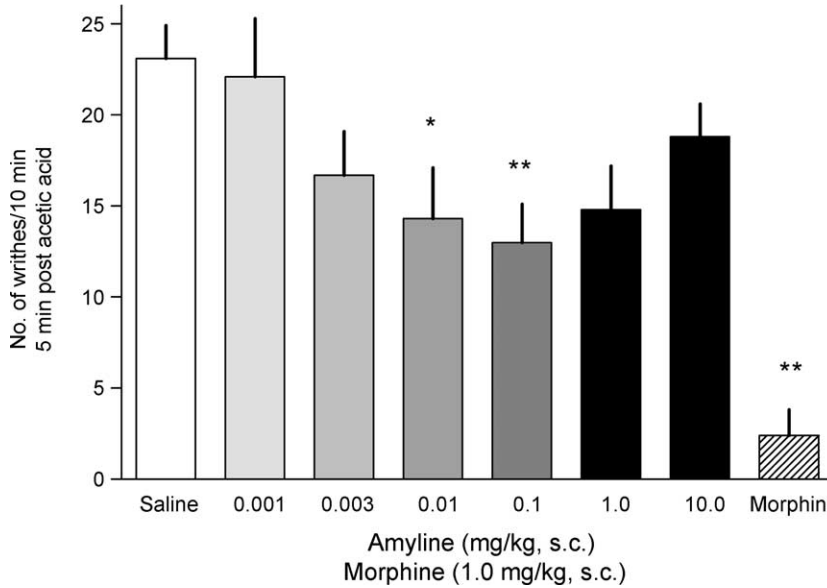

FIGURE 2 Dose response for analgesic effect of rat amylin in acetic acid-induced writhing in mice. Redrawn from U.S. Patent 5,677,279 (Young, 1997).

acid. Intraperitoneal and subcutaneous dose responses were similar and indicated a detectable analgesic effect at a dose of 10 μg/kg. By comparison, the lowest effective dose of morphine in the same test system was 300 μg/kg. The effect diminished with each route at higher doses, perhaps explaining another study's lack of effect at high doses (Bouali *et al.*, 1995) (Fig. 2).

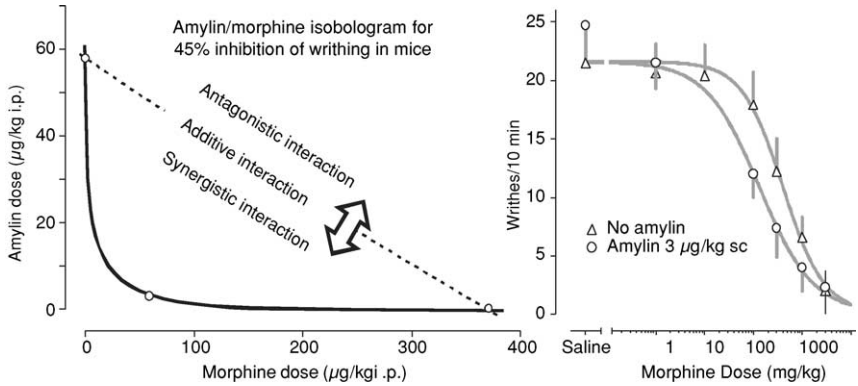

FIGURE 3 Opiate-sparing effect of amylin on morphine analgesia. Adding a small dose of amylin markedly reduced the morphine required to attain a given level of analgesia, as illustrated in the isobologram (left panel). From U.S. Patent 5,677,279 (Young, 1997) and unpublished data.

The analgesic effect of amylin was not mediated via opiate receptors, since it was not diminished by the opiate antagonist naloxone (Young, 1997). On the other hand, there was a synergistic interaction between amylin and morphine in this model, such that much lower doses of morphine were required to invoke an equivalent degree of analgesia when amylin was present. The opiate-sparing synergy was shown by isobolar analysis (Fig. 3).

An analgesic effect of the amylinomimetic agent salmon calcitonin has been demonstrated in animals and humans, the latter principally in relation to bone pain (Szanto *et al.*, 1986). In animals, the analgesic effect of salmon calcitonin differed from that of morphine in that it did not diminish with repeated dosing and did not involve opiate receptors (Braga *et al.*, 1978). Involvement of amylin-like receptors is suggested by a weaker effect of human calcitonin (versus salmon calcitonin).

The site at which amylin exerts its analgesic activity is unknown. In a study using *in situ* hybridization, immunocytochemistry, and immunochemistry to determine its distribution, amylin itself was found to be expressed in a population of small- to medium-sized nerve cell bodies in dorsal root ganglia from all levels and in the jugular-nodose and trigeminal ganglion, and included cells also expressing CGRP, substance P, and pituitary adenylate cyclase-activating polypeptide. Amylin-immunoreactive fibers were localized in the dorsal horns of the spinal cord (sensory input), and to a lesser extent in peripheral tissues receiving sensory innervation. It was concluded that amylin was expressed in sensory neurons and was thus a novel sensory neuropeptide candidate (Mulder *et al.*, 1995).

Mice with deletion of the amylin gene displayed a reduced pain response in the paw formalin test, leading the authors to conclude that amylin had a pro-nociceptive function in primary sensory neurons (Gebre-Medhin *et al.*,

1998). That contrasts with the anti-nociceptive effects observed when it is injected peripherally (Young, 1997).

VIII. Effects on Inflammation

A potential anti-inflammatory activity of amylin was studied in different models of inflammation and compared to that of CGRP (Clementi *et al.*, 1995). Both peptides were active against mouse ear oedema induced by croton oil and acetic acid-induced peritonitis in the rat. CGRP was more potent than amylin in both models. Pretreatment with the CGRP antagonist CGRP[8–37] blocked the anti-inflammatory activity of both peptides in croton oil ear oedema. No effect was seen on inflammation produced by serotonin (rat paw oedema) or dextran (plasma protein extravasation in rat skin). Thus, amylin exerted anti-inflammatory activity only in models characterized by a vascular component. Blockade of these effects with CGRP [8–37] suggests the involvement of CGRP receptors.

References

Balasubramaniam, A., Zhang, F. S., Thomas, I., and Chance, W. T. (1991). Amylin increases transport of tyrosine and tryptophan into brain. *Soc. Neurosci. Abstr.* **17**, 976.

Baldo, B. A., and Kelley, A. E. (1999). Effects of insulin or amylin infusion into the nucleus accumbens shell on unconditioned exploratory and ingestive behaviors. *Soc. Neurosci. Abstr.* **25**, 2141.

Baldo, B. A., and Kelley, A. E. (2001). Amylin infusion into rat nucleus accumbens potently depresses motor activity and ingestive behavior. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**, R1232–R1242.

Banks, W. A., Kastin, A. J., Maness, L. M., Huang, W. T., and Jaspan, J. B. (1995). Permeability of the blood-brain barrier to amylin. *Life Sci.* **57**, 1993–2001.

Bouali, S. M., Wimalawansa, S. J., and Jolicoeur, F. B. (1995). *In vivo* central actions of rat amylin. *Regul. Pept.* **56**, 167–174.

Braga, P., Ferri, S., Santagostino, A., Olgiati, V. R., and Pecile, A. (1978). Lack of opiate receptor involvement in centrally induced calcitonin analgesia. *Life Sci.* **22**, 971–977.

Carlini, V. P., Monzon, M. E., Varas, M. M., Cragnolini, A. B., Schioth, H. B., Scimonelli, T. N., and de Barioglio, S. R. (2002). Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem. Biophys. Res. Commun.* **299**, 739–743.

Chance, W. T., Balasubramaniam, A., Zhang, F.-S., and Fischer, J. E. (1991). Hyperthermia following the intrahypothalamic administration of amylin. *Surg. Forum* **42**, 84–86.

Chance, W. T., Balasubramaniam, A., Thomas, I., and Fischer, J. E. (1992). Amylin increases transport of tyrosine and tryptophan into the brain. *Brain Res.* **593**, 20–24.

Clementi, G., Caruso, A., Cutuli, V. M. C., Prato, A., Debernardis, E., Fiore, C. E., and Amicoroxas, M. (1995). Anti-inflammatory activity of amylin and CGRP in different experimental models of inflammation. *Life Sci.* **57**, PL193–PL197.

Clementi, G., Valerio, C., Emmi, I., Prato, A., and Drago, F. (1996). Behavioral effects of amylin injected intracerebroventricularly in the rat. *Peptides* **17**, 589–591.

Dilts, R. P., Phelps, J., Koda, J., and Beaumont, K. (1995). Comparative distribution of amylin and calcitonin gene related peptide (CGRP): Immunoreactivities in the adult rat brain. *Soc. Neurosci. Abstr.* **21**, 1116.

Edwards, B. J. A., and Morley, J. E. (1992). Amylin. *Life Sci.* **51**, 1899–1912.

Flood, J. F., and Morley, J. E. (1992). Differential effects of amylin on memory processing using peripheral and central routes of administration. *Peptides* **13**, 577–580.

Gebre-Medhin, S., Mulder, H., Zhang, Y. Z., Sundler, F., and Betsholtz, C. (1998). Reduced nociceptive behavior in islet amyloid polypeptide (amylin) knockout mice. *Mol. Brain Res.* **63**, 180–183.

Gulpinar, M. A., and Yegen, B. C. (2004). The physiology of learning and memory: Role of peptides and stress. *Curr. Protein Pept. Sci.* **5**, 457–473.

Kovacs, A. M., and Telegdy, G. (1996). The effects of amylin on motivated behavior in rats. *Physiol. Behav.* **60**, 183–186.

Morley, J. E., and Flood, J. F. (1994). Effects of amylin and CGRP on appetite regulation and memory. *Can. J. Physiol. Pharmacol.* **72**, 32.

Morley, J. E., Flood, J., and Silver, A. J. (1992). Effects of peripheral hormones on memory and ingestive behaviors. *Psychoneuroendocrinology* **17**, 391–399.

Morley, J. E., Flood, J. F., Farr, S. A., Perry, H. J., Kaiser, F. E., and Morley, P. M. K. (1995). Effects of amylin on appetite regulation and memory. *Can. J. Physiol. Pharmacol.* **73**, 1042–1046.

Mulder, H., Leckstrom, A., Uddman, R., Ekblad, E., Westermark, P., and Sundler, F. (1995). Islet amyloid polypeptide (amylin) is expressed in sensory neurons. *J. Neurosci.* **15**, 7625–7632.

Sellami, S., and de Beaurepaire, R. (1993). Medial diencephalic sites involved in calcitonin-induced hyperthermia and analgesia. *Brain Res.* **616**, 307–310.

Szanto, J., Jozsef, S., Rado, J., Juhos, E., Hindy, I., and Eckhardt, S. (1986). Pain killing with calcitonin in patients with malignant tumours. *Oncology* **43**, 69–72.

Young, A. A. (1997). Amylin Pharmaceuticals Inc. inventor, Methods and compositions for treating pain with amylin or agonists thereof. U.S. Patent 5,677,279.

Andrew Young

Clinical Studies

I. Summary

Recognizing that type 1 diabetes was characterized not only by insulin deficiency, but also by amylin deficiency, Cooper (Cooper, 1991) predicted that certain features of the disease could be related thereto, and he proposed amylin/insulin co-replacement therapy. Although the early physiological rationale was flawed, the idea that glucose control could be improved over that attainable with insulin alone without invoking the ravages of worsening insulin-induced hypoglycemia was vindicated. The proposal spawned a first-in-class drug development program that ultimately led to marketing approval by the U.S. Food and Drug Administration of the amylinomimetic pramlintide acetate in March 2005. The prescribers' package insert (Amylin Pharmaceuticals Inc., 2005), which includes a synopsis of safety and efficacy of pramlintide, is included as Appendix 1.

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Pramlintide exhibited a terminal $t_{½}$ in humans of 25–49 min and, like amylin, was cleared mainly by the kidney.

The dose-limiting side effect was nausea and, at some doses, vomiting. These side effects usually subsided within the first days to weeks of administration.

The principal risk of pramlintide co-therapy was an increased probability of insulin-induced hypoglycemia, especially at the initiation of therapy. This risk could be mitigated by pre-emptive reduction in insulin dose.

Pramlintide dosed at 30–60 μg three to four times daily in patients with type 1 diabetes, and at doses of 120 μg twice daily in patients with type 2 diabetes, invoked a glycemic improvement, typically a decrease in HbA_{1c} of 0.4–0.5% relative to placebo, that was sustained for at least 1 year. This change relative to control subjects treated with insulin alone typically was associated with a reduction in body weight and insulin use, and was not associated with an increase in rate of severe hypoglycemia other than at the initiation of therapy.

Effects observed in animals, such as slowing of gastric emptying, inhibition of nutrient-stimulated glucagon secretion, and inhibition of food intake, generally have been replicated in humans. A notable exception appears to be induction of muscle glycogenolysis and increase in plasma lactate.

II. Pharmacokinetics

Human amylin was first administered to humans by Gilbey (Gilbey *et al.*, 1989) and others (Bretherton-Watt *et al.*, 1990; Ghatei *et al.*, 1990; Wilding *et al.*, 1994) working with Bloom at the Hammersmith Hospital in 1989. The material showed no activity in those studies, perhaps consistent, as it turned out, with a lack of effect on glucose disposal in clamp experiments, but also consistent with the propensity of human amylin to precipitate from solution and lose activity. Some of the same authors subsequently observed hypocalcemic activity with human amylin in patients with Paget's disease (Gilbey *et al.*, 1991), an observation that was soon repeated by others (Wimalawansa *et al.*, 1992), illustrating that human amylin could retain biological activity under some conditions. Human amylin (designated AC001) was produced at Amylin Pharmaceuticals Inc. and was used in several sponsored studies of the renin-angiotensin-aldosterone system in 1993–1994 (Cooper *et al.*, 1995; McNally *et al.*, 1994a,b; Nuttall *et al.*, 1995a,b; Young *et al.*, 1995) in which the use of the human hormone in humans was used to examine human physiology. But the adverse physicochemical properties of AC001 and the loss of several manufactured batches compelled the development of the analog, pramlintide (designated AC137), which was stable in solution and equally active with human amylin.

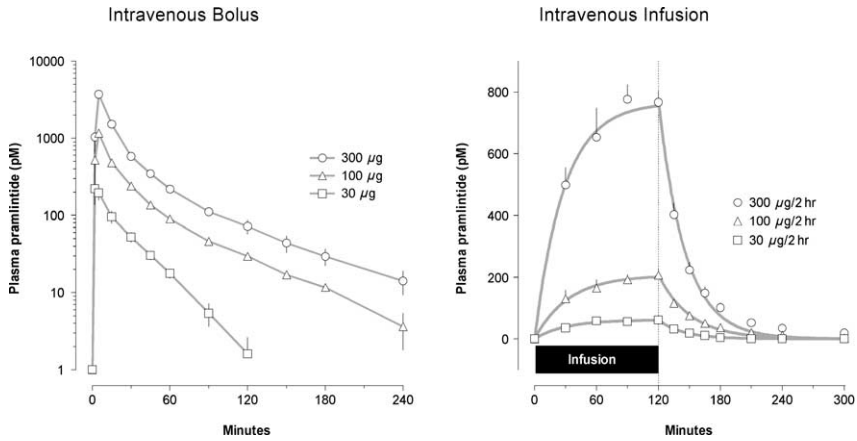

FIGURE 1 Plasma concentration profiles of pramlintide following intravenous infusion of doses of 30, 100, and 300 μg over 2 min (left panel) or 2 hr (right panel). Data from Colburn *et al.* (1996).

Pramlintide was first administered to healthy volunteers in 1993 (Moyses *et al.*, 1993, 1994) in a study examining safety, tolerability, and pharmacokinetics. Over a subcutaneous dose range of 300 to 10,000 μg, the $t_{½}$ for pramlintide ranged from 26 to 42 min (median of 35 min). The C_{max} was monotonically related to dose, derived here as C_{max} (pM) = $10^{(1.34 \log \text{dose } \mu g - 1.38)}$ (Moyses *et al.*, 1993).

A subsequent pharmacokinetic study of pramlintide in non-fasted subjects with type 1 diabetes (Colburn *et al.*, 1996) that used doses in the therapeutic range was enabled by the development of sensitive two-site linear immunometric assays (Koda *et al.*, 1993; Percy *et al.*, 1993; Petry *et al.*, 1995). In the pharmacokinetic study (Colburn *et al.*, 1996), the terminal $t_{½}$ for pramlintide was 24–40 min for intravenous bolus injections of 30–300 μg and was 25–49 min for the same doses infused over 2 hr. The volume of distribution was similar to extracellular water space (Fig. 1).

One pharmacokinetic study found that pramlintide could be syringe-mixed with 70/30 insulin without adversely affecting pharmacokinetic measures (Redalieu *et al.*, 1996). Other studies reported that pharmacokinetics was altered, and for this reason mixing was not recommended. Efficacy was nonetheless maintained when pramlintide was immediately mixed with isophane insulin (Redalieu *et al.*, 1997a,b) and regular NPH insulin (Schoenfeld *et al.*, 1998).

III. Tolerability

Initial studies in humans anticipated doses that were higher than those subsequently shown to be effective in humans. As a consequence, safety and

tolerability were tested across a broader dose range than might otherwise have been employed. In a dose-rising tolerability study in non-diabetic volunteers, the highest doses were 10 mg, ~80- to 300-fold higher than anti-diabetic doses. Dose-limiting side effects were nausea and vomiting at 5 mg and 10 mg doses, with no effects reported at doses of 0.3, 1, and 3 mg (Moyses *et al*., 1993). At doses closer to the therapeutic range, and depending upon timing of doses in relation to meals, several studies reported no adverse events with acute pramlintide administration in patients with type 1 diabetes (Weyer *et al*., 2003b) or type 2 diabetes (Burrell *et al*., 2003; Maggs *et al*., 2002, 2004; Weyer *et al*., 2003a,b).

Other studies reported minimal nausea (0/15 of non-diabetic subjects, 1/11 of type 2 diabetic subjects administered with 120 μg pramlintide) (Chapman *et al*., 2004).

However, in most long-term studies using large numbers of subjects, nausea (typically transient and mild to moderate; Ratner *et al*., 2004) did emerge as the principal side effect.

A. Type 1 Diabetes

In a 14-day study of patients with type 1 diabetes, doses of 30 μg resulted in no difference from placebo in adverse event reporting (Kolterman *et al*., 1996b). At doses of 60 and 90 μg at frequencies of two to four times per day, the most common side effects were mild nausea and anorexia, with both occurring during the initial week (Thompson *et al*., 1996, 1997f) or weeks of treatment and dissipating over time (Gottlieb *et al*., 2000a; Whitehouse *et al*., 2002). At doses of 60 μg up to 90μg three times per day, the mild nausea generally dissipating during the initial 8 weeks of therapy (Fineman *et al*., 1999c). In a 14-day study, at doses of 100 and 300 μg 1/23 and 8/21 subjects, respectively, withdrew due to nausea (Kolterman *et al*., 1996b).

Data from long-term studies in type 1 diabetes were pooled. Overall, the most common adverse event associated with pramlintide was transient nausea, which began to resolve within 2 weeks of initiating treatment (Fineman *et al*., 2001). Gradual titration of doses up to 60 μg allowed 76% of pramlintide-treated patients to progress with minimal nausea (Kolterman *et al*., 2003d).

B. Type 2 Diabetes

The incidence of nausea was generally less in patients with type 2 diabetes than in those with type 1 diabetes. At doses of 30, 75, and 150 μg three times per day (but using another formulation with slightly differing bioavailability), nausea was mild to moderate and dissipated early in treatment (Ratner *et al*., 2002). The same transient, mild-to-moderate nausea occurred with doses of 60 μg three times daily, and 90 or 120 μg twice daily

(Hollander *et al.*, 2003b). Transient nausea began to resolve within 2 weeks of initiating treatment and was independent of weight loss (Maggs *et al.*, 2001a). In a mixed race study, nausea was the most common adverse event associated with pramlintide treatment; it was mild and was confined mostly to the first 4 weeks of therapy (25% pramlintide versus 16% placebo). Patterns in the three ethnic groups were comparable (Kolterman *et al.*, 2003a; Maggs *et al.*, 2003). At total daily doses of 180–270 μg in type 2 diabetes, mild nausea generally dissipated during the initial 8 weeks of therapy (Gottlieb *et al.*, 1999). At doses of 90 μg twice daily, 120 μg twice daily, or placebo, nausea, the most common drug-related side effect, dissipated over time. Severe nausea was reported in 1.2, 4.1, and 2.4% of subjects, respectively (Fineman *et al.*, 2000a,b).

In a primary practice use (Want *et al.*, 2004), 56% of patients reported no nausea when on pramlintide. The remainder reported intermittent nausea occurring principally during initiation of therapy or dose titration, typical of other reports (Weyer *et al.*, 2001b). No patient vomited or discontinued pramlintide due to nausea (Want *et al.*, 2004).

IV. Safety

In a review of several studies (Fineman *et al.*, 2000b; Gottlieb *et al.*, 2000a; Ratner *et al.*, 2002; Whitehouse *et al.*, 2002), there was no evidence of cardiac, hepatic, or renal toxicity; no changes in serum lipid parameters; no clinically relevant changes in laboratory tests, vital signs, and electrocardiograms; and no abnormal findings upon physical examinations in patients treated with pramlintide (Buse *et al.*, 2002).

A. Hypoglycemia in Absence of Hypoglycemic Agents

On its own, pramlintide did not cause hypoglycemia in non-diabetic volunteers (Moyses *et al.*, 1993), even at doses 83- to 300-fold higher than those used in the treatment of diabetes. Nor was hypoglycemia observed in 160 non-diabetic obese patients (Weyer *et al.*, 2005a,b). Severe hypoglycemia was not observed in patients with type 2 diabetes who were treated with pramlintide and metformin alone (that is, not treated concomitantly with hypoglycemic agents, such as insulin or sulfonylureas) (Weyer *et al.*, 2005).

B. Hypoglycemia in Type 1 Diabetes

Without a pre-emptive reduction in insulin dose, the initiation of pramlintide therapy could be associated with a transient increase in the risk of hypoglycemia that subsequently decayed. In a 2 year study of 480 patients

with type 1 diabetes (1 year blinded, 1 year open label), the event rate of severe hypoglycemia was 2.12 ± 0.35 events per patient-year on pramlintide, not different from the 2.00 ± 0.34 rate for patients on placebo, although the event rate was higher at the initiation of therapy (Whitehouse *et al.*, 2002). The finding of no overall increase in the rate of hypoglycemia for type 1 diabetes patients on pramlintide was consistent with several other reports (Kolterman *et al.*, 2002, 2003c,d; Maggs *et al.*, 2001b; Thompson *et al.*, 1996; Want, 2002).

In one report of patients with type 1 diabetes approaching the glycemic target of 7% HbA1c, a group in which increased risk of hypoglycemia can frustrate further glucose-lowering therapy, the risk of hypoglycemia was, if anything, less with pramlintide than with placebo (1.4 versus 1.9 events per patient-year) (Kolterman *et al.*, 2002). In a meta-analysis of 1154 patients with type 1 diabetes, in those treated with insulin alone, the hypoglycemic risk was ~4-fold higher over 26 weeks in those who achieved a <8% HbA1c target compared to those who did not achieve the target (2.00 ± 0.2 versus 0.52 ± 0.06 events per patient-year) (Maggs *et al.*, 2001b). Meanwhile, in those who had achieved the glycemic target with pramlintide co-therapy, including some patients using a non-indicated 90 μg dose, the hypoglycemic risk was reduced (1.16 ± 0.1 events per patient-year) (Maggs *et al.*, 2001b).

C. Hypoglycemia in Type 2 Diabetes

Rates of severe hypoglycemia in insulin-treated patients with type 2 diabetes were typically much lower than in patients with type 1 diabetes, and were not increased by concomitant therapy with pramlintide (Fineman *et al.*, 2000a,b; Gottlieb *et al.*, 1999; Maggs *et al.*, 2003; Ratner *et al.*, 2002; Thompson *et al.*, 1997d, 1998). In a cohort with type 2 diabetes that was approaching the American Diabetes Association glycemic target (HbA1c < 7%), co-therapy with pramlintide was associated with a 0.1 event per patient-year rate of hypoglycemia, while the rate in controls was 0.2 (Weyer *et al.*, 2003a). The event rate in a 1-year study was 0.3 ± 0.05 events per patient-year in patients injecting insulin + placebo, and 0.1 ± 0.03 events per patient-year in those injecting insulin + pramlintide 90 μg twice daily (Hollander *et al.*, 2003b). A higher pramlintide dose, 120 μg twice daily resulted in no difference from placebo overall (0.3 ± 0.05 events per patient-year) but did invoke an increased rate within the 4 weeks after initiation of therapy (Hollander *et al.*, 2003b).

D. Summary of Hypoglycemic Risk

Pivotal clinical studies of pramlintide effect generally required insulin dose to be maintained constant at the initiation of pramlintide therapy. Some analyses showed that if pre-emptive reductions in insulin dose to

accommodate the insulin-pramlintide synergy were not made, there was the potential for an increased risk of hypoglycemia in the first 4 weeks after addition of pramlintide to insulin therapy (Anonymous, 2003).

Variability in definitions and perceptions of hypoglycemia has confounded calculation of true event rates. The most rigorous and standardized definition of severe hypoglycemia, as used in the landmark Diabetes Control and Complications Trial (DCCT Research Group, 1986) is "assisted," requiring third party intervention such as glucagon or intravenous glucose. Severe hypoglycemia thus defined was higher in pramlintide-treated patients with type 1 diabetes in the 3 months after initiating therapy (0.50 versus 0.19 events per patient-year) but contributed no significant risk thereafter (0.27 versus 0.24) in blinded studies. In open-label studies, in which mealtime insulin was reduced, the risk of medically assisted hypoglycemia dropped to 0.1 events per patient-year on initiation of therapy, and to 0.04 thereafter (Amylin Pharmaceuticals Inc., 2005) (see package insert at http://www.symlin.com).

In patients with type 2 diabetes, rates of medically assisted hypoglycemia were universally low at initiation of placebo (0.06), or pramlintide in blinded (0.09) or open-label (0.05) studies, and were as low, or lower, after 3 months of therapy (0.07, 0.02, 0.03 events per patient-year, respectively) (Amylin Pharmaceuticals Inc., 2005).

Currently recommended therapy is to reduce current mealtime insulin doses by 50% and begin pramlintide with doses of 15 μg, gradually increasing to 60 μg in patients with type 1 diabetes, and to titrate up from 60 μg to 120 μg in patients with type 2 diabetes (see http://www.symlin.com).

E. Effects on Counterregulatory Responses

In general, there was no adverse effect of pramlintide on hormonal, metabolic, or symptomatic responses to insulin-induced hypoglycemia in patients with type 1 diabetes (Amiel *et al.*, 2005). There was no effect of pramlintide on the glycogenic effect of glucagon administered to patients with type 1 diabetes (Orskov *et al.*, 1997a, 1999). In one study there was no effect of pramlintide (versus placebo) on catecholamines, cortisol, or growth hormone concentrations during hypoglycemia (Orskov *et al.*, 1999); in another report, cortisol and growth hormone were even increased during hypoglycemia (Nyholm *et al.*, 1996; Schmitz *et al.*, 1997). Pramlintide therapy at doses up to 300 μg three times per day for 14 days did not affect glucose, free insulin, glucagon, epinephrine, and norepinephrine concentrations following a standard (40 mU/kg/hr) insulin challenge (Kolterman *et al.*, 1996b). Stepped hypoglycemia in non-diabetic volunteers evoked catecholamine responses, other autonomic responses, and perceptual responses (recorded by visual analog scale) that did not differ between pramlintide-

and placebo-treated cohorts (Heinemann *et al.*, 2003; Heise *et al.*, 2003, 2004).

In summary, the principal risk associated with amylin replacement therapy, an increase in the risk of severe hypoglycemia, especially in patients with type 1 diabetes at the initiation of therapy, appeared to occur only in association with other hypoglycemic agents. Pramlintide itself was not hypoglycemic and did not impair counterregulatory defenses against hypoglycemia.

V. Effects on Glycemic Indices

Effects of amylinomimetics on glycemic indices have been observed over a range of time domains. These range from acute effects immediately after dosing, associated or unassociated with meals, to glucose profiles measured periodically for up to 24 hours, and to longer-term surrogates such as fructosamine (approximating a 2 week average) and hemoglobin A1c (for which the time constant following a step glucose change is ~35 days).

A. Postprandial Glucose Profiles

The effects of amylinomimetics to reduce appearance of glucose and other nutrients after meals led to identification of effects on gastric emptying and other mechanisms, and eventually to elucidation of amylin's physiology as described in this volume. Effects of pramlintide on postprandial hyperglycemia were first appreciated in a clinical study in patients with type 1 diabetes infused with pramlintide at 150 μg/hr during breakfast (Kolterman *et al.*, 1994a,b,c, 1995b,c). Effects were equally apparent following a Sustacal challenge with pramlintide infused at 50 μg/hr (plasma concentration of 225 pM) (Kolterman *et al.*, 1995c). Pramlintide exhibited such an effect when nutrient was delivered orally but not when infused intravenously (Kolterman *et al.*, 1995a,c). The implication was that the "glucose smoothing" could not be due to metabolic events that follow glucose appearance in plasma (Figs. 2 and 3).

In patients with type 1 diabetes, the reduction in postprandial hyperglycemia (after Sustacal) was dose dependent over the subcutaneous dose range of 30, 100, 300 μg pramlintide prior to each meal. The lowest dose was effective with peak plasma concentrations of 21–29 pM, close to plasma concentrations of native amylin in non-diabetic subjects. Effects were undiminished after 7 and 14 days of therapy (Kolterman *et al.*, 1996b). Similar effects to reduce postprandial glucose excursions also were observed when added to Lispro therapy in patients with type 1 diabetes (Weyer *et al.*, 2003b). Lispro is a fast-acting insulin analog designed to reduce postprandial hyperglycemia (Anonymous, 1996).

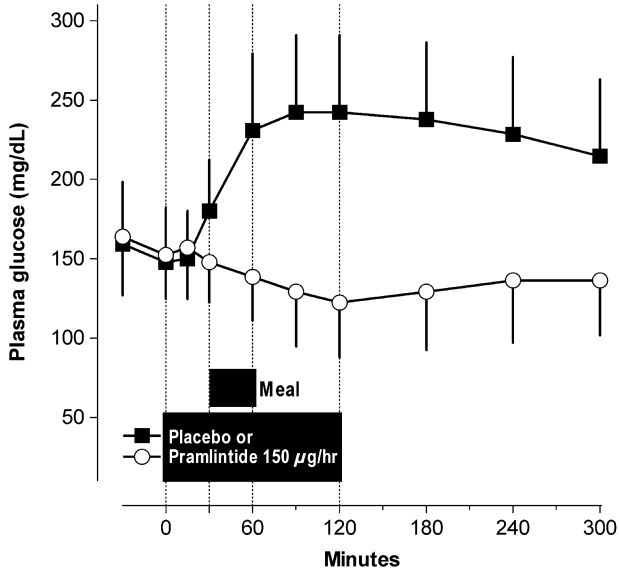

FIGURE 2 When pramlintide was infused intravenously at 150 μg/hr during the course of a meal in patients with type 1 diabetes, the postprandial hyperglycemia observed during the placebo arm of this crossover study did not occur. Data from Kolterman *et al.* (1995c).

Pramlintide reduced postprandial hyperglycemia similarly in patients with type 2 diabetes (Kolterman *et al.*, 1995d; Thompson and Kolterman, 1997; Thompson *et al.*, 1995a,b, 1997e), including when added to Lispro therapy (Maggs *et al.*, 2002, 2004; Weyer *et al.*, 2003b) (Figs. 4 and 5).

B. Twenty-four-hour Glucose Profiles

In patients with type 1 diabetes glucose concentrations measured over 24-hr were significantly reduced (Thompson *et al.*, 1997b) with doses of 30 μg four times per day (Kolterman and Schoenfeld, 1997b; Nyholm *et al.*, 1999; Thompson *et al.*, 1997g). Reductions of mean 24-hour glucose of 1 mM or greater occurred more frequently in pramlintide-treated patients with type 1 diabetes, regardless of HbA1c at initiation of therapy (Kolterman *et al.*, 1996a) (Fig. 6).

C. Fructosamine

Glucose molecules are joined to protein molecules to form stable keto-amines, or fructosamines, through non-enzymatic glycation. The reaction rate is proportionate to glucose concentration. Fructosamine reflects the

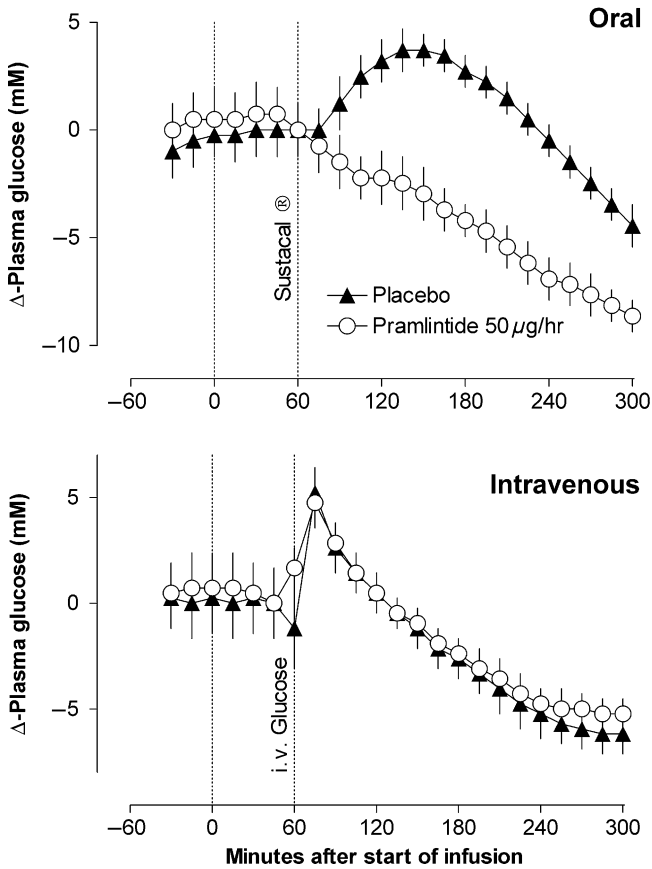

FIGURE 3 Absence of an effect of pramlintide on the glucose profile when glucose was delivered intravenously indicated that its effect was on processes affecting assimilation from the meal, rather than on disposal from the plasma. Data from Kolterman *et al.* (1995c).

average blood sugar concentration over the past 2 to 3 weeks (Armbruster, 1987). Reductions in fructosamine were observed after 4 weeks of pramlintide therapy in patients with type 1 diabetes (Kolterman and Schoenfeld, 1997b; Nyholm *et al.*, 1999; Thompson *et al.*, 1996, 1997, 1997a,b) and in insulin-using patients with type 2 diabetes (Thompson *et al.*, 1997d, 1998) (Fig. 7).

D. Hemoglobin A1c, Type 1 Diabetes

Glucose reacts non-enzymatically with the amino-terminal amino of the β chain of human hemoglobin via a ketoamine linkage to form hemoglobin A1c. Hemoglobins are formed continuously throughout the 120-day life

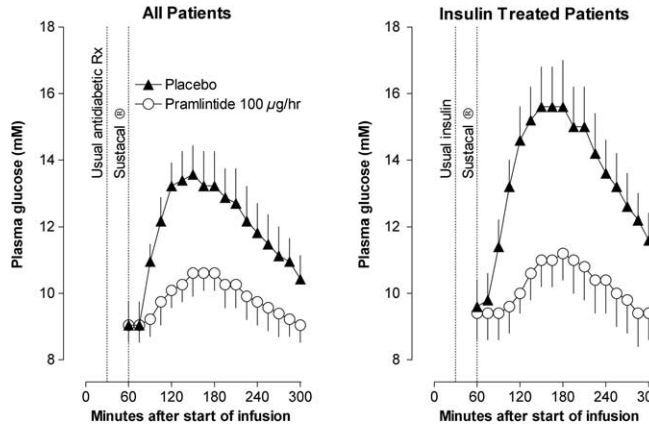

FIGURE 4 Effect of pramlintide to reduce postprandial glucose excursions in subjects with type 2 diabetes. Effects were as great in patients treated with supplemental insulin as they were in a mixed-treatment cohort, indicating that the effects were not dependent upon insulin action. Data from Thompson *et al.* (1997e).

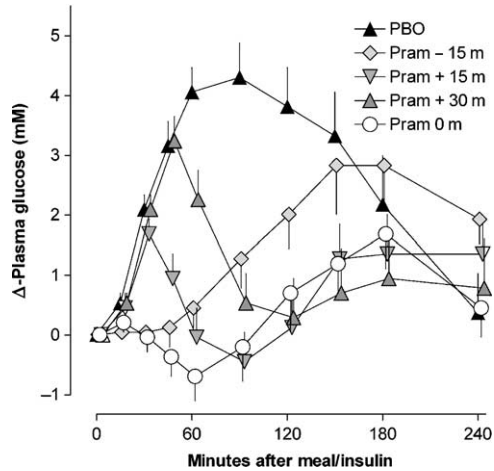

FIGURE 5 Dose timing study exploring the sequence of dosing of pramlintide and short-acting insulin (Lispro). The least glycemic excursion occurred when the insulin and amylin mimetics were administered concurrently with the ingestion of the test meal. Data from Maggs *et al.* (2004).

span of red cells. Glycosylated hemoglobins provide an integrated measurement of blood glucose that is useful in assessing the degree of diabetic control (Bunn *et al.*, 1978) and can predict the risk of progression of complications in type 1 [Diabetes Control and Complications Trial (DCCT) Research Group, 1995, 1995a,b,c,d] and type 2 diabetes [U.K.

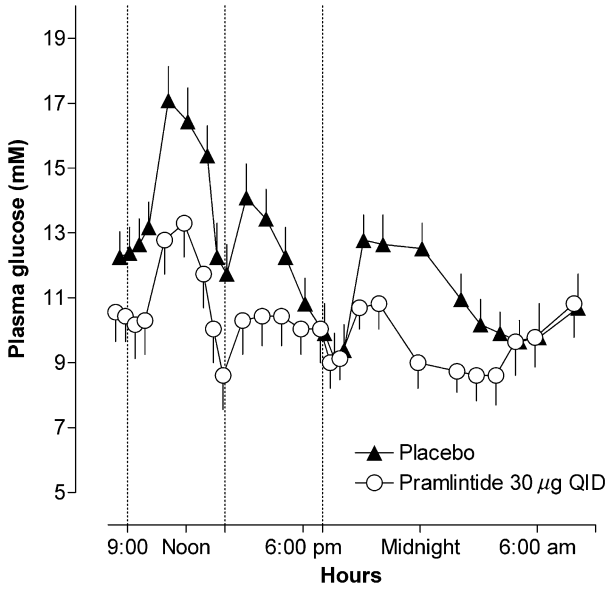

FIGURE 6 Reduction in 24-hr glucose profiles with 30 μg pramlintide administered four times daily to subjects with type 1 diabetes. Data from Thompson *et al.* (1997g).

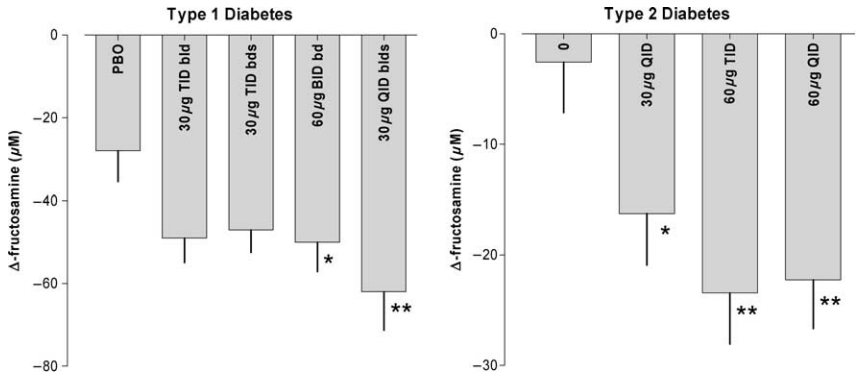

FIGURE 7 Effect of pramlintide on plasma fructosamine in patients with type 1 (Thompson *et al.*, 1997) and type 2 diabetes (Thompson *et al.*, 1998).

Prospective Diabetes Study (UKPDS) Group, 1998, 1998]. For example, in patients with type 2 diabetes treated with metformin, HbA1c was reduced from 8 to 7.4% and all-cause mortality was reduced by 36% [UK Prospective Diabetes Study (UKPDS) Group, 1998a].

In several reports (Rosenstock *et al.*, 1998a,b), pramlintide therapy lowered HbA1c, whether measured after 6 months of therapy (Fineman *et al.*, 1999a) or after 2 years (Kolterman *et al.*, 1999). Reductions in HbA1c of 0.67% relative to placebo-treated controls were observed after

13 weeks of therapy, and were sustained to 52 weeks (Whitehouse *et al.*, 2002). Other 1 year studies (Gottlieb *et al.*, 2000a,b) reported similar changes in HbA1c. In all long-term studies in patients with type 1 diabetes, 44% of all patients showed an improvement in both HbA1c and weight (versus 22% in placebo-treated groups), and 90% showed an improvement in either HbA1c or weight (Weyer *et al.*, 2001a).

In patients with type 1 diabetes who were approaching the glycemic goal of <7% HbA1c, 40% of those on pramlintide achieved it (versus 22% of placebo-treated patients), and they did so without an increase in overall severe hypoglycemia risk (1.4 versus 1.9 events per patient-year of exposure) (Kolterman *et al.*, 2002, 2003c,d). In another study, three times the proportion of pramlintide- compared to placebo-treated patients achieved an HbA1c of < 7% (Ratner *et al.*, 2004).

Lesser changes in HbA1c, such as 0.29 and 0.34% for 1 year dosing with 60 μg three and four times daily, respectively, have been reported (Ratner *et al.*, 2004). It appeared that in some circumstances, the glucose-lowering effect of pramlintide per se was masked by a concomitant reduction in insulin use. Since freedom to vary insulin dose has been standard practice in the treatment of diabetes, isolation of the pramlintide-specific effect was a challenge of clinical trial design. Analysis of HbA1c changes in a prospectively defined "insulin-stable" cohort, which maintained insulin use within a 10% range of initial use, revealed reductions in HbA1c that could be twice as great as in the total intent-to-treat population (Ratner *et al.*, 2004) (Fig. 8).

E. Hemoglobin A1c, Type 2 Diabetes

In patients with type 2 diabetes, improvements in HbA1c were similar to those observed in patients with type 1 diabetes (Thompson *et al.*, 1997d, 1998). Improvements were apparent after 6 months (Fineman *et al.*, 1999b) and 1 year (Ratner *et al.*, 1998). HbA1c response after 4 weeks was predictive of longer term response (Gottlieb *et al.*, 1999). Reduction in HbA1c of 0.9–1.0% from baseline after 13 weeks was associated with a 0.6% reduction relative to placebo-treated controls after 1 year (Fineman *et al.*, 2000a, b; Ratner *et al.*, 2002), and with weight loss and no increase in severe hypoglycemia (Ratner *et al.*, 2002).

After 52 weeks of pramlintide therapy, 48% of patients had an improvement of weight and glycemic control (versus 16% of placebo-treated patients) (Ratner *et al.*, 2002). With the 120 μg twice daily dose, 42% of patients with type 2 diabetes achieved the treatment target of a 0.8% reduction in HbA1c (versus 27% of placebo-treated patients, who on average achieved that goal with increased insulin dosing and weight gain; Maggs *et al.*, 2001a). Similarly, at the same dose, 46% of pramlintide-treated patients (versus 28% of placebo-treated) achieved an HbA1c of <8.0%

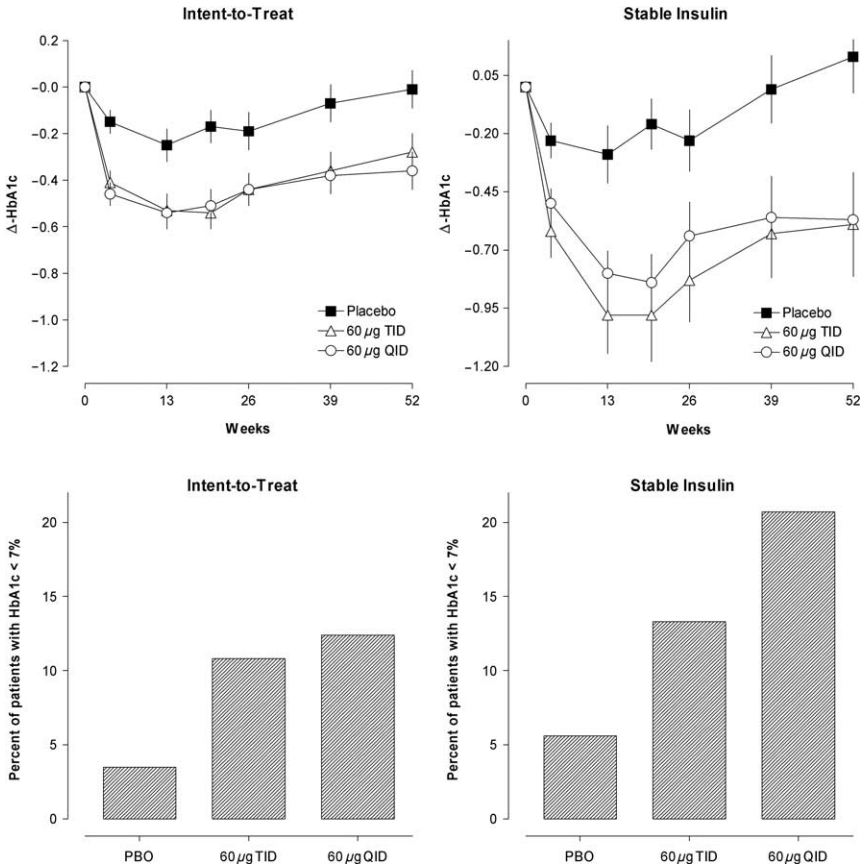

FIGURE 8 Changes in hemoglobin A1c (upper panels) and proportion of patients achieving an HbA1c target of <7% (lower panels) in patients with type 1 diabetes during 1 year of therapy with pramlintide 60 μg three or four times per day in addition to optimized insulin therapy (placebo groups). The intent-to-treat analysis (left panels) included all patients started on pramlintide. To account for the tendency of pramlintide-treated subjects to reduce insulin dose, the independent glucose-lowering effect of pramlintide was assessed in a cohort of patients in whom the latter was varied by <10% (right panel). Data from Ratner *et al.* (2004).

after 1 year of therapy (Hollander *et al.*, 2003b). This glycemic benefit was associated with 1.4 kg loss (versus a 0.7 kg gain in placebo-treated patients) (Hollander *et al.*, 2003b) (Fig. 9).

Plasma insulin and C-peptide concentrations were lowered by pramlintide in patients with type 2 diabetes (Kolterman and Schoenfeld, 1997a; Thompson and Kolterman, 1997; Thompson *et al.*, 1995b, 1997e), consistent with an effect of pramlintide to reduce demand on β-cells. This is distinct, for example, from the effect of therapies that increase β-cell secretion.

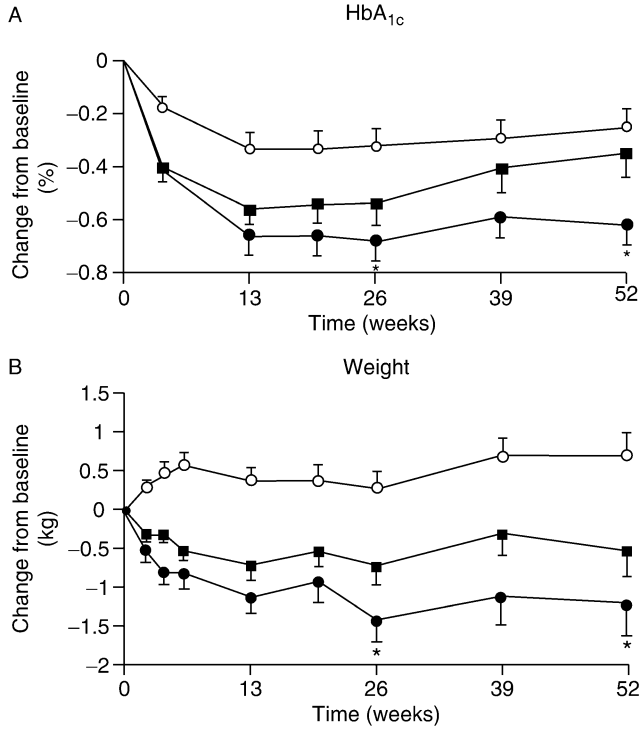

FIGURE 9 Reduction in hemoglobin A1c with 1 year of pramlintide therapy in insulin-treated subjects with type 2 diabetes. Data from Hollander *et al.* (2003b).

VI. Effects on Body Weight

A. Body Weight, Type 1 Diabetes

Weight loss in association with anti-diabetic effect has been a consistent finding in long-term placebo-controlled studies of pramlintide (Jeffcoate *et al.*, 1998; Kolterman *et al.*, 1998a,b; Whitehouse *et al.*, 1998, 2002) and has been reviewed in several papers (Buse *et al.*, 2002; Edelman and Weyer, 2002; Heise *et al.*, 2002; Kruger and Gloster, 2004; Weyer *et al.*, 2001b).

Weight loss, relative to changes in placebo-treated subjects, has been reported as the following:

- 1.8 kg at 26w in a pooled analysis of three studies (Kolterman *et al.*, 2002, 2003c)
- 1.4–1.7 kg at 52w (placebo increased) (Gottlieb *et al.*, 2000a)
- 1.9 kg, 1.0 kg, and 1.9 kg for the 60 μg thrice daily, 90 μg twice daily, and 90 μg thrice daily groups, respectively (Fineman *et al.*, 1999c)

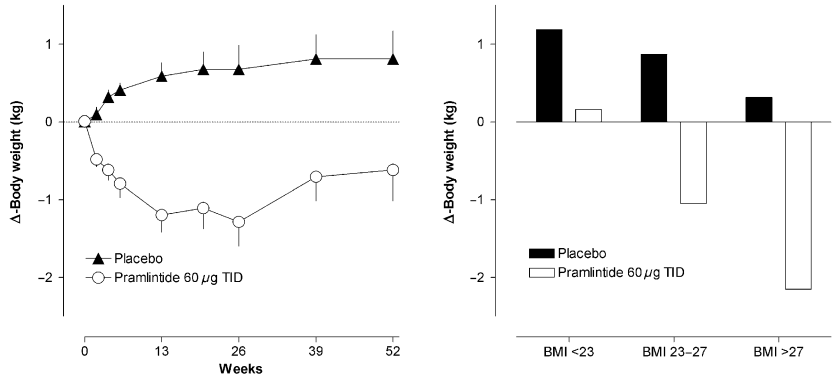

FIGURE 10 Effect of pramlintide on body weight in patients with type 1 diabetes. Weight loss was greatest in those with higher BMI. Data from Ratner *et al.* (2004).

- 1.2 kg in the 60 μg three or four times per day at 52 weeks (Ratner *et al.*, 2004)

Stratification by body mass index (BMI) revealed that the weight-lowering effect of pramlintide was most pronounced in obese patients (Weyer *et al.*, 2001a). After 26 weeks, loss was 2.2 kg in those with a BMI > 27, 1.5 kg in those with a BMI of 23–27, and only 0.05 kg in those with BMI < 23. Insulin therapy alone resulted in weight gain in each of these cohorts (0.2, 0.8, 0.9 kg, respectively) (Fineman *et al.*, 2001).

The presence of nausea in long-term studies of patients with type 1 diabetes did not predict subsequent weight loss (Fineman *et al.*, 2001) (Fig. 10).

B. Body Weight, Type 2 Diabetes

There are several reports of sustained weight loss in patients with type 2 diabetes following pramlintide co-therapy (Fineman *et al.*, 2000b; Gottlieb *et al.*, 1999; Hollander *et al.*, 2004; Jeffcoate *et al.*, 1998; Kolterman *et al.*, 1998a), and several of these studies have been reviewed (Buse *et al.*, 2002; Edelman and Weyer, 2002; Heise *et al.*, 2002; Kruger and Gloster, 2004; Weyer *et al.*, 2001b).

The first evidence of weight loss was detected in a 4 week study of patients with type 2 diabetes, in which body weight decreased in groups treated with 60 μg pramlintide three and four times per day, but the trend did not achieve statistical significance (Thompson *et al.*, 1998).

Mean loss in body weight after 26 weeks of pramlintide co-therapy was 0.9, 1.4, and 1.5 kg for the 90 twice daily, 90 thrice daily, and 120 twice daily groups, respectively, compared to the insulin alone group (Gottlieb *et al.*, 1999).

In a pooled post hoc analysis of two trials in patients with type 2 diabetes who were approaching the ADA treatment guideline (HbA1c between 7 and 8.5%), weight reduction after 26 weeks of pramlintide 120 μg twice daily was 2.0 kg more than in placebo + insulin controls (Hollander *et al*., 2003a; Weyer *et al*., 2003a).

In a meta-analysis comparing either placebo or pramlintide 120 μg twice daily in combination with insulin, changes in HbA1c, body weight, and insulin use were stratified by achievement of specific glucose control targets at 26 weeks. The higher proportion of patients treated with pramlintide who achieved glucose control targets did so with weight loss and no change in insulin use. The smaller fraction of patients achieving the same glycemic target with insulin and placebo did so with increased insulin use and without losing weight (Maggs *et al*., 2001a). In a pooled analysis of pivotal trials in patients with type 2 diabetes, 51% of subjects showed an improvement in both weight and glycemia after 26 weeks (versus 26% in PBO groups), and 90% had an improvement in either HbA1c or weight (Maggs *et al*., 2001).

In a pooled post hoc analysis of two long-term trials that included all patients who were overweight/obese at baseline (BMI > 25), reduction in body weight after 26 weeks of pramlintide 120 μg twice daily was 1.8 kg more than in placebo + insulin-treated subjects. Stratification by baseline BMI revealed that the greatest weight loss occurred in the most obese patients, averaging 3.2 kg in those with a BMI > 40 (Hollander *et al*., 2004; Weyer *et al*., 2002). Three times the number of patients using pramlintide (9% versus 3% using placebo) experienced a 5% or more weight loss, which was proportionate to reduction in insulin use (Hollander *et al*., 2004).

In each pivotal study, reduction in body weight was durable, with weight either being maintained or still decreasing 1 year after initiation of pramlintide co-therapy. After 1 year of therapy with 30, 75, or 150 μg doses of pramlintide twice daily in patients with insulin-treated type 2 diabetes, body weight was reduced in all dose groups compared to placebo, and three times the proportion of subjects in the 150 μg pramlintide group compared to the placebo group achieved a concomitant reduction in both HbA1c and body weight (48 versus 16%) (Ratner *et al*., 2002).

In another study, mean weight at 52 weeks decreased 1.9 and 1.2 kg for patients using 120 μg and 90 μg twice daily pramlintide doses, respectively, compared to placebo (who gained 0.7 kg) (Fineman *et al*., 2000a,b). With a 120 μg twice daily pramlintide dose in similar patients in an additional trial, mean weight loss was 1.4 kg (versus a 0.7 kg gain with placebo) after 52 weeks (Hollander *et al*., 2003b).

Glycemic improvement and weight loss after 52 weeks were most pronounced in African Americans (–0.7%, 4.1 kg) but were significant across other ethnic groups (Caucasian and Hispanic) in patients with type 2 diabetes (Kolterman *et al*., 2003a,b; Maggs *et al*., 2003) (Fig. 11).

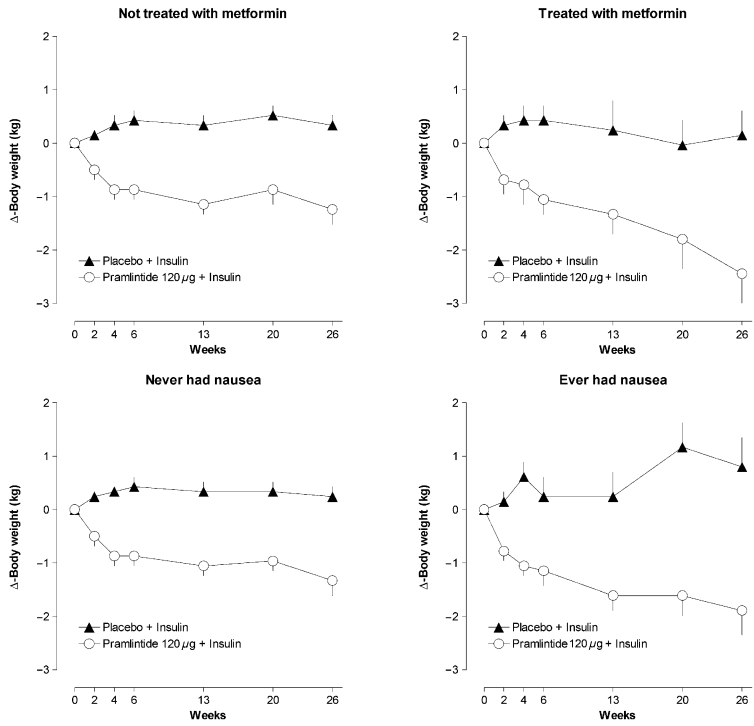

FIGURE 11 Illustration that weight loss effect of pramlintide in patients with type 2 diabetes was not dependent upon concomitant biguanide therapy, nor was it dependent upon the presence of nausea. Data from Hollander *et al.* (2004).

VII. Effects on Specific Actions

A. Food Intake

Effects of pramlintide on *ad libitum* food intake were determined in a blinded crossover study in obese non-diabetic men and in men with insulin-treated type 2 diabetes. Over all 26 subjects, energy intake at a buffet meal was reduced by 19 ± 5% following a single injection of pramlintide 120 μg compared to PBO (818 ± 73 versus 1002 ± 62 kcal) (Chapman *et al.*, 2004).

B. Gastric Emptying

Effects of pramlintide on rate of gastric emptying have been determined in non-diabetic subjects, and in those with type 1 diabetes and type 2 diabetes. Subcutaneous injections in patients with type 1 diabetes slowed

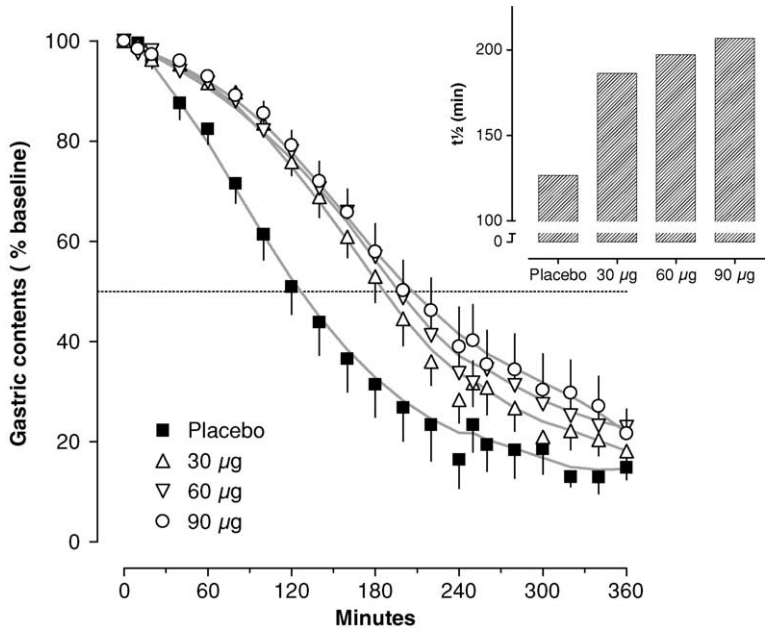

FIGURE 12 Effects of s.c. administration of pramlintide on scintigraphically measured emptying of stomachs from subjects with type 1 diabetes mellitus. Data from Kong *et al.* (1997c) and Parker *et al.* (1998).

emptying measured by scintigraphy (Macdonald *et al.*, 1995). Pramlintide infused intravenously in patients with type 1 diabetes at 25 μg/hr delayed solid and liquid gastric emptying (Kong *et al.*, 1997a,b) (Fig. 12).

The slowing of gastric emptying following single subcutaneous injections of 30, 60, or 90 μg in subjects with type 1 diabetes was dose dependent, with emptying times of 187, 200, and 215 min (versus 129 min in placebo-injected controls). The effects endured throughout one meal, but were no longer detectable 4 hr later (Kong *et al.*, 1997c; Parker *et al.*, 1998).

Pramlintide doses of 30 or 60 μg each delayed gastric emptying as measured by $^{13}CO_2$ expiration after ingestion of a ^{13}C-enriched test meal in patients with type 1 or type 2 diabetes (Vella *et al.*, 2002). Effects were similar at each dose for each form of diabetes, were similarly associated with suppression of pancreatic polypeptide, and were similarly unassociated with clinically detectable complications (Vella *et al.*, 2002). Changes in pancreatic polypeptide mark vagal activation, and indicated that effects of pramlintide likely occurred via vagal inhibition. In non-diabetic volunteers, doses of 30 and 60 μg delayed gastric emptying and suppressed pancreatic polypeptide without affecting small bowel or colonic transit (Samsom *et al.*, 2000a,b).

C. Glucagon Suppression

In several reports of studies in patients with type 1 diabetes, postprandial secretion of glucagon was elevated and was inhibited by administration of pramlintide (Fineman *et al.*, 1997a,b,c,d, 1998a,b; Levetan *et al.*, 2003; Nyholm *et al.*, 1997a,b,c, 1999; Thompson *et al.*, 1997a).

Pramlintide also inhibited postprandial glucagon secretion in patients with type 2 diabetes (Fineman *et al.*, 1998, 2002). It should be noted that reduced glucagon secretion could result from slowing amino acid appearance by slowing gastric emptying or protein digestion, for example. In none of the human studies, however, has there been an attempt to distinguish pramlintide's glucagon suppressive effect from that on gastric emptying or digestive secretions.

In a crossover study in patients with type 1 diabetes, pramlintide inhibited glucagon secretion during normoglycemia but not during insulin-induced hypoglycemia (Nyholm *et al.*, 1996). Concomitant pramlintide administration did not affect the glycogenic response to a glucagon challenge in patients with type 1 diabetes (Orskov *et al.*, 1997b).

Type 1 diabetes mellitus is characterized by relative or absolute hyperglucagonemia (Müller *et al.*, 1970; Unger *et al.*, 1970) and overresponsiveness of glucagon secretion in response to amino acid (Raskin *et al.*, 1976; Unger *et al.*, 1970) or protein stimuli (Kawamori *et al.*, 1985; Müller *et al.*, 1970). Exaggerated glucagon secretion complicates the course of the disease (Dobbs *et al.*, 1975), and one therapeutic goal has been the suppression of glucagon secretion. In several respects, the selective actions of amylin on glucagon secretion identified in animals are also seen with pramlintide in humans. Amylinergic restraint of nutrient-stimulated glucagon secretion could prove useful in restoring metabolic control in diabetes characterized by glucagon excess (Fig. 13).

D. Glucose Variability

Pramlintide administration to patients with type 1 diabetes reduced the variability of plasma glucose concentration as measured by a continuous glucose-monitoring system. While on placebo, patients maintained blood glucose readings within the target (euglycemic) range 28% of the time. While on pramlintide, this increased 1.3-fold to 37% (Levetan *et al.*, 2002; Want *et al.*, 2002), while meal time insulin use was reduced by 17% (Levetan *et al.*, 2003) (Fig. 14).

In another study using a continuous glucose monitoring system in patients with type 1 diabetes, the primary analysis was glucose rate of change, a parameter that can predict acute changes in mood and cognitive symptoms. The glucose rate of change was significantly reduced following 4 weeks of pramlintide co-therapy compared to placebo treatment. The

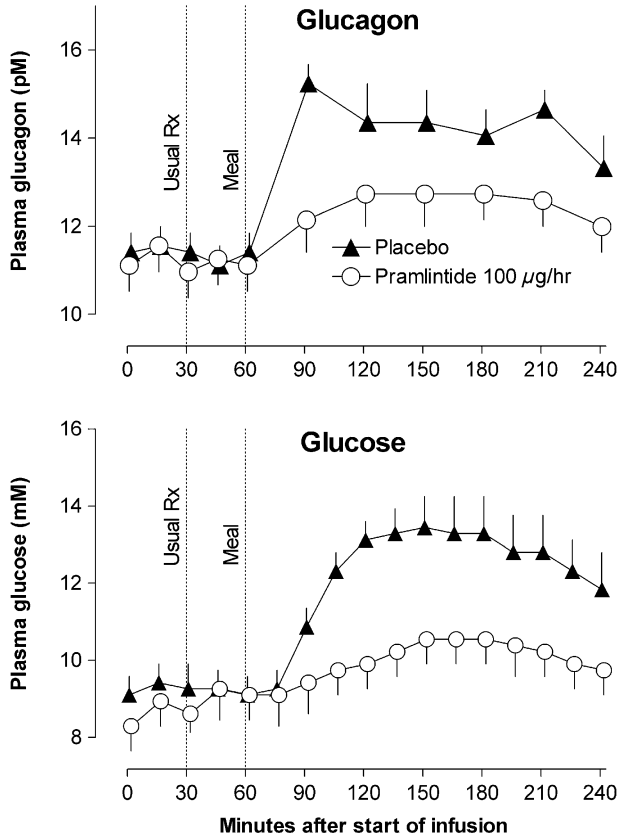

FIGURE 13 Effects of pramlintide infused at 100 μg/hr on glucose and glucagon profiles following a test meal in patients with type 2 diabetes treated with either insulin or sulfonylurea. Data from Fineman *et al.* (2002).

groups did not differ at baseline or after a 2-week washout period (Kovatchev *et al.*, 2004; McCall *et al.*, 2004b).

E. Bone Markers

In a 1 year study of patients with type 1 diabetes, there was no consistent overall effect of pramlintide on bone mineral density or markers of bone turnover (Bone *et al.*, 1999). Bone alkaline phosphatase was reduced in the postmenopausal subset of patients.

In a separate study, there was no consistent change in bone density, serum calcium, parathyroid hormone (PTH), osteocalcin, or pyridinium cross-links. Only osteocalcin decreased (from 7.2 to 5.8 ng/ml), but this change was not statistically significant (Borm *et al.*, 1999).

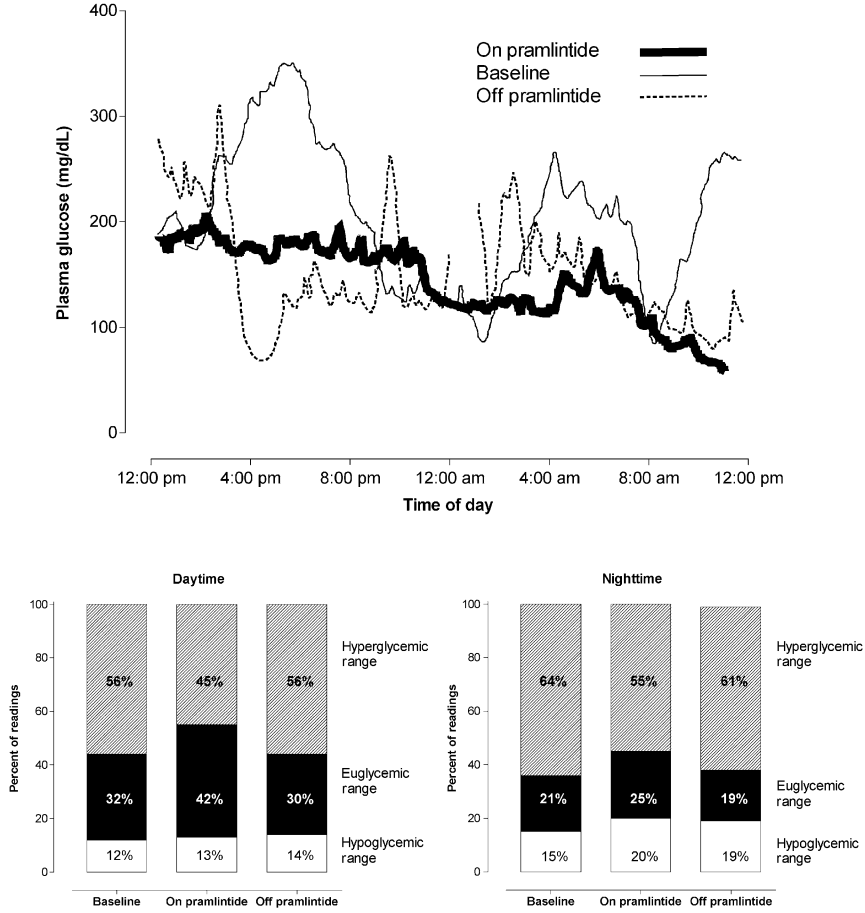

FIGURE 14 Example of the effect of pramlintide on glucose excursions, as measured by a wearable continuous glucose-monitoring system, in a patient with type 1 diabetes. Data from Levetan *et al.* (2003).

F. Patient Satisfaction

In a patient satisfaction survey, subjects perceived that the benefits of pramlintide outweighed the need for additional injections (Marrero *et al.*, 2004a,b).

G. Renin/Angiotensin/Aldosterone System

In a dose–response study, human amylin dose-dependently elevated plasma renin activity (Cooper *et al.*, 1995; McNally *et al.*, 1994b; Nuttall *et al.*, 1995b; Young *et al.*, 1995) and aldosterone concentration (Nuttall

et al., 1995a). These changes were not associated with any effect on blood pressure (Young *et al*., 1999).

H. Insulin Sensitivity

Pramlintide exerted no acute effect on insulin sensitivity in humans, as measured by glucose infusion rate required to maintain euglycemia in the face of hyperinsulinemia (glucose clamp) (Nyholm *et al*., 1995a,b, 1996; Schmitz *et al*., 1997).

I. Lactate Flux

In contrast to effects in rats, lactate flux (Cori cycle activity) was a comparatively minor feature of the response to pramlintide in humans. Nonetheless, pramlintide augmented forearm lactate output and some counterregulatory hormones during insulin-induced hypoglycemia (Nyholm *et al*., 1995a,b, 1996; Schmitz *et al*., 1997).

References

Anonymous (1996). Humalog: Insulin Lispro Injection (rDNA origin) [package insert]. Eli Lilly and Company, Indianapolis, IN.

Anonymous (2003). Pramlintide: (AC 137, AC 0137, Symlin trade mark, Tripro-Amylin). *BioDrugs* **17**, 73–79.

Amiel, S. A., Heller, S. R., Mac Donald, I. A., Schwartz, S. L., Klaff, L. J., Ruggles, J. A., Weyer, C., Kolterman, O. G., and Maggs, D. G. (2005). The effect of pramlintide on hormonal, metabolic, or symptomatic responses to insulin-induced hypoglycaemia in patients with type 1 diabetes. *Diabet. Obes. Metab.* **7, 504–516.**

Amylin Pharmaceuticals, Inc. (2005). Symlin™ (pramlintide acetate) injection [package insert]. Amylin Pharmaceuticals Inc, San Diego, CA.

Armbruster, D. A. (1987). Fructosamine: Structure, analysis, and clinical usefulness. *Clin. Chem.* **33**, 2153–2163.

Bone, H. G., Hurley, M. A., Goldstein, H., Fineman, M. S., and Kolterman, O. G. (1999). Effects of administration of pramlintide for 12 months on bone metabolism markers in people with type 1 diabetes. *Program and Abstracts, Endocrine Society 81st Annual Meeting, June 12–15, San Diego, Calif*, 447.

Borm, A. K., Klevesath, M. S., Borcea, V., Kasperk, C., Seibel, M. J., Wahl, P., Ziegler, R., and Naworth, P. P. (1999). The effect of pramlintide (amylin analogue) treatment on bone metabolism and bone density in patients with type 1 diabetes mellitus. *Horm. Metab. Res.* **31**, 472–475.

Bretherton-Watt, D., Gilbey, S. G., Ghatei, M. A., Beacham, J., and Bloom, S. R. (1990). Failure to establish islet amyloid polypeptide (amylin) as a circulating beta cell inhibiting hormone in man. *Diabetologia* **33**, 115–117.

Bunn, H. F., Gabbay, K. H., and Gallop, P. M. (1978). The glycosylation of hemoglobin: Relevance to diabetes mellitus. *Science* **200**, 21–27.

Burrell, T. A., Fineman, M. S., Deckhut, D., Weyer, C., McIntyre, S., Wang, Y., Lutz, K., Nielsen, L. L., and Kolterman, O. G. (2003). Mealtime subcutaneous (SC) injection of

pramlintide slows without arresting gastric emptying in patients with type 2 diabetes. *Diabet. Metab.* **29**, 4S124–4S125.

Buse, J. B., Weyer, C., and Maggs, D. G. (2002). Amylin replacement with pramlintide in type 1 and type 2 diabetes: A physiological approach to overcome barriers with insulin therapy. *Clin. Diabet.* **20**, 137–144.

Chapman, I., Parker, B., Doran, S., Feinle-Bisset, C., Wishart, J., Wang, Y., Gao, H.-Y., McIntyre, S., Burrell, T., Deckhut, D., Weyer, C., and Horowitz, M. (2004). Effect of pramlintide on ad-libitum food intake in obese subjects and subjects with type 2 diabetes: A randomized, double-blind, placebo-controlled, cross-over study. *Diabetes* **53**(suppl. 2), A82.

Colburn, W. A., Gottlieb, A. B., Koda, J., and Kolterman, O. G. (1996). Pharmacokinetics and pharmacodynamics of AC137 (25,28,29 tripro-amylin, human) after intravenous bolus and infusion doses in patients with insulin-dependent diabetes. *J. Clin. Pharmacol.* **36**, 13–24.

Cooper, G. J. S. (1991). Amylin and insulin co-replacement therapy for insulin-dependent (type I) diabetes mellitus. *Med. Hypotheses* **36**, 284–288.

Cooper, M. E., McNally, P. G., Phillips, P. A., and Johnston, C. I. (1995). Amylin stimulates plasma renin concentration in humans. *Hypertension* **26**, 460–464.

DCCT Research, Group (1986). The Diabetes Control and Complications Trial (DCCT): Design and methodologic considerations for the feasibility phase. *Diabetes* **35**, 530–545.

DCCT Research, Group (1995a). The effect of intensive diabetes therapy on the development and progression of neuropathy. *Ann. Intern. Med.* **122**, 561–568.

DCCT Research, Group (1995b). Effect of intensive diabetes treatment on nerve conduction in the Diabetes Control and Complications Trial. *Ann. Neurol.* **38**, 870–880.

DCCT Research, Group (1995c). The effect of intensive diabetes treatment on the progression of diabetic retinopathy in insulin-dependent diabetes mellitus. *Arch. Ophthalmol.* **113**, 36–51.

DCCT Research, Group (1995d). Progression of retinopathy with intensive versus conventional treatment in the Diabetes Control and Complications Trial. *Ophthalmology* **102**, 647–661.

Diabetes, Control and Complications, Trial (DCCT) Research, Group (1995). Effect of intensive diabetes management on macrovascular events and risk factors in the Diabetes Control and Complications Trial. *Am. J. Cardiol.* **75**, 894–903.

Dobbs, R., Sakurai, H., Sasaki, H., Faloona, G., Valverde, I., Baetens, D., Orci, L., and Unger, R. (1975). Glucagon: Role in the hyperglycemia of diabetes mellitus. *Science* **187**, 544–547.

Edelman, S. V., and Weyer, C. (2002). Unresolved challenges with insulin therapy in type 1 and type 2 diabetes: Potential benefit of replacing amylin, a second β-cell hormone. *Diabet. Technol. Ther.* **4**, 175–189.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1997a). Glucagon secretion in patients with type I diabetes was inhibited by the human amylin analogue, pramlintide. *Diabet. Med.* **14**, S29.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1997b). The human amylin analogue pramlintide inhibited glucagon secretion in type I diabetic subjects. *79th Annual Meeting of the Endocrine Society, Program and Abstracts*, 472.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1997c). Pramlintide, a human amylin analogue, inhibited glucagon secretion in patients with type I diabetes. *Can. J. Diabet. Care* **21**, 26.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1997d). The human amylin analogue pramlintide inhibited glucagon secretion in type I diabetic subjects. *Diabetes* **40**, 30A.

Fineman, M., Koda, J., and Kolterman, O. (1998a). Subcutaneous administration of a human amylin analogue suppresses postprandial plasma glucagon concentrations in type I diabetic patients. *Diabetes* **47**, A89.

Fineman, M., Kolterman, O., Thompson, R., and Koda, J. (1998b). Pramlintide, a human amylin analogue, inhibited glucagon secretion in patients with type 1 diabetes. *AACE Back to the Future: A Renaissance in Endocrinology, 1998 Syllabus American Association of Clinical Endocrinologists Seventh Annual Meeting and Clinical Congress, April 28–May 3, 1998, Buena Vista Palace, Orlando, Florida*, 112.

Fineman, M., Organ, K., and Kolterman, O. (1998). The human amylin analogue pramlintide suppressed glucagon secretion in patients with type 2 diabetes. *Diabetologia* **41**, A167.

Fineman, M., Bahner, A., Gottlieb, A., and Kolterman, O. (1999a). Effects of six months administration of pramlintide as an adjunct to insulin therapy on metabolic control in people with type 1 diabetes. *Diabetes* **48**(suppl. 1), A113.

Fineman, M., Bahner, A., Gottlieb, A., and Kolterman, O. (1999b). Effects of six months' administration of pramlintide as an adjunct to insulin therapy on metabolic control in people with type 2 diabetes. *Endocrine Society 81st Annual Meeting Program and Abstracts*, 471–472.

Fineman, M., Gottlieb, A., Bahner, A., Parker, J., Waite, G., and Kolterman, O. (1999c). Pramlintide therapy in addition to insulin in type 1 diabetes: Effect on metabolic control after 6 months. *Diabetologia* **42**(suppl. 1), A232.

Fineman, M., Gottlieb, A., Skare, S., and Kolterman, O. (2000a). 52 weeks of pramlintide therapy as an adjunct to insulin improved metabolic control in people with type 2 diabetes. *Diabetologia* **43,** (abstract 783).

Fineman, M., Gottlieb, A., Skare, S., and Kolterman, O. (2000b). Pramlintide as an adjunct to insulin therapy improved glycemic and weight control in people with type 2 diabetes during treatment for 52 weeks. *Diabetes* **49**, A106.

Fineman, M., Maggs, D., Burrell, T., Velte, M., and Kolterman, O. (2001). Addition of pramlintide to insulin therapy in type 1 diabetes: Impact on glycemic and weight control stratified by BMI. *Diabetes* **50**(suppl. 2), A112.

Fineman, M., Weyer, C., Maggs, D. G., Strobel, S., and Kolterman, O. G. (2002). The human amylin analog, pramlintide, reduces postprandial hyperglucagonemia in patients with type 2 diabetes mellitus. *Horm. Metab. Res.* **34**, 504–508.

Ghatei, M. A., Datta, H. K., Zaidi, M., Bretherton-Watt, D., Wimalawansa, S. J., Mac Intyre, I., and Bloom, S. R. (1990). Amylin and amylin-amide lack an acute effect on blood glucose and insulin. *J. Endocrinol.* **124**, R9–R11.

Gilbey, S. G., Bretherton-Watt, D., Beacham, J., Ghatei, M. A., and Bloom, S. R. (1989). High dose amylin in man: Unexpected failure to affect intravenous glucose tolerance. *BDA Diabet. Med.* **6**, 5A.

Gilbey, S., Ghatei, M. A., Bretherton-Watt, D., Jones, P. M., Beacham, I., Perera, T., Girgis, S., Bloom, S. R., and Zaidi, M. (1991). Amylin lowers serum calcium in Paget's bone disease: Further evidence for a role in calcium metabolism. *J. Bone Miner. Res.* **6**, S293.

Gottlieb, A., Fineman, M., Bahner, A., Parker, J., Waite, G., and Kolterman, O. (1999). Pramlintide therapy in addition to insulin in type 2 diabetes: Effect on metabolic control after 6 months. *Diabetologia* **42**(suppl. 1), A232.

Gottlieb, A., Velte, M., Fineman, M., and Kolterman, O. (2000a). Pramlintide as an adjunct to insulin therapy improved glycemic and weight control in people with type 1 diabetes during treatment for 52 weeks. *Diabetes* **49**, A109.

Gottlieb, A., Velte, M., Fineman, M., and Kolterman, O. (2000b). Pramlintide treatment for 52 weeks improved glycemic and weight control in people with type 1 diabetes. *Diabetologia* **43**, A47.

Heinemann, L., Heise, T., Maggs, D., McKay, R., Ruggles, J., Wang, Y., Burrell, T., and Weyer, C. (2003). Pramlintide does not impair symptomatic or catecholaminergic responses to hypoglycemia. *Diabet. Metab.* **29**, 4S264.

Heise, T., Heinemann, L., and Weyer, C. (2002). Amylin-substitution with pramlintide as conjunction to insulin therapy. *Diabet. Stoffwechsel* **11**, 233–244.

Heise, T., Heinemann, L., Maggs, D., McKay, R., Ruggles, J., Wang, Y., Burrell, T., and Weyer, C. (2003). Pramlintide does not impair symptomatic or catecholaminergic responses to hypoglycemia. *Diabetes* **52**(suppl. 1), A463.

Heise, T., Heinemann, L., Heller, S., Weyer, C., Wang, Y., Strobel, S., Kolterman, O., and Maggs, D. (2004). Effect of pramlintide on symptom, catecholamine, and glucagon responses to hypoglycemia in healthy subjects. *Metabolism* **53**, 1227–1232.

Hollander, P., Ratner, R., Fineman, M., Strobel, S., Shen, L., Maggs, D., Kolterman, O., and Weyer, C. (2003a). Addition of pramlintide to insulin therapy lowers HbA_{1c} in conjunction with weight loss in patients with type 2 diabetes approaching glycaemic targets. *Diabet. Obes. Metab.* **5**, 408–414.

Hollander, P. A., Levy, P., Fineman, M. S., Maggs, D. G., Shen, L. Z., Strobel, S. A., Weyer, C., and Kolterman, O. G. (2003b). Pramlintide as an adjunct to insulin therapy improves long-term glycemic and weight control in patients with type 2 diabetes: A 1-year randomized controlled trial. *Diabet. Care* **26**, 784–790.

Hollander, P., Maggs, D. G., Ruggles, J. A., Fineman, M., Shen, L., Kolterman, O. G., and Weyer, C. (2004). Effect of pramlintide on weight in overweight and obese insulin-treated type 2 diabetes patients. *Obes. Res.* **12**, 661–668.

Jeffcoate, S., Schoenfeld, S., Blonde, L., and Kolterman, O. (1998). Sustained weight loss after 1 year of pramlintide therapy in both type 1 and type 2 diabetes. *Int. J. Obes. Relat. Metab. Disord.* **22**, S268.

Kawamori, R., Shichiri, M., Kikuchi, M., Yamasaki, Y., and Abe, H. (1985). The mechanism of exaggerated glucagon response to arginine in diabetes mellitus. *Diabet. Res. Clin. Pract.* **1**, 131–137.

Koda, J., Fineman, M., Percy, A., Blase, E., and Lillioja, S. (1993). Use of a new two-site immunoassay for amylin to characterize amylin hormone response in Pima Indians. *Diabetologia* **36**, A137.

Kolterman, O., Gottlieb, A., and Moyses, C. (1994a). Amylin agonist, AC-137, reduces postprandial hyperglycemia in subjects with insulin dependent diabetes mellitus (IDDM). *Diabetes* **43**, 78A.

Kolterman, O. G., Gottlieb, A. B., and Moyses, C. J. (1994c). Administration of tripro-amylin reduces postprandial hyperglycemia in subjects with juvenile-onset diabetes. *Diabetologia* **37**, A72.

Kolterman, O. G., Gottlieb, A., Moyses, C., and Colburn, W. (1995c). Reduction of postprandial hyperglycemia in subjects with IDDM by intravenous infusion of AC137, a human amylin analogue. *Diabet. Care* **18**, 1179–1182.

Kolterman, O. G., Gottlieb, A. B., Organ, K. A., and Thompson, R. G. (1995d). Reduction of postprandial hyperglycemia in patients with type II diabetes by the human amylin analogue AC137. *Diabetologia* **38**, A193.

Kolterman, O., Fineman, M., Burrell, T., Strobel, S., Shen, L., and Maggs, D. (2003c). Adjunctive therapy with pramlintide lowered A1c without an increase in overall severe hypoglycemia event rate in patients with type 1 diabetes approaching ADA glycemic targets. *Diabetes* **52**(suppl. 1), A124.

Kolterman, O., Kisicki, J. C., Peltier, L., Gottlieb, A., and Moyses, C. (1994b). Infusion of amylin agonist AC-0137 reduces postprandial hyperglycemia in subjects with type 1 diabetes (IDDM). *Clin. Res.* **42**, 87A.

Kolterman, O., Organ, K., and Gottlieb, A. (1995a). Intravenous (IV) infusion of the human amylin analogue, AC 137, reduces postprandial hyperglycemia (PPH) in subjects with type I diabetes mellitus receiving oral nutrients but not IV glucose. *Diabetes* **44**, 127A.

Kolterman, O. G., Pearson, L., Peterson, J., Gottlieb, A., and Thompson, R. G. (1996a). Pramlintide, a human amylin analog, improved glucose control independent of entry HbA1c. *Diabetes* **45**, 289A.

Kolterman, O., Peterson, J., and Gottlieb, A. (1995b). Subcutaneous administration of AC137, a human amylin analogue, reduces postprandial hyperglycemia (PPH) in subjects with type I diabetes mellitus. *Diabetes* **44**, 57A.

Kolterman, O. G., Schwartz, S., Corder, C., Levy, B., Klaff, L., Peterson, J., and Gottlieb, A. (1996b). Effect of 14 days' subcutaneous administration of the human amylin analogue, pramlintide (AC137), on an intravenous insulin challenge and response to a standard liquid meal in patients with IDDM. *Diabetologia* **39**, 492–499.

Kolterman, O. G., and Schoenfeld, S. L. (1997a). Intravenous pramlintide reduces postprandial hyperglycemia and C-peptide concentrations in people with type II diabetes mellitus. *American Association of Clinical Endocrinologists (AACE) Sixth Annual Meeting and Clinical Congress*, 124.

Kolterman, O. G., and Schoenfeld, S. L. (1997b). Pramlintide reduces 24-hour glucose and serum fructosamine concentrations in people with type I diabetes mellitus. *American Association of Clinical Endocrinologists (AACE) Sixth Annual Meeting and Clinical Congress* (abstract 8).

Kolterman, O., Whitehouse, F., Ratner, R., Rosenstock, J., Schoenfeld, S., and Jeffcoate, S. (1998a). Positive effects on body weight resulting from pramlintide therapy in type 1 and type 2 diabetes. *Diabetologia* **41**, A214.

Kolterman, O., Whitehouse, F., Ratner, R., Rosenstock, J., Schoenfeld, S., and Jeffcoate, S. (1998b). Positive effects on body weight resulting from pramlintide therapy in type 1 and type 2 diabetes. *Can. J. Diabet. Care* **22**, A13.

Kolterman, O., Bahner, A., Gottlieb, A., and Fineman, M. (1999). Pramlintide (human amylin analogue) as an adjunct to insulin therapy in patients with type 1 diabetes improved glycemic control over 2 years. *Diabetes* **48**, A104–A105.

Kolterman, O., Maggs, D., Fineman, M., Burrell, T., Strobel, S., Shen, L., and Weyer, C. (2002). Adjunctive therapy with pramlintide lowers HbA_{1c} without concomitant weight gain and increased risk of severe hypoglycemia in patients with type1 diabetes approaching glycemic targets. *Diabetologia* **45**(suppl. 2), A240.

Kolterman, O., Maggs, D., Burrell, T., Strobel, S., Brown, D., and Weyer, C. (2003a). Pramlintide, as an adjunct to insulin therapy, led to improved control of both glycemia and weight in African American and Hispanic patients with type 2 diabetes. *Diabetes* **52** (suppl. 1), A124.

Kolterman, O., Maggs, D., Burrell, T., Strobel, S., Brown, D., and Weyer, C. (2003b). Pramlintide, as an adjunct to insulin therapy, led to improved control of both glycemia and weight in African American and Hispanic patients with type 2 diabetes. *Diabet. Metab.* **29**, 4S265.

Kolterman, O., Burrell, T., Shen, L., *et al.* (2003d). Initiation of pramlintide using dose-titration in intensively-treated patients with type 1 diabetes resulted in mitigation of nausea and hypoglycemia. Late-breaking abstract presented at: American Diabetes Association 63rd Scientific Sessions; June 13–17, 2003; New Orleans, LA.

Kong, M. F., King, P., Macdonald, I., Stubbs, T., Perkins, A., and Tattersall, R. (1997a). Pramlintide reduced postprandial hyperglycaemia by slowing the delivery of meal-derived glucose. *Diabet. Med.* **14**, S47–S48.

Kong, M. F., King, P., Macdonald, I. A., Stubbs, T. A., Perkins, A. C., Blackshaw, P. E., Moyses, C., and Tattersall, R. B. (1997a). Infusion of pramlintide, a human amylin analogue, delays gastric emptying in men with IDDM. *Diabetologia* **40**, 82–88.

Kong, M. F., Stubbs, T., King, P., Lambourne, J., Madonald, I., Blackshaw, E., Perkins, A., and Tattersall, R. (1997b). The effect of single doses of pramlintide on gastric emptying of two meals in IDDM. *Diabetes* **40**, 154A.

Kovatchev, B., Cox, D. J., McCall, A., Crean, J., Gloster, M., and Whitehouse, F. (2004). Effects of pramlintide on the magnitude and speed of postprandial blood glucose fluctuations in patients with type 1 diabetes. *Diabetes* **53**(suppl. 2), A133.

Kruger, D. F., and Gloster, M. A. (2004). Pramlintide for the treatment of insulin-requiring diabetes mellitus: Rationale and review of clinical data. *Drugs* **64**, 1419–1432.

Levetan, C., Want, L., Weyer, C., Crean, J., Strobel, S., Wang, Y., Maggs, D. G., Schoenamsgruber, E., Kolterman, O. G., Chandran, M., Mudaliar, S., and Henry, R. R. (2002). Reduced glucose fluctuations following 4 weeks of pramlintide treatment in patients with type 1 diabetes intensively treated with insulin pumps. *Diabetes* **51**(suppl. 1), A106.

Levetan, C., Want, L. L., Weyer, C., Strobel, S. A., Crean, J., Wang, Y., Maggs, D. G., Kolterman, O. G., Chandran, M., Mudaliar, S. R., and Henry, R. R. (2003). Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglycerides excursions among patients with type 1 diabetes intensively treated with insulin pumps. *Diabet. Care* **26**, 1–8.

Macdonald, I., King, P., Kong, M.-F., Stubbs, T., Perkins, A., Moyses, C., and Tattersall, R. (1995). Infusion of the human amylin analogue, AC137, delays gastric emptying in men with IDDM. *Diabetologia* **38**(suppl. 1), A32.

Maggs, D., Burrell, T., Fineman, M., and Kolterman, O. (2001). Pramlintide as adjunctive treatment to insulin in type 2 diabetes resulted in improved glycemic control and concomitant weight loss. *Diabetes* **50**(suppl. 2), A124–A125.

Maggs, D., Fineman, M., Burrell, T., Gottlieb, A., and Kolterman, O. G. (2001). In type 1 diabetes, addition of pramlintide to insulin therapy resulted in long-term glycemic improvement without the increase in severe hypoglycemia noted in patients treated with insulin alone. *Diabetes* **50**(suppl. 2), A442.

Maggs, D. G., Weyer, C., Burrell, T., Gottlieb, A. D., Shen, L. Z., and Kolterman, O. G. (2001). Amylin replacement with pramlintide as an adjunct to insulin therapy facilitates a combined improvement in glycemic and weight control in type 2 diabetes. *Diabetologia* **44**(suppl. 1), A237.

Maggs, D., Weyer, C., Crean, J., Wang, Y., Burrell, T., Fineman, M., Kornstein, J., Schwartz, S., Guiterrez, M., and Kolterman, O. (2002). Mealtime amylin replacement with pramlintide markedly improves postprandial glucose excursions when added to insulin lispro in patients with type 2 diabetes: A dose-timing study. *Diabetologia* **45**(suppl. 2), A264.

Maggs, D., Shen, L., Strobel, S., Brown, D., Kolterman, O., and Weyer, C. (2003). Effect of pramlintide on A1C and body weight in insulin-treated African Americans and Hispanics with type 2 diabetes: A pooled post hoc analysis. *Metabolism* **52**, 1638–1642.

Maggs, D. G., Fineman, M., Kornstein, J., Burrell, T., Schwartz, S., Wang, Y., Ruggles, J. A., Kolterman, O. G., and Weyer, C. (2004). Pramlintide reduces postprandial glucose excursions when added to insulin lispro in subjects with type 2 diabetes: A dose timing study. *Diabet. Metab. Res. Rev.* **20**, 55–60.

Marrero, D., Kruger, D., Burrell, T., Gloster, M., Crean, J., Herrmann, K., and Kolterman, O. (2004a). Patients with type 1 diabetes: Perceptions associated with pramlintide as an adjunctive treatment to insulin. *Endocr. Pract.* **10**(suppl. 1), 44.

Marrero, D., Kruger, D., Burrell, T., Gloster, M., Crean, J., Herrmann, K., and Kolterman, O. (2004b). Patients with type 1 diabetes: Perceptions associated with pramlintide as an adjunctive treatment to insulin. *Diabetes* **53**(suppl. 2), A137–A138.

McCall, A., Kovatchev, B. P., Cox, D. J., Crean, J., and Gloster, M. (2004b). Assessing glucose variability using CGMS in pramlintide- and placebo-treated subjects with type 1 diabetes mellitus. *Diabetes* **53**(suppl. 2), A138.

McNally, P. G., Phillips, P. A., Johnston, C. I., and Cooper, M. E. (1994a). Human amylin increases plasma renin in man: A possible explanation for the association between hypertension and insulin resistance? Program and Abstracts, the Endocrine Society, 76th Annual Meeting; 1994 Jun 15–16; Ahaheim, CA, Abstract 286.

McNally, P. G., Phillips, P. A., Johnston, C. I., Kolterman, O. G., and Cooper, M. E. (1994b). Human amylin increases plasma renin in man: A possible link between hypertension and insulin resistance? *Diabetologia* **37**, A46.

Moyses, C., Kolterman, O., and Mant, T. (1993). Pharmacokinetics and hyperglycaemic effects of the amylin analogue, AC137, in man. *Diabet. Med.* **10**, S25.

Moyses, C., Kolterman, O., Nuttall, A., and Mant, T. (1994). First administration to man of the human amylin analogue tripro-amylin. *Diabetologia* **37**, A72.

Müller, W. A., Faloona, G. R., Aguilar-Parada, E., and Unger, R. H. (1970). Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. *N. Engl. J. Med.* **283**, 109–115.

Nuttall, A., Bryan, G. L., and Moyses, C. (1995a). Administration of human amylin increases plasma renin activity and plasma aldosterone in man. *Am. J. Hypertens.* **8**, 108A.

Nuttall, A., Bryan, G. L., and Moyses, C. (1995b). Intravenous human amylin increases plasma renin activity and plasma aldosterone in man. *Eur. Heart J.* **16**, 66.

Nyholm, B., Moller, N., Bryan, G., Moyses, C., and Alberti, K. G. M. M. (1995a). Acute effects of the amylin analogue AC-0137 on fuel metabolism in patients with IDDM. *Diabetes* **44**, 255A.

Nyholm, B., Moller, N., Bryan, G., Moyses, C., Alberti, K. G. M. M., and Schmitz, O. (1995b). Acute metabolic effects of the amylin analogue AC137 in patients with insulin-dependent diabetes mellitus. *Diabetologia* **38**, A193.

Nyholm, B., Moller, N., Gravholt, C. H., Orskov, L., Mengel, A., Bryan, G., Moyses, C., Alberti, K. G., and Schmitz, O. (1996). Acute effects of the human amylin analog AC137 on basal and insulin-stimulated euglycemic and hypoglycemic fuel metabolism in patients with insulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* **81**, 1083–1089.

Nyholm, B., Orskov, L., Hove, K., Gravholt, C., Moller, N., Alberti, N., and Schmitz, O. (1997a). The amylin analogue pramlintide decreases post-prandial plasma glucose and glucagon in IDDM. *Diabetes* **46**, 155A.

Nyholm, B., Orskov, L., Hove, K. Y., Gravholt, C. H., Moller, N., Alberti, K. G. M. M., and Schmitz, O. (1997b). The amylin analogue pramlintide decreases postprandial plasma glucose and glucagon in IDDM patients. *Diabetologia* **40**, A46.

Nyholm, B., Orskov, L., and Schmitz, O. (1997c). The amylin analogue pramlintide decreased post-prandial plasma glucose and glucagon in patients with type I diabetes. *Diabet. Med.* **14**, S29.

Nyholm, B., Orskov, L., Hove, K., Gravholt, C., Moller, N., Alberti, K., Moyses, C. K. O., and Schmitz, O. (1999). The amylin analog pramlintide improves glycemic control and reduces postprandial glucagon concentrations in patients with type 1 diabetes mellitus. *Metabolism* **48**, 935–941.

Orskov, L., Nyholm, B., Hove, K., Gravholt, C., Moller, N., Kolterman, O., Alberti, K., and Schmitz, O. (1997a). Effects of the amylin analogue pramlintide on the glucose response to a glucagon challenge in IDDM. *Diabetologia* **40**, A355.

Orskov, L., Nyholm, B., Hove, K. Y., Gravholt, C. H., Moller, O., Kolterman, O., Alberti, K. G. M. M. A., and Schmitz, O. (1997b). Effects of the amylin analogue pramlintide on the glucose response to a glucagon challenge in IDDM. *Diabetes* **46**, 155A.

Orskov, L., Nyholm, B., Yde Hove, K., Gravholt, C. H., Moller, N., and Schmitz, O. (1999). Effects of the amylin analogue pramlintide on hepatic glucagon responses and intermediary metabolism in Type 1 diabetic subjects. *Diabet. Med.* **16**, 867–874.

Parker, J., Kong, M. F., Stubbs, T., King, P., Lambourne, J., Macdonald, I., Blackshaw, E., Perkins, A., and Tattersall, R. (1998). Single subcutaneous doses of pramlintide and gastric emptying of two meals in type I diabetes. *Diabet. Med.* **15**(suppl. 1), S50.

Percy, A. J., Trainor, D., and Koda, J. (1993). Development of a sensitive two-site immunoassay and its use for monitoring amylin levels in normal individuals. Abstract

presented at American Diabetes Association Southern California Research Symposium; Irvine, CA.

Petry, C. J., Percy, A., Koda, J. E., and Hales, C. N. (1995). Development of sensitive two-site immunoassays for rat amylin. Proceedings of the ACB National Meeting, p. 75.

Raskin, P., Aydin, I., and Unger, R. H. (1976). Effect of insulin on the exaggerated glucagon response to arginine stimulation in diabetes mellitus. *Diabetes* **25**, 227–229.

Ratner, R., Levetan, C., Schoenfeld, S., Jeffcoate, S., and Kolterman, O. (1998). Effects of pramlintide therapy: A 1-year study in insulin-requiring type 2 diabetes. *Diabetologia* **41**, A61.

Ratner, R. E., Want, L. L., Fineman, M. S., Velte, M. J., Ruggles, J. A., Gottlieb, A., Weyer, C., and Kolterman, O. G. (2002). Adjunctive therapy with the amylin analogue pramlintide leads to a combined improvement in glycemic and weight control in insulin-treated subjects with type 2 diabetes. *Diabet. Technol. Ther.* **4**, 51–61.

Ratner, R. E., Dickey, R., Fineman, M., Maggs, D. G., Shen, L., Strobel, S. A., Weyer, C., and Kolterman, O. G. (2004). Amylin replacement with pramlintide as an adjunct to insulin therapy improves long-term glycaemic and weight control in Type 1 diabetes mellitus: A 1-year randomized controlled trial. *Diabet. Med.* **21**, 1204–1212.

Redalieu, E., Thompson, R. G., Dean, E., Petrella, E., Musunuri, S., and Gottlieb, A. (1996). Pharmacokinetics of pramlintide and free insulin following combined or separate injections in patients with type I diabetes. *Diabetes* **45**, 220A.

Redalieu, E., Blake, D., Nuttall, A., and Thompson, R. (1997a). Pharmacokinetic effects of syringe mixing pramlintide, isophane insulin, and soluble insulin. *Diabetologia* **40**, A356.

Redalieu, E., Dean, E., Schoenfeld, S., and Thompson, R. (1997b). Effects of syringe mixing pramlintide with regular and NPH insulin upon plasma glucose control in patients with type I diabetes. *Can. J. Diabet. Care* **21**, 26.

Rosenstock, J., Whitehouse, F., Schoenfeld, S., Jeffcoate, S., and Kolterman, O. (1998a). Results of 1-year study with pramlintide therapy in type 1 diabetes: Effect on metabolic control and safety profile. *Can. J. Diabet. Care* **22**, A13.

Rosenstock, J., Whitehouse, F., Schoenfeld, S., Jeffcoate, S., and Kolterman, O. (1998b). Results of 1-year study with pramlintide therapy in type 1 diabetes: Effect on metabolic control and safety profile. *Diabetologia* **41**, A239.

Samsom, M., Szarka, L. A., Camilleri, M., Vella, A., Zinsmeister, A. R., and Rizza, R. A. (2000a). Pramlintide, an amylin analog, selectively delays gastric emptying: Potential role of vagal inhibition. *Am. J. Physiol.* **278**, G946–G951.

Samsom, M., Szarka, L. A., Vella, A., Daniel, D. A., Burton, D., Thomforde, G. M., Zinsmeister, A. R., Rizza, R. A., and Camilleri, M. (2000b). Effects of the amylin analog, pramlintide, on gastrointestinal and colonic transit in healthy subjects: Association with vagal inhibition. *Gastroenterology* **118**, A–625.

Schmitz, O., Nyholm, B., Orskov, L., Gravholt, C., and Moller, N. (1997). Effects of amylin and the amylin agonist pramlintide on glucose metabolism. *Diabetic Med.* **14**, S19–S23.

Schoenfeld, S., Dean, E., Blonde, L., and Kolterman, O. (1998). Effects of syringe mixing pramlintide with regular and NPH insulin upon plasma glucose control in people with type 1 diabetes [in French]. *Diabet. Metab.* **24**, LXXVIII.

Thompson, R., and Kolterman, O. (1997). Intravenous pramlintide reduced postprandial hyperglycaemia and C-peptide concentrations in patients with type II diabetes mellitus [in French]. *Diabet. Metab.* **23**, P054.

Thompson, R. G., Gottlieb, A. B., Organ, K., and Kolterman, O. G. (1995a). The human amylin analogue (AC137) reduces glucose following Sustacal in patients with type II diabetes. *Diabetes* **44**, 127A.

Thompson, R. G., Organ, K., Gottlieb, A., and Kolterman, O. G. (1995b). Pramlintide (AC137) reduced postprandial hyperglycaemia, insulin, and C-peptide in patients with type II diabetes. *Diabet. Med.* **12**, S46.

Thompson, R., Pearson, L., Schoenfeld, S., and Kolterman, O. (1997d). Pramlintide improves glycemic control in patients with type II diabetes requiring insulin. *Diabetologia* **40**, A355.

Thompson, R. G., Gottlieb, A., Organ, K., Koda, J., Kisicki, J., and Kolterman, O. G. (1997e). Pramlintide: A human amylin analogue reduced postprandial plasma glucose, insulin, and C-peptide concentrations in patients with type 2 diabetes. *Diabet. Med.* **14**, 547–555.

Thompson, R. G., Peterson, J., Gottlieb, A., and Mullane, J. (1997g). Effects of pramlintide, an analog of human amylin, on plasma glucose profiles in patients with IDDM: Results of a multicenter trial. *Diabetes* **46**, 632–636.

Thompson, R. G., Pearson, L., Gottlieb, A., and Kolterman, O. G. (1996). Pramlintide, an analog of human amylin, reduced fructosamine in patients with Type I diabetes. *Diabetes* **45**, 222A.

Thompson, R., and Kolterman, O. G. (1997). Intravenous pramlintide reduced postprandial hyperglycaemia and C-peptide concentrations in patients with type II diabetes mellitus. *Diabetic Med.* **14**, S41.

Thompson, R., Kolterman, O., Peterson, J., and Pearson, L. (1997a). Pramlintide reduced 24-hour glucose and serum fructosamine concentrations in people with type I diabetes mellitus. *Can. J. Diabet. Care* **21**, 26.

Thompson, R., Kolterman, O., Peterson, J., and Pearson, L. (1997b). Pramlintide reduced 24-hour glucose concentrations and serum fructosamine in patients with Type I diabetes mellitus [in French]. *Diabet. Metab.* **23**, P053.

Thompson, R., Kolterman, O. G., Peterson, J., and Pearson, L. (1997c). Pramlintide reduced 24-hour glucose concentrations and serum fructosamine in patients with type I diabetes mellitus. *Diabet. Med.* **14**, S2.

Thompson, R. G., Pearson, L., and Kolterman, O. G. (1997f). Effects of 4 weeks' administration of pramlintide, a human amylin analogue, on glycaemia control in patients with IDDM: Effects on plasma glucose profiles and serum fructosamine concentrations. *Diabetologia* **40**, 1278–1285.

Thompson, R. G., Pearson, L., Schoenfeld, S. L., and Kolterman, O. G. (1998). Pramlintide, a synthetic analog of human amylin, improves the metabolic profile of patients with type 2 diabetes using insulin. The Pramlintide in Type 2 Diabetes Group. *Diabet. Care* **21**, 987–993.

U.K. Prospective Diabetes, Study (UKPDS), Group (1998a). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* **352**, 837–853.

UK Prospective Diabetes, Study (UKPDS), Group (1998b). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* **352**, 854–865.

Unger, R. H., Aguilar-Parada, E., Muller, W. A., and Eisentraut, A. M. (1970). Studies of pancreatic alpha cell function in normal and diabetic subjects. *J. Clin. Invest.* **49**, 837–848.

Vella, A., Lee, J. S., Camilleri, M., Szarka, L. A., Burton, D. D., Zinsmeister, A. R., Rizza, R. A., and Klein, P. D. (2002). Effects of pramlintide, an amylin analogue, on gastric emptying in type 1 and 2 diabetes mellitus. *Neurogastroenterol. Motil.* **14**, 123–131.

Want, L. L. (2002). Pramlintide reduced postprandial glucose excursions without increasing severe hypoglycemia in patients with type 1 diabetes treated with CSII. Program Book AADE's 29th Annual Meeting and Exhibition, pp. 125–129.

Want, L. L., Levetan, C., Weyer, C., Maggs, D. G., Crean, J., Wang, Y., Strobel, S., Schoenamsgruber, E., Kolterman, O. G., Chandran, M., Henry, R. R., and Mudaliar, S. (2002). Reduced postprandial glucose, glucagon and triglyceride excursions following 4

weeks of pramlintide treatment in patients with type 1 diabetes treated intensively with insulin pumps. *Diabetes* **51**(suppl. 2), A117.

Want, L. L., Ratner, R. E., and Uwaifo, G. I. (2004). Safety and tolerability of long-term pramlintide therapy. *Diabetes* **53**(suppl. 2), A150.

Weyer, C., Maggs, D. G., Fineman, M. S., Burrell, T., and Kolterman, O. G. (2002). The human amylin analog, pramlintide, reduces body weight in insulin-treated patients with type 2 diabetes. *Int. J. Obes.* **26**(suppl. 1), abstract 508.

Weyer, C., Kim, D., Burrell, T., Wang, Y., Kornstein, J., Bicsak, T., Fineman, M., Ruggles, J., Schwartz, S., and Kolterman, O. (2003). Mealtime amylin replacement with pramlintide markedly reduced postprandial glucose excursions when added to insulin lispro in patients with type 1 or type 2 diabetes: A dose-timing study. *Diabetes* **52**(suppl. 1), A16.

Weyer, C., Fineman, M., Burrell, T., Strobel, S., Shen, L., and Kolterman, O. (2003a). Adjunctive therapy with pramlintide lowers A1c without concomitant weight gain in patients with type 2 diabetes approaching ADA glycemic targets. *Diabetes* **52**(suppl. 1), A138.

Weyer, C., Fujioka, K., Aroda, V., Edelman, S., Chen, K., Lush, C., Wang, Y., Burns, C., Lutz, K., McIntyre, S., Kornstein, J., Wintle, M., and Baron, A. (2005a). Safety, dose-tolerance, and weight-related effects of pramlintide in obese subjects with or without type 2 diabetes. *Endocrine Society Program & Abstracts 87th Annual Meeting, San Diego, June 4–7*.

Weyer, C., Aronne, L., Fujioka, K., Aroda, V., Edelman, S., Chen, K., Lush, C., Wang, Y., Burns, C., Lutz, K., McIntyre, S., Kornstein, J., Wintle, M., and Baron, A. (2005b). Safety, dose-tolerance, and weight-related effects of pramlintide in obese subjects with or without type 2 diabetes. Abstract presented at 14th European Congress on Obesity; 2005 Jun 1–4; Athens, Greece.

Whitehouse, F., Kruger, D. F., Fineman, M., Shen, L., Ruggles, J. A., Maggs, D. G., Weyer, C., and Kolterman, O. G. (2002). A randomized study and open-label extension evaluating the long-term efficacy of pramlintide as an adjunct to insulin therapy in type 1 diabetes. *Diabet. Care* **25**, 724–730.

Wilding, J. P. H., Khandan-Nia, N., Bennet, W. M., Gilbey, S. G., Beacham, J., Ghatei, M. A., and Bloom, S. R. (1994). Lack of acute effect of amylin (islet associated polypeptide) on insulin sensitivity during hyperinsulinaemic euglycaemic clamp in humans. *Diabetologia* **37**, 166–169.

Wilding, J. P. H., Khandan-Nia, N., Gilbey, S. G., Bennet, W. M., Beacham, J., Ghatei, M. A., and Bloom, S. R. (1993). Amylin does not affect insulin sensitivity in man. *Br. Diabet. Assn* .

Wimalawansa, S. J., Gunasekera, R. D., and Datta, H. K. (1992). Hypocalcemic actions of amylin amide in humans. *J. Bone Miner. Res.* **7**, 1113–1116.

Weyer, C., Maggs, D. G., Fineman, M., Gottlieb, A. D., Shen, L. Z., and Kolterman, O. G. (2001a). Amylin replacement with pramlintide as an adjunct to insulin therapy facilitates a combined improvement in glycemic and weight control in type 1 diabetes. *Diabetologia* **44**(suppl. 1), A237.

Weyer, C., Maggs, D. G., Young, A. A., and Kolterman, O. G. (2001b). Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: A physiological approach toward improved metabolic control [published correction appears in Curr. Pharm. Des. 2001; 7, 1967]. *Curr. Pharm. Des.* **7**, 1353–1373.

Young, A., Nuttall, A., Moyses, C., Percy, A., Vine, W., and Rink, T. (1995). Amylin stimulates the renin-angiotensin-aldosterone axis in rats and man. *Diabetologia* **38**, A225.

Young, A., Kolterman, O., and Hall, J. (1999). Amylin innocent in essential hypertension? *Diabetologia* **42**, 1029.

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