ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY
Volume 298

SENSORY NERVES AND NEUROPEPTIDES IN GASTROENTEROLOGY

From Basic Science to Clinical Perspectives

Edited by Marcello Costa, Calogero Surrenti, Sergio Gorini, Carlo Alberto Maggi, and Alberto Meli

SENSORY NERVES AND NEUROPEPTIDES IN GASTROENTEROLOGY

From Basic Science to Clinical Perspectives

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Recent Volumes in this Series

Volume 291
FUEL HOMEOSTASIS AND THE NERVOUS SYSTEM
Edited by Mladen Vranic, Suad Efendic, and Charles H. Hollenberg

Volume 292
MECHANISMS OF LYMPHOCYTE ACTIVATION AND IMMUNE REGULATION III
Edited by Sudhir Gupta, William E. Paul, Max D. Cooper, and Ellen V. Rothenberg

Volume 293
MOLECULAR BIOLOGY AND PHYSIOLOGY OF INSULIN AND INSULIN-LIKE GROWTH FACTORS
Edited by Mohan K. Raizada and Derek LeRoith

Volume 294
KYNURENINE AND SEROTONIN PATHWAYS: Progress in Tryptophan Research
Edited by Robert Schwarcz, Simon N. Young, and Raymond R. Brown

Volume 295
THE BASAL FOREBRAIN: Anatomy to Function
Edited by T. Celeste Napier, Peter W. Kalivas, and Israel Hanin

Volume 296
PLASTICITY AND REGENERATION OF THE NERVOUS SYSTEM
Edited by Paola S. Timiras, Alain Privat, Ezio Giacobini, Jean Lauder, and Antonia Vernadakis

Volume 297
NEW ASPECTS OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES
Edited by W. H. Hörl and P. J. Schollmeyer

Volume 298
SENSORY NERVES AND NEUROPEPTIDES IN GASTROENTEROLOGY: From Basic Science to Clinical Perspectives
Edited by Marcello Costa, Calogero Surrenti, Sergio Gorini, Carlo Alberto Maggi, and Alberto Meli

---

SENSORY NERVES AND NEUROPEPTIDES IN GASTROENTEROLOGY

From Basic Science to Clinical Perspectives

Edited by

Marcello Costa
Flinders University of South Australia
Adelaide, Australia

Calogero Surrenti
University of Florence
Florence, Italy

Sergio Gorini
Fondazione Internazionale Menarini
Florence, Italy

Carlo Alberto Maggi and
Alberto Meli
A. Menarini Pharmaceuticals
Florence, Italy

Springer Science+Business Media, LLC

Library of Congress Cataloging in Publication Data

International Meeting on Sensory Nerves and Neuropeptides in Gastroenterology (1st: 1989: Florence, Italy)

Sensory nerves and neuropeptides in gastroenterology: from basic science to clinical perspectives / edited by Marcello Costa . . . [et al.].

p. cm. — (Advances in experimental medicine and biology; v. 298)

"Proceedings of the First International Meeting on Sensory Nerves and Neuropeptides in Gastroenterology, held December 4–5, 1989, in Florence, Italy" — T.p. verso.

Includes bibliographical references and index.

1. Gastrointestinal system — Innervation — Congresses. 2. Afferent pathways — Congresses. 3. Gastrointestinal hormones — Congresses. 4. Neuropeptides — Congresses. I. Costa, Marcello. II. Title. III. Series.

[DNLM: 1. Gastrointestinal System — innervation — congresses. 2. Neurons, Afferent — congresses. 3. Neuropeptides — congresses. 4. Receptors, Sensory — congresses. WI I613s 1989]

QP145.I48 1989

599'.013 — dc20

DNLM/DLC 91-3586

for Library of Congress CIP

Proceedings of the First International Meeting on
Sensory Nerves and Neuropeptides in Gastroenterology
held December 4–5, 1989, in Florence, Italy

ISBN 978-1-4899-0746-2 ISBN 978-1-4899-0744-8 (eBook)
DOI 10.1007/978-1-4899-0744-8

Originally published by Plenum Press, New York in 1991.
Softcover reprint of the hardcover 1st edition 1991

PREFACE

In the past 15 years much evidence has accumulated which indicates the paramount importance of sensory nerves in regulating functions of the gastrointestinal tract. In parallel, the attention of researchers in this field has been increasingly attracted to the role played by neuropeptides in the normal and diseased gut. Basic research on the peculiar properties of capsaicin, the pungent ingredient from plants of the genus Capsicum, has allowed the gap between these two areas of research to be bridged. Since then, the study of gut afferents and neuropeptides has become more and more interconnected and recognized as a major avenue to understanding the pathophysiology of various human diseases. It is widely recognized that a certain subset of primary afferents synthesize, store and release neuropeptides (such as tachykinnins and calcitonin gene-related peptide) from their central and peripheral endings, the latter being widely distributed in the alimentary canal and related organs (liver, pancreas).

The First International Meeting on Sensory Nerves and Neuropeptides in Gastroenterology, held in Florence from December 4-5, 1989, sponsored and organized by Fondazione Internazionale Menarini, aimed to focus the current status of research in this field. The contributions presented at the meeting and in this book delineate a suggestive scenario in which sensory nerves of the gut, and the multiple messages they carry through the release of neuropeptides, are to be considered as a major target for the development of new drugs potentially useful in a number of diseases of the gastrointestinal tract.

We are indebted to Donata Brugioni and Katherine Fay Loparco for the editorial assistance they provided. This volume could not have been published had we not had the cooperation of each of the contributors, for which we are sincerely thankful.

The Editors

C O N T E N T S

SENSORY NERVES AND NEUROPEPTIDES IN GASTROENTEROLOGY

From Basic Science to Clinical Perspectives

CAPSAICIN AS A TOOL FOR STUDYING SENSORY NEURON FUNCTIONS

Peter Holzer

Department of Experimental and Clinical Pharmacology
University of Graz
Graz, Austria

Introduction

It was only by the middle of this century that the extent of the sensory innervation of visceral organs including the gastrointestinal tract was revealed. Quantitative analysis showed that as much as 90% of the fibers in the vagus nerve are of afferent nature and that also in the splanchnic and pelvic nerves the afferent-to-efferent fiber ratio is 3:1 and 1:1, respectively (see Leek, 1977). These sensory neurons are primary afferent neurons, the vagal afferents having their cell bodies in the nodose ganglion and the splanchnic and pelvic afferents arising from the dorsal root (spinal) ganglia. The sensory nervous system is thought of as a receptive and afferent system that reflexly activates efferent pathways and thereby enables the organism to react to changes in the external and internal environment and to maintain homeostasis. In addition, there is evidence that a population of peptide-containing afferent neurons can act as an effector system by itself (see Szolcsányi, 1984b; Holzer, 1988; Maggi and Meli 1988).

Much of the current information on the histochemistry, physiology and pathophysiology of primary afferent neurons has been obtained in the last 15 years. This resulted not only from the advent of new experimental techniques but also from the availability of a special pharmacological tool with which to study the functional implications of sensory neurons. This tool is capsaicin. The present article attempts to briefly review the value of capsaicin as a tool in sensory neuroscience and the selectivity with which it acts on a population of sensory neurons and to address some of the limitations of its usefulness.

Capsaicin is the pungent ingredient in a wide variety of red peppers of the genus Capsicum. Although capsaicin had long been recognized as a strong irritant to the skin, it was not before the middle of this century that the Hungarian investigator N. Jancsó realized that capsaicin defunctionalizes a certain group of sensory neurons (see Szolcsányi, 1984a). Based on this discovery, a large number of morphological, histochemical and neuropharmacological studies has established capsaicin as an important probe for sensory neuron mechanisms. Certain aspects of this research on capsaicin have been summarized in various re-

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

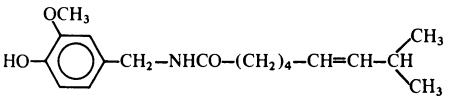


Fig. 1. Chemical structure of capsaicin

views (Nagy, 1982; Szolcsányi, 1982, 1984a, 1984b; Fitzgerald, 1983; Russell and Burchiel, 1984; Marley and Livett, 1985; Buck and Burks, 1986; Chahl, 1988; Holzer, 1988; Maggi and Meli, 1988) which the interested reader is referred to.

Capsaicin-Sensitive Afferent Neurons

Chemically, capsaicin is a derivative of vanillyl-amide, 8-methyl-N-vanillyl-6-nonenamide (Figure 1). It is two actions which this drug exerts on sensory neurons, an acute excitatory and a long-term neurotoxic effect. I am first defining the neurons which are affected by capsaicin and then will move on to describe the actions of capsaicin in some detail. It has become common usage to refer to those neurons which are stimulated and subsequently defunctionalized by capsaicin as being capsaicin-sensitive sensory neurons. Inherent in this way of classification is a quantitative definition of the doses or concentrations of capsaicin that are active on these neurons. This issue will be addressed in the section describing the actions of capsaicin. It furthermore is important to characterize capsaicin-responsive afferent neurons within the framework of established morphological and neurophysiological criteria. Capsaicin-sensitive sensory neurons are primary afferent neurons the majority of which has small dark cell bodies (Szolcsányi et al., 1975; Jancsó et al., 1977, 1985; Lawson and Harper, 1984; Kai-Kai et al., 1986; Arvidsson and Ygge, 1986; Hiura and Sakamoto, 1987; Winter, 1987) although a minority of afferent neurons with large light cell bodies also is sensitive to capsaicin (Lawson and Harper, 1984). The cell bodies of capsaicin-sensitive sensory neurons lie in the spinal and cranial sensory ganglia and give rise to either unmyelinated or thinly myelinated nerve fibers (Jancsó et al., 1977; Scadding, 1980; Nagy et al., 1981, 1983; Lawson and Harper, 1984; Arvidsson and Ygge, 1986; Hiura and Sakamoto, 1987) which conduct in the C- and Aδ-fiber range, respectively (Szolcsányi, 1977, 1984b, 1987; Foster and Ramage, 1981; Kenins, 1982; Petsche et al., 1983; Handwerker et al., 1984; Lawson and Harper, 1984; Lynn et al., 1984; Baranowski et al., 1986; Szolcsányi et al., 1988). A further characteristic of capsaicin-sensitive afferent neurons is that they contain a variety of peptides which are thought to play a transmitter or mediator role. The best-known among these peptides are substance P, neurokinin A and CGRP but there is a long list of other peptides, including somatostatin, vasoactive intestinal polypeptide and galanin, which are associated with these afferent neurons (see Marley and Livett, 1985; Holzer, 1988; Maggi and Meli, 1988).

These features of capsaicin-sensitive afferent neurons exemplify the difficulties and limitations in their classification. The main problem consists in the fact that capsaicin-sensitive afferent neurons do not completely overlap with any population of afferent neurons that have been classified according to morphological, neurochemical or functional criteria. Thus, not all

unmyelinated afferent neurons conducting in the C-fiber range are sensitive to capsaicin (Jancsó et al., 1977; Scadding, 1980; Nagy et al., 1981, 1982; Handwerker et al., 1984; Lawson and Harper, 1984; Lynn et al., 1984; Arvidsson and Ygge, 1986; Hiura and Sakamoto, 1987). Furthermore, not all afferent neurons containing substance P, CGRP or other peptides also are sensitive to capsaicin (see Marley and Livett, 1985; Holzer, 1986; Maggi and Meli, 1988). As a corollary, these peptides cannot be used as exclusive neurochemical markers of capsaicin-sensitive afferent neurons, and there is in fact no specific histochemical marker for this group of neurons (see Lawson and Harper, 1984; Kirchgessner et al., 1988). Finally, capsaicin-sensitive afferent neurons are heterogeneous in terms of their sensory modality and the functions they subserve. The only consensus that has emerged is that capsaicin-sensitive sensory neurons are chemosensitive neurons (Jancsó et al., 1977; Hayes and Tyers, 1980; Gamse, 1982; Lundberg and Saria, 1983; Szolcsányi, 1984b; Stein et al., 1986; Saria et al, 1988; Bevan and Yeats, 1989). The somatic capsaicin-sensitive afferents correspond to a large degree with polymodal nociceptors sensitive to chemical, thermal and mechanical stimuli (Szolcsányi, 1977, 1984b, 1987; Foster and Ramage, 1981; Kenins, 1982; Petsche et al., 1983, Handwerker et al., 1984; Lawson and Harper, 1984; Lynn et al., 1984; Szolcsányi et al., 1988). Visceral capsaicin-sensitive afferent neurons have been found to respond to noxious (Cervero and McRitchie, 1982; Longhurst et al., 1980; Szolcsányi, 1984b; Stein et al., 1986) as well as non-noxious "physiological" stimuli (Raybould and Taché, 1988; South and Ritter, 1988; Rózsa and Jacobson, 1989).

Given the uncertainties with which capsaicin-sensitive sensory neurons are defined it appears appropriate to sharpen their classification by specifically naming those neurons which are capsaicin-insensitive. There is consistent evidence that afferent neurons with thickly myelinated axons, conducting in the Aα and Aβ range, are not sensitive to capsaicin, at least not to the long-term neurotoxic action of this compound (for reviews see Handwerker et al., 1984; Lawson and Harper, 1984; Lynn et al., 1984; Russell and Burchiel, 1984; Szolcsányi, 1984b; Buck and Burks, 1986). However, capsaicin does exert a transient effect on some thickly myelinated afferents (Williams and Zieglgänsberger, 1982; Lynn et al., 1984; Baranowski et al., 1986; Marsh et al., 1987). Central neurons also seem to lack any sensitivity to capsaicin, with the exception of a group of thermosensitive neurons in the preoptic region of the hypothalamus (Szolcsányi et al., 1971) and some neurons in other areas of the brain (Ritter and Dinh, 1988). There is thus far no indication that efferent motor neurons and efferent neurons of the autonomic nervous system are directly sensitive to the neurotoxic action of capsaicin (Cervero and McRitchie, 1982; Gamse et al., 1982; Handwerker et al., 1984; Stein et al., 1986). The enteric nervous system also is believed to be insensitive to capsaicin (see Barthó and Holzer, 1985) although two reports suggest that a minority of enteric neurons may be sensitive to the drug (Fehér and Vajda, 1982; Kirchgessner et al., 1988).

The conclusion to be drawn from all these findings is that the selectivity with which capsaicin acts on a group of primary afferent neurons is exceptional but not absolute. The "non-selective" effects of capsaicin can be differentiated into three different types of action. (1) Capsaicin can affect neural and

non-neural systems that are functionally related to primary afferent neurons. Capsaicin-induced changes in these systems are interpreted to be secondary to the action of capsaicin on primary afferent neurons. These effects of capsaicin will be discussed in more detail in the section describing the long-term neurotoxic action of capsaicin on sensory neurons. (2) Certain actions of capsaicin on non-sensory neural systems can at present not be explained as being secondary consequences of an effect on primary sensory neurons. Examples for this category of "non-selective action" of capsaicin are the effects of capsaicin on certain neurons in the hypothalamus (Szolcsányi et al., 1971; Panerai et al., 1983) and other brain areas (Ritter and Dinh, 1988) and on enteric neurons (Fehér and Vajda, 1982; Kirchgessner et al., 1988). (3) Finally, capsaicin can directly influence a number of non-neural systems. This type of "non-selective" action of capsaicin includes, among others, changes in the contractility of vascular and visceral smooth muscle (Donnerer and Lembeck, 1982; Duckles, 1986; Barthó et al., 1987; Maggi et al., 1987).

Taken together, the acute excitatory and long-term neurotoxic action of capsaicin is not specific for a discrete group of sensory neurons but is highly selective for a subpopulation of thin primary afferent neurons. The majority of capsaicin-sensitive sensory neurons is constituted by neurons having small dark cell bodies and unmyelinated (C fiber) axons.

Acute Excitatory Action of Capsaicin on Sensory Neurons

As already briefly mentioned, there are basically two actions which capsaicin exerts on sensory neurons. On first contact with the drug, sensory neurons are invariably stimulated by capsaicin, and there seems to be no difference whether the drug is applied to the peripheral or central endings or to the cell bodies of sensory neurons. Capsaicin is quite potent in this respect, its typical actions being produced by μg/kg doses in vivo (see Szolcsányi, 1977, 1987) or concentrations in the range of 0.03-10 μM in vitro (Barthó and Szolcsányi, 1978; Maggi et al., 1987; Marsh et al., 1987; Saria et al., 1988; Wood et al., 1988). Characteristically, excitation is soon followed by desensitization to the drug (Barthó and Szolcsányi, 1978; Maggi and Meli, 1988). Like administration of capsaicin to the peripheral endings (Szolcsányi, 1977, 1987; Szolcsányi et al., 1988), perineural application of the drug also depolarizes sensory nerve fibers (Such and Jancsó, 1986; Marsh et al., 1987), an effect that is soon followed by a block of nerve conduction (Petsche et al., 1983; Lynn et al., 1984; Baranowski et al., 1986; Marsh et al., 1987). Similar responses are observed when capsaicin is applied to the cell bodies (Williams and Zieglgänsberger, 1982; Heyman and Rang, 1985; Bevan et al., 1987; Bevan and Yeats, 1989; Petersen et al., 1989) or dorsal roots of sensory neurons (Ault and Evans, 1980; Williams and Zieglgänsberger, 1982).

Ever since the selectivity of capsaicin for a subpopulation of afferent neurons was unravelled investigators were intrigued by the mechanism of its action. The finding of a structure-activity relationship pointed to a specific capsaicin receptor (see Szolcsányi, 1982) the existence of which, however, has not yet been proved because of a number of reasons including technical problems. Very recently, though, specific binding of resiniferatoxin, an ultrapotent analog of capsaicin (Szallasi and Blum-

berg, 1989a), to dorsal root ganglia membranes has been reported (Szallasi and Blumberg, 1989b). It is not the intent of this article to review the mechanisms of action of capsaicin in detail, but recent studies indicate that the selective excitatory and subsequent neurotoxic action of capsaicin on thin sensory neurons involves an increase in the membrane permeability for cations and an overwhelming accumulation of calcium within the cell (Bevan et al., 1987; Marsh et al., 1987; Wood et al., 1988; Bevan and Yeats, 1989). Calcium removal or administration of ruthenium red, a compound interfering with the cellular handling of calcium at multiple sites, is able to selectively block the excitatory and desensitizing effects of capsaicin (Marsh et al., 1987; Maggi et al., 1988; Amann et al., 1989).

Functionally the excitatory action of capsaicin on sensory neurons has two immediate consequences: on the one hand, nerve activity is conducted to the central nervous system and, on the other hand, peptides are released from the activated peripheral nerve endings themselves (for review of this concept see Szolcsányi, 1984b; Holzer, 1988; Maggi and Meli, 1988). The afferent function enables information being transmitted to the central nervous system. The local release of peptide mediators from their peripheral nerve endings reflects a local effector function (Holzer, 1988) of these neurons, as the released peptides are able to influence a variety of local tissue functions. These include local blood flow, vascular permeability, cardiac and smooth muscle activity, tissue growth and repair and immunologic processes (see Holzer, 1988; Maggi and Meli, 1988).

Further analysis of the local effector function of sensory neurons has revealed two different mechanisms depending on whether or not nerve conduction is involved (see Szolcsányi, 1984a). The first mechanism implies that excitation of sensory nerve endings results in the release of peptides from the nerve endings stimulated by capsaicin. This process does not involve conduction of nerve activity and is therefore not inhibited by local anesthetics or tetrodotoxin (Jancsó et al., 1968; Maggi et al., 1987). The second mechanism depends on the assumption that sensory nerve fibers in the periphery give off several branches. When one of these branches is stimulated by capsaicin, nerve activity will travel along this branch to the branching point and then can travel both to the central nervous system and to the periphery in the other branches. When nerve impulses arrive in the terminal region of these branches they again will release peptides from the nerve ending varicosities. This process is called an axon reflex and enables excitation to spread to all nerve branches of a given sensory fiber. As this process involves nerve conduction it is blocked by tetrodotoxin (see Holzer, 1988).

Long-Term Neurotoxic Effect of Capsaicin on Sensory Neurons

The long-term neurotoxic action of capsaicin is produced by local or systemic administration of capsaicin. For experimental purposes, either high doses of capsaicin (in the range of 50 mg/kg or more) are administered by the subcutaneous route (Jancsó et al., 1967, 1977; Szolcsányi et al., 1975; Gamse et al., 1980; Hayes and Tyers, 1980; Nagy et al., 1981, 1983; Buck et al., 1983) or a 1% solution of capsaicin is administered perineurally (see below). Systemic administration of capsaicin produces, after an initial excitation, a long-lasting elimina-

tion of primary afferent neurons sensitive to this drug (Jancsó et al., 1967, 1977; Szolcsányi et al., 1975; Gamse et al., 1980; Hayes and Tyers, 1980; Nagy et al., 1981, 1983; Buck et al., 1983). This ablation lasts for months, if it is not permanent, and is reflected by functional, neurochemical and morphological alterations of the respective neurons. Analysis of the temporal sequence with which these changes take place showed that the neurotoxic action of capsaicin first becomes manifest by defunctionalization of, and morphologic damage to, sensory neurons (Jancsó et al., 1967, 1977; Szolcsányi et al, 1975; Lembeck and Donnerer, 1981; Petsche et al., 1983; Lynn et al., 1984; Marsh et al., 1987). The resulting sensory and functional deficits are used experimentally to obtain information as to the physiological and/or pathophysiological implications of capsaicin-sensitive sensory neurons (for recent reviews see Buck and Burks, 1986; Maggi and Meli, 1986, 1988; Lundberg and Saria, 1987; Chahl, 1988; Holzer, 1988). The neurochemical alterations produced by capsaicin are reflected by a long-lasting depletion of neuropeptide and other markers from sensory neurons (see e.g. Jessell et al., 1978; Gamse et al., 1980; Jancsó et al., 1981a; Nagy et al., 1981, 1983; Buck et al., 1983; Lundberg et al., 1985; Skofitsch and Jacobowitz, 1985; Theodorsson-Norheim et al., 1985; Kirchgessner et al., 1988). In the rat, these neurochemical responses to capsaicin become evident much later than the defunctionalization of sensory neurons (Lembeck and Donnerer, 1981).

The severity of long-term morphological changes produced by capsaicin in sensory neurons of the rat varies with the age of the animals at the time they are treated with the drug. When capsaicin is administered to newborn rats, in which sensory and other neurons are not yet fully developed, up to 95% of all unmyelinated afferent neurons may degenerate (Jancsó et al., 1977; Scadding, 1980; Nagy et al., 1981, 1983). In contrast, administration of neurotoxic doses of capsaicin to adult rats produces degeneration of only a minority of cell bodies in the sensory ganglia and their fibers (Jancsó et al., 1985; Lynn et al., 1987) whereas the peripheral axons of unmyelinated sensory fibers appear to undergo extensive degeneration (Hoyes and Barber, 1981; Chung et al., 1985). In addition, ultrastructural changes in the surviving sensory neurons also are evident (Szolcsányi et al., 1975; Handwerker et al., 1984; Lynn et al., 1984; Chiba et al., 1986; Marsh et al., 1987). It is not unexpected, therefore, that functionally and neurochemically the degrees of sensory neuron ablation do not necessarily differ after treatment of newborn or adult rats. There is some evidence, though, that functional ablation of sensory neurons induced by capsaicin given to newborn rats is more profound than when given to adult rats (see Maggi and Meli, 1988).

The different morphological consequences of capsaicin administration to newborn or adult rats are important to consider in another respect. Substantial degeneration of a population of neurons at a stage when the nervous system is not yet fully developed is likely to have a much more significant impact on the whole system than ablation of these neurons at a stage when the nervous system has been fully developed. Indeed, capsaicin treatment of newborn rats changes cellular systems that are in functional connection with either the central or peripheral terminals of primary sensory neurons. Such changes have been observed e.g. in the spinal cord (Virus et al., 1983; Hajós et

al., 1986; Réthelyi et al., 1986), the spinothalamic tract (Saporta, 1986) and the somatotopic maps of the cerebral cortex (Wall et al., 1982). Histologic alterations were also seen in peripheral tissues including the cornea (Shimizu et al., 1987) and lung (Ahlstedt et al., 1986). These changes are thought to be secondary to the capsaicin-induced degeneration of sensory neurons. When treating rats with capsaicin as neonates one has to be aware, therefore, that not only primary afferent neurons but also other sensory pathway-related systems might be altered to a significant extent.

It is important to note that there are profound species differences in the sensitivity to capsaicin. Most of the studies on capsaicin-sensitive sensory neurons have been performed in small rodents (rat, mouse, guinea-pig). In these species, treatment of adult animals with doses of capsaicin in the range of 50-125 mg/kg is sufficient to produce maximal ablation of capsaicin-sensitive neurons. There is no need to use higher doses, and the use of several hundreds of mg/kg capsaicin (Jessell et al., 1978) may in fact be prone to produce avoidable non-selective effects of capsaicin. Treatment of newborn rats with a dose of 50 mg/kg capsaicin appears to be selective for unmyelinated afferent nerve fibers and small dark cell bodies in the sensory ganglia (Jancsó et al., 1977; Nagy et al., 1981) whereas doses in excess of 50 mg/kg also cause degeneration of some myelinated afferents and large light cell bodies (Nagy et al., 1983; Lawson and Harper, 1984). The capsaicin sensitivity of cat (Gamse et al., 1982) and man (Jancsó et al., 1968; Szolcsányi , 1977; Carpenter and Lynn, 1981) also is well established whereas rabbits are clearly less sensitive to capsaicin (Gamse et al., 1982; Baranowski et al., 1986; Lynn and Shakhanbeh, 1988) than rats or guinea-pigs. Birds and frogs are insensitive to capsaicin (see Szolcsányi et al., 1986).

Systemic treatment with capsaicin has been used extensively to investigate the functional implications of sensory neurons. It is clear that this route of administration will eliminate all somatic and visceral afferent neurons sensitive to the drug. There is another possibility by which one can ablate capsaicin-sensitive afferent neurons in a region-selective manner, namely by local periaxonal treament of nerves supplying the region under study (Jancsó et al., 1981b; Gamse et al., 1982; Petsche et al., 1983; Raybould and Taché, 1988). Treatment of the vagus nerve with a 1% solution, for instance, has enabled to study the role of vagal afferent nerve fibers in cardiovascular (Jancsó and Such, 1983) and gastric (Raybould and Taché, 1988) functions.

Acute versus Long-Term Effects of Capsaicin

The investigation of sensory neuron functions can make use of both the acute excitatory and long-term neurotoxic actions of capsaicin. I have to point out, though, that the degree of selectivity with which capsaicin influences sensory neurons differs for the acute and long-term actions of capsaicin. The acute effects of capsaicin are less selective for thin sensory neurons in that the drug also can transiently stimulate and block thickly myelinated afferents (Williams and Zieglgänsberger, 1982; Lynn et al., 1984; Baranowski et al., 1986; Such and Jancsó, 1986; Barthó et al., 1987; Maggi et al., 1987). Thus, whenever acute actions of capsaicin are used to probe functions of thin

sensory neurons it is necessary to check whether these actions are indeed due to stimulation of thin afferent neurons. Many of the non-selective actions of capsaicin do not show desensitization and are not abolished after ablation of capsaicin-sensitive afferent neurons (Barthó et al., 1987; Maggi et al., 1987).

Summary

The exceptional selectivity with which capsaicin acts on a population of peptide-containing thin primary afferent neurons has made this drug an important tool with which to investigate the neuroanatomical, neurochemical and functional implications of these neurons. As a consequence, the use of capsaicin has enabled a substantial furthering of our understanding of the physiological and pathophysiological roles of thin primary sensory neurons. With appropriate controls, both the acute excitatory and long-term neurotoxic actions of capsaicin can be utilized in these studies but it is important to know the advantages and disadvantages and the limitations of each of the different experimental approaches. Table 1 is a brief checklist of the caveats that should be considered and that have been dealt with in this article.

TABLE 1

CAPSAICIN AS A PROBE FOR SENSORY NEURON FUNCTIONS: CAVEATS TO BE CONSIDERED

1. Limitations in the classification of capsaicin-sensitive afferent neurons
   - 1.1. Not all unmyelinated afferent neurons are capsaicin-sensitive.
   - 1.2. Not all afferent neurons containing substance P, CGRP and other peptide markers are capsaicin-sensitive.
   - 1.3. Capsaicin-sensitive afferent neurons are heterogeneous in terms of their sensory modality.
   - 1.4. The group of capsaicin-sensitive afferent neurons does not overlap with any group of afferent neurons defined according to morphological, neurochemical or functional criteria.
2. There are species differences in the sensitivity to capsaicin
3. The acute effects of capsaicin are less selective for thin sensory neurons than its long-term effects
   - 3.1. Capsaicin has a transient excitatory and blocking action on some thickly myelinated afferent neurons and possibly other neuronal systems.
   - 3.2. Capsaicin can have a transient influence on the activity of non-neuronal cells. These effects do not show desensitization.
   - 3.3. The acute effects of capsaicin may be considered selective for thin afferent neurons when they are abolished after pretreatment with capsaicin.
4. Differences in the neurotoxic effects of capsaicin administered to newborn versus adult rats
   - 4.1. The sensory neurons in newborn rats are not yet fully developed and up to 95% of the unmyelinated afferents can degenerate in response to capsaicin.
   - 4.2. Deafferentation in newborn rats may result in secondary

changes in the neuronal and non-neuronal systems that are functionally linked to sensory neurons.

4.3. In adult rats capsaicin also produces functional and neurochemical ablation of thin sensory neurons. Extensive degeneration, however, is seen only with the peripheral axons of sensory neurons. Secondary changes in sensory neuron-related systems are less likely to occur.

Acknowledgements

The research performed in the author's laboratory was supported by the Austrian Scientific Research Funds.

References

Ahlstedt, S., Alving, K., Hesselmar, B., Olaisson, E., 1986, Enhancement of the bronchial reactivity in immunized rats by neonatal treatment with capsaicin, **Int. Arch. Allergy Appl. Immunol.**, 80:262.

Amann, R., Donnerer, J., Lembeck, F., 1989, Capsaicin-induced stimulation of polymodal nociceptors is antagonized by ruthenium red independently of extracellular calcium, **Neuroscience**, 32:255.

Arvidsson, J., Ygge, J., 1986, A quantitative study of the effects of neonatal capsaicin treatment and of subsequent peripheral nerve transection in the adult rat, **Brain Res.**, 397:130.

Ault, B., Evans, H., 1980, Depolarizing action of capsaicin on isolated dorsal root fibres of the rat, **J. Physiol. (London)**, 306:22P.

Baranowski, R., Lynn, B., Pini, A., 1986, The effects of locally applied capsaicin on conduction in cutaneous nerves of four mammalian species, **Br. J. Pharmacol.**, 89:267.

Barthó, L., Holzer, P., 1985, Search for a physiological role of substance P in gastrointestinal motility, **Neuroscience**, 16:1.

Barthó, L. Pethö, G., Antal, A., Holzer, P., Szolcsányi J., 1987, Two types of relaxation due to capsaicin in the guinea-pig isolated ileum, **Neurosci. Lett.**, 81:146.

Barthó, L., Szolcsányi, J., 1978, The site of action of capsaicin on the guinea-pig isolated ileum, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 305:75.

Bevan, S.J., James, I.F., Rang, H.P., Winter, J. Wood, J.N., 1987, The mechanism of action of capsaicin - a sensory neurotoxin, in: "Neurotoxins and Their Pharmacological Implications", P. Jenner, ed., Raven Press, New York.

Bevan, S., Yeats, J.C., 1989, Protons activate a sustained inward current in a subpopulation of rat isolated dorsal root ganglion (DRG) neurones, **J. Physiol. (London)**, 417:81P.

Buck, S.H. Burks, T.F., 1986, The neuropharmacology of capsaicin - review of some recent observations, **Pharmacol. Rev.**, 38:179.

Buck, S.H., Walsh, J.H., Davis T.P., Brown, M.R., Yamamura, H.I., Burks, T.F., 1983, Characterization of the peptide and sensory neurotoxic effects of capsaicin in the guinea pig, **J. Neurosci.**, 3:2064.

Carpenter, S.E., Lynn, B., 1981, Vascular and sensory response of human skin to mild injury after topical treatment with capsaicin, **Br. J. Pharmacol.**, 73:755.

Cervero, F., McRitchie, H.A., 1982, Neonatal capsaicin does not affect unmyelinated efferent fibers of the autonomic nervous system: functional evidence, **Brain Res.**, 239:283.
Chahl, L.A., 1988, Antidromic vasodilation and neurogenic inflammation, **Pharmacol. Ther.**, 37:275.
Chiba, T., Masuko, S., Kawano, H., 1986, Correlation of mitochondrial swelling after capsaicin treatment and substance P and somatostatin immunoreactivity in small neurons of dorsal root ganglion in the rat, **Neurosci. Lett.**, 64:311.
Chung, K., Schwen, R.J., Coggeshall, R.E., 1985, Ureteral axon damage following subcutaneous administration of capsaicin in adult rats, **Neurosci. Lett.**, 53:221.
Donnerer, J., Lembeck, F., 1982, Analysis of the effects of intravenously injected capsaicin in the rat, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 320:54.
Duckles, S.P., 1986, Effects of capsaicin on vascular smooth muscle, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 333:59.
Fehér, E., Vajda, J., 1982, Effect of capsaicin on the nerve elements of the small intestine, **Acta Morphol. Acad. Sci. Hung.**, 30:57.
Fitzgerald, M., 1983, Capsaicin and sensory neurones - a review, **Pain**, 15:109.
Foster, R.W., Ramage, A.G., 1981, The action of some chemical irritants on somatosensory receptors of the cat, **Neuropharmacology**, 20:191.
Gamse, R., 1982, Capsaicin and nociception in the rat and mouse. Possible role of substance P, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 320.205.
Gamse, R., Holzer, P., and Lembeck, F., 1980, Decrease of substance P in primary sensory neurones and impairment of neurogenic plasma extravasation by capsaicin, **Br. J. Pharmacol.**, 68:207.
Gamse, R., Petsche, U., Lembeck, F., Jancsó, G., 1982, Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin, **Brain Res.**, 239:447.
Hajós, M., Svensson, K., Nissbrandt, H., Obál, F., Carlsson, A., 1986, Effects of capsaicin on central monoaminergic mechanisms in the rat, **J. Neural. Transm.**, 66:221.
Handwerker, H.O., Holzer-Petsche, U., Heym, C., Welk, E., 1984, C-fibre functions after topical application of capsaicin to a peripheral nerve and after neonatal capsaicin treatment, in: "Antidromic Vasodilatation and Neurogenic Inflammation", L.A. Chahl, J. Szolcsányi, F. Lembeck, eds., Akadémiai Kiadó, Budapest.
Hayes, A.G., Tyers, M.B., 1980, Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat, **Brain Res.**, 189:561.
Heyman, I., Rang, H.P., 1985, Depolarizing responses to capsaicin in a subpopulation of rat dorsal root ganglion cells, **Neurosci. Lett.**, 56:69.
Hiura, A., Sakamoto, Y., 1987, Quantitative estimation of the effect of capsaicin on the mouse primary sensory neurons, **Neurosci. Lett.**, 76:101.
Holzer, P., 1988, Local effector functions of capsaicin-sensitive nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.
Hoyes, A.D., Barber, P., 1981, Degeneration of axons in the ureteric and duodenal nerve plexuses of the adult rat following in vivo treatment with capsaicin, **Neurosci. Lett.**, 25:19.
Jancsó, G., Hökfelt, T., Lundberg, J.M., Király, E., Halász, N.,

Nilsson, G., Terenius, L., Rehfeld, J. Steinbusch, H., Verhofstad, A.E.R., Said, S., Brown, M., 1981a, Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptide and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin and 5-hydroxytryptamine, **J. Neurocytol.**, 10: 963.

Jancsó, G., Király, E., Jancsó-Gábor, A., 1977, Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones, **Nature**, 270:741.

Jancsó, G., Király, E., Jancsó-Gábor, A., 1981b, Direct evidence for an axonal site of action of capsaicin, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 313:91.

Jancsó, G., Király, E., Joó, F., Such, G., Nagy, A., 1985, Selective degeneration by capsaicin of a subpopulation of primary sensory neurones in the adult rat, **Neurosci. Lett.**, 59:209.

Jancsó, G., Such, G., 1983, Effects of capsaicin applied perineurally to the vagus nerve on cardiovascular and respiratory functions in the cat, **J. Physiol. (London)**, 341:359.

Jancsó, N., Jancsó-Gábor, A., Szolcsányi, J., 1967, Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin, **Br. J. Pharmacol.**, 31:138.

Jancsó, N., Jancsó-Gábor, A., Szolcsányi, J., 1968, The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat, **Br. J. Pharmacol.**, 32:32.

Jessell, T.M., Iversen, L.L., Cuello, A.C., 1978, Capsaicin-induced depletion of substance P from primary sensory neurones, **Brain Res.**, 152:183.

Kai-Kai, M.A., Anderton, B.H., Keen, P., 1986, A quantitative analysis of the interrelationships between subpopulations of rat sensory neurons containing arginine vasopressin or oxytocin and those containing substance P, fluoride-resistant acid phosphatase or neurofilament protein, **Neuroscience**, 18:475.

Kenins, P., 1982, Responses of single nerve fibres to capsaicin applied to the skin, **Neurosci. Lett.**, 29:83.

Kirchgessner, A.L., Dodd, J., Gershon, M.D., 1988, Markers shared between dorsal root and enteric ganglia, **J. Comp. Neurol.**, 276:607.

Lawson, S.N., Harper, A.A., 1984, Neonatal capsaicin is not a specific neurotoxin for sensory C-fibres or small dark cells of rat dorsal root ganglia, in: "Antidromic Vasodilatation and Neurogenic Inflammation", L.A. Chahl, J. Szolcsányi, F. Lembeck, eds., Akadémiai Kiadó, Budapest.

Leek, B.F., 1977, Abdominal and pelvic visceral receptors, **Br. Med. Bull.**, 33:163.

Lembeck, F., Donnerer, J., 1981, Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content, **Naunyn-Schiedeberg's Arch. Pharmacol.**, 316:240.

Longhurst, J.C., Ashton, J.W., Iwamoto, G.A., 1980, Cardiovascular reflexes resulting from capsaicin-stimulated gastric receptors in anesthetized dogs, **Circ. Res.**, 46:780.

Lundberg, J.M., Franco-Cereceda A., Hua, X.-Y., Hökfelt, T., Fischer, J., 1985, Co-existence of substance P and calcitonin gene-related peptide immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin, **Eur. J. Pharmacol.**, 108:315.

Lundberg, J.M., Saria, A., 1983, Capsaicin-induced desensitiza-

tion of the airway mucosa to cigarette smoke, mechanical and chemical irritants, **Nature**, 302:251.
Lundberg, J.M., Saria, A., 1987, Polypeptide-containing neurons in airway smooth muscle, **Ann. Rev. Physiol.**, 49:557.
Lynn, B., Carpenter, S.E., Pini, A., 1984, Capsaicin and cutaneous afferents, in: "Antidromic Vasodilatation and Neurogenic Inflammation", L.A. Chahl, J. Szolcsányi, F. Lembeck, eds., Akadémiai Kiadó, Budapest.
Lynn, B., Pini, A., Baranowski, R., 1987, Injury of somatosensory afferents by capsaicin: selectivity and failure to regenerate, in: "Effects of Injury on Trigeminal and Spinal Somatosensory Systems", L.M. Pubols, B. Sessle, eds., Alan R. Liss, New York.
Lynn, B., Shakhanbeh, J., 1988, Substance P content of the skin, neurogenic inflammation and numbers of C-fibres following capsaicin application to a cutaneous nerve in the rabbit, **Neuroscience**, 24:769.
Maggi, C.A., Meli, A., 1988, The role of neuropeptides in the regulation of the micturition reflex, **J. Auton. Pharmacol.**, 6:133.
Maggi, C.A., Meli, A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, **Gen. Pharmacol.**, 19:1.
Maggi, C.A., Meli, A., Santicioli, P., 1987, Four motor effects of capsaicin on guinea-pig distal colon, **Br. J. Pharmacol.**, 90:651.
Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Geppetti, P., Meli, A., 1988, Protective action of ruthenium red toward capsaicin desensitization of sensory fibers, **Neurosci. Lett.**, 88:201.
Marley, P., Livett, B.G., 1985, Neuropeptides in the autonomic nervous system, **CRC Crit. Rev. Clin. Neurobiol.**, 1:201.
Marsh, S.J., Stansfeld, C.E., Brown, D.A., Davey, R., McCarthy, D., 1987, The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro, **Neuroscience**, 23:275.
Nagy, J.I., 1982, Capsaicin: a chemical probe for sensory neuron mechanisms, in: "Handbook of Psychopharmacology", Vol. 15, L.L. Iversen, S.D. Iversen, S.H. Snyder, eds., Plenum, New York.
Nagy, J.I., Hunt, S.P., Iversen, L.L., Emson, P.C., 1981, Biochemical and anatomical observations on the degeneration of peptide containing primary afferent neurons after neonatal capsaicin, **Neuroscience**, 6:1923.
Nagy, J.I., Iversen, L.L., Goedert, M., Chapman, D., Hunt, S.P., 1983, Dose-dependent effects of capsaicin on primary sensory neurons in the neonatal rat, **J. Neurosci.**, 3:399.
Panerai, A.E., Martini, A., Locatelli, V., Mantegazza, P., 1983, Capsaicin decreases b-endorphin hypothalamic concentrations in the rat, **Pharmacol. Res. Commun.**, 15:825.
Petersen, M., Wagner, G., Pierau, F.-K., 1989, Modulation of calcium-currents by capsaicin in a subpopulation of sensory neurons of guinea pig, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 339:184.
Petsche, U., Fleischer, E., Lembeck, F., Handwerker, H.O., 1983, The effect of capsaicin application to a peripheral nerve on impulse conduction in functionally identified afferent nerve fibres, **Brain Res.**, 265:233.
Raybould, H.E., Taché, Y., 1988, Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats, **Am. J. Physiol.**, 255:G242.
Réthelyi, M., Salim, M.Z., Jancsó, G., 1986, Altered distribution of dorsal root fibers in the rat following neonatal capsaicin treatment, **Neuroscience**, 18:749.

Ritter, S., Dinh, T.T., 1988, Capsaicin-induced neuronal degeneration: silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat, **J. Comp. Neurol.**, 271:79.

Rózsa, Z., Jacobson, E.D., 1989, Capsaicin-sensitive nerves are involved in bile-oleate induced intestinal hyperemia, **Am. J. Physiol.**, 256:G476.

Russell, L.C., Burchiel, K.J., 1984, Neurophysiological effects of capsaicin, **Brain Res. Rev.**, 8:165.

Saporta, S., 1986, Loss of spinothalamic tract neurons following neonatal treatment of rats with the neurotoxin capsaicin, **Somatosens. Res.**, 4:153.

Saria, A., Martling, C.-R., Yan, Z., Theodorsson-Norheim, E., Gamse, R., Lundberg, J.M. 1988, Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation, **Am. Rev. Respir. Dis.**, 137:1330.

Scadding, J.W., 1980, The permanent anatomical effects of neonatal capsaicin on somatosensory nerves, **J. Anat.**, 131:473.

Shimizu, T., Izumi, K., Fujita, S., Koya, T., Sorimachi, M., Ohba, N., Fukuda, T., 1987, Capsaicin-induced corneal lesions in mice and the effects of chemical sympathectomy, **J. Pharmacol. Exp. Ther.**, 243:690.

Skofitsch, G., Jacobowitz, D.M., 1985, Galanin-like immunoreactivity in capsaicin-sensitive sensory neurons and ganglia, **Brain Res. Bull.**, 15:191.

South, E.H., Ritter, R.C., 1988, Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety, **Peptides**, 9:601.

Stein, R.D., Genovesi, S., Demarest, K.T., Weaver, L.C., 1986, Capsaicin treatment attenuates the reflex excitation of sympathetic activity caused by chemical stimulation of intestinal afferent nerves, **Brain Res.**, 397:145.

Such, G., Jancsó, G., 1986, Axonal effects of capsaicin: an electrophysiological study, **Acta Physiol. Hung.**, 67:53.

Szallasi, A., Blumberg, P.M., 1989a, Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper, **Neuroscience**, 30:515.

Szallasi, A., Blumberg, P.M., 1989b, Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, to dorsal root ganglia membranes, **Pharmacologist**, 31:P353.

Szolcsányi, J., 1977, A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain, **J. Physiol. (Paris)**, 73:251.

Szolcsányi, J., 1982, Capsaicin type pungent agents producing pyrexia, in: "Pyretics and Antipyretics", Handbook of Experimental Pharmacology, Vol. 60, A.S. Milton, ed., Springer, Berlin.

Szolcsányi, J., 1984a, Capsaicin and neurogenic inflammation: history and early findings, in: "Antidromic Vasodilatation and Neurogenic Inflammation", L.A. Chahl, J. Szolcsányi, F. Lembeck, eds., Akadémiai Kiadó, Budapest.

Szolcsányi, J., 1984b, Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function, in: "Antidromic Vasodilatation and Neurogenic Inflammation", L.A. Chahl, J. Szolcsányi, F. Lembeck, eds., Akadémiai Kiadó, Budapest.

Szolcsányi, J., 1987, Selective responsiveness of polymodal nociceptors of the rabbit ear to capsaicin, bradykinin and ultraviolet irradiation, **J. Physiol. (London)**, 388:9.

Szolcsányi, J., Anton, F., Reeh, P.W., Handwerker, H.O., 1988, Selective excitation by capsaicin of mechano-heat sensitive nociceptors in rat skin, **Brain Res.**, 446:262.
Szolcsányi, J., Jancsó-Gábor, A., Joó, F., 1975, Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 287:157.
Szolcsányi, J., Joó, F., Jancsó-Gábor, A.,1971, Mitochondrial changes in preoptic neurones after capsaicin desensitization of the hypothalamic thermodetectors in rats, **Nature**, 229:116.
Szolcsányi, J., Sann, H., Pierau, F.-K., 1986, Nociception in pigeons is not impaired by capsaicin, **Pain**, 27:247.
Theodorsson-Norheim, E., Hua, X.-Y., Brodin, E., Lundberg, J.M., 1985, Capsaicin treatment decreases tissue levels of neurokinin A-like immunoreactivity in the guinea-pig, **Acta Physiol. Scand.**, 124:129.
Virus, R.M. McManus D.Q., Gebhart, G.F., 1983, Capsaicin treatment in adult Wistar-Kyoto and spontaneously hypertensive rats: neurochemical effects in the spinal cord, **Eur. J. Pharmacol.**, 92:1.
Wall, P.D., Fitzgerald, M., Nussbaumer, J.C., Van der Loos, H., Devor, M., 1982, Somatotopic maps are disorganized in adult rodents treated neonatally with capsaicin, **Nature**, 295:691.
Williams, J.T., Zieglgänsberger, W., 1982, The acute effects of capsaicin on rat primary afferent and spinal neurons, **Brain Res.**, 253:125.
Winter, J., 1987, Characterization of capsaicin-sensitive neurones in adult rat dorsal root ganglion cultures, **Neurosci. Lett.**, 80:134.
Wood, J.N., Winter, J., James, I.F., Rang, H.P., Yeats, J., Bevan, S., 1988, Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture, **J. Neurosci.**, 8:3208.

CHEMICAL CODING OF NEURONS IN THE GASTROINTESTINAL TRACT

M. Costa, S. Brookes, P. Steele, and J. Vickers

Centre for Neuroscience, Department of Physiology
The Flinders University of South Australia
Bedford Park, Australia

The gastrointestinal tract, unlike most of the other internal organs, contains a rich and complex neuronal system embedded within its wall. The first description of this system dates back to the middle of the last century when Meissner and Auerbach first described the rich neuronal network made of microganglia and nerve bundles forming the myenteric and submucous plexuses (Meissner, 1857; Auerbach, 1864). By the turn of the century Bayliss and Starling (1889) first, followed by Langley (1921), provided evidence that this system, named then the Enteric Nervous System (ENS) controls coordinated motor functions in isolation from the central nervous system. In addition the intestine receives inputs from the central nervous system via sympathetic and parasympathetic nerves and sends sensory information via vagal and spinal afferent neurons. Between the beginning of this century and around twenty years ago the attempts to identify neuroanatomically the different classes of enteric neurons involved in the control of intestinal functions were hampered by the inability of the neurohistological techniques to distinguish specific neuronal groups within the apparently homogeneous maze of nerve cells and nerve fibres which form the ENS. Over the last twenty years the diversity of enteric neurons has become more apparent in functional, pharmacological, morphological, electrophysiological and neurochemical studies. It is only in the last few years that considerable progress has been made to unravel the neuronal circuitries of the enteric nervous system by correlating these studies to provide an integrated view of how the enteric nervous system is organized.

Functional Classes of Enteric Neurons and Their Transmitters

Evidence that there is a functional diversity of enteric neurons (Table I) has accumulated from studies in intact animals and in isolated preparations of intestine since the beginning of the century but it is only in the last twenty-five years that the diversity of transmitters in the enteric nervous system has become apparent.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

TABLE I

FUNCTIONAL CLASSES OF ENTERIC NEURONS IN THE SMALL INTESTINE

	Intrinsic
Motorneurons:	excitatory to the longitudinal muscle inhibitory to the longitudinal muscle excitatory to the circular muscle inhibitory to the circular muscle
Interneurons:	excitatory orally directed interneurons within the myenteric plexus excitatory anally directed interneurons within the myenteric plexus excitatory local interneurons within the myenteric plexus excitatory and inhibitory myenteric neurons to the submucous ganglia excitatory interneurons within submucous plexus excitatory neurons from myenteric plexus to prevertebral sympathetic ganglia (intestinofugal)
Secretomotor:	secretomotor submucous neurons secretomotor myenteric neurons
Vasomotor:	vasodilator submucous neurons
Sensory Neurons:	mechano and chemoreceptors in the myenteric ganglia mechano and chemoreceptors in submucous ganglia
	Extrinsic
Motor:	inhibitory and excitatory parasympathetic pathways in the vagus and the sacral nerves inhibitory sympathetic neurons to myenteric ganglia excitatory sympathetic neurons to muscle sphincters vasoconstrictor sympathetic neurons inhibitory sympathetic neurons to submucous ganglia
Sensory:	mechano and chemoreceptor vagal sensory neurons mechano and chemoreceptor spinal sensory neurons

Enteric Neurons Involved in Motility

The intestine displays a repertoire of patterns of motility. These include the migrating myoelectric motor complex, a cyclic activity of enteric motor neurons which does not depend on sensory stimulation nor on central connections and that slowly mi-

grates from the stomach to the end of the small intestine; peristalsis, i.e. a coordinated sequence of reflexes initiated by distension of the intestine responsible for the aboral propulsion of intestinal content; mixing movements resulting from irregular contractions of circular and longitudinal muscles. All these patterns of motility are mediated probably by the same final motor neurons. There are both excitatory and inhibitory neurons to all muscle layers. Acetylcholine is a major transmitter of the excitatory motor neurons for muscarinic antagonist drugs inhibit all motor pattern of motility. Furthermore either the mechanical contractions or the excitatory junction potentials recorded from smooth muscles in response to activation of motor neurons are inhibited by muscarinic antagonists (Furness and Costa, 1987). However there is convincing evidence that the peptide substance P or a related tachykinin is also a neurotransmitter of exitatory motor neurons (Costa et al., 1985). These cholinergic and non-cholinergic enteric excitatory motor neurons supply both the longitudinal and circular muscle layers and probably also the muscularis mucosa.

The existence of enteric inhibitory motor neurons was demonstrated in functional studies at the turn of the century but the realization that the transmitter was not adrenergic only came in the 1960s when the term non adrenergic-non cholinergic (NANC) neurons was proposed. The enteric inhibitory neurons relax the intestinal muscle layers, in particular the circular muscle, by two mechanisms; one mediated by a fast acting unknown transmitter, possibly a purine, responsible for the inhibitory junction potential recorded electrophysiologically, the other by a slow acting transmitter, probably vasoactive intestinal peptide (VIP) (see Furness and Costa, 1987). Nicotinic blocking drugs have a profound inhibitory effect on all patterns of motility and act on enteric ganglia indicating that acetylcholine is also a major transmitter from enteric neurons to other enteric neurons (see Furness and Costa, 1987). Transmission from cholinergic neurons in enteric ganglia is mediated by fast synaptic potentials via nicotinic receptors and also by slow synaptic potentials via muscarinic receptors (see North, 1982). Both types of receptors are involved in the enteric reflexes which underlie peristalsis (Tonini and Costa, 1990). In addition electrophysiological evidence indicates that there are several types of non-cholinergic transmission within the enteric ganglia mediating slow synaptic potentials. There is evidence for non-cholinergic transmission in ganglia to be involved in enteric reflexes (Barthó et al., 1989; Holzer, 1989; Tonini and Costa, 1990), and substance P or a related tachykinin may mediate it. The enteric motorneurons and some of the interneurons are part of orally directed excitatory and anally directed inhibitory reflex pathways. These reflexes can be activated by distension of the gut, by mechanical stimulation of the mucosa or by chemical stimuli in the lumen (Tonini and Costa, 1990; Smith et al., 1990). These studies of enteric reflexes point to the existence of enteric sensory neurons which synapse on interneurons to form complex polyneuronal reflex pathways. There is evidence to suggest that some of these sensory neurons may utilize acetylcholine as a transmitter in addition to non-cholinergic substances (Tonini and Costa, 1990). The enteric neurons of these motor pathways are mostly located in the myenteric plexus.

Enteric Neurons Involved in Mucosal Transport and Blood Flow

Secretomotor effects mediated by both cholinergic and non-cholinergic neurons have been demonstrated by electrical stimulation and by reflex activation (Lundgren, 1989; Cook, 1989). VIP has been proposed to be involved in the non-cholinergic secretomotor functions. Most of the secretomotor neurons are located in the submucous ganglia (see Bornstein and Furness, 1988).

Much of the evidence for the role of enteric neurons in the control of local blood flow comes from experiments in cats (Lundgren, 1989). A vasodilator response can be demonstrated in response to mechanical mucosal stimulation and two mechanisms have been proposed, i.e. a local reflex response involving a local VIP vasodilator neuron and an axon reflex possibly involving a tachykinin. In the guinea-pig recent evidence demonstrates that there are both cholinergic and non-cholinergic vasodilator fibres to the submucous arterioles (Neild et al., 1990; Galligan et al., 1990).

Functional Connections between Myenteric and Submucous Plexus

Although there appears to be a segregation of functions, i.e. the motor neurons to muscles are in the myenteric ganglia and most secretomotor-vasomotor neurons are in the submucous ganglia, it is likely that coordination of motor, secretory and circulatory functions occurs. There is good electrophysiological evidence that submucous neurons receive cholinergic and non-cholinergic excitatory input and non-cholinergic inhibition inputs from myenteric ganglia (Bornstein et al., 1986). The role of these interneuronal pathways in coordinating different functions remains to be established. Similarly a population of cholinergic submucous neurons send processes to the myenteric ganglia (Bornstein et al., 1989; Kirchgessner and Gershon, 1988).

Extrinsic Connections

The enteric nervous system and its target tissues receive inputs from the sympathetic and parasympathetic pathways. The intestine is also supplied by vagal and spinal afferent neurons. The sympathetic noradrenergic neurons independently exert control of motor, secretory and circulatory functions. Thus activation of sympathetic pathways inhibits motility by inhibiting the activity of myenteric neurons and by direct excitation of sphincteric smooth muscle. Sympathetic vasoconstrictor neurons reduce intestinal blood flow. The sympathetic neurons that control mucosal transport inhibit the activity of non-cholinergic secretomotor submucous neurons (Furness and Costa, 1987; Bornstein and Furness, 1988).

There is ample evidence that intestino-intestinal reflexes activate the sympathetic pathways that control motility. The afferent pathways can be spinal sensory neurons (intestino-intestinal spinal reflex) or intestinofugal cholinergic myenteric neurons synapsing directly on sympathetic noradrenergic postganglionic neurons (intestino-intestinal peripheral reflex) (Furness and Costa, 1987).

The spinal sensory neurons respond to distension, to chemicals in the lumen and to capsaicin, and can also mediate pain sensations (Dockray, Green and Varro, 1989).

Vagal inputs to the small intestine have been less well studied than those in the stomach (see Grundy, 1989). In rats Kirchgessner and Gershon (1989) have demonstrated rare efferent vagal fibres in the myenteric plexus of the small intestine after injections of an anterograde tracer in the dorsal motor nucleus of the vagus nerve.

There is ample evidence that vago-vagal reflexes occur in response to mechanical and chemical stimuli from the upper digestive tract (Mei, 1983). Inhibitory and excitatory efferent vagal pathways are composed of preganglionic cholinergic parasympathetic neurons activating either the enteric inhibitory or the enteric excitatory motorneurons in myenteric ganglia.

More than half of the nerve fibres in the vagus are afferent and many must supply the gastrointestinal tract. While there is evidence from retrograde tracing for vagal sensory fibres supplying the stomach and the pancreas (Dockray et al., 1989), little information is available on vagal sensory fibres to the small intestine.

TABLE II

HISTOCHEMICAL MARKERS OF TRANSMITTER RELATED ENTERIC NEURONS

Potential Transmitters	Histochemical Method
Acetylcholine (Ach)	not available
Noradrenaline (NA)	aldehyde induced fluorescence
Serotonin (5HT)	immunohistochemistry (IM)
Histamine	not available
Gamma amino butyric acid (GABA)	IM
Adenosin triphosphate (ATP)	not available
Cholecystokinin (CCK)	IM
Calcitonin gene-related peptide (CGRP)	IM
Dynorphin (DYN)	IM
Enkephalin (ENK)	IM
Galanin (GAL)	IM
Neurokinin A (NKA)	IM
Neuromedin U (NMU)	IM
Somatostatin (SOM)	IM
Substance P (SP)	IM
Vasoactive intestinal peptide (VIP)	IM
Enzymes	
Aromatic Aminoacid Decarboxylase (AACD)	IM
Cholineacetyltransferase (ChAT)	IM
Dopamine β-Hydroxylase (DBH)	IM
Histidine Decarboxylase (HD)	IM
Monoamineoxidase A (MAO A)	IM and histochemical reaction
Tyrosine Hydroxylase (TH)	IM
Glutamic Acid Decarboxylase (GAD)	IM

Neurochemical Classes of Enteric Neurons

The pharmacological and functional studies have unveiled a range of non-adrenergic non-cholinergic transmitter mechanisms in the enteric nervous system, and a large number of functional classes of enteric neurons. This diversity is matched by the wealth of neurochemicals found over the last twenty years in the enteric nervous system. Histochemical methods have been developed in the sixties to visualize noradrenaline and from the 1970s a large number of potential neurotransmitter substances have been visualized in enteric neurons including amines, aminoacids and neuropeptides (Table II). Transmitter related enzymes have also been demonstrated histochemically in enteric neurons and a number of neurochemicals unrelated to transmitters have been described in enteric neurons (Table III). A major challenge for neuroscientists of the enteric nervous system has been to correlate the classes of histochemically identifiable enteric neurons with their morphology, connections, electrophysiological properties and functions. One of the important clues in the unravelling of the organization and function of the enteric neuronal circuits has been the finding that most enteric neurons contain several neurochemical markers and that each group of enteric neurons is coded by a particular combination of neurochemicals and by its projections (Costa et al., 1986). For example, although the postganglionic sympathetic neurons are all noradrenergic, those which inhibit secretion also contain somatostatin, dynorphin and neurofilaments whereas the vasoconstrictor noradrenergic neurons contain neuropeptide Y but not neurofilaments (Costa et al., 1986; Vickers et al., 1989). Similarly the submucous neurons can be divided into four classes and each subserve a different function (Table IV.

TABLE III

HISTOCHEMICAL MARKERS OF ENTERIC NEURONS NOT RELATED TO SPECIFIC TRANSMITTERS

Acetylcholinesterase (AChE)
Alkaline phosphatase (AP)
Amine Handling (AH)
Calcineurin (CN)
Calcium Binding Proteins (CaBP)
Carbonic anhydrase (CA)
Cytochrome oxidase (CO)
Methylene Blue (MB)
Microtubules Associated Proteins (MAP)
Monoamineoxidase B (MAO B)
Nicotinamide adenine dinucleotide diaphorase (NADH-D)
Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-D)
Neurofilament proteins (NF)
Neuron specific enolase (NSE)
Cyclic AMP Dependent Protein Kinase (cAMP PK)
Ca^{++} Calmodulin Dependent Protein Kinase II (CaMKII)
Quinacrine
Synaptic Vescicular Proteins

TABLE IV

FUNCTION AND NEUROCHEMICAL CLASSES OF SUBMUCOUS NEURONS

Neurochemical Coding	Function
VIP/DYN/GAL	non-cholinergic secretomotor and vasodilator
Ach/CCK/CGRP/NPY/SOM	cholinergic secretomotor
Ach/Calretinin	cholinergic vasodilator
Ach/SP	sensory

The neurochemical coding of the myenteric neurons, their connections and their functions is still incomplete. The connectivity and projections of enteric neurons has been studied by lesions of the neuronal pathway (Costa et al., 1987; Ekblad et al., 1989) and by retrograde tracing method to identify enteric motor neurons recently developed in our laboratory (Brookes and Costa, 1990). By combining these results with the functional studies discussed above, a number of classes of myenteric neurons could be identified. There appear to be four classes of enteric motor neurons to the circular muscle. Two are orally directed excitatory cholinergic motor neurons: one short, also containing substance P, and one long, containing substance P, enkephaline, neurofilaments and the enzyme Ca^{++}/calmodulin dependent protein Kinase II (CamK). Thus the same motor neurons contain and release the two excitatory transmitters described in the functional studies, i.e. Ach and SP. This is one of the best examples of contransmission in the nervous system. There are two anally directed inhibitory neurons: one long, containing VIP/CYN/GRP/NF/CamK/NADPH-diaphorase/Alkaline phosphatase, and one short, containing VIP/ENK. VIP relaxes the muscle and these two neurons correspond to the non-adrenergic non-cholinergic inhibitory motor neurons. All these motor neurons show a typical morphology of Dogiel type I with several short dendrites and a long axonal process.

The longitudinal muscle is supplied by two classes of motor neurons with Ach and Calretinin or SP (Costa et al., 1990) and these would account for both cholinergic and non-cholinergic excitatory neurons and a third minor population of inhibitory neurons with VIP. The enteric motor neurons on the two muscle layers represent about half of all myenteric neurons.

About a third of myenteric neurons have the morphology of Dogiel type II neurons with a smoother cell body and numerous long processes and are likely to have sensory function (Pompolo et al., 1989) and some of these contain Ach, SP and Somatostatin (Steele and Costa, 1990). These neurons may account for the cholinergic and non cholinergic ganglionic transmission in enteric reflexes (Tonini and Costa, 1990). A number of myenteric Dogiel type I neurons act as interneurons. One population of orally directed interneurons contains Ach/SP/ENK/DYN/NF/CamK and may represent the main interneuron in the excitatory enteric reflexes underlying peristalsis. At least four anally directed interneurons could be identified. Their neurochemical coding is still being investigated.

All these populations of motor, inter and sensory neurons account for about 90% of myenteric neurons and it is anticipated

that a complete account of functional and neurochemical classes of myenteric neurons will be achieved.

The neurochemical coding is correlated also with the morphology and the electrophysiology of myenteric neurons. Thus all motor neurons belong to the Dogiel type I cells with several short dendrites and a single long axonal process (Brookes and Costa, 1990). Similarly the proposed sensory neurons with calbindin have a Dogiel type II morphology (Iyer et al., 1988). All Dogiel type I cells are electrophysiologically S neurons, i.e. receive fast nicotinic excitatory synaptic potentials (Bornstein, et al., 1984; Lees, et al., 1989; Katayama et al., 1986) while the Dogiel type II cells all have the characteristic of AH cells (afterhyperpolarizing) and do not receive fast synaptic input (Iyer et al., 1988; Lees et al., 1989).

The knowledge of the neurochemical coding of sensory neurons supplying the intestine is still incomplete. Histochemical evidence has demonstrated that spinal sensory neurons with substance P and calcitonin gene-related peptide (CGRP) supply the small intestine (Gibbins et al., 1985) where they branch in the myenteric plexus, submucous plexus in the mucosa and form a perivascular plexus around submucous blood vessels (Costa et al., 1985). Experiments using the fluorescent retrograde label DiI applied to the guinea-pig distal small intestine showed that a small number of dorsal root ganglia are retrogradely labelled (Vickers and Costa unpublished results). The vast majority of the labelled neurons (90%) are medium size and show immunoreactivity for SP and a faint immunoreactivity for neurofilament proteins. A small proportion of labelled neurons are large with intense NF immunoreactivity. These results imply that most sensory inputs from the intestine are mediated by medium size SP/CGRP neurons. Similar results have been reported by Dockray et al. (1989) showing that 80% of spinal sensory neurons to the stomach contain SP and CGRP.

These sensory neurons are sensitive to capsaicin (Papka et al., 1984) and are also involved in motor responses of the intestine elicited by antidromic activation of sensory fibres (Holzer, 1988). The physiological role of these sensory neurons in the intestine has not been established nor has it been ascertained whether axon reflexes may elicit motor responses and vasodilatation of the intestinal blood vessels in physiological conditions.

CONCLUSIONS

The combination of functional, morphological and neurochemical studies have led to considerable advances in the understanding of the neural control of intestinal functions. It is now possible to link such studies to the investigations of physiology, pharmacology, neurochemistry and pathophysiology of the sensory innervation of the intestine. A similar picture emerges in the autonomic and visceral sensory systems indicating that each class of functionally different neurons has specific features and these include connectivity, morphology, projections, biophysical properties and neurochemistry. This suggests that there is a highly ordered and chemically coded organization of the autonomic and sensory systems.

Acknowledgements

This work has been supported by the NH&MRC of Australia. We are indebted to Joy Davis for the typing of the manuscript.

References

Auerbach, L., 1864, Fernere vorlaufige Mitteilung uber den Nervenapparate des Darmes, **Arch. Pathol. Anat. Physiol.**, 30:457.

Bartho, L., Holzer, P., Leander, S., and Lembeck, F., 1989, Evidence for an involvement of substance P, but not cholecystokinin-like peptides, in hexamethonium-resistant intestinal peristalsis, **Neuroscience**, 28:211.

Bayliss, W.M., and Starline, E.H., 1899, The movements and innervation of the small intestine, **J. Physiol. (Lond.)**, 24:100.

Bornstein, J.C., Costa, M., and Furness, J.B., 1986, Synaptic inputs to immunohistochemically identified neurons in the submucous plexus of the guinea-pig small intestine, **J. Physiol.**, 381:465.

Bornstein, J.C., Costa, M., Furness, J.B., and Lees, G.M., 1984, Electrophysiology and enkephalin immunoreactivity of identified myenteric plexus neurones of guinea-pig small intestine, **J. Physiol.**, 351:313.

Bornstein, J.C., and Furness, J.B., 1988, Correlated electrophysiological and histochemical studies of submucous neurons and their contribution to understanding enteric neural circuits, **J. Autonom. Nerv. System**, 25:1.

Bornstein, J.C., Furness, J.B., and Costa, M., 1989, An electrophysiological comparison of substance P immunoreactive neurons with other neurons in the guinea-pig submucous plexus, **J. Autonom. Nerv. Syst.**, 26:113.

Brookes, S.J.H., and Costa, M., 1990, Identification of motor neurones in the guinea pig small intestine by retrograde transport in vitro and immunohistochemistry, **Proc. Aust. Neurosc. Soc.**, 1:59.

Cooke, H.J., 1989, Neuroeffector relations in the intestinal mucosa in vitro, in: "Nerves and the Gastrointestinal Tract" Section III, M.V. Singer and H. Goebell, eds., MTP Press, Netherlands.

Costa, M., Furness, J.B., and Gibbins, I.L., 1986, Chemical coding of enteric neurons, **Progress in Brain Res.**, 68:217.

Costa, M., Furness, J.B., and Llewellyn-Smith, I.J., 1987, Histochemistry of the enteric nervous system, in: "Physiology of the Gastrointestinal Tract," L.R. Johnson, ed., Raven Press, New York.

Costa, M., Furness, J.B., Llewellyn-Smith, I.J., Murphy, R., Bornstein, J.C., and Keast, J.R., 1985, Functional roles for substance P-containing neurons in the gastrointestinal tract, in: "Metabolism and Biological Actions," C.C. Jordan and P. Oehme, eds., Taylor & Francis, London.

Costa, M., Furness, J.B., Pullin, C.O., and Bornstein, J., 1985, Substance P neurons mediate non-cholinergic transmission to the circular muscle of the guinea-pig intestine, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 328:446.

Costa, M., Steele, P., and Brookes, S., 1990, Immunohistochemical evidence for the presence of the calcium binding protein calretinin in enteric neurons, **Proc. Aust. Neurosc. Soc.**, 1:67.

Dockray, G.J., Green, T., and Varro, A., 1989, The afferent peptidergic innervation of the upper gastrointestinal tract, in: "Nerves and the Gastrointestinal Tract" Section I, M.V. Singer and H. Goebell, eds., MTP Press, Netherlands.
Ekblad, E., Hakanson, R., and Sundler, F., 1989, Projections of enteric peptide-containing neurons in the rat, in: "Nerves and the Gastrointestinal Tract" Section I, M.V. Singer and H. Goebell, eds., MTP Press, Netherlands.
Furness, J.B. and Costa, M., 1987, "The Enteric Nervous System," Churchill-Livingstone.
Galligan, J.J., Jiang, M.-M., Shen, K.-Z., and Surprenant, A., 1990, Substance P mediates neurogenic vasodilatation in extrinsically denervated guinea-pig submucosal arterioles, **Journal of Physiology**, 420:247.
Gibbins, I.L., Furness, J.B., Costa, M., Macintyre, I., Hillyard, C.J., and Girgis, S., 1985, Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea-pigs, **Neurosci. Lett.**, 57:125.
Grundy, D., 1988, Vagal control of gastrointestinal function, **Baillieres Clinical Gastroenterology**, 2:23.
Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.
Holzer, P., 1989, Ascending enteric reflex: multiple neurotransmitter systems and interactions, **Am. J. Physiol.**, 256:G540.
Iyer, V., Bornstein, J.C., Costa, M., Furness, J.B., Takahashi, Y., and Iwanga, T., 1988, Electrophysiology of guinea-pig myenteric neurons correlated with immunoreactivity for a calcium-binding protein, **J. Autonom. Nerv. Sys.**, 22:141.
Katayama, Y., Lees, G.M., and Pearson, G.T., 1986, Electrophysiology and morphology of vasoactive intestinal peptide immunoreactive neurones of the guinea-pig ileum, **J. Physiol.**, 378:1.
Kirchgessner, A.L., and Gershon, M.D., 1988, Projections of submucosal neurons to the myenteric plexus of the guinea pig intestine: In vitro tracing of microcircuits by retrograde and anterograde transport,**Journal of Comparative Neurology**, 277:487.
Kirchgessner, A.L., and Gershon, M.D., 1989, Identification of vagal and submucosal inputs to the myenteric plexus by retrograde and anterograde transport, in: "Nerves and the Gastrointestinal Tract," Section I, M.V. Singer and H. Goebell, eds., MTP Press, Netherlands.
Langley, J.N., 1921, "The Autonomic Nervous System," Part 1, Cambridge.
Lees, G.M., Leishman, D.J., and Pearson, G.T., 1989, Electrophysiological characteristics of guinea-pig myenteric plexus neurons immunoreactive for dynorphin A(1-8), in: "Nerves and the Gastrointestinal Tract," Section I, M.V. Singer and H. Goebell, eds., MTP Press, Netherlands.
Lundgren, O., 1989, Enteric nervous control of mucosal functions of the small intestine in vivo, in: "Nerves and the Gastrointestinal Tract," Section III, M.V. Singer and H. Goebell, eds., MTP Press, Netherlands.
Mei, N., 1983, Sensory structures in the viscera, in: "Progress in Sensory Physiology," Vol. 4, D. Ottoson, ed., Springer-Verlag, Berlin.
Meissner, G., 1857, Über die Nerven der Darmwand, **Z. Ration. Med. N.F.**, 8:364.

Neild, T.O., Shen, K.-Z., and Surprenant, A., 1990, Vasodilatation of arterioles by acetylcholine released from single neurones in the guinea-pig submucosal plexus, **Journal of Physiology**, 420:247.

North, R.A., 1982, Electrophysiology of the enteric nervous system, **Neuroscience**, 7:315.

Papka, R.E., Furness, J.B., Della, N.G., Morphy, R., and Costa, M., 1984, Time course of effect of capsaicin on ultrastructure and histochemistry of substance P immunoreactive nerves associated with the cardiovascular system of the guinea-pig, **Neuroscience**, 12:1277.

Pompolo, S., Furness, J.B., Bornstein, J.C., Hendriks, R., and Trussel, D.C., 1989, Dogiel type II neurons in the guinea-pig small intestine: ultrastructure in relation to other characteristics, in: "Nerves and the Gastrointestinal Tract," Section I, M.V. Singer and H. Goebell, eds., MTP Press, Netherlands.

Smith, T.K., Bornstein, J.C., and Furness, J.B., 1990, Distension-evoked ascending and descending reflexes in the circular muscle of guinea-pig ileum: an intracellular study, **Journal of the Autonomic Nervous System**, 29:203.

Steele, P.A., and Costa, M., 1990, Immunohistochemical identification of cholinergic neurons in the guinea-pig ileum, **Proc. Aust. Neurosc. Soc.**, 1:130.

Tonini, M., and Costa, M., 1990, A pharmacological analysis of the neuronal circuitry involved in distension-evoked enteric excitatory reflex, **Neuroscience**, in press.

Vickers, J.C., Costa, M., Vitadello, M., and Dahl, D., 1989, Neurofilament immunoreactivity in the coeliac ganglia of the guinea-pig, **Australian Neuroscience Society**, February, Abstract.

OCCURRENCE AND DISTRIBUTION OF SUBSTANCE P- AND CGRP-CONTAINING NERVE FIBERS IN GASTRIC MUCOSA: SPECIES DIFFERENCES

F. Sundler[1], E. Ekblad[1], and R. Håkanson[2]

Departments of Medical Cell Research[1] and Pharmacology[2]
University of Lund
Lund, Sweden

Introduction

The gastric mucosa is rich in acetylcholinesterase-positive nerve fibers (c.f. Schofield, 1968: Yamaguchi et al., 1974; Lechago and Barajas, 1976) and in fibers storing neuropeptides (Schultzberg et al., 1980; Ekblad, 1985; Keast et al., 1985; Tsutsumi and Hara, 1989). In the mucosa of the rat stomach, fibers containing VIP and GRP/bombesin immunoreactivity are notably numerous (Dockray et al., 1979; Ekblad et al, 1985; Iwanaga et al., 1988), while fibers storing other neuropeptides such as substance P (SP), neuropeptide Y and enkephalin are less frequent (Schultzberg et al., 1980; Ekblad et al, 1985). Keast et al. (1985) noted marked species differences in the density of SP-containing nerve fibers in the gastric mucosa. Thus, such fibers were fairly numerous in man, moderate in number in rat and guinea pig and absent altogether from the mucosa of the dog stomach. Also the density of calcitonin gene-related peptide (CGRP)-containing fibers in the gastric mucosa varies greatly among species (own unpublished observations). In the muscle layers and the myenteric ganglia the density of fibers storing SP and CGRP displays less species variation. Generally, SP-containing fibers are numerous in the intramural ganglia and in the smooth muscle (Schultzberg et al., 1980; Ekblad et al., 1985), whereas CGRP-storing fibers are much less numerous (Fig. 1). SP-containing nerve cell bodies are regularly seen in the myenteric ganglia (Schultzberg et al., 1980; Kuwahara et al., 1983; Ekblad et al., 1985; Green and Dockray, 1988) whereas CGRP-containing cell bodies are lacking (c.f. Green and Dockray, 1988; Dockray et al., 1989).

SP and CGRP in Gastric Mucosa

Previous work has linked neuronal SP and CGRP in the gastric mucosa and submucosa - at least in the rat - to sensory fibers, as defined by their sensitivity to capsaicin (Sharkey et al., 1984; Sternini et al., 1987; Green and Dockray, 1988; Dockray et al., 1989). Nonetheless we thought it worthwhile to examine more carefully the occurrence and distribution of SP- and CGRP-containing nerve fibers in the oxyntic and antral mucosa of several mammals, including man. The following is a description of the findings (summarized in Table I).

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

Table I
Relative density of SP- and CGRP-containing nerve fibers in the gastric mucosa of some mammals.

	Corpus		Antrum	
	SP	CGRP	SP	CGRP
Mouse	(+)	+	(+)	++
Rat	(+)	+	+	++
Mole	+++	++	++	++
Hamster	(+)	++	+	++
Guinea Pig	(+)	(+)	+	(+)
Ferret	(+)	+++	0	+++
Cat	(+)	+	+	+
Pig	+++	0	+++	(+)
Man	+	0	++	0

(+) an occasional fiber; + few fibers; ++ moderate number of fibers; +++ numerous fibers

In the gastric mucosa of the mouse and rat, SP-containing fibers were few to moderate in number, with scattered fibers running along the glands, sometimes high up in the mucosa (see also Sharkey et al., 1984; Ekblad et al., 1985) (Fig. 1). The fibers were somewhat more numerous in the antrum than in the body of the stomach. CGRP-containing fibers had the same distribution as those storing SP but were somewhat more numerous. The majority of the mucosal SP-containing fibers contained CGRP and vice versa (Fig. 1). Similar findings were made in the hamster and guinea pig, although in the guinea pig SP and CGRP-storing fibers were notably few. In the mole SP-containing fibers were numerous, while those storing CGRP were moderate in number (Fig. 2a and b). SP-storing fibers were particularly numerous in the oxyntic mucosa. All fibers storing CGRP seemed to contain SP as well. In the ferret (Fig. 3) CGRP-containing fibers were numerous in the gastric mucosa, more so than in any of the other species examined. The fibers formed dense networks beneath the foveolar surface epithelium and around the neck of the glands. SP-containing fibers, on the other hand, were virtually absent from the mucosa. Only occasionally could a weakly SP-immunoreactive fiber be detected in the oxyntic mucosa. Also in the cat (Fig. 2c and d) were CGRP-containing fibers found to predominate over those storing SP, although the general density of CGRP-containing fibers was lower than in the ferret. In the cat, as in the ferret, all SP-containing fibers seemed to harbour in addition CGRP (see also Parkman et al., 1989).

In pig and man CGRP-containing fibers were virtually absent from the mucosa. Only at the very base of the glands and in the submucosa could an occasional CGRP-storing fiber be seen in the porcine antrum. By contrast SP-containing fibers were moderate in number to numerous, both in the oxyntic and antral mucosa (Fig. 4). Interestingly, in the porcine stomach nerve cell bodies storing SP were quite numerous in small ganglia located within the mucosa, just beneath the base of the glands (Fig. 4a and b). Occasionally, SP-containing nerve cell bodies were seen also in submucous ganglia. These findings suggest that mucosal SP-containing fibers in this species could have a local origin rather than being primary sensory afferents. It should be mentioned in this context that small intramucosal ganglia were de-

scribed in the porcine stomach already in the thirties by Vau (1937). These mucosal ganglia were observed also in goat, sheep and cow, but they could not be demonstrated in the cat and dog. In the human stomach SP-containing fibers were more numerous in the antral than in the oxyntic mucosa. These findings are in line with reports of higher SP concentrations in antral than in oxyntic mucosa (c.f. Ferri et al., 1989). In the human stomach we failed to find SP-containing nerve cell bodies in the mucosa. In the submucosa, however, SP-containing nerve cell bodies were

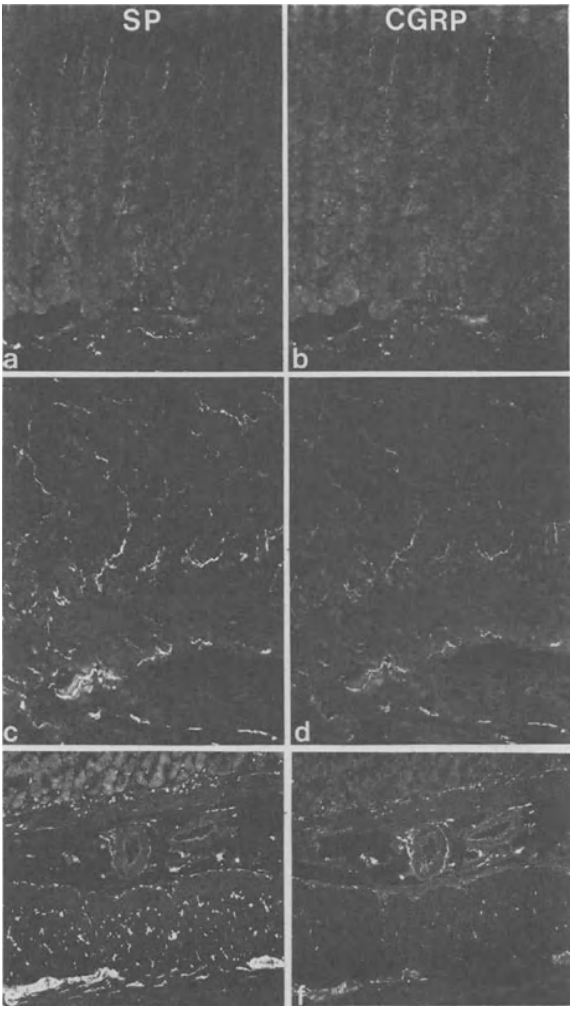


Fig. 1. Cryostat sections from rat stomach double stained for SP (TRITC) and CGRP (FITC). a and b oxyntic mucosa, c and d antral mucosa, e and f submucosa and smooth muscle from oxyntic region. Note almost complete coexistence of SP and CGRP in nerve fibers in the mucosa as well as around blood vessels. In the smooth muscle and myenteric ganglia SP- and CGRP-containing fibers constitute separate nerve fiber populations. a and b X 120, c and d X 150, e and f X 100.

regularly seen, which at least in part may account for the supply of SP-containing fibers in the mucosa. Hence, in pig, and possibly also in man, SP-containing fibers in the gastric mucosa (that do not contain CGRP) may have a local origin in ganglia located in the mucosa/submucosa (pig) and submucosa (man). However, studies of the effects of specific denervations are needed before it is possible to exclude the possibility that primary sensory afferents contribute to the mucosal SP innervation in these two species.

For other species than those examined here data are available from e.g. the dog stomach. In this species SP-storing fibers were reported to be totally absent from the mucosa (Keast et al., 1985). Of interest in this context is that canine chief cells have been reported to express SP receptors, exemplifying the frequent mismatch between neuropeptide and receptor distribution.

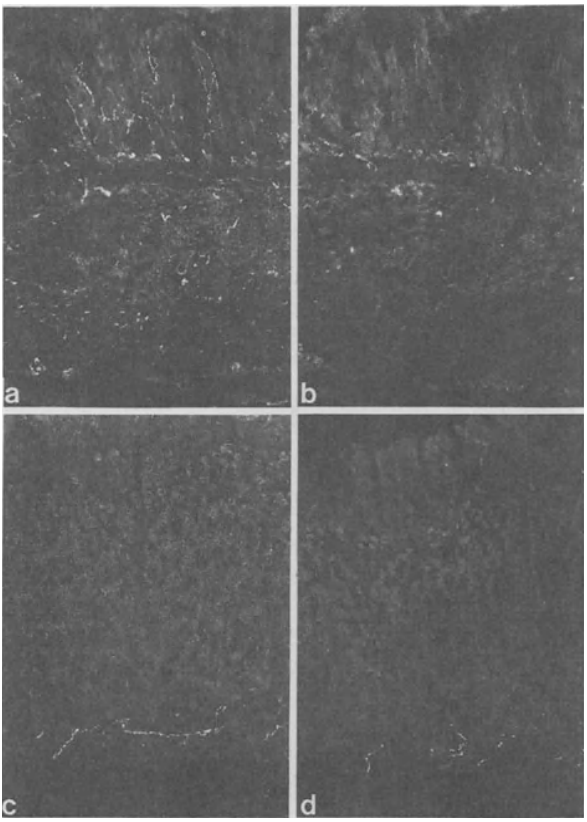


Fig. 2. Cryostat sections from the stomach (acid-producing region) from mole (a and b) and cat (c and d). Sections in a and c are immunostained for SP, in b and d for CGRP. In the mole, mucosal SP-containing fibers are numerous, while those storing CGRP are fewer. The muscle coat (lower part of fig.) is also rich in SP fibers but lacks CGRP fibers. In the cat, both SP- and CGRP-containing fibers are few. a and b X 100, c and d X 110.

In conclusion, fibers storing both SP and CGRP, probably sensory in origin, can readily be demonstrated in the gastric mucosa of several mammals, including many rodents. The ferret is special in that the gastric mucosa harbours numerous CGRP-containing fibers that are devoid of SP, whereas in pig and man numerous SP-containing fibers are devoid of CGRP.

The origin of the rich CGRP nerve supply in the ferret gastric mucosa is obscure. Since the intramural ganglia lack CGRP-immunoreactive nerve cell bodies, the fibers are likely to be extrinsic in origin, presumably sensory, as has been shown to be the case in the rat (c.f. Dockray et al., 1989). We have tested the possibility that the fibers are primary sensory afferents by giving multiple injections of capsaicin. The treatment resulted in a slight reduction in the intensity of the CGRP immunostaining which may support the idea that the fibers are primary afferents.

Interestingly, the dense network of CGRP-containing fibers beneath the foveolar epithelium and around the neck of the glands in the ferret stomach coincides with the occurrence of numerous mast cells in the connective tissue stroma around the

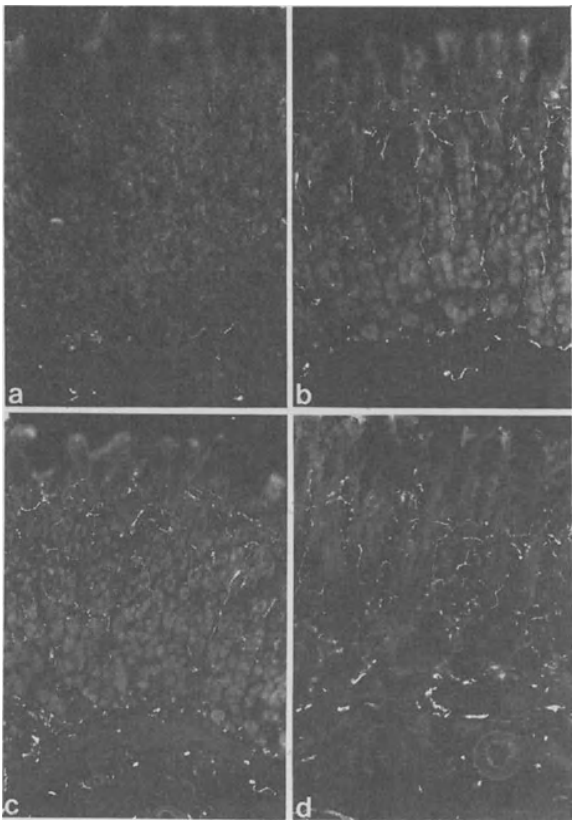


Fig. 3. Cryostat sections from ferret stomach. a-c show oxyntic mucosa, d antral mucosa. SP-containing fibers are few (a) while the CGRP-containing ones are quite numerous (b and c). For comparison the CGRP innervation of antral mucosa is shown in d. a-d X 110.

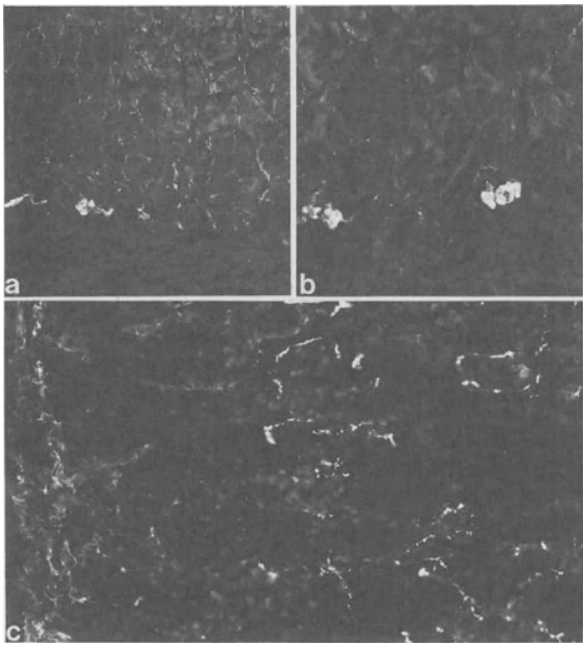


Fig. 4. Cryostat sections from pig oxyntic mucosa (a and b) and human antral mucosa (c). Numerous delicate SP-containing fibers in the mucosa as well as nerve cell bodies in small ganglia located beneath the glands. a and b X 110, c X 160.

foveolae and neck portion of the glands. Thus, there is a morphological basis for suggesting a possible interaction between CGRP-containing nerve fibers and superficial mast cells in the ferret gastric mucosa.

Possible Functional Role

The sensory innervation of the stomach has attracted much attention during recent years. The magnitude of this innervation is reflected in the fact that 80-90% of the fibers in the abdominal vagus are afferents (c.f. Andrews, 1986). Moreover, capsaicin-sensitive mechanisms are known to operate in the stomach. Thus, in rats pretreated with capsaicin histamine-induced gastric acid secretion was found to be reduced whereas the responses to charbachol and pentagastrin were unaffected (Alföldi et al., 1986). Further, elimination of capsaicin-sensitive vagal afferents (by application of capsaicin directly to the cervical vagus one week before the experiments) resulted in a 40% reduction of gastric secretion induced by gastric distension in rats (Raybould and Taché, 1989). The secretory response to histamine was reduced to about the same degree whereas the responses to pentagastrin and bethanechol were unaffected. Systemic capsaicin pretreatment in addition increases the incidence of ulcerogen-induced duodenal ulcers (Maggi et al., 1987). Acute intragastric capsaicin administration, on the other hand, stimulates gastric acid secretion and increases mucosal blood flow in the rat (Limlomwongse et al., 1979) and protects against experimental ulcer formation (Szolcsányi and Barthó, 1981; Holzer and Sametz, 1986; Maggi and Meli, 1988; Holzer et al., 1989). The afferent neuron-

mediated gastric mucosal protection is thought to result from local release of vasodilatory peptides such as SP and CGRP from afferent nerve endings within the stomach. This view is supported by the demonstration by Lippe et al. (1989) of a protective effect of CGRP administered by close arterial infusion into the rat stomach against ethanol- and aspirin-induced mucosal damage. Rozsa et al, (1988) demonstrated a capsaicin-sensitive viscero-circulatory reflex in the rat, possibly mediated by SP. Thus, warming of the gastric mucosa causes hypotension, tachycardia, and a reduction in mesenteric blood flow. These responses were inhibited by systemic or topical capsaicin. Finally, it ought to be mentioned that intravenous infusions of SP or CGRP cause inhibition of gastric secretion (Konturek et al., 1981; Kraenzlin et al., 1985; Lenz et al., 1985).

Conclusion

Capsaicin-sensitive primary afferents and protective mechanisms of such fibers on the gastric mucosa have previously been demonstrated in the rat and SP and CGRP are firmly linked to such fibers. Our studies of the SP and CGRP innervation of the gastric mucosa in different mammals revealed remarkable species differences in the occurrence of these peptides and in the nature of the fibers that store them. The data suggest that in some species messengers other than SP and CGRP may mediate the protective effects of primary afferents on the gastric mucosa. It may be mentioned, finally, that the mucosal SP innervation displays marked variation among species also in the intestines. Thus, SP-containing fibers are numerous in the intestinal mucosa of for instance man, whereas such fibers are notably few in e.g. the cat (Brodin et al., 1983).

Acknowledgements

Grant support from the Swedish Medical Research Council (projects no 4499 and 1007), and the Påhlsson Foundation.

References

Alföldi, P., Obal, F. Jr., Toth, E., and Hideg, J., 1986, Capsaicin pretreatment reduces the gastric acid secretion elicited by histamine but does not affect the responses to carbachol and pentagastrin, **Eur. J. Pharmacol.**, 123:321.

Andrews, P.L.R., 1986, Vagal afferent innervation of the gastrointestinal tract, **Progr. Brain Ras.**, 67:65.

Brodin, E., Sjölund, K., Håkanson, R., and Sundler, F., 1983, Substance P-containing nerve fibers are numerous in human intestinal mucosa, **Gastroenterology**, 85:557.

Dockray, G.J., Green, T., and Varro, A., 1989, The afferent peptidergic innervation of the upper gastrointestinal tract, in: "Nerves and the Gastrointestinal Tract," M.V. Singer and H. Goebell, eds., Kluwer Acad. Publ., Dordrecht, Netherlands.

Dockray, G.J., Vaillant, C., and Walsh, J.H., 1979, the neuronal origin of bombesin-like immunoreactivity in the rat gastrointestinal tract, **Neuroscience**, 4:1561.

Ekblad, E., Ekelund, M., Graffner, H., Håkanson, R., and Sundler, F., 1985, Peptide-containing nerve fibers in the stomach wall of rat and mouse, **Gastroenterology**, 89:73.

Ferri, G.-L., Adrian, T.E., Soimero, L., Blank, M., Cavalli, D., Biliotti, G., Polak, J.M., Bloom, S.R., 1989, Intramural distribution of immunoreactive vasoactive intestinal polypeptide (VIP), substance P, somatostatin and mammalian bombesin in the oesophago-gastro-pyloric region of the human gut, **Cell Tissue Res.**, 256:191.

Green, T., and Dockray, G.J., 1988, Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig, **Neuroscience**, 25:181.

Holzer, P., Pabst, M.A., and Lippe, I.Th., 1989, Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa, **Gastroenterology**, 96:1425.

Holzer, P., and Sametz, W., 1986, Capsaicin-sensitive afferent neurons involved in gastric mucosal protection, **Br. J. Pharmacol.**, 89:562P.

Keast, J.R., Furness, J.B., and Costa, M., 1985, Distribution of certain peptide-containing nerve fibers and endocrine cells in the gastrointestinal mucosa in five mammalian species, **J. Comp. Neurol.**, 236:403.

Konturek, S.J., Jaworek, J., Tasler, J., Cieszkowski, M., and Pawlik, W., 1981, Effect of substance P and its C-terminal hexapeptide on gastric and pancreatic secretion in the dog, **Am. J. Physiol.**, 241:G74.

Kraenzlin, M.E., Ching, J.L.C., Mulderry, P.K., Ghatei, M.A., and Bloom, S.R., 1985, Infusion of a novel peptide, calcitonin gene-related peptide (CGRP) in man. Pharmacokinetics and effects on gastric acid secretion and on gastrointestinal hormones, **Regul. Pept.**, 10:189.

Kuwahara, A., Ishikawa, T., Mikami, S.-I., and Yanaihara, N., 1983, Distribution of neurons containing immunoreactivity for gastrin-releasing peptide (CGRP), substance P., and vasoactive intestinal polypeptide (VIP) in the rat gastric wall, **Biomed. Res.**, 4:473.

Lechago, J., and Barajas, L., Cholinergic innervation of the Simian oxyntic mucosa, **Am. J. Anat.**, 147:147.

Lenz, H.J., Mortud, M.T., Rivier, J.E., and Brown, M.R., 1985, Calcitonin gene-related peptide inhibits basal, pentagastrin, histamine, and bethanecol stimulated gastric acid secretion, **Gut**, 26:550.

Limlomwongse, L., Chaitauchawong, C., and Tongyai, S., 1979, Effects of capsaicin on gastric acid secretion and mucosal blood flow in the rat, **J. Nutr.**, 109:773.

Lippe, I.T., Lorbach, M., and Holzer, P., 1989, Close arterial infusion of calcitonin gene-related peptide into the rat stomach inhibits aspirin- and ethanol-induced hemorrhagic damage, **Regul. Pept.**, 26:35.

Maggi, C.A., Evangelista, S., Abelli, S., Abelli, L., Somma, V., and Meli, A., 1987, Capsaicin-sensitive mechanisms and experimentally induced duodenal ulcers in rats, **J. Pharm. Pharmacol.**, 39:559.

Maggi, C.A., and Meli, A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, **Gen. Pharmacol.**, 19:1.

Parkman, H.P., Reynolds, J.C., Elfman, K.S., and Ogorek, C.P., 1989, Calcitonin gene-related peptide: a sensory and motor neurotransmitter in the feline lower esophageal sphincter, **Regul. Pept.**, 25:131.

Raybould, H.E., and Taché, Y., 1989, Capsaicin-sensitive vagal afferent fibers and stimulation of gastric acid secretion in anesthetized rats, **Eur. J. Pharmacol.**, 167:237.

Rosza, Z., Mattila, J., and Jacobson, E.D., 1988, Substance P mediates a gastrointestinal thermoreflex in rats, **Gastroenterology**, 95:265.

Schofield, G.C., 1968, Anatomy of muscular and neural tissues in the alimentary canal, in: Handbook of Physiology, Sect. 6 Alimentary Canal, Vol. IV Motility, Ch. F. Code, section ed.

Schultzberg, M., Hökfelt, T., Nilsson, G., Terenius, L., Rehfeld, J.F., Brown, M., Elde, R., Goldstein, M., and Said, S., 1980, Distribution of peptide- and catecholamine- containing neurons in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies witih antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurontensin and dopamine-β-hydroxylase, **Neuroscience**, 5:689.

Sternini, C., Anderson, K., Frantz, G., Krause, J.E., and Brecha, N., 1989, Expression of substance P/Neurokinin A-encoding preprotachykinin messenger ribonucleic acids in the rat enteric nervous system, **Gastroenterology**, 97:348.

Sternini, C., Reeve, J.R. Jr., and Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.

Szolcsányi, J., and Barthó, L., 1981, Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat, **Adv. Physiol. Sci.**, 29:39.

Taché, Y., Pappas, T., Lauffenburger, M., Goto, Y., Walsh, J.H., and Debas, H., 1984, Calcitonin gene-related peptide: Potent peripheral inhibitor of gastric acid secretion in rats and dogs, **Gastroenterology**, 87:344.

Tsutsumi, Y., and Hara, M., Peptidergic nerve fibers in human stomach and duodenal bulb: immunohistochemical demonstration of changes in fiber distribution in chronic gastritis, **Biomed. Res.**, 10:209.

Vau, E., 1937, Über die subglandulären Gangliezellen in der Magenwant einiger Haussäugetiere, **Anat. Anz.**, 73:380.

Vigna, S.R., Mantyh, C.T., Soll, A.H., Maggio, J.E., and Mantyh, P., 1989, Substance P receptors on canine chief cells: Localization, characterization, and function, **J. Neurosci.**, 9:2878.

Yamaguchi, I., Mori, J., Honda, F., Nishizaki, H., and Kumada, S., 1974, A histochemical approach to the cholinergic innervation of endocrine-like cells in dog antro-pyloric mucosa, **Japan J. Pharmacol.**, 24:721.

TACHYKININ AND CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITIES AND mRNAs IN THE MAMMALIAN ENTERIC NERVOUS SYSTEM AND SENSORY GANGLIA

Catia Sternini

Department of Medicine and Brain Research Institute
UCLA School of Medicine and Center for Ulcer Research
and Education, Veterans' Administration Medical Center
Los Angeles, California, USA

INTRODUCTION

The enteric nervous system, which extends with continuity from the esophagus to the anal canal, including the hepatobiliary pathway and pancreas, comprises a large number of intrinsic (enteric) neurons embedded within the alimentary tract itself and the processes of extrinsic (efferent and afferent) neurons (Furness and Costa, 1987). It contains a heterogeneous population of neurochemically distinct types of neurons, which are characterized by different morphologies, projection patterns, pharmacological and electrophysiological properties and functions (Costa et al., 1987; Furness and Costa, 1987). During the past decade, a variety of chemical messengers, including bioactive peptides, or different combinations of them, have been recognized in enteric neurons and their terminals, and in extrinsic nerves supplying the enteric nervous system as well (Costa et al., 1986; Furness et al., 1988; Llewellyn-Smith, 1989; Sternini, 1988). The same chemical messengers can occur also in other neuronal structures, including primary sensory neurons and terminals (Gibbins et al., 1987; Ju et al., 1987). Substance P (SP) or related tachykinins and calcitonin gene-related peptide (CGRP), which are the most generalized peptide sensory markers, being present in a substantial number of small- to medium-size neurons in sensory ganglia, are expressed in a considerable proportion of enteric and afferent neurons innervating the digestive system (Costa et al., 1985; Gibbins et al., 1987; Goodman and Iversen, 1986; Jessell, 1983; Ju et al., 1987; Kruger et al., 1989; Pernow, 1983; Rosenfeld et al., 1983). These neuropeptides possess a variety of biological effects and might subserve nociceptive functions and non-sensory roles in the periphery.

This chapter will focus on the distribution of enteric and sensory neuropeptides and their mRNAs in the enteric nervous system, as exemplified by the tachykinins and CGRP. Most of the data presented here refer to experiments on rats.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

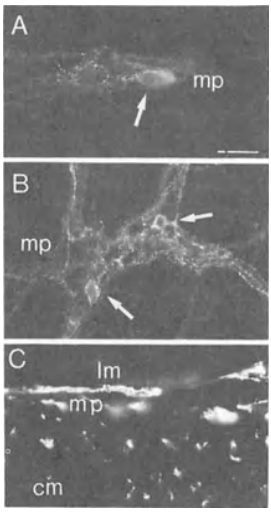


Fig. 1. A and B: whole mount preparations of the rat stomach (A)and duodenum (B) showing tachykinin immunoreactive fibers and ganglion cells (arrows) in the myenteric plexus (mp). C: cryostat section of the rat stomach showing the abundance of tachykinin immunoreactive fibers in the muscularis externa, particularly the circular muscle layer (cm) and myenteric plexus. Tissue was processed for immunohistochemistry using the immunofluorescence method as previously described (Sternini et al., 1987). Tachykinin immunostaining was obtained with a rabbit polyclonal NKA antiserum, kindly provided by Dr. J.H. Walsh and H. Wong. Immunostaining was blocked by preincubating the NKA antiserum with an excess of synthetic SP, NKA or NKB, indicating that this antiserum is a generalized marker for the tachykinin peptides. lm: longitudinal muscle. Calibration bar: 50 μm.

Tachykinin and CGRP Immunoreactivities in the Enteric Nervous System

The tachykinins are a family of structurally related peptides, which have been proposed as neurotransmitters, neuromodulators and perhaps growth factors (Erspamer, 1981; Maggio, 1988). In mammals, this family includes SP, which is one of the best characterized bioactive peptides and has been regarded as a major excitatory transmitter in the gut (Barthó and Holzer, 1985; Costa et al., 1985; Dockray, 1987), neurokinin A (NKA), neurokinin B (NKB) and the N-terminally extended forms of NKA, neuropeptide K and neuropeptide-gamma, all sharing the same COOH-terminal sequence (Erspamer, 1981; Kage et al., 1988; Maggio, 1988; Tatemoto et al., 1985). The precursors of the tachykinins are generated by distinct genes, the preprotachykinin (PPT)I or PPT A gene, which encodes SP, NKA, neuropeptide K and neuropeptide-gamma, and the PPT II or PPT B gene encoding only NKB (Kage et al., 1988; Maggio, 1988). The PPT I gene generates three mRNAs, by alternative splicing, the α, β and gamma; the α-mRNA encodes only SP, whereas the β- and gamma-mRNAs encode both SP and NKA. NPK and neuropeptide-gamma are posttranslational

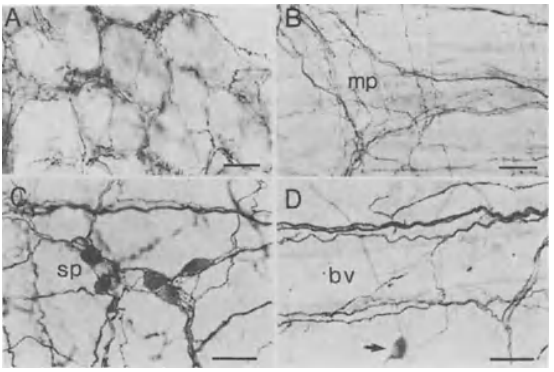


Fig. 2. Whole mount preparations of the rat duodenum (A, C, D) and stomach (B) processed for CGRP immunohistochemistry using the avidin biotin peroxidate-anti-peroxidase method, as previously described (Sternini et al., 1987), and rabbit polyclonal antisera raised against [Tyr] rat α-CGRP$_{23-37}$ (Sternini and Brecha, 1986). CGRP immunoreactive fibers innervate the duodenal glands (A), the myenteric plexus (mp) of the stomach (B) and the submucosal plexus (sp) of the duodenum (C), and are associated with blood vessels (bv) of the submucosa of the duodenum (D). The presence of CGRP immunoreactivity in ganglion cells within the intestinal enteric plexuses is shown in C and D (arrow in D). Calibration bars: 50 μm.

processing products of β- and gamma-PPT I mRNAs, respectively (Kage et al., 1988; Krause et al., 1987). CGRP was originally discovered as the result of alternative RNA processing of the primary transcript of the rat calcitonin gene (Amara et al., 1982; Rosenfeld et al., 1983). Subsequently, a family of CGRPs, the α (or I) and the β (or II), which differ in their sequences by only a few amino acids and which are generated by separate genes, have been described in both rats and humans (Amara et al., 1985; Rosenfeld et al., 1984; Steenbergh et al., 1985).

Tachykinin and CGRP immunoreactivities are widely distributed throughout the body, including the digestive system (Figures 1 and 2) (Fisher and Born, 1985; Goodman and Iversen, 1986; Jessel, 1983; Pernow, 1983; Rosenfeld et al., 1983), as shown by immunohistochemical methods. These studies have relied on antisera directed against the highly conserved C-terminal sequence of tachykinins or against α-CGRP, therefore recognizing all tachykinin peptides and both α and β CGRP forms, respectively. In the enteric nervous system, tachykinins and CGRP label two distinct populations of neurons: a population of intrinsic neurons located in the enteric plexuses (Figures 1 A and B, Figure 2 C), and a population of extrinsic, sensory neurons, which are mostly located in dorsal root ganglia (Costa et al., 1985; Gibbins et al., 1985; Green and Dockray, 1988; Sternini et al., 1987; Sternini 1990; Su et al., 1987). Tachykinin- and CGRP-containing enteric and afferent neurons give rise to processes that innervate all the layers of the gut and vasculature (Figure 1 C; Figures 2 A and D), with a slightly different pattern and density in different layers and regions. For example, in the muscle layers SP-containing fibers are usually more abundant than the

CGRP immunoreactive fibers, particularly in the circular muscle. In the mucosa, both tachykinin- and CGRP-positive fibers are more numerous in the intestine than in the stomach. Rich networks of tachykinin- and CGRP-containing processes are associated with the enteric plexuses (Figures 1 A and B; Figures 2 B and C) and vasculature (Figure 2 D). Tachykinin immunoreactivity is present in intrinsic neurons along the entire length of the alimentary tract, whereas CGRP immunoreactivity has a differential distribution in different regions. It is present in intrinsic neurons and terminals in the intestine, but it is restricted to extrinsic, afferent nerve fibers in the esophagus, stomach, hepatobiliary tract, and pancreas (Clague et al., 1985; Goehler et al., 1988a,b; Mulderry et al., 1985; Sternini and Brecha, 1986; Sternini et al., 1987). The CGRP-containing perivascular and a portion of the non-vascular fibers in the intestinal wall also are extrinsic, afferent in origin. These observations are supported by several lines of evidence, which include 1) the lack of intrinsic CGRP-containing neurons in the enteric plexuses of the esophagus and stomach (Figure 2 B), in the ganglionated plexus of the gallbladder, and in the intrapancreatic ganglia, even after pretreatment with the neurotoxin colchicine, which is commonly used to increase peptide levels in cell bodies, 2) the depletion of these populations of fibers by neonatal treatment with the sensory neurotoxin capsaicin, which primarily affects small-diameter primary sensory neurons and 3) the failure to affect CGRP immunostained fibers with the sympathetic neurotoxin, 6-hydroxydopamine (Goehler et al., 1988a,b;

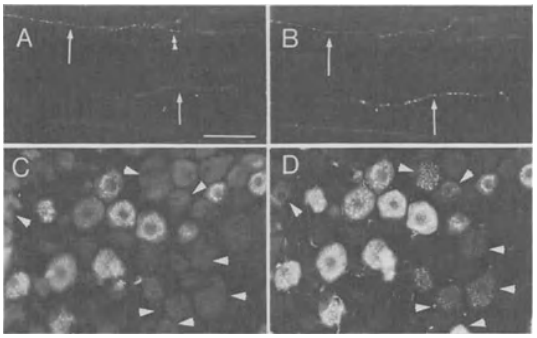


Fig. 3. Simultaneous visualization of tachykinin (A and C) and CGRP (B and D) immunoreactivities in fibers innervating the circular muscle layer of the rat duodenum (A and B, whole mount preparation) and in neurons of rat thoracic dorsal root ganglion (C and D). Tachykinin and CGRP immunostainings were obtained using a mixture of polyclonal antisera raised and in rabbits against the C-terminal region of SP (provided by Drs. R. Murphy and J.B. Furness) and in guinea pigs against the [Tyr] rat α-CGRP (Sternini, 1990) with double labeling immunofluorescence methods, as previously described (Goehler et al., 1988a). Note the extensive colocalization of tachykinin and CGRP immunoreactivities in primary sensory neurons. SP immunostaining was blocked by preabsorbing the antiserum with an excess of SP, NKA or NKB, indicating that this antiserum is a generalized marker for tachykinins. Arrowheads indicate cells immunoreactive for CGRP (D), but lacking tachykinin immunoreactivity (C). Calibration bar: 50μm.

Sternini and Brecha, 1986; Sternini et al., 1987; Su et al., 1987). The extrinsic CGRP visceral afferents form a major component of the sensory innervation of the alimentary tract (Green and Dockray, 1987; 1988; Sternini, 1990; Su et al., 1987), as demonstrated by axonal transport studies (see below). SP/tachykinin innervation also comprises an extrinsic, sensory component, which is mainly confined to a perivascular location and to the submucosa in the gut, and includes fibers innervating the hepatobiliary pathway and pancreas (Costa et al., 1985; Gibbins et al., 1985; Sternini, unpublished; Su et al., 1987).

Tachykinin and CGRP immunoreactivities colocalize in a subpopulation of fibers, mainly those innervating the vasculature, but also some of those innervating the mucosa, submucosa and muscle layers (Figures 3 A and B) and a few of those found in the enteric plexuses (Gibbins et al., 1985; Sternini, 1990). Colocalization in intrinsic neurons has not been reported, suggesting that only extrinsic fibers express both peptides.

Tachykinin and CGRP Immunoreactive Afferents to the Gut

Tachykinin and CGRP immunoreactivities extensively colocalize in sensory neurons of the vagal and dorsal root ganglia (Figures 3 C and D) (Gibbins et al., 1987; Helke and Hill, 1988; Ju et al., 1987). Recent work has demonstrated that these sensory neuropeptides, particularly CGRP, are the most reliable markers for the identification of a subpopulation of afferents innervating the digestive system (Green and Dockray, 1987; 1988; Sternini, 1990; Su et al., 1987). The extrinsic tachykinin- and CGRP-containing fibers mostly originate from neurons located in the spinal sensory ganglia, as indicated by surgical denervation experiments. Thus, celiac and superior mesenteric ganglionectomy, but not bilateral vagotomy, modifies the tachykinin and CGRP

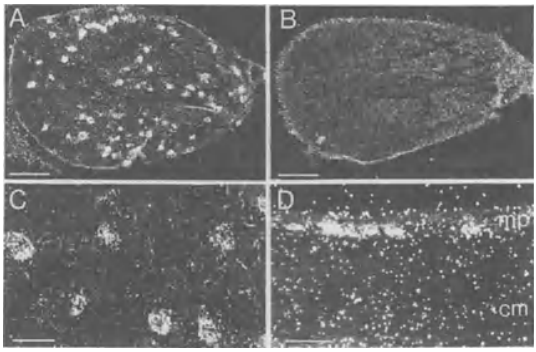


Fig. 4. Dark field photomicrographs of autoradiograms of rat dorsal root ganglia (A-C) and stomach (D) showing SP/NKA-encoding mRNAs in primary sensory neurons (A and C) and in ganglion cells of the myenteric plexus (mp) (D) as visualized using ^{35}S-β-PPT antisense RNA probe (1 ng/slide in A and C and 2 ng/slide in D) as previously published (Sternini et al., 1989). B: section adjacent to A and hybridized with ^{35}S-labeled PPT RNA probe (1 ng/slide) in the sense orientation; note the lack of specific signal in this section. Exposure times: 8 days for A-C and 7 days for D. Calibration bars: 200 μm in A and B; 50 μm in C and D.

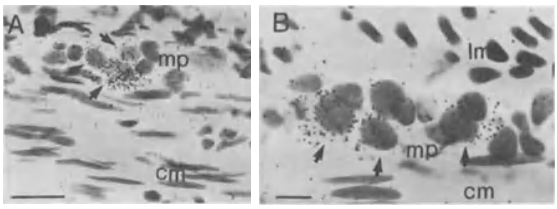


Fig. 5. Cellular localization of SP/NKA-encoding transcripts inneuronal cells (arrows) of the myenteric plexus (mp) of the rat stomach (A) and duodenum (B) as visualized by hybridizing sections with ^{35}S-β-PPT antisense RNA probe (2 ng/slide). Bright field photomicrographs. Exposure times for A and B: 14 and 10 days, respectively. Calibration bars: 25 μm in A and 10 μm in B.

innervation of the gut, pancreas and hepatobiliary tract (Gibbins et al., 1985; Goehler et al., 1988b; Sternini, unpublished; Su et al., 1987). These observations have been confirmed by retrograde transport studies, which rely on the peripheral injection and uptake of fluorescent tracers (e.g. Fast Blue, True Blue, Fluoro-Gold) that are axonally transported to the cell bodies of origin. These investigations have directly demonstrated that the vast majority of afferents supplying the gut and pancreas and displaying tachykinin and CGRP immunoreactivities (or both) have their cell bodies of origin in spinal sensory ganglia (Dockray and Sharkey, 1986; Green and Dockray, 1987; 1988; Sternini, 1990; Su et al., 1987).

Cellular Localization of SP/NKA- and CGRP-Encoding mRNAs in the Enteric Nervous System and Sensory Ganglia

The application of in situ hybridization histochemistry with radio-labeled probes complementary to mRNAs encoding tachykinin- and CGRP-containing precursors enables to establish which tachykinins and CGRPs are synthesized in the enteric nervous system and sensory ganglia. For the identification of SP/NKA- or CGRP-encoding mRNAs we have used ^{35}S-labeled single-stranded RNA probes complementary to rat β- and gamma-PPT I mRNAs (Sternini et al., 1989) and to specific sequences of α- and β-rat CGRPs, containing the 3' non-coding regions (Sternini and Anderson, 1990). Due to the extensive sequence homologies among the three processed PPT transcripts, both β- and gamma-SP/NKA antisense probes recognize all three PPT I mRNA species (Krause et al., 1987). However, these probes are specific for the PPT I-encoding mRNAs and do not recognize mRNAs from the PPT II gene, therefore allowing for the identification of sites of transcription of the PPT I gene. The significant divergencies among the 5' and 3' non-coding regions of α- and β-rat CGRP mRNAs, on the other hand, allow for the generation of α- and β-CGRP specific hybridization probes.

These studies have demonstrated that SP/NKA-encoding transcripts are expressed in selected neurons within the sensory ganglia (Figure 4 A and C), mainly characterized by small- to medium-sizes, and in neurons within the enteric plexuses of the esophagus, stomach (Figures 4 D and 5 A) and intestine (Figure 5 B). The lack of specific signal above background level in sections incubated with sense RNA probes (Figure 4 B) or pretreated

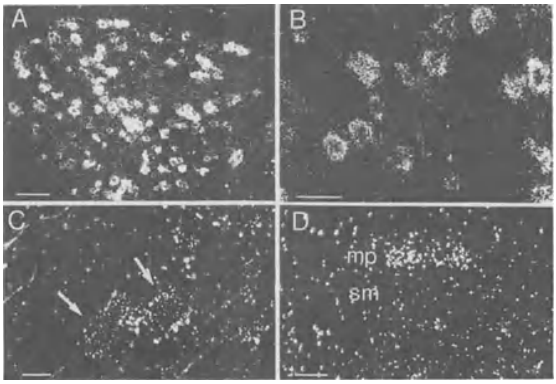


Fig. 6. Dark field photomicrographs of rat dorsal root ganglia (A-C) and ileum (D) showing α-CGRP mRNA (A and B) and β-CGRP mRNA (C; arrows) in primary sensory neurons, and β-CGRP mRNA in ganglion cells of the ileum myenteric plexus (mp) (D) as visualized using ^{35}S-α- and β-CGRP RNA probes. RNA probes were used at 4 ng/slide (A-C) and 8 ng/slide (D). Exposure times: 30 days for sensory ganglia and 50 days for gut sections. Calibration bars: 100 μm in A, 50 μm in B and 25 μm in C and D.

with RNase A prior to hybridization, along with the identification of a single band of hybridization in RNA blot analysis by the same antisense RNA probe, indicate the specificity of the in situ hybridization experiments (Sternini et al., 1989). Solution hybridization-nuclease protection assays, which are highly specific and sensitive for the detection and quantitation of α-, β- and gamma-PPT mRNAs, show multiple PPT-encoding transcripts in RNA extracts from the sensory ganglia and gut, with an abundance level of gamma-mRNA >β-mRNA >> α-mRNA (Sternini et al., 1989). We were not able to detect hybridization signal in the same ganglia and gut tissues incubated with RNA probes complementary to NKB-encoding mRNAs (Sternini, unpublished), which we used successfully in central nervous system structures, such as the retina (Brecha et al., 1989).

Both α- and β-CGRP mRNAs are expressed in sensory ganglia (Figures 6 A-C, 7 A), but to a different extent; α-CGRP mRNA is much more abundant than β-CGRP mRNA. These mRNAs are found mainly in small-to medium-size cell bodies, but occasionally in larger cells. On the contrary, only β-CGRP mRNA is expressed in enteric neurons (Figures 6 D and 7 B) of the myenteric and submucosal plexuses of the intestine, but not in intrinsic ganglion cells of the esophagus and stomach. α-CGRP mRNA is not detected in the enteric nervous system (Figure 7 C). As for the PPT probes, the lack of hybridization with non-complementary sense RNA probes suggests specificity of the hybridization signal; additional evidence of specificity is provided by the different labeling patterns obtained with the two CGRP RNA probes.

SUMMARY

Tachykinins and CGRP label two distinct populations of neurons innervating the digestive system: intrinsic and extrinsic,

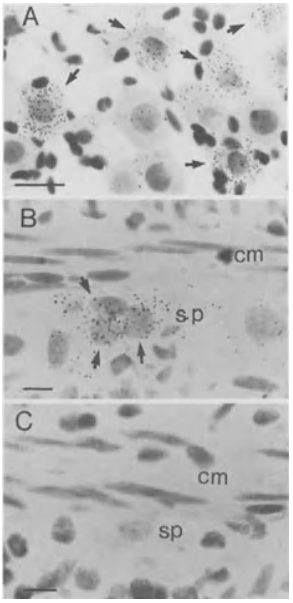


Fig. 7. Cellular localization of α-CGRP mRNA in sensory neurons ofrat dorsal root ganglion (A) and of β-CGRP mRNA in neuronal cells of the submucosal plexus (sp) of the rat ileum (B); arrows). Sections were hybridized with 4 ng/slide and 8 ng/slide of radiolabeled RNA probes and exposed for 7 and 50 days, respectively. C: section consecutive to B hybridized with radiolabeled α-CGRP antisense RNA probe (8 ng/slide) and exposed for 50 days; note the lack of specific signal over ganglion cells. Calibration bars: 25 μm in A and 10 μm in B and C.

afferents. The bulk of SP/tachykinin innervation originates from intrinsic neurons, even though a minor component of this innervation derives from afferent neurons, which are mostly located in dorsal root ganglia. Afferent SP/ tachykinin fibers are mainly confined to a perivascular location and to the submucosa in the gut, but are distributed also to the hepatobiliary pathway and pancreas. On the contrary, the extrinsic CGRP-containing afferents form a major component of the sensory innervation of the alimentary tract, including the rich CGRP innervation of the esophagus, stomach, hepatobiliary tract, pancreas, and vasculature, as well as a portion of non-vascular fibers distributed to the intestinal wall. Tachykinin and CGRP immunoreactivities appear to be colocalized in a population of nerve fibers, which are likely to be extrinsic, afferent, since colocalizaiton of these peptide immunoreactivities has not been reported in intrinsic neurons.

The presence of SP/NKA-encoding transcripts in the enteric nervous system and sensory ganglia and the lack of hybridization signal with RNA probes complementary to NKB mRNA indicate that the PPT I gene, but not the PPT II gene, is transcribed in these structures. This observation, along with receptor binding sites and radioimmunoassay data, which have failed to detect NKB receptor binding sites or immunoreactivity (Eysselein et al., 1990; Maggio, 1988; Mantyh et al., 1988; 1989) in the intestine

of several mammals, is consistent with a differential expression of the two PPT genes in the periphery and in the central nervous system (Brecha et al., 1989; Warden and Young, 1988). A differential expression of the tachykinin-encoding genes, the existence of multiple tachykinin receptor subtypes (Mantyh et al., 1988; 1989), and the findings that tachykinins can be differentiated on the basis of the potency of their activities (Galligan et al., 1987; Maggio, 1988), support the possibility that each tachykinin is expressed in separate, and perhaps functionally distinct neuronal systems. α- and β-CGRP genes also are differentially expressed according to the neuronal populations: α-CGRP mRNA is the most prominent form in sensory ganglia, and β-CGRP mRNA is the only form detected in enteric neurons (Mulderry et al., 1988; Sternini and Anderson, 1990). In addition, distinct distributions of mRNAs generated from the two CGRP genes have been reported in the central nervous system (Amara et al., 1985). The differential expression patterns of α- and β-CGRP mRNAs are consistent with a differential regulation of the α- and β-CGRP genes. The different level of expression in sensory ganglia also might indicate that the two genes are differentially regulated by cell-specific factors or other stimuli.

The physiological function of tachykinins and CGRP has not been firmly established, although neuroanatomical and pharmacological findings suggest that these peptides might subserve nociceptive functions and nonsensory roles in the periphery (Barthó and Holzer, 1985; Fisher and Born, 1985; Goodman and Iversen, 1986; Holzer, 1988; Kruger et al., 1989; Pernow, 1983). Additionally, tachykinins and CGRP are powerful vasoactive peptides and also exert numerous effects within the digestive system, influencing gastric and pancreatic secretions and food intake, coordinating gastrointestinal motility and exciting myenteric neurons (Barthó and Holzer, 1985; Goodman and Iversen, 1986; Holzer et al., 1989, Holzer, 1988; Palmer et al., 1986; Takaki and Nakayama, 1989). Tachykinins and CGRP have been regarded as possible mediators of afferent fibres implicated in gastric mucosa protection and could be involved in neurogenic inflammatory processes and trophic effects, in addition to influencing autonomic and visceral activities (Holzer, 1988). The apparent colocalization of tachykinin and CGRP immunoreactivities in afferent fibers supplying the enteric nervous system, along with the finding that tachykinins and CGRP are co-stored in the same large vesicles in peripheral nerves (Gulbenkian et al., 1986), is consistent with, but does not prove, a co-release of these peptides by afferent endings after stimulation. Whether they act in concert and influence each other's effect is not known. It is likely that both enteric and afferent SP/tachykinin- and CGRP-containing neurons innervating the digestive tract contribute to the control of a variety of functions by either an indirect effect via neuronal or hormonal stimulation or a direct effect on target cells.

Acknowledgements

Original work presented here was supported by NIH grants DK38752 and DK40469. The author would like to acknowledge the important contribution of K. Anderson and the skillful technical assistance of M. Lai. The author is also grateful to Drs. R. Murphy and J.B. Furness for the SP antiserum (RMSP-4), to Dr. J.H. Walsh and H. Wong for the NKA antiserum (SK8701), to Dr. Krause for the SP/NKA cDNAs (pG1β-PPT and pG2SP31-1) and NKB cDNA

(pG1NKB17-1), to Dr. T. Bonner for the NKB cDNA (pRNK p4), to Dr. S. Amara for the α- and β-rat CGRP cDNAs (pSP64pACGRP and pSP64NV), and to Dr. N. Brecha for his comments on the manuscript.

References

Amara, S.G., Jones, V., Rosenfeld, M.G., Ong., E.S., and Evans, R.M., 1982, Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products, **Nature**, 298:240.

Amara, S.G., Arriza, J.L., Leff, S.E., Swanson, L.W., Evans, R.M., Rosenfeld, M.G., 1985, Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide, **Science**, 229:1094.

Barthó, L., Holzer, P., 1985, Search for a physiological role of substance P in gastrointestinal motility, **Neuroscience**, 16:1.

Brecha, N.C., Sternini, C., Anderson, K., Krause, J.E., 1989, Expression and cellular localization of substance P/neurokinin A and neurokinin B mRNAs in the rat retina, **Visual Neurosci.**, 3:527.

Clague, J.R., Sternini, C., Brecha, N.C., 1985, Localization of calcitonin gene-related peptide-like immunoreactivity in neurons of the rat gastrointestinal tract, **Neurosci. Lett.**, 56:63.

Costa, M., Furness, J.B., Llewellyn-Smith, I.J., Murphy, R., Bornstein, J.C., Keast, J.R., 1985, Functional roles for substance P-containing neurons in the gastrointestinal tract, in: "Substance P. Metabolism and Biological Actions," C.C. Jordan, P. Oehme, eds., Taylor and Francis, London.

Costa, M., Furness, J.B., Gibbins, I.L., 1986, Chemical coding of enteric neurons, **Prog. Brain Res.**, Volume 68, "Coexistence of Neuronal Messengers: a New Principle of Chemical Transmission," T. Hökfelt, K. Fuxe, B. Pernow, eds., Elsevier, New York.

Costa, M., Furness, J.B., Llewellyn-Smith, I.J., 1987, Histochemistry of the enteric nervous system, in: "Physiology of the Gastrointestinal Tract," L.R. Johnson, ed., Raven, New York.

Dockray, G.J., 1987, Physiology of enteric neuropeptides in: "Physiology of the Gastrointestinal Tract," L.R. Johnson, ed., Raven, New York.

Dockray, G.J., Sharkey, K.A., 1986, Neurochemistry of visceral afferent neurons, **Prog. Brain Res.**, Volume 67, "Visceral Sensation," F. Cervero, J.F.B. Morrison, eds., Elsevier, New York.

Erspamer, V., 1981, The tachykinin peptide family, **Trends Neurosci.**, 4:267.

Eysselein, V.E., Khan, H., Davis, M., Sternini, C., Brecha, N., Cominelli, F., Williams, R., Snape, W., 1990, Neurokinin B is an abundant tachykinin form in bovine but not in human, rabbit and rat intestine: species differences in the expression of the preprotachykinin-II precursor, **Gastroenterology**, 98:A493.

Fisher, J.A., Born, W., 1985, Novel peptides from the calcitonin gene: Expression, receptors and biological function, **Peptides**, 6:265.

Furness, J.B., Costa, M., 1987, "The Enteric Nervous System," Churchill Livingstone, London.

Furness, J.B., Llewellyn-Smith, I.J., Bornstein, J.C., Costa, M., 1988, Chemical neuroanatomy and the analysis of neuronal circuitry in the enteric nervous system, in: "Handbook of Chemical Neuroanatomy," Volume 6: **The Peripheral Nervous System**, A. Björklund, T. Hökfelt, C. Owman, eds., Elsevier, New York.

Galligan, J.J., Tokimasa, T., North, R.A., 1987, Effects of three mammalian tachykinins on single enteric neurons, **Neurosci. Lett.**, 82:167.

Gibbins, I.L., Furness, J.B., Costa, M., MacIntyre, I., Hillyard, C.J. Girgis, S., 1985, Colocalization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of the guinea pig, **Neurosci. Lett.**, 57:125.

Gibbins, I.L. Furness, J.B., Costa, M., 1987, Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig, **Cell Tissue Res.**, 248:417.

Goehler, E.L., Sternini, C., Brecha, N., 1988a, Calcitonin gene-related peptide immunoreactivity in the biliary pathway and liver of the guinea-pig: distribution and colocalization with substance P, **Cell Tissue Res.**, 253:145.

Goehler, E.L. Sternini, C., 1988b, Effect of extrinsic denervation on calcitonin gene-related peptide immunoreactivity (CGRP-IR) in the rat hepatobiliary system, Soc. Neurosci. Abs., 14:984.

Goodman, E.C., Iversen, L.L., 1986, Calcitonin gene-related peptide: novel neuropeptide, **Life Sci.**, 38:2169.

Green, T., Dockray, G.J., 1987, Calcitonin gene-related peptide and substance P in afferents to the upper gastrointestinal tract in the rat, **Neurosci. Lett.**, 76:151.

Green, T., Dockray, G.J., 1988, Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig, **Neuroscience**, 25:181.

Gulbenkian, S., Merighi, A., Wharton, J., Varndell, I.M., Polak, J.M., 1986, Ultrastructural evidence for the coexistence of calcitonin gene-related peptide and substance P in secretory vesicles of peripheral nerves in the guinea pig, **J. Neurocytol.**, 15:535.

Helke, C.J., Hill, K.M., 1988, Immunohistochemical studies of neuropeptides in vagal and glossopharyngeal afferent neurons in the rat, **Neuroscience**, 26:539.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.

Holzer, P., Barthó, L., Matusaki, O., Bauer, V., 1989, Calcitonin gene-related peptide action on intestinal circular muscle, **Am. J. Physiol.**, 256:G546.

Jessell, T.M., 1983, Substance P in the nervous system, in: "Handbook of Psychopharmacology" Volume 16, L.L. Iversen, S.D. Iversen, S.H. Snyder, eds., Plenum, New York.

Ju, G., Hökfelt, T., Brodin, E., Fahrenkrug, J., Fisher, J.A., Frey, P., Elde, R.P., Brown, J.C., 1987, Primary sensory neurons of the rat showing calcitonin gene-related peptide immunoreactivity and their relation to substance P-, somatostatin-, vasoactive intestinal polypeptide- and cholecystokinin-immunoreactive ganglion cells, **Cell Tissue Res.**, 247:417.

Kage, R., McGregor, G.P., Thim, L., Conlon, J.M., 1988, Neuro-

peptide-gamma: a peptide isolated from rabbit intestine that is derived from gamma-preprotachykinin, **J. Neurochem.**, 50:1412.

Krause, J.E., Chirgwin, J.M., Carter, M.S., Xu, Z.S., Hershey, A.D., 1987, Three rat preprotachykinin mRNAs encode the neuropeptide substance P and neurokinin A, **Proc. Natl. Acad. Sci. USA**, 84:881.

Kruger, L., Silverman, J.D., Mantyh, P.W., Sternini, C., Brecha, N., 1989, Peripheral patterns of calcitonin gene-related peptide general somatic sensory innervation: cutaneous and deep terminations, **J. Comp. Neurol.**, 280:291.

Llewellyn-Smith, I.J., 1989, Neuropeptides and the microcircuitry of the enteric nervous system, in: "Regulatory Peptides," J.M. Polak, ed., Verlag.

Maggio, J.E., 1988, Tachykinins, **Ann. Rev. Neurosci.**, 11:13.

Mantyh, P.W., Gates, T., Mantyh, C.R., Maggio, J.E., 1989, Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues, **J. Neurosci.**, 9:258.

Mantyh, P.W., Mantyh, C.R., Gates, T., Vigna, S.R., Maggio, J.E., 1988, Receptor binding sites for substance P and substance K in the canine gastrointestinal tract and their possible role in inflammatory bowel disease, **Neuroscience**, 25:817.

Mulderry, P.K., Ghatei, M.A., Bishop, A.E., Allen, Y.S., Polak, J.M., Bloom, S.R., 1985, Distribution and chromatographic characterization of CGRP-like immunoreactivity in the brain and gut of the rat, **Reg. Pept.**, 12:133.

Mulderry, P.K., Ghatei, M.A., Spokes, R.A., Jones, P.M., Pierson, A.M., Hamid, Q.A., Kanse, S., Amara, S.G., Burrin, J.M., Legon, S., Polak, J.M., Bloom, S.R., 1988, Differential expression of α-CGRP and β-CGRP by primary sensory neurons and enteric autonomic neurons of the rat, **Neuroscience**, 25:195.

Palmer, J.M., Schemann, M., Tamura, K., Wood, J.D., 1986, Calcitonin gene-related peptide excites myenteric neurons, **Eur. J. Pharmacol.**, 132:163.

Pernow, B., 1983, Substance P, **Pharmacol. Rev.**, 35:85.

Rosenfeld, M., Mermod, J.-J., Amara, S.G., Sawcenko, P.E., Rivier, J., Vale, W.W., Evans, R.M., 1983, Production of a novel neuropeptide encoded by the calcitonin gene via tissue specific RNA processing, **Nature**, 304:129.

Rosenfeld, M.G., Amara, S.G., Evans, R.M., 1984, Alternative RNA processing: determining neuronal phenotype, **Science**, 225:1315.

Steenbergh, P.H., Höppener, J.W.M., Zandberg, J., Lips, C.J.M., Jansz, H.S., 1985, A second human calcitonin/CGRP gene, **FEBS Lett.**, 183:402.

Sternini, C., 1988, Structural and chemical organization of the myenteric plexus, **Ann. Rev. Physiol.**, 50:81.

Sternini, C., 1990, Neurochemistry of spinal afferents supplying the upper gastrointestinal tract and pancreas, in: "Brain and Gut Interactions," Y. Taché, D. Wingate, eds., CRC Press, in press.

Sternini, C., Anderson, K., 1990, Differential distribution of α- and β-calcitonin gene-related peptide (CGRP) mRNAs in enteric and sensory neurons, **Gastroenterology**, 98:A526.

Sternini, C., Anderson, K., Frantz, G., Krause, J.E., 1989, Expression of substance P/neurokinin A-encoding preprotachykinin messenger ribonucleic acids in the rat enteric nervous system, **Gastroenterology**, 97:348.

Sternini, C., Brecha, N., 1986, Immunocytochemical identification of islet cells and nerve fibers containing calcitonin gene-related peptide-like immunoreactivity in the rat pancreas, **Gastroenterology**, 90:1155.

Sternini, C., Reeve, J.R., Jr., Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.

Su, H.C., Bishop, A.E., Power, R.F., Hamada, Y., Polak, J.M., 1987, Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas, **J. Neurosci.**, 7:2674.

Takaki, M., Jin, J.-G., Nakayama, S., 1989, Possible involvement of calcitonin gene-related peptide (CGRP) in non-cholinergic, non-adrenergic relaxation induced by mesenteric nerve stimulation in guinea pig ileum, **Brain Res.**, 478:199.

Tatemoto, K., Lundberg, J.M., Jornvall, H., Mutt, V., 1985, Neuropeptide K: isolation, structure and biological activities of a novel brain tachykinin, **Biochem. Biophys. Res. Commun.**, 128:947.

Warden, M.K., Young, W.S., III, 1988, Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system, **J. Comp. Neurol.**, 272:90.

PEPTIDES AND THEIR RECEPTORS ON AFFERENT NEURONS TO THE UPPER GASTROINTESTINAL TRACT

G.J. Dockray, E.R. Forster, and S.M. Louis

MRC Secretory Control Research Group,
Physiological Laboratory, University of Liverpool
Liverpool, England

INTRODUCTION

The two morphological divisions of the afferent innervation of the upper gastrointestinal tract, the vagal and splanchnic nerves, are distinguishable in functional and in neurochemical terms. It has been known since the early part of the century that the vagal pathway mediates physiological reflexes involved in the normal control of gastric motility and secretion, and that the splanchnic pathway mediates the effects of noxious stimulation of the gut (Hertz, 1911). Peptides are involved in these functions in two ways - as mediators of some of the effects of nerve stimulation, and as modulators of afferent discharge by acting at peptide receptors expressed on afferent nerve fibres (Dockray, 1988). Splanchnic afferents are a rich source of neuropeptides and provide a good illustration of how peptides might mediate the function of some visceral afferents, while vagal fibres express receptors for several peptides and so illustrate modulatory functions. The present account will deal with the physiological significance of these actions.

PEPTIDERGIC VISCERAL AFFERENT SYSTEMS

Organization

Quantitative information on the neurochemistry of peptidergic visceral afferents has come from the combined use of retrograde tracing and immunohistochemistry. An illustration of the data obtained from this approach is shown in Fig. 1. These data were obtained by injection of the retrograde tracer True Blue into the stomach in anesthetized rats; the tracer is taken up by afferent fibres and retrogradely transported to nerve cell bodies in the dorsal root ganglia and nodose ganglia. Sections of ganglia processed for peptide immunohistochemistry allow the localization of those peptidergic neurons that project to the stomach. The data from these and similar studies indicate that approximately 80% of the spinal afferent neurons to the stomach contain calcitonin gene related peptide-(CGRP) and approximately

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

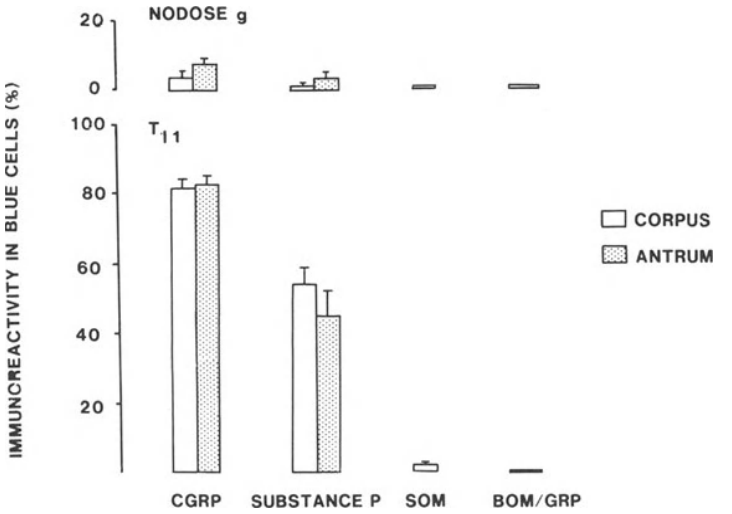


Fig. 1. Relative proportions of different neurochemically identified neurons projecting to the stomach from the vagus (top), and spinal (T_{11} - bottom) ganglia in the rat. The results were obtained from the combined use of retrograde tracing and immunohistochemistry. Note that CGRP and substance P are well represented in the spinal afferent innervation to both corpus and antrum, neither peptide is well represented in the gastric projection from the nodose ganglia, and that other peptides are poorly represented in the gastric spinal projection.

50% contain substance P-immunoreactivity (Sharkey et al., 1983; Green and Dockray, 1988; Dockray and Sharkey, 1986): less than about 5% of vagal afferents to the stomach contain these two peptides. Other peptides that are known to occur in primary afferent neurons, including cholecystokinin (CCK), somatostatin, vasoactive intestinal polypeptide (VIP), opioid peptides and gastrin releasing peptide are poorly represented in the gastric spinal afferents of the rat. Within afferent neurons, peptides are synthesized in the cell body and transported both centrally and peripherally. The rate of transport of a variety of peptides in the vagal afferents of several species is about 2 $mm.hr^{-1}$, and the transport of CGRP in splanchnic afferents to the rat upper gastrointestinal tract is also of this order (Dockray and Sharkey, 1986; Varro et al., 1988).

Substance P in the stomach occurs in both afferent fibres and intrinsic neurons, but CGRP is solely of afferent origin (Sharkey et al., 1983) Green and Dockray, 1988; Su et al., 1987; Sternini et al., 1987). Radioimmunoassays using antibodies specific for the C-terminus of rat α CGRP show almost complete loss of immunoreactivity in extracts of the stomach after coeliac ganglionectomy (which lesions splanchnic afferents), and complete loss after pretreatment with the sensory neurotoxin capsaicin. CGRP is therefore an excellent marker for the distribution of peripheral terminals of gastric splanchnic afferents (Varro et al., 1988). These fibres can be found around blood vessels in the submucosa, in the myenteric plexus, circular

smooth muscle and occasionally in the mucosa (Green and Dockray, 1988; Sternini et al., 1987). It appears that the α form of CGRP is localized to afferent fibres and that the β form (which is found more distally in the gut) may occur in intrinsic gut neurons (Mulderry et al, 1988).

Functional significance

The peptides found in the peripheral terminals of gastrointestinal afferents can be released in response to capsaicin, electrical field stimulation in vitro, or antidromic nerve stimulation. Evidence for this comes from detection of the released peptides by radioimmunoassay of tissue perfusates in vitro (Maggi et al., 1989; Renzi et al., 1988), by motility studies in vitro (Barthó et al, 1982), and in vivo (Delbro et al., 1983) and by electrophysiological recordings from prevertebral ganglia neurons which are supplied by the collaterals of primary afferents passing through the ganglia (Tsunoo et al., 1982). Electrophysiological studies suggest that splanchnic afferents constitute a homogeneous population of fibres, that are often silent in physiological conditions, and that often have punctate receptive fibres in the serosa or mesentery (Janig and Morrison, 1985). Whether or not the fibres identified electrophysiologically correspond to those peptidergic afferents detected in immunohistochemistry is uncertain. One interpretation of the electrophysiological and morphological data is that mechanical stimulation of the serosa or mesentery might act via axon reflexes to cause release of peptides from collaterals at any of several levels within the gut or prevertebral ganglia. However the precise functional significance of this system in the control of digestion in the upper gastrointestinal tract is uncertain. Most of the presently available data on the electrophysiological properties of the mammalian spinal visceral afferents deal with the distal gut, and studies on the stomach are quite sparce; it may be that further studies will clarify the physiological questions outlined above. Quite recently, however, evidence has emerged to suggest that gastric splanchnic afferents might function in the control of local responses during tissue damage. This would, of course, be quite compatible with the notion that splanchnic, as compared with vagal, afferents mediate the effects of noxious stimuli to the gut.

Holzer and Sametz (1986) have shown that capsaicin-sensitive afferents appear to play a role in protecting the gastric mucosa from a variety of damaging agents. In the rat, the hemorrhagic lesions produced by intragastric ethanol were exacerbated in animals pretreated with capsaicin. Moreover, acutely administered capsaicin (which stimulates small diameter afferents) reversed the effects of intragastric ethanol and of aspirin (Holzer et al., 1989; Holzer and Lippe, 1988). Since CGRP and substance P are well represented in capsaicin-sensitive afferents they are good candidates for mediating the protective action of these neurons in the gastric mucosa. To test this idea, we have examined the hemorrhagic lesions produced by ethanol in rats pretreated with antibodies to substance P or CGRP in order to neutralize the endogenous peptides. The validity of this type of immunoneutralization approach has previously been established in studies of the role of substance P and CGRP in mediating the inflammatory effects of mustard oil, or capsaicin, in the skin (Louis et al., 1989 a,b). We have now found that passive immunization with either substance P or CGRP antibodies increased the

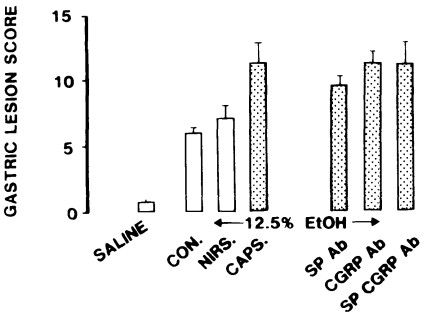


Fig. 2. Effects of CGRP and substance P antibodies on hemorrhagic lesions (see Holzer and Sametz, 1986, for scoring system) produced by intragastric instillation of 3 ml of 12.5% ethanol in urethane anesthetized rats. On the left are shown control data (a) for saline in place of ethanol, (b) ethanol in control rats (con), (c) after non-immune serum (NIRS) and (d) in capsaicin-treated rats. On the right the effects of i.v. injection of antibody (Ab) to substance P (SP) or CGRP, or both, are shown. Note that there is a significant increase ($p<0.05$) in the lesion score compared to control after administration of either antibody. Administration of both antibodies produces no further increase; the lesion score in passively immunized rats is similar to that seen in capsaicin-treated rats.

effect of ethanol in causing hemorrhagic mucosal lesions in the rat (Fig. 2). These data therefore point to a role for substance P and CGRP as protective agents following their release from splanchnic afferents by noxious stimuli. Direct evidence for a cytoprotective action of exogenous tachykinins has been provided by Evangelista et al. (1989). The relevant sites of action remain unknown, but several possibilities are worth mentioning. First, CGRP and substance P are potent vasodilators and they might act by increasing blood flow in the mucosa; second, CGRP inhibits acid secretion, perhaps by releasing somatostatin, and third, substance P stimulates HCO_3-secretion (Taché et al., 1984; Dunning et al., 1987; Beglinger et al., 1988; Fändriks and Delbro, 1983).

PEPTIDES AS MODULATORS OF AFFERENT FUNCTION

The electrophysiological studies of Paintal were the first to establish that vagal afferents respond to a variety of chemical substances, including 5HT and adrenaline (Paintal, 1953). Since then both electrophysiological and autoradiographic work has provided evidence for the presence of receptors for other biologically active substances on vagal fibres, e.g. glucose, CCK, neurotensin, atrial natriuretic peptide (Niijima, 1981, 1983; Zarbin et al., 1981; Moran et al, 1987; Schultz et al., 1988; Kessler and Beaudot, 1989). In addition, there is evidence that afferent discharge can be evoked secondary to changes in smooth muscle movements caused by experimentally applied substances including CCK-like peptides (Cottrell and Iggo, 1984). In most cases, the normal function of the receptors that occur on

vagal afferents is unknown, but in the case of CCK, evidence now available from electrophysiological studies of the vagal afferent fibres themselves or of dorso-medial medulla neurons with a gastric mechanoreceptor input, concomitant motility recordings, lesioning (surgical or capsaicin) experiments, as well as autoradiographic data, indicate that CCK receptors are likely to occur on vagal fibres that also function as gastric mechanoreceptors (Raybould et al., 1985, 1988; Davison and Clarke, 1988). There are a number of actions of CCK that are blocked by vagotomy or by capsaicin-pretreatment and so might be mediated by vagal afferents; these include inhibition of gastric emptying, inhibition of food intake, supression of somatic functions, increased memory retention, inhibition of prolactin release, stimulation of oxytocin release and inhibition of dopamine release (Yamagishi and Debas, 1978; Kawasaki et al., 1983; Smith et al., 1985; Ritter et al., 1986; Flood et al., 1987; Raybould and Taché, 1988; Hodson et al., 1986; McCann et al., 1988; Hamamura et al., 1989). The physiological significance of some of these actions is uncertain, but at least in the case of gastric emptying, there is an increasing body of evidence to suggest that the mechanisms are of physiological relevance.

The idea that CCK acts physiologically to control gastric emptying is supported by assays in which plasma concentrations of exogenous and endogenous peptide required for a particular response are matched (Debas et al, 1975; Liddle et al., 1986; Kleibeuker et al., 1988). In addition, good progress has been made recently in this area because excellent specific CCK antagonists have become available, notably the benzodiazepine L-364,718 and the glutaramic acid derivative CR1409, which are both highly selective for the CCK-A receptor (Freidinger, 1989). Protein rich meals both release CCK and delay gastric emptying. The inhibition of gastric emptying by protein is inhibited by CCK antagonists; this is a specific effect because liquid meals that delay gastric emptying but do not release CCK are not inhibited (Green et al., 1988; Meyer et al., 1989). There are several possible sites of action of CCK that might be relevant for control of gastric emptying. Thus, in addition to possible reflex effects mentioned above, CCK also stimulates smooth muscle directly, e.g. pyloric antral muscle (Isenberg and Csendes, 1982; Morgan et al., 1978) and has prejunctional effects e.g. release of Ach. In the dog, CCK acts partly on the distal stomach to delay gastric emptying (Yamagishi and Debas, 1978), but our data suggests that in the rat pyloroplasty has little or no effect on the gastric emptying of protein solutions (Bremner et al., 1990). The action of CCK on gastric emptying does, however, depend on intact small diameter afferents because it is inhibited in rats pretreated with capsaicin; since lesion of splanchnic afferents by coeliac ganglionectomy does not influence the action of CCK-releasing meals, the most likely capsaicin-sensitive afferents to be involved are those of the vagus (Dimaline et al., 1988; Forster et al., 1990). In capsaicin treated rats, L-364,718, far from reversing the inhibition of gastric emptying by CCK actually enhances it: this is attributable to the direct effect of CCK on gastric smooth muscle which increases tension and so speeds up gastric emptying (Forster et al., 1990). In the intact rat, this mechanism is probably overridden by the reflex which is activated when CCK stimulates vagal afferents to evoke vago-vagal reflex relaxation of the body of the stomach, which in turn delays gastric emptying (Raybould et al, 1987; Raybould and Taché, 1988). The final efferent pathway in this reflex

could include inhibition of a tonically active cholinergic excitatory pathway, or activation of a VIP-mediated relaxation pathway. Direct evidence for a role of VIP as a final mediator is provided by the recent finding that emptying of peptone, but not other liquid test meals, was impaired in rats with circulating VIP antibodies (Forster, 1990).

Taken as a whole, the available experimental data can be summarized as follows: in order to delay gastric emptying CCK-releasing solutions need to penetrate the distal duodenum or beyond (which is where CCK is found), but do not require either splanchnic afferents or those vagal afferents that supply the distal intestine (and pass through the coeliac ganglia) and neither do they need an intact pylorus or sympathetic adrenergic neurons. The effect is, however, reduced by VIP antibodies. It is of interest that different mechanisms must mediate the actions of other solutions on gastric emptying (Table). The results of applying the treatments mentioned above to the emptying of hyperosmolal solutions and acid are summarized in the Table. The results suggest that while all three solutions act reflexly to delay emptying (i.e. are inhibited in capsaicin-treated rats) there are different afferent and efferent mechanisms involved. Thus, hyperosmolal solutions require access to the small intestine, where presumably they activate (either vagal or splanchnic) afferents that pass centrally through the coeliac ganglia; the efferent side of this reflex does not require VIP, an intact pylorus or adrenergic neurons. In contrast, acid acts at a site in the stomach or proximal duodenum, does not require splanchnic afferents, VIP or adrenergic neurons, but does require an intact pylorus. Evidently there are likely to be many extrinsic reflexes that mediate the action of a mixed meal on gastric emptying. Whether or not humoral agents acting on afferent fibres mediate the effects of acid and hyperosmolal solutions, in the same way that CCK mediates protein effects, remains to be determined.

TABLE 1

The action of various treatments on the gastric emptying of different substances in the rat.*

	Liquid Test Meals		
Treatment	**Peptone**	**Hyperosmolal ($900\ mosmol.kg^{-1}$)**	**Saline Acid (50 mM HCl)**
Capsaicin	+	+	+
Access to distal duodenum	+	+	-
CCK antagonists	+	-	-
VIP immunoneutralization	+	-	-
Coeliac ganglionectomy	-	+	-
Pyloroplasty	-	-	+
Adrenergic inhibition	-	-	-

* +, reverses the inhibition of meal emptying; -, no effect. Adrenergic blockade was produced by 6 OH dopamine, guanethidine or reserpine treatment. Capsaicin was administered to neonatal rats. Note that while all three solutions require intact small diameter afferents, there are marked differences in the actions of other treatments, indicating that different reflex pathways are involved in mediating their effects on gastric emptying. See text for references and details.

References

Barthó, L., Holzer, P., Lembeck, F. and Szolcsány, J., 1982, Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to release of substance P, **J. Physiol.**, 332:157.

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.

Bremner, A.J., Dockray, G.J., and Forster, E.R., 1990, The mechanism of action of liquid test meals on gastric emptying in rat, **J. Physiol.**, 422:63P.

Cottrell, D.F., and Iggo, A., 1984, The responses of duodenal tension receptors in sheep to pentagastrin, cholecystokinin and some other drugs, **J. Physiol.**, 354:477.

Davison, J.S., and Clarke G.D., 1988, Mechanical properties and sensitivity to CCK of vagal gastric slowly adapting mechanoreceptors, **Am. J. Physiol.**, 255:G55.

Debas, H.T., Farooq, O., and Grossman, M.I., 1975, Inhibition of gastric emptying is a physiological action of cholecystokinin, **Gastroenterology**, 68:1211.

Delbro, D., Fändriks, L., Rosel, S., and Folkers, K., 1983, Inhibition of antidromically induced stimulation of gastric motility by substance P receptor blockade, **Acta Physiol. Scand.**, 118:309.

Dimaline, R., Dockray, G.J., and Green, T., 1988, The role of the coeliac ganglion in the control of gastric emptying in the rat, **J. Physiol.**, 403:53P.

Dockray, G.J., 1988, Regulatory peptides and the neuroendocrinology of gut-brain relations, **Quart. J. Expl. Physiol.**, 73:703.

Dunning, B.E., and Taborsky, G.J., 1987, Calcitonin gene-related peptide: A potent and selective stimulator of gastrointestinal somatostatin secretion, **Endocrinology**, 120:1774.

Evangelista, S., Lippe, I.Th., Rovero, P., Maggi, C.A., and Meli, A., 1989, Tachykinins protect against ethanol-induced gastric lesions in rats, **Peptides**, 79:81.

Fändriks, L., and Delbro, D., 1983, Neural stimulation of gastric bicarbonate secretion in the cat. An involvement of vagal axon-reflexes and substance P? **Acta Physiol. Scand.**, 118:301.

Flood, J.F., Smith, G.E., and Morley, J.E., 1989, Modulation of memory processing by cholecystokinin: Dependence on the vagus nerve, **Science**, 236:832.

Forster, E.R., Green, T., Elliot, M., Bremner, A., and Dockray, G.J., 1990, The role of afferent neurons in the action of cholecystokinin, acid and hyperosmolal solutions on gastric emptying in the rat, **Am. J. Physiol.**, in press.

Forster, E.R., 1990, The role of vasoactive intestinal polypeptide in the control of gastric emptying in the rat, **J. Physiol.**, in press.

Freidinger, R.M., 1989, Cholecystokinin and gastrin antagonists, **Med. Res. Rev.**, 9:271.

Green, T., Dimaline, R., Peikin, S., and Dockray, G.J., 1988, Action of the cholecystokinin antagonist L364,718 on gastric emptying in the rat, **Am. J. Physiol.**, 255:G685.

Green, T., and Dockray, G.J., 1988, Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea pig, **Neuroscience**, 25:181.

Hamamura, T., Kazahaya, Yl, and Otsuki, S., 1989, Ceruletide suppresses endogenous dopamine release via vagal afferent system, studied by in vivo intracerebral dialysis, **Brain Res.**, 483:78.

Hertz, A.F., 1911, "The sensibility of the alimentary canal", Oxford University Press.

Hodson, C.A., Burden, H.W., and Lawrence, I.E. Jr., 1986, Intraperitoneal cholecystokinin administration inhibits prolactin release: an effect prevented by vagotomy, **IRCS Medical Science**, 14:551.

Holzer, P., and Lippe, I.Th., 1988, Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa, **Neuroscience**, 27:981.

Holzer, P., Pabst, M.A., and Lippe, I.Th., 1989, Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa, **Gastroenterology**, 96:1425.

Holzer, P., and Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons, **Gastroenterology**, 91:975.

Isenberg, J.I., and Csendes, A., 1972, Effect of octapeptide of cholecystokinin on canine pyloric pressure, **Am. J. Physiol.**, 222:428.

Janig, W., and Morrison, J.F.B., 1986, Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception, **Progress in Brain Research**, 67:87.

Kessler, J.P., and Beaudet, A., 1989, Association of neurotensin binding sites with sensory and visceromotor components of the vagus nerve, **J. Neurosci.**, 9:466.

Kleibeuker, J.H., Beekhuis, H., Jansen, J.B.M.J., Piers, D.A., and Lamers, C.B.H.W., 1988, Cholecystokinin is a physiological hormonal mediator of fat-induced inhibition of gastric emptying in man, **Eur. J. Clin. Invest.**, 18:173.

Kawasaki, K., Kodama, M., and Matsushita, A., 1983, Caerulein, a cholecystokinin-related peptide, depresses somatic function via the vagal afferent system, **Life Sciences**, 33:1045.

Liddle, R.A., Morita, E.T., Conrad, C.F., and Williams, J.A., 1986, Regulation of gastric emptying in humans by cholecystokinins, **J. Clin. Invest.**, 77:992.

Louis, S.M., Jamieson, A., Russell, N., and Dockray, G.J., 1989a, The role of substance P and CGRP in neurogenic plasma extravasation and vasodilatation in the rat, **Neuroscience**, 32:581.

Louis, S.M., Johnstone, D., Russell, N.J.W., Jamieson, A., and Dockray, G.J., 1989b, Antibodies to calcitonin gene-related peptide reduce inflammation induced by topical mustard oil but not that due to carrageenin in the rat, **Neurosci. Lett.**, 102:257.

McCann, M.J., Verbalis, J.G., and Sticker, E.M., 1988, Capsaicin pretreatment attenuates multiple responses to cholecystokinin in rats, **J. Autonomic Ner. System**, 23:265.

Maggi, C.A., Santicioli, P., Renzi, D., Patachini, R., Surrenti, G., and Meli, A., 1989, Release of substance P- and calcitonin gene-related peptide-like immunoreactivity and motor response of the isolated guinea pig gallbladder to capsaicin, **Gastroenterology**, 96:1093.

Meyer, B.M., Beglinger, C., Jansen, J.B.M.J., Rovati, L.C., Werth, B.A., Hildebrand, P., Zach, D., Stalder, G.A., 1989, Role of cholecystokinin in regulation of gastrointestinal motor functions, **The Lancet**, 1:12.

Moran, T.H., Smith, G.P., Hostetler, A.M., and McHugh, P.R., 1987, Transport of cholecystokinin (CCK) binding sites in subdiaphragmatic vagal branches, **Brain Research**, 415:149.

Morgan, K.G., Schmalz, P.F., Go, V.L.W., and Szurszewski, J.H., 1978, Electrical and mechanical effects of molecular variants of CCK on antral smooth muscle, **Am. J. Physiol.**, 235:E324.

Mulderry, P.K., Ghatei, M.A., Spokes, R.A., Jones, P.M., Pierson, A.M., Hamid, Q.A., Kanse, S., Amara, S.G., Burrin, J.M., Legon, S., Polak, J.M. and Bloom, S.R., 1988, Differential expression of α-CGRP and β-CGRP by primary sensory neurons and enteric autonomic neurons of the rat, **Neuroscience**, 25:195.

Niijima, A., 1981, Visceral afferents and metabolic function, **Diabetologia** 20:325.

Niijima, A., 1983, Glucose-sensitive afferent nerve fibres in the liver and their role in food intake and blood glucose regulation, **J. Aut. Nerv. System**, 9:207.

Paintal, A.S., 1953, Impulses in vagal afferent fibres from stretch receptors in the stomach and their role in the peripheral mechanism of hunger, **Nature**, 172:1194.

Raybould, H.E., Roberts, M.E., and Dockray, G.J., 1987, Reflex decreases in intragastric pressure in response to cholecystokinin in rats, **Am. J. Physiol.**, 253:G165.

Raybould, H.E., Gayon, R., and Dockray, G.J., 1985, CNS effects of circulating CCK8 - involvement of brainstem neurones responding to gastric distension, **Brain Res.**, 342:187.

Raybould, H.E., Gayton, R.J., and Dockray, G.J., 1988, Mechanisms of action of peripherally administered cholecystokinin octapeptide on brain stem neurones in the rat, **J. Neurosci.**, 8:3018.

Raybould, H.E., and Taché, Y., 1988, cholecystokinin inhibits gastric motilitiy and emptying via a capsaicin-sensitive vagal pathway in rats, **Am. J. Physiol.**, 255:G242.

Renzi, D., Santicioli, P., Maggi, C.A., Surrenti, C., Pradelles, P., and Meli, A., 1988, Capsaicin-induced release of substance P-like immunoreactivity from the guinea pig stomach in vitro and in vivo, **Neuroscience Letters**, 92:254.

Ritter, R.C., Kalivas, P., and Bernier, S., 1986, Cholecystokinin-induced suppression of locomotion is attenuated in capsaicin pretreated rats, **Peptides**, 7:587.

Schultz, H.D., Gardner, D.G., Deschepper, C.F., Coleridge, H.M., and Coleridge, J.C.G., 1988, Vagal C-fiber blockade abolishes sympathetic inhibition by atrial natriuretic factor, **Am. J., Physiol.**, 255:R6.

Sharkey, K.A., Williams, R.G., Dockray, G.J., 1983, Sensory substance P innervation of the stomach and pancreas: Demonstration of capsaicin-sensitive sensory neurones in the rat by combined immunohistochemistry and retrograde tracing, **Gastroenterology**, 87:914.

Smith, G.P., Jerome, C., and Norgren, R., 1985, Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats, **Am. J. Physiol.**, 249:R638.

Sternini, C., Reeve, J.R., Jr., and Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.

Su, H.C., Bishop, A.E., Power, R.F., Hamada, Y, and Polak, J.M., 1987, Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of the rat gut and pancreas, **J. Neurosci.**, 7:2674.

Taché, Y., Pappas, T., Lauffenburger, M., Goto, Y., Walsh, J.H., and Debas, H., 1984, Calcitonin gene-related peptide; potent peripheral inhibitor of gastric acid secretion in rats and dogs, **Gastroenterology**, 87:344.

Tsunoo, A., Konishi, S., and Otsuka, M., 1982, Substance P as an excitatory transmitter of primary afferent neurons in guinea-pig sympathetic ganglia, **Neuroscience**, 7:2025.

Varro, A., Green, T., Holmes, S., and Dockray, G.J., 1988, Calcitonin gene-related peptides in visceral afferent nerve fibres: quantification by radioimmunoassay and transmission of axonal transport rates, **Neuroscience**, 26:927.

Yamagishi, T., and Debas, H.T., 1978, Cholecystokinin inhibits gastric emptying by acting on both proximal stomach and pylorus, **Am. J. Physiol.**, 234:E375.

Zarbin, M.A., Wamsley, J.K., Innis, R.B., and Kuhar, M.J., 1981, Cholecystokinin receptors; presence and axonal flow in the rat vagus nerve, **Life Sciences**, 29:697..cp255

ACTIVATION OF SENSORY NERVES BY KININS: PHARMACOLOGIC TOOLS FOR STUDYING KININ RECEPTORS

D. Regoli, N.-E. Rhaleb, N. Rouissi, C. Tousignant, D. Jukic, and C. Drapeau

Department of Pharmacology, Medical School
University of Sherbrooke, Sherbrooke, Canada

INTRODUCTION

Bradykinin and kallidin are released in blood and tissues by several stimuli and produce a variety of local effects by acting as autacoids. Some of these effects appear to be initiated by stimulation of afferent fibers and produce tissue responses of reflex nature. Indeed, bradykinin has been shown to be the most active endogenous agent to produce pain sensation when applied to blister base in man (1, 2, 3): this peptide has also been found to activate nociceptive responses in laboratory animals (Table 1), among others the writhing syndrome in the mouse (4, 5) the vocalization response in the dog (6), the reflex head flexion reaction in the rat (7, 8) and other painful responses (see Garcia-Leme (9) and Clark (50), for reviews). In other in vivo assays in the dog, the cat and the rat, bradykinin has been used to stimulate sensory fibers and activate cardiovascular reflexes. Thus, Lombardi et al. (10) and Malliani (11) have applied bradykinin in the coronary arteries of anesthetized cats to activate reflex responses of the sympathetic and vagal cardiac sensory nerves. They have observed an increase of the left ventricular and of the systemic pressures, together with a significant increase in the sympathetic efferent impulses. In the cat, Longhurst et al. (16) recorded a reflexly increase of heart rate, blood pressure and myocardiac contractility after application of capsaicin or bradykinin to sensory endings in abdominal viscera (stomach, duodenum, jejunum, ileum, pancreas, liver, gallbladder). Furthermore, Baker et al. (12), recorded a significant increase of afferent impulses from single fibers of the thoraxic nerves in anesthetized cats after application of bradykinin to the heart, the great vessels, the pericardium and pleura (Table I).

In anesthetized dogs, Staszewska et al (13) and Staszewska et al. (14) measured a positive chronotropic and inotropic effect following the application of bradykinin on the epicardium. A similar protocol was applied by Ushida and Murow (15) who recorded an increase of afferent impulses from sensory fibers of anesthetized dogs. Recently Rioux et al. (17) have performed similar experiments in the guinea-pig. In this study, the most ac-

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

tive stimulants of chronotropism and inotropism were found to be neurotensin and capsaicin: bradykinin was less active and the effect of neurotensin was prevented by pretreatment with capsaicin. In the same species, similar results were obtained by Geppetti et al. (18) who showed that capsaicin and bradykinin promoted the release of SP and of the calcitonin gene-related peptide from the sensory nerves: pretreatment with capsaicin inhibited the effect of bradykinin (Table I).

Table I
Sensory nerves and functions activated by kinins

Species	Anatomic Site	Response/Effect	Reference
Man	blister base (skin)	pain	1,2,3
	peritoneum	visceral pain	24
	bronchi	bronchoconstriction	21,23
	trachea/bronchi	bronchoconstriction	22
Dog	heart	positive chronotropism and inotropism	14
	trachea/bronchi	bronchoconstriction	20
	knee joint	algesia	48
Cat	heart	positive chronotropism cardiovascular reflexes	12
Rabbit	eye	pupillary constriction	42
	ear	hypotension (pain)	43
	hindlimb	flexion reflex (algesia)	44
Guinea-Pig	myenteric plexus longitudinal muscle (small intestine)	release of ^{3}H-Ach	45
	heart	positive chronotropism	18
	heart	positive chronotropism and inotropism	17
	urinary bladder	contraction	40
Rat	spinal cord	depolarization of sensory C-fibers	37
	injection into the carotid artery	rotation of the head	7,8
	tail flick test	algesic	46
Mice	intraperitoneal application	writhing syndrome	4,47

In the respiratory system, work by Kaufman et al. (19), in anesthetized dogs showed that bradykinin is a potent bronchoconstrictor. In an extensive review on this topic, Coleridge and Coleridge (20) have analyzed the various activities of bradykinin in the respiratory system and the ability of this peptide to activate local reflexes by acting on sensory C-fibers in the dog and in other species (for instance the cat). Other investigators (Simonsson et al. (21) and more recently Fuller et al. (22) and Barnes et al. (23) have proposed an interesting interpretation on the possible role of kinins in asthma (see Fig. 1)

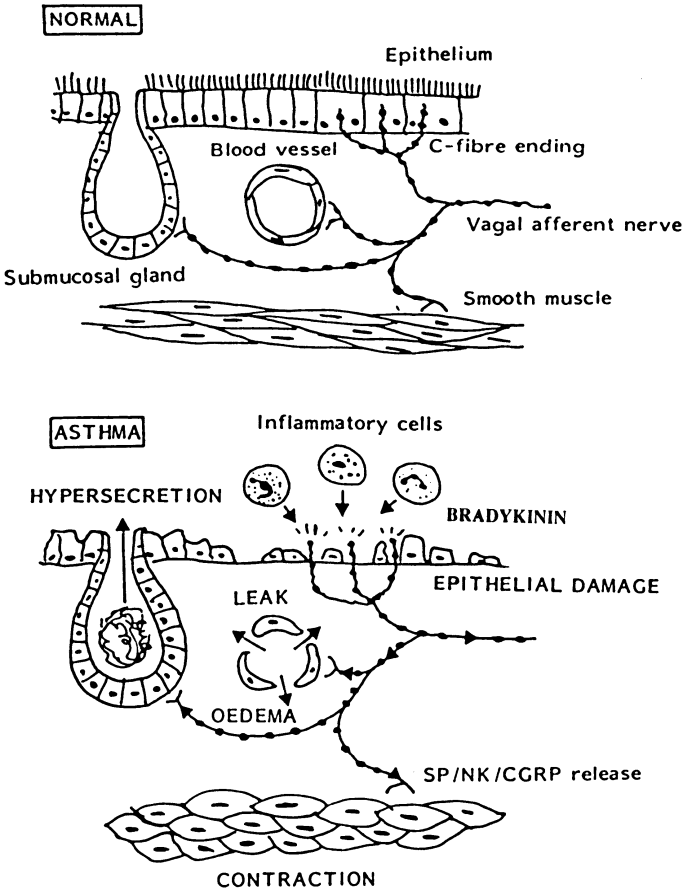


Fig. 1. Axon reflex mechanisms in asthma. Damage to airway epithelium in asthma exposes non-myelinated nerve endings. They may be triggered by bradykinin, resulting in release of sensory neuropeptides such as substance P (SP), neurokinins (NKs) and calcitonin gene-related peptide (CGRP), which together contribute to the pathology of asthma (modified from Barnes (23)).

The painful effect of bradykinin when applied in the peritoneal cavity in humans has been known for many years (Linn et al., 1967 (24)). Visceral pain has also been demonstrated in the cat by Longhurst et al. (16).

Other tests in the rabbit, the rat and the guinea-pig have been used to demonstrate the activity of kinins on sensory nerves. A summary of literature is presented in Table I.

Complex biological actions of kinins in relation to inflammation and tissue repair

Bradykinin and related kinins have been shown to act directly on sensory nerves, where receptors have been localized recently by Steranka et al. (8) using ^{3}H bradykinin and the technique of historadiography. Kinins may also act indirectly

through the intermediary of prostaglandins (25) and possibly other mediators. In this context, it is worthy of mention that kinins are candidates for the generation and maintenance of the inflammatory reaction in its whole complexity. In fact, kinins are generated locally from kallikreins that are brought and released (in active form) in the tissues by blood cells. Kallikreins have a relatively long half life and interact with low molecular weight kininogen (which is ubiquitous) to generate a large amount of kinins which, in their turn, promote the release of a variety of other endogenous agents, as illustrated in Fig. 2. These agents include prostaglandins, prostacyclin, hisatamine, catecholamines, leukotrienes, acetylcholine, sensory neuropeptides and possibly other agents. In this way, kinins act as typical autacoids and set into work a cascade of events that lead to pain, inflammation and tissue reactions to noxious stimuli. All these effects are believed to be due to the activation of B_2 and possibly B_1 receptors, which may be originated **de novo** in pathological conditions (26), as well as to the intervention of post receptor mechanisms (27). Indeed, kinin receptors of the B_2 type have been shown to be present in a variety of nervous structures, particularly sensory nerve terminals, ganglia and the dorsal horn of the spinal cord. Kinin receptors have also been identified in other tissues, particularly in endothelia where they subserve important biological functions, both in the arteries (vasodilation through release of EDRF) (28, 29) or in the capillaries, where they may contract the endothelial cells and open holes in the capillary wall to promote transfer of fluid from the blood to the tissues and the accumulation of edema (30).

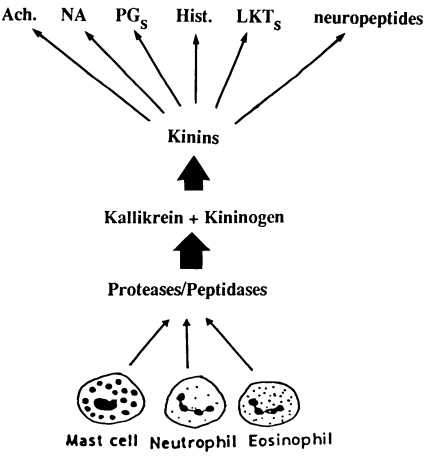


Fig. 2. When leaving the blood stream, blood cells may be activated to release proteases and proteinases. Among these proteolytic enzymes, kallikreins interact with low molecular weight kininogen to release kinins, particularly kallidin. Kinins have been shown to be able to promote the release of a variety of neurotransmitters, autacoids and sensory neuropeptides.
Ach: Acetylcholine; NA: Noradrenaline; PG_s: Prostaglandins; His: Histamine; LKT_s: Leukotrienes; Neuropeptides: Sensory neuropeptides such as SP, NKA and calcitonin gene-related peptides.

To assess the role of kinins in pathophysiological conditions, various pharmacological approaches have been used. First, kinins have been applied in segmental circulations, for instance in the kidney (31), in the coronary arteries (11, 32) or have been given by intravenous infusions or by local injections (33). The effects of kinins have been potentiated using inhibitors of the converting enzyme (kininase II) which appears to be very important for the degradation of kinins, particularly in the lung circulation (34, 35). Because of their rapid inactivation in the lung and in blood (by several other proteolytic enzymes), concentrations of circulating kinins are very low and questions have been raised about their relevance for hormonal functions by these peptides (36). In recent publications, kinins are proposed as paracrine hormones (36) since they may reach biologically active concentration only in tissues where they are produced.

Pharmacological approaches to studying the role of the kallikrein-kinin system

From the above, it appears that the most promising approach for evaluating the possible role of kinins in physiology and pathology will be through inhibition of their release (for instance by aprotinin and other similar compounds) or by the antagonism of their receptors. Both approaches have been utilized with some success and the results obtained with antagonists are summarized in Table II. In general, B_2 receptor antagonists have been used, most frequently the compounds analysed in Table III. All compounds except [Leu^8]desArg^9-BK are B_2 receptor antagonists of various potency, from the weak [Thi^{5,8},D-Phe^7]-BK to the more potent DArg[Hyp^3,Thi^{5,8},D-Phe^7]-BK which has been shown to have less agonistic activity **in vivo** than the others (Scicli, personal communication).

When applied to blister base in men, D-Arg[Hyp^3,Thi^{5,8},D-Phe^7]-BK.TFA prevents the analgesia induced by bradykinin (3). The compound is also active in preventing the increase of firing in the rat neonatal spinal cord produced by bradykinin when applied to the rat tail: similar results have been obtained by two groups of investigators (37, 38). Various compounds (see Table II) were used by Steranka et al. for preventing hyperalgesia induced by bradykinin injected in the rat paw (5, 8). The compounds were also active in preventing the head rotation reflex that followed the injection of bradykinin into the carotid arteries in rats (5, 8). A B_1 receptor antagonist, [Leu^8]desArg^9-BK was found to be active in preventing the release of enkephalin induced by BK on the tooth pulp in the rat (39). Using B_2 antagonists, Steranka et al. (5, 8) were able to prevent the writhing syndrome induced by acetic acid in the mouse.

D-Arg[Hyp^3,Thi^{5,8},D-Phe^7]-BK antagonizes the contraction of the guinea-pig urinary bladder in response to bradykinin (40). This response appears to be largely indirect, since it is markedly reduced by indomethacin. B_2 receptor antagonists and in particular Lys-Lys[Hyp^3,Thi^{5,8},D-Phe^7]-BK have been used by Griesbacher and Lembeck (41) to counteract various effects of bradykinin in the rabbit; namely a) plasma extravasation in the skin (40), contraction of the iris sphincter, and the reflex hypotension that follows the injection of bradykinin into the ear artery (41). Finally, Staszewska-Wolley et al. (14) have shown that B_2 receptor antagonists prevent the positive chronotropic and inotropic responses of the dog heart to topically applied bradykinin.

Table II
Algesic/analgesic effects of kinins

Species	Organs	Agonist	Effect inhibited	Antagonist	Receptor	Reference
Man	skin blister base	BK	pain	DArg[Hyp^3,Thi^{5,8},DPhe^7]BK-TFA	B_2	37
Rat	spinal cord	BK	depolarisation of spinal ventral root	DArg[Hyp^3,Thi^{5,8},DPhe^7]BK	B_2	38
	paw, intradermal	urate BK	hyperalgesia	DArg[Hyp^3,Thi^{5,8},DPhe^7]BK DArg[Hyp^3,DPhe^7]BK Lys-Lys[Hyp^3,Thi^{5,8},DPhe^7]BK [Leu^{5,8},Gly^6,DPhe^7]BK	B_2	5, 8
	tooth pulp	BK	Met-enkephalin-like release	[Leu^8]DesArg^{9}BK	B_1	39
	intraarterial	BK	head rotation	Lys-Lys[Hyp^3,Thi^{5,8},DPhe^7]BK [Leu^{5,8},Gly^6,DPhe^7]BK	B_2	8
	intrathecal	BK	nociception	[Thi^{5,8},DPhe^7]BK	B_2	46
Mice	intraperitoneal	acetic acid	writhing	DArg[Hyp^3,Thi^{5,8},DPhe^7]BK DArg[Hyp^3,DPhe^7]BK	B_2	5
Rabbit	skin	BK	plasma extravasation	Lys-Lys[Hyp^3,Thi^{5,8},DPhe^7]BK	B_2	41
	iris sphincter	BK	contraction	Lys-Lys[Hyp^3,Thi^{5,8},DPhe^7]BK	B_2	41
	isolated ear	BK	venoconstriction	Lys-Lys[Hyp^3,Thi^{5,8},DPhe^7]BK	B_2	41
	intra-ear arterial	BK	reflex hypotension (nociception)	Lys-Lys[Hyp^3,Thi^{5,8},DPhe^7]BK	B_2	41
Guinea pig	urinary bladder	BK	contraction	DArg[Hyp^3,Thi^{5,8},DPhe^7]BK	B_2	40
Dog	heart	BK	positive chronotropic positive inotropic	DArg[Hyp^3,Thi^{5,8},DPhe^7]BK	B_2	14

From the above, it is evident that the activation of the sensory nerves by kinins in various tissues is mediated by B_2 receptors: these receptors have been characterized by using antagonists (52, 56) and therefore the following will be devoted to this group of compounds and in particular to recent work performed to improve their pharmacologic features.

B_2 receptor antagonists: history, discovery, improvements, utilization

Identified in 1985 by Vavrek and Stewart (56), B_2 receptor antagonists have been utilized by numerous investigators and further improved by Stewart and Vavrek (58) and by others (27, 52, 54). Published data indicate that these compounds antagonize kinin effects in vivo and in vitro and act as analgetics (5, 8, 27, 38, 46, 52) but they maintain agonistic effects and are potent histamine releasers (27, 49, 51, 52). In various smooth muscle preparations, several of the compounds that are listed in Table III act as partial agonists while in other tissues they are inactive (see examples of the rabbit jugular vein and the rabbit aorta in Table III).

Work has been performed to increase antagonist affinity and eliminate the partial agonistic effects (27, 52, 56, 57) with some success. As shown in Table III, affinity has been increased

Table III
Apparent affinity (pA_2) of kinin receptor antagonists on rabbit jugular vein, rabbit aorta and their ability to release histamine from rat mast cells

	Rabbit jugular vein		Histamin release	Rabbit aorta	
	pA_2	A.A.	potency[(a)]	pA_2	A.A.
1) [Thi^{5,8},DPhe^7]BK	6.70	+	+++	6.23	--
2) DArg[Hyp^3,Thi^{5,8},DPhe^7]BK	7.86	++	++	6.16	--
3) AcDArg[Hyp^3,Thi^{5,8},DPhe^7]BK	7.51	--	+	5.47	--
4) DArg[Hyp^3,DPhe^7]BK	8.01	++	N.D.	6.41	--
5) AcDArg[Hyp^3,DPhe^7]BK	7.78	+	N.D.	6.41	--
6) [Leu^{5,8},Gly^6,DPhe^7]BK	P.Ag.	+++	N.D.	5.68	±
7) DArg[Hyp^3,Leu^{5,8},Gly^6,DPhe^7]BK	8.84	±	N.D.	6.19	--
8) AcDArg[Hyp^3,Leu^{5,8},Gly^6,DPhe^7]BK	8.07	±	N.D.	5.97	--
9) DArg[Hyp^3,Leu^8,DPhe^7]BK	8.86	±	N.D.	5.76	--
10)AcDArg[Hyp^3,Leu^8,DPhe^7]BK	8.07	--	N.D.	5.77	--
11)[Leu^8]DesArg^{9}BK	Inac.	--	±	7.27	--

(a): semiquantitative evaluation of histamin release by kinin antagonists on rat peritoneal mast cells (49, 51).

pA_2: -Log of concentration of antagonist that reduces the effect of a double dose of agonist to that of a single one.

A.A.: Agonistic activity; P.Ag.: Partial agonist; N.D.: Not determined.

--: inactive; ±, +: weak; ++: intermediate; +++: strong.

with modification in position 3 (replacement of Pro by hydroxyproline (Hyp)) or the extension of the peptide chain with a D-Arg (57) (see compound no 2 in Table III). Affinity has been further improved by eliminating Thi in positions 5 and 8 and by replacing Phe^8 with Leu (see compounds no 7 and 9, which are the most active B_2 receptor antagonists to date)(27, 52). The partial agonistic effect, for instance on the rabbit jugular vein, and the histamine releasing activity, are reduced or eliminated by the acetylation of the N-terminal amine (see compounds 3, 5, 8 and 10 in Table III) (27, 52, 54). All compounds are active on the B_1 receptor system (the rabbit aorta) because they are broken down by kininase I into C-terminal desArg metabolites which act on the B_1 receptor (53). Affinities on the B_1 receptor are generally lower than those observed on the B_2 of the rabbit jugular vein but not of other preparations (27, 52). Affinity for the B_1 receptor appears to be further reduced by the utilisation of Leu in position 8 (27, 52). This makes the B_2 receptor antagonists more selective.

B_2 receptor antagonists have been shown to be specific for kinins since they are inactive against substance P, angiotensin and other peptides in various isolated smooth muscle preparations (52, 54, 57).

CONCLUSIONS

- Kinins are the most active endogenous agents that activate sensory afferent nerves to produce pain, raise cardiovascular reflexes and local segmental vascular responses.
- The effects of kinins on the sensory system appear to be mediated by receptors of the B_2 type which have been identified by historadiography and characterized by the use of agonists and antagonists. Kinins may act directly on sensory nerves or indirectly by the intermediary of other agents such as prostaglandins.
- Effects of kinins on sensory nerves and reflexly evoked responses have been found to be reduced or eliminated by specific B_2 receptor antagonists. These compounds have been recently improved by chemical modifications that have brought to increase of affinity, elimination of agonistic and partial agonistic activities and protection against the degradation from kininase I, which converts B_2 into B_1 receptor antagonists.

References

1. D. Armstrong, Pain, in: "Handbook of Experimental Pharmacology, Vol. 25, Bradykinin, Kallidin and Kallikrein, E.G. Erdös, ed., Springer-Verlag, Berlin, pp.434-481 (1970).
2. D.C. Manning, A.A. Khan, J.N. Campbell, R.A. Meyer and S.N. Raja, Bradykinin induced cutaneous pain is independent of wheal and flare, **Pain (Suppl.)** 4:S16 (abstr.) (1987).
3. E.T. Whalley, S. Clegg, J.M. Stewart and R.J. Vavrek, The effect of kinin agonists and antagonists on the pain response of the human blister base, **Naunyn Schmeideberg's Arch.Pharmacol.**, 336:652 (1987)
4. J.M. Emerle and J.E. Shanaman, Bradykinin writhing: a method for measuring analgesia, **Pro. Soc. Exp. Biol. Med.** 114:680 (1963).
5. L.R. Steranka, C.J. Detlaas, R.J. Vavrek, J.M. Stewart, S.J.

Enna and S.H. Snyder, Antinociceptive effects of bradykinin antagonists, **Eur. J. Pharmacol.**, 136:261 (1987).
6. G.D. Potter, F. Guzman and R.K.S. Lim, Visceral pain evoked by intra-arterial injection of substance P, **Nature**, 193:983 (1962).
7. G. Deffenu, L. Pegrassi and B. Lumachi, The use of bradykinin-induced effects in rats as an assay for analgesic drugs, **J. Pharm. Pharmacol.**, 18:135 (1966).
8. L.R. Steranka, D.C. Manning, C.J. DeHaas, J.W. Ferkany, S.A. Borosky, J.R. Connor, R.J. Vavrek, J.M. Stewart and S.H. Snyder, Bradykinin as a pain mediator: Receptors are localized to sensory neurons and antagonists have analgesic actions, **Proc. Natl. Acad. Sci.**, 85:3245 (1988).
9. J. Garcia-Leme, Bradykinin-System, in: "Handbook of Experimental Pharmacology", Vol. 50/1, J.R. Vane, S.H. Ferreira, eds. Inflammation, Springer Verlag, Berlin (1978).
10. F. Lombardi, C.P. Patton, P.D. Bella, M. Pagani and A. Malliani, Cardiovascular and sympathetic responses reflexly elicited through the excitation with bradykinin of sympathetic and vagal cardiac sensory endings in the cat, **Circulation Res.**, 57:65 (1982).
11. A. Malliani, Cardiovascular sympathetic afferent fibers, **Rev. Physiol. Biochem. Pharmacol.**, 94:11 (1982).
12. D.G. Baker, H.M. Coleridge, J.C.G. Coleridge and T. Nerdrum, Search for a cardiac nociceptor: stimulation by bradykinin of sympathetic afferent nerve endings in the heart of the cat, **J. Physiol. London**, 306:519 (1980).
13. J. Staszewska-Barzak, S.H. Ferreira and J.R. Vane, An excitatory nociceptive cardiac reflex elicited by bradykinin and potentiated by prostaglandins and myocardial ischaemia, **Card.Res.**, 10:314 (1980)
14. J. Staszewska-Woolley, G. Woolley and D. Regoli, Specific receptors for bradykinin-induced cardiac sympathetic chemoreflex in the dog, **Eur. J. Pharmacol.**, 156:309 (1980).
15. Y. Uchida and S. Murao, Bradykinin-induced excitation of afferent cardiac sympathetic nerve fibers, **Jap. Heart J.**, 15:84 (1974).
16. J.C. Longhurst, M.P. Kaufman, G.A. Ordway and T.J. Mush, Effects of capsaicin and bradykinin on endings of afferent fibers from abdominal visceral organs, **Am. J. Physiol.**, 245:R552 (1984).
17. F. Rioux, H. Bachelard, S. St-Pierre and J. Barabé, Epicardial application of bradykinin elicits pressor effects and tachycardia in guinea-pigs, Possible mechanisms, **Peptides**, 8:863 (1987).
18. P. Geppetti, C.A. Maggi, F. Perretti, S. Frilli and S. Manzini, Simultaneous release by bradykinin of substance P and calcitonin gene-related peptide immunoreactivities from capsaicin-sensitive structures in guinea-pig heart, **Br. J. Pharmacol.**, 94:288 (1988).
19. M.P. Kaufman, H.M. Coleridge, J.C.G. Coleridge and D.G. Baker, Bradykinin stimulates afferent vagal C-fibres in intrapulmonary airways of dogs, **J. Appl. Physiol.**, 48:511 (1980).
20. J.C.G. Coleridge and H.M. Coleridge, Afferent vagal C-fibre innervation of the lungs and airways and its functional significance, **Rev. Physiol. Biochem. Pharmacol.**, 99:1 (1984).
21. B.G. Simonsson, B.E. Skoogh, N.P. Bergh, R. Andersson and N. Svedmyr, In vivo and in vitro effect of bradykinin on bronchial motor tone in normal subjects and patients with airways obstruction, **Respiration**, 30:378 (1973).

22. R.W. Fuller, C.M.S. Dixon, F.M.C. Cuss and P.I. Barnes, Bradykinin-induced bronchoconstriction in man: mode of action, **Am. Rev. Respir. Dis.**, 135:176 (1987).
23. P.J. Barnes, Airway neuropeptides and asthma, **TIPS**, 8:24 (1987).
24. R.K.S. Lim, D.G. Miller, F. Guzman, D.W. Rodgers, R.W. Rogers, S.K. Wang, P.Y. Chao and T.Y. Shih, Pain and analgesia evaluated by the intraperitoneal bradykinin-evoked pain method in man, **Clin. Pharmacol. Ther.**, 8:521 (1967).
25. A. Nasjletti and K.U. Malik, Relationships between the kallikrein-kinin and prostaglandin systems, **Life Sci.**, 25:99 (1979).
26. D. Regoli, J. Barabé and W.K. Park, Receptors for bradykinin in rabbit aortae, **Can. J. Physiol. Pharmacol.**, 55:855 (1977).
27. D. Regoli, N.E. Rhaleb, S. Dion and G. Drapeau, Bradykinin receptors: B_1 and B_2 and what else? **TIPS**, in press (1990).
28. C.P. Sung, A.J. Arleth, K. Shikano and B.A. Berkowitz, Characterization and function of bradykinin receptors in vascular endothelial cells, **J. Pharmacol. Exp. Ther.**, 247:8 (1988).
29. P. D'Orléans-Juste, G. de Nucci and J.R. Vane, Kinins act on B_1 or B_2 receptors to release conjointly endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells, **Br. J. Pharmacol.**, 96:920 (1989).
30. F. Marceau, A. Lussier, D. Regoli and J.-P. Giroud, Pharmacology of kinins: their relevance to tissue injury and inflammation, **Gen. Pharmacol.**, 14:209 (1983).
31. K.U. Malik and A. Nasjletti, Attenuation by bradykinin of adrenergically-induced vasoconstriction in the isolated perfused kidney of the rabbit: relationship to prostaglandin synthesis, **Br. J. Pharmacol.**, 67:269 (1979).
32. F. Lombardi, P.D. Bella, R. Casati and A. Malliani, Effects of intracoronary administration of bradykinin on the impulse activity of afferent sympathetic unmyelinated fibers with left ventricular endings in the cat, **Circulation Res.**, 48:69 (1981).
33. J. Barabé, F. Marceau, B. Thériault, J.-N. Drouin and D. Regoli, Cardiovascular actions of kinins in the rabbit, **Can. J. Physiol. Pharmacol.**, 57:78 (1979.
34. S.H. Ferreira and J.R. Vane, The disappearance of bradykinin and eledoisin in the circulation of vascular beds of the cat, **Br. J. Pharmacol.**, 30:417 (1967).
35. G. Bönner, S. Preis, V. Shunk and D. Iwèrsen, Hemodynamic effects of kinins and their role in blood pressure regulation in "The Kallikrein-Kinin System in Health and Disease", H. Fritz et al. eds., Limbach-Verlag, Braunschweig, Germany (1989).
36. O.A. Carretero and A.G. Scicli, Kinins paracrine hormone, in "The Kallikrein-Kinin System in Health and Disease", H. Fritz et al. eds., Limbach-Verlag, Braunschweig, Germany (1989).
37. A. Dray and M.N. Perkins, Bradykinin activates peripheral capsaicin-sensitive fibres via a second messenger system, **Agents and Actions**, 25:214 (1988).
38. P.M. Dunn and H.P. Rang, Bradykinin activation of primary afferent terminals in the neonate rat spinal cord in vitro, **Br. J. Pharmacol.**, 98:827P (abs.)(1989).
39. T. Kudo, H.L. Chang, M. Kuroi, S. Wakisaka, M. Akai and R. Inoki, Influences of bradykinin and substance P on the Met-enkephalin-like peptide content in the rat incisor pulp, **Neuropeptides**, 7:399 (1986).

40. C.A. Maggi, R. Patacchini, P. Santicioli, P. Geppetti, R. Cecconi, S. Giuliani and A. Meli, Multiple mechanisms in the motor response of the guinea-pig isolated urinary bladder to bradykinin, **Br. J. Pharmacol.**, 98:619 (1989).
41. T. Griesbacher and F. Lembeck, Effect of bradykinin antagonists on bradykinin-induced plasma extravasation, venoconstriction, prostaglandin E_2 release, nociceptor stimulation and contraction of the iris sphincter muscle in the rabbit, **Br. J. Pharmacol.**, 92:333 (1987).
42. J.M. Buttler and B. Hammond, Neurogenic responses of the eye injury: effect of sensory denervation on the responses of the rabbit eye to bradykinin and prostaglandin E_1, **Trans. Ophthalmol. Soc. U.K.**, 97:668 (1977).
43. H. Juan and F. Lembeck, Action of peptides and other algesic agents on paravascular pain receptor of the isolated perfused rabbit ear, **Naunyn-Schmiedebergs Arch. Pharmacol.** 283:151 (1974).
44. T. Doi, A. Akaike, M. Ohashi, M. Satoh and H. Takaji, Bradykinin-induced flexor reflex of rabbit hindlimb for comparing analgesics, **Jap. J. .Pharmacol.**, 26:634 (1976).
45. W.M. Yau, J.A. Dorsett and M.L. Youther, Bradykinin releases acetylcholine from myenteric plexus by a prostaglandin-mediated mechanism, **Peptides**, 7:289 (1986).
46. O. Laneuville and R. Couture, Bradykinin analogue blocks bradykinin-induced inhibition of a spinal nociceptive reflex in the rat, **Eur. J. Pharmacol.**, 137:281 (1987).
47. T.N. Bhalla, S.N. Sinha, R.P. Kohli and K.P. Bhargava, A comparative study of the writhing response induced by phenylquinone, bradykinin and aconitine for the assessment of analgesic agents, **Jap. J. Pharmacol.**, 23:609 (1973).
48. S.H. Ferreira, S. Moncada and J.R. Vane, Potentiation by prostaglandins of the nociceptive activity of bradykinin in the dog knee joint, **Br. J. Pharmacol,**, 50:461P (abst.)(1974).
49. P. Devillier, M. Renoux, G. Drapeau and D. Regoli, Histamine release from rat peritoneal mast cells by kinin antagonists, **Eur. J. Pharmacol.**, 149:137 (1988).
50. W.C. Clark, Kinins and the peripheral and central nervous system, **Handb. Exp. Pharmacol.** 25 (suppl.):311 (1979).
51. P. Devillier, G. Drapeau, M. Renoux and D. Regoli, Role of the N-terminal arginine in the histamine-releasing activity of substance P, bradykinin and related peptides, **Eur. J. Pharmacol.**, 168:53 (1989).
52. N.E. Rhaleb, S. Télémaque, N. Rouissi, S. Dion, D. Jukic, G. Drapeau and D. Regoli, Structure-activity studies of bradykinin and related peptides. II. Antagonists., **Br. J. Pharmacol.**, (submitted)(1990).
53. D. Regoli, G. Drapeau, P. Rovero, S. Dion, N.-E. Rhaleb, J. Barabé, P. D'Orléans-Juste and P. Ward, Conversion of kinins and their antagonists into B_1 receptor activators and blockers in isolated vessels, **Eur. J. Pharmacol,**, 127:219 (1986).
54. D. Regoli, N.-E. Rhaleb, G. Drapeau, S. Dion, C. Tousignant and P. D'Orléans-Juste, Basic pharmacology of kinins.in: "Pharmacologic receptors and other mechanisms, Kinins V.," Plenum Press, New York (1989).
55. D. Regoli, N.-E. Rhaleb, G. Drapeau, S. Dion, Kinin receptor subtypes, **J. Cardiovasc. Pharmacol.**, (in press)(1990).
56. R.J. Vavrek and J.M. Stewart, Competitive antagonists of bradykinin, **Peptides**, 6:161 (1985).

57. J.M. Stewart and R.J. Vavrek, Design of bradykinin antagonists in: Peptides, Chemistry and Biology. Proceedings of the Tenth American Peptide symposium, G.R. Marshall ed., Escom Leiden (1988).

EVOLUTIONARY ASPECTS IN THE PERIPHERAL PEPTIDERGIC SIGNALS: CRF-LIKE PEPTIDES AND MODULATION OF G.I. FUNCTIONS

Giovanna Improta

Institute of Pharmacology
University "La Sapienza", Rome, Italy

INTRODUCTION

In studies of peptide structure-function relationships evolutionary changes are important tools. A study of the differences between species helps one to understand the importance of functional and receptor addressing domains in the sequence as well as of the side chains of some amino acid residues. Intra- and interpeptide relationships reveal the presence of preserved sequences and relate many peptides in families. In biologically active peptides, evolutionary changes may reflect additional complexity since these peptides interact with receptors and therefore participate in a system of co-evolving protein structures and inter-cell signaling. In evolutionary studies of the most basic properties of a peptide family, it is essential to include variants that differ widely from one another. This is because in closely related forms, differences are often trivial or random, and therefore of little general value. To enable one to draw conclusions about fundamental properties conserved during evolution an appropriate choice would involve peptides that are present in phylogenetically distant species. Thus, despite marked phylogenetic differences, in their primitive functional sequences these peptides clearly appear as homologues. Furthermore, in order to link the molecular evolution with the changes in biological activity the comparison should include peptides with a known three-dimensional structure.

The family of CRF-like peptides underwent wide reorganization of the encoding genes throughout the phylogenetic tree, from fishes to mammals. The expression products of these genes are urotensin I (UI) in fishes, sauvagine (SV) in amphibians and corticotropin-releasing factor (CRF) in mammals. A loss of biological activities and a reduction in the extent of tissue localizations is paralleled by evolutionary changes in the peptide sequences. However, some amino acid sequences in the primary structure of UI and SV have been preserved during evolution and can still be found in the primary structure of mammalian CRF (Fig. 1). Most probably, the preserved sequences represent the message and the address domains, essential for the biological activity of these peptides.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

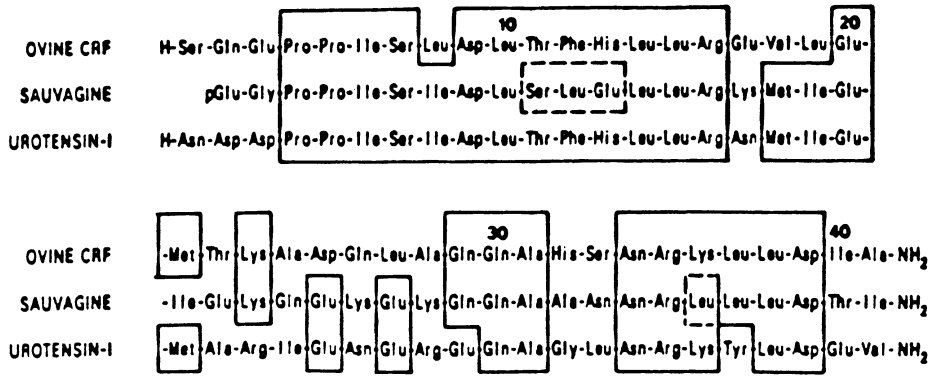


Fig. 1. Primary structures of ovine corticotropin-releasing factor (CRF), sauvagine and urotensin I. Boxed areas represent homologies of amino acids between peptides.

Analogies can also be found in the secondary structure of SV and CRF (Fig. 2). The fundamental conformation for receptor binding is the alpha-helical structure and any amino acid substitution in the primary sequence that destabilizes such a structure, deprives the molecules of their biological activity.

What is surprising in the evolution of the CRF-like peptide family is that, although many point mutations occurred in the functional domains of the primary sequence, none of these destroyed the alpha-helical structure required for receptor fitting. Selection during evolution thus appears to be guided by the conformational rules for inter-molecular recognition.

PHYLOGENY OF A PEPTIDERGIC SENSORY SYSTEM

Fishes

The main localization of UI in teleosts is the caudal neurosecretory system. A role of this system and its neurosecretory products in osmoregulation in fishes has been discussed at length in reviews of the physiological functions of urotensin (Bern, 1985). The caudal neurosecretory system consists of neurons located at the caudal end of the spinal cord. Their processes enter the urophysis, a neurohemal organ on the ventral surface of the posterior spinal cord. At least two types of immunoreactive UI neurons are known to exist in the caudal neurosecretory system and their size and shape has been described. Distal to the urophysis there is a population of very large IR-UI cells with polymorphic nuclei, formerly called Dahlgren cells. These neurons display thick processes, which project to the ventral surface of the spinal cord and form two compact bilateral pathways, which enter the urophysis. A second group of smaller IR-UI neurons with mostly regular shaped nuclei are located just dorsal to the urophysis. These bipolar cells are arranged in a compact mass and display ventrally directed processes similar to those described above. A rostrally directed pathway made up of these neurons runs parasagittally along the whole length of the spinal cord, in a parallel arrangement. The thin, beaded fibres of these IR-UI neurons enter the brainstem and two parasagittal clusters of fibres are visible in the mesencephalon, one located

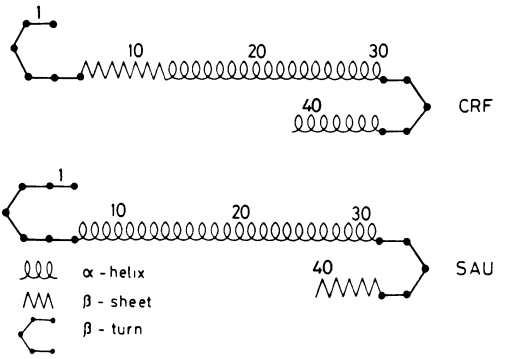


Fig. 2. Prediction of the secondary structures of sauvagine and CRF, according to the Chou and Fasman method

just below the base of the cerebellum, and the other more ventrally near the interpeduncular nucleus. In the diencephalon, the fibres surround the mammillary body and become distributed mainly into the posterior tuberal nucleus, lateral recessus nucleus and inferior lobe. Epithalamic fibres are seen in the area pretectalis and also in the ventral region of the habenular nucleus. Many fibres are present in the thalamus, where they form part of the fasciculus medialis telencephali between the thalamus and the telencephalon. In the preoptic region they form a plexus in the nucleus anterior periventricularis just caudal to the preoptic nucleus. IR-UI fibres are also found in the dorsorostral and dorso-caudal telencephalic regions.

Immunocytochemical studies have recently demonstrated that a prompt and intense decrease of IR-UI content in the caudal neurosecretory system occurs when environmental osmotic pressure is increased. In contrast, fishes transferred to hypoosmotic milieu showed a fast and dramatic increase in IR-UI in neural endings of the urophysis (Minniti, 1989). Radioimmunoassay of UI in the venous drainage (caudal vein) of the urophyseal region demonstrates a prompt release of the hormone upon exposure of the fish to a hyperosmotic environment. Urophysectomy leads to behavioural and metabolic changes suggestive of osmoregulatory insufficience. Administration of UI results in alterations in blood ion levels and prolonged survival of the fish in a hyperosmotic environment. These observations indicate possible responses to alterations in hydromineral metabolism by the caudal neurosecretory system and a role of UI in osmo-ionoregulation (Lederis, 1985).

In updating and reasserting the potential role of UI in osmotic and ionic changes, one should realise that this neuropeptide could intervene physiologically in at least three major ways. First, by virtue of its CRF-like activity, UI may be involved in the regulation of ACTH release by fish pituitary and consequential, in the control of cortisol secretion by the adrenocortical homolog, the interrenal. Teleosts are considered to depend primarily upon cortisol for osmoregulation. Second, in fishes, UI almost certainly modulates transepithelial transport directly across a variety of osmoregulatory surfaces, such as intestine, kidney, urinary bladder, gill, swim bladder and skin

(Lederis, 1985). Between some of these osmoregulatory organs and the urophysis direct vascular connections exist. The renal portal system (caudal vein) carries blood from urophysis to the renal field for example. However, some branches of the caudal vein are known to bypass the kidneys and connect with the liver, urinary bladder, swim bladder and intestine. Hence "direct" targets of UI released by urophysis could include the kidney, urinary bladder, swim bladder, intestine, and liver. Third, UI receptors have been traced in these organs as well as in synaptosomal preparations of urophysis (Lederis, 1985). Moreover, infusion of 1-5 ng of UI into the caudal vein produced firing of the bipolar neurons located dorsal to urophysis (Lederis, 1985). By activating the same terminals that are stimulated by environmental osmotic changes, UI secreted by nerve terminals into urophyseal vessels may therefore function as a local transmitter. In this sense, UI is a sensory neuropeptide.

Amphibia

Sauvagine was first isolated from amphibian skin (Montecucchi, 1979) and sauvagine-like immunoreactivity (IR-SV) has been detected in the skin, GI tract, pancreas, retina and CNS of amphibians (Erspamer, 1981). In the GI tract, IR-SV has been traced to nerve fibres of the stomach wall and small intestine. In the hypothalamus, IR-SV is present in the magnocellular perykaria, in the dorsal and ventral regions of the preoptic nucleus, and in the hypothalamic-hypophyseal projections to pars nervosa and in fibres running from the pars nervosa to pars intermedia of the pituitary. Encephalic sensitive ganglia of Xenopus levis numerous perykaria have been shown to contain IR-SV. No IR-CRF staining has been found in neurons containing IR-SV, but IR-CRF has been distinctly localized in neurons and fibres. The use of UI specific antisera failed to give a positive response in the frog brain and GI tract. In the frog brain, two anatomically different systems, CRF-like and SV-like are thought to co-exist, just as IR-UI and IR-CRF neuronal systems are reported to coexist in fish brain (Lederis, 1985). However, albeit varying degrees of reliability, until recently the functional role of sauvagine has never been clarified.

Mammals

In rats, dogs and humans, UI, SV and CRF possess a common spectrum of biological activities on the central nervous system, anterior pituitary, cardiovascular system and gastrointestinal tract (Broccardo, 1982; Brown, 1982; Erspamer, 1980; Improta, 1988; Lenz, 1985; Melchiorri, 1981; Melchiorri, 1982). CRF, SV and UI evoke a practically equipotent hypophysiotropic response. However, SV acts within the brain and is more potent than CRF in increasing plasma levels of catecholamines and glucose and in elevating mean arterial pressure (Brown, 1982). Administered icv, SV and UI, but not CRF, produče a long-lasting increase in mesenteric blood flow in conscious dogs (Lenz, 1985). Similarly, outside the brain, SV is more potent than CRF in increasing superior mesenteric artery flow and plasma glucose and in decreasing mean arterial pressure (Brown, 1982). In the gastrointestinal tract, CRF, SV and UI evoke anorexogenic responses in rats and exert a potent inhibitory effect on gastric acid secretion and motility. At least in part, these actions appear to be mediated via the central nervous system (Taché, 1983; Lenz, 1985; Improta, 1988); however, evidence also supports the importance

of peripheral mechanisms (Taché, 1984; Konturek, 1985; Improta, 1988).

By the subcutaneous (sc) route, only SV (3-10 μg/kg) and UI (10-20 μg/kg) inhibited feeding in 18 hr deprived rats (Negri, 1985). The anorexogenic effects of subcutaneous injections of SV are apparently taste dependent, being 5 times more intense for a salty food than for a low sodium diet. Peripherally injected SV modulates drinking behaviour in the rat, favouring the choice of tap water instead of saline solution.

Peripheral and central administration of SV, UI and CFR inhibits gastric acid secretion evoked by vagus-dependent stimuli (pylorus ligature, carbachol, pentagastrin) but does not modify the gastric response to histamine (Improta, 1988; Lenz, 1986; Taché, 1983).

SV, UI and CRF injected sc and icv produce a dose-related and long-lasting decrease of gastric emptying (Broccardo, 1982; Taché, 1987).

The relative potency (calculated on the basis of ED_{50}) for sc injected CRF-like peptides as concerning the above mentioned effects is reported in Table I.

TABLE I

RELATIVE POTENCY OF SC INJECTED CRF-LIKE PEPTIDES IN FEEDING, GASTRIC SECRETION (GAS) AND GASTRIC EMPTYING (GE) INHIBITION

PEPTIDES	FEEDING	GAS	GE
CRF	< 0.1	2	7
UROTENSIN I	40	35	45
SAUVAGINE	100	100	100

The relative potency (on a molar basis) was calculated on the ED_{50}.

SV appears to be 100, 50 and 20 times more potent than CRF in decreasing food intake, acid secretion and gastric emptying, respectively. Comparing the relative potency of the three peptides given by icv route, SV is again more potent (10, 20 and 50 times) than CRF in modifying the studied functions (Table II).

TABLE II

RELATIVE POTENCY OF ICV INJECTED CRF-LIKE PEPTIDES IN FEEDING, GASTRIC SECRETION (GAS) AND GASTRIC EMPTYING (GE) INHIBITION

PEPTIDES	FEEDING	GAS	GE
CRF	10	5	2
UROTENSIN I	50	25	45
SAUVAGINE	100	100	100

The relative potency (on a molar basis) was calculated on the ED_{50}.

As both sc and icv doses of SV produced the observed effects, the question arises as to which site of action, central or peripheral, contributes more in regulating these functions. To answer this question, IR-SV levels were measured by RIA in plasma and CSF after sc and icv administration of the peptide. Plasma concentration of IR-SV after sc injection was higher than that measured in CSF (Table III). In contrast, after icv injection, CSF levels of IR-SV were 10,000 times higher than blood levels.

TABLE III
CSF AND PLASMA IMMUNOREACTIVITY OF S C AND I C V INJECTED SAUVAGINE (5 μg/rat)

ROUTES	CSF	PLASMA
Subcutaneous (sc)	0.2±0.01	108±12.5
Intracerebroventricular (icv)	3500±345	0.3±0.02

Data are means expressed in fmol/ml.

Thus, the effects of sc SV on food intake, acid secretion and gastric emptying must be peripherally mediated and not due to the entry of the peptide in the CNS. Similarly, the effects observed after icv injections are mediated through the CNS and not by leakage of SV into the peripheral blood. Clearly, therefore, SV induces satiety and promotes feeding and drinking choices through two distinct mechanisms, central and peripheral. Examination of the effects of peripherally injected SV on gastric emptying, acid secretion and ingestive behaviour, suggests that they are due to messages that the peptide sends to the CNS through peripheral afferent pathways. In order to establish the peripheral localization of the pathways through which these signals are centrally transferred, an attempt was made to detect SV-containing fibres in the GI tract. In particular, RIA, with a specific antibody capable of distinguishing SV from UI and CRF (Lederis, 1987), demonstrated SV-like immunoreactivity in extracts of rat gastric submucosa and muscolaris mucosa layers (Table IV). In addition, six days after a subdiaphragmatic bilateral vagotomy, a sharp decrease in IR-SV was measured both in the submucosa and in the muscolaris mucosa (Table IV). This demonstrates that IR-SV is contained in nervous terminal of peptidergic fibres running along the vagus nerve.

TABLE IV
IMMUNOREACTIVITY OF SAUVAGINE-LIKE PEPTIDES IN STOMACH WALLS OF CONTROL (CO) AND VAGOTOMIZED (VAG-X) RATS

RAT STOMACH	CO	VAG-X
Mucosa	< 0.1	< 0.1
Submucosa	26 (8-41)	0.5 (0.1-1.3)
Muscolaris Mucosa	86 (12-98)	0.7 (0.2-18)

Values are means expressed as fmol/g.

Moreover, as Table V indicates, vagotomy completely antagonizes SV inhibitory effects on foraging and food intake. Vagotomy, which alone does not modify the gastric emptying in rats, does abolish the inhibition of gastric emptying in SV-treated animals (Table VI). Similarly, whereas vagotomy does not change the secretory response to bethanechol in pylorus-ligated rats, it does block the SV antisecretory response in bethanechol-stimulated animals (Table VII).

The fact that after vagotomy endogenous IR-SV content of GI nerve fibres fell dramatically and sc-injected SV failed to evoke satiety or to modulate feeding and drinking choices, confirms that the peptide is present in sensory terminals and needs the integrity of vagal afferent pathways to transfer the signals evoked in periphery to the CNS.

TABLE V

EFFECTS OF VAGOTOMY ON SAUVAGINE-INDUCED FEEDING INHIBITION

TREATMENT	FORAGING	FOOD-INTAKE
Saline+Vagotomy	28±0.8	9.8±2.6
Sauvagine (5μg/Kg, sc)	98±1.7*	2.7±0.9*
SV+Vagotomy	3.2±1.6	10.2±3.7

Values are means ± SE expressed as min.(foraging) and g. (food intake). *P<0.001 compared with saline.

TABLE VI

SV-INDUCED GASTRIC EMPTYING INHIBITION IN SHAM AND VAGOTOMIZED (VAG-X) PHENOL RED FED RATS

TREATMENT	SHAM-RATS	VAG-X RATS
Saline	81.8±2.2	81.5±1.4
Sauvagine (20μg/Kg.sc)	23.2±4.1*	73.0±5.9

Data are means ± SE expressed as percent of gastric emptying.
* P<0.05 compared with Saline.

TABLE VII

SV-INDUCED INHIBITION OF BETHANECHOL (BET) STIMULATED GASTRIC SECRETION IN SHAM AND VAGOTOMIZED (VAG-X) 2h-PYLORUS LIGATED RATS

TREATMENT	SHAM-RATS	VAG-X RATS
Saline	152±8	< 1
Bethanechol (4mg/Kg,sc	296±42	267±58
BET + SV (2.5μg/Kg,sc)	47±4*	187±29

Data are means ± SE expressed as μEq/2h.
* P < 0.001 compared with saline.

CONCLUDING REMARKS

Apart from their hypophysiotropic activity, UI and SV appear to represent a new class of sensory peptides. In fishes, amphibians and mammals, the sensory neurons that contain these peptides are not sensitive to capsaicin, and are thus distinguishable from SP-containing neurons. Nevertheless, UI and SV meet the general criteria of sensory neuropeptides. They are released by the same sensory terminals that are activated by environmental stimuli. Specific auto-receptors located on the same terminal from which these peptides are released have been traced in sensory fibres of fishes amd mammals. Binding of endogenously released or injected UI and SV to these receptors decreases the threshold of the activation of the sensory terminals by environmental stimuli. More generally, the specific function of the peptidergic system throughout the phylogenetic tree may be the initiation and modulation of neuroendocrine reflexes, with the purpose of activating signals necessary for the maintenance of chemical homeostasis of the body under stress conditions.

References

Bern, H.A., Pearson, D., Larson, B.A., Nishioka, R.S., 1985, Neurohormone from fish tails: the caudal neurosecretory system, **Recent Progress in Hormone Research**, 41:533.

Broccardo, M., Improta, G., Melchiorri, P., 1982, Effect of Sauvagine on gastric emptying in conscious rats, **Eur. J. Pharmacol.**, 85:111.

Brown, M.R., Fisher, A.L., Spiess, J., Rivier, J., Rivier, C., Vale, W., 1982, Comparison of the biological actions of corticotropin-releasing factor and sauvagine, **Regul. Peptides**, 4:107.

Erspamer, V., Falconieri Erspamer, G., Improta, G., Negri, L., de Castiglione, R., 1980, Sauvagine, a new polypeptide from Phyllomedusa sauvagei skin. Occurrence in various Phyllomedusa species and pharmacological actions on rat blood pressure and diuresis, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 312:265.

Erspamer, V., Melchiorri, P., Broccardo, M., Falconieri Erspamer, G., Falaschi, P., Improta, G., Negri, L., Renda, T., 1981, The brain-gut-skin triangle: new peptides, **Peptides**, 2:7.

Improta, G., Broccardo, M., 1988, Sauvagine: effects on gastric acid secretion in rats, **Peptides**, 9:843.

Konturek, S.J., Bilski, J., Pawlik, W., Thor, P., Czarnobiliski, K., Szoke, B., Schally, A.V., 1985, Gastrointestinal secretory, motor and circulatory effects of corticotropin releasing factor (CRF), **Life Sciences**, 37:1231.

Lederis, K., Fryer, J., Rivier, J., MacCannell, K.L., Kobayashi, Y., Woo, N., Wong, K.L., 1985, Neurohormones from fish tails: actions of urotensin I in mammals and fishes, **Recent Progress in Hormone Research**, 41:553.

Lederis, K., Fryer, J.N., Yulis, C.R., 1985, The fish neuropeptide urotensin I: its physiology and pharmacology, **Peptides**, 6:353.

Lederis, K., Ko, D., Rivier, J., Melchiorri, P., Negri, L., 1987, Specificity and sensitivity of antisera produced against non-coniugated urotensin I and related peptides, **Proc. West. Pharmacol. Soc.**, 30:187.

Lenz, H.J., Fisher, L.A., Vale, W.W., Brown, M.R., 1985, Corti-

cotropin-releasing factor, Sauvagine and Urotensin I: effects on blood flow, **Am. J. Physiol.**, 249:R85.
Lenz, H.J., Klapdor, R., Hester, S.E., Webb, V.J., Galyean, R.F., Rivier, J.E., Brown, M.R., 1986, Inhibition of gastric acid secretion by brain peptides in the dog, **Gastroenterology**, 91:905.
Melchiorri, P., Negri, L., 1981, Action of sauvagine on the mesenteric vascular bed of the dog, **Regul. Peptides**, 2:1.
Melchiorri, P., Negri, L., Erspamer, V., 1982, Effects of sauvagine on release of prolactin, adrenocorticotropin, beta-endorphin and corticosterone in rats, in: "Neuroendocrinology of Vasopressin Corticoliberin and Opiomelanocortins," A.J. Baerschi and J.J. Dreifuss, eds., Academic Press, London.
Minniti, F., Donato, A., D'este, L., Renda, T., 1989, Sauvagine/urotensin I-like immunoreactivity in the caudal and neurosecretory system of a sea water fish Diplodus sargus L. in normal and hyposmotic milieu, **Peptides**, 10:383.
Montecucchi, P.C., Henschen, A., Erspamer, V., 1979, Structure of sauvagine, a vasoactive peptide from the skin of a frog, **Hoppr-Seyler's Z. Physiol. Chem.**, 360:1178.
Negri, L., Noviello, L., Noviello V., 1985, Effects of sauvagine, urotensin I and CRF on food intake in rats, **Peptides**, 6:59.
Taché, Y., Goto, Y., Gunion, M.W., Vale, W., Brown, M., 1983, Inhibition of gastric acid secretion in rats by intracerebral injection of corticotropin-releasing factor, **Science**, 222:935.
Taché, Y., Goto, Y., Gunion, M., Rivier, J., Debas, H., 1984, Inhibition of gastric acid secretion in rats and in dogs by corticotropin-releasing factor, **Gastroenterology**, 86:281.
Taché, Y., Maeda-Hagiwara, M., Turkelson, C.M., 1987, Central nervous system action of corticotropin releasing factor to inhibit gastric emptying in rats, **Am. J. Physiol.**, 253:G241.

GASTRIC AND JEJUNAL ULTRASTRUCTURE IN CAPSAICIN-TREATED RATS WITH AND WITHOUT EXPERIMENTAL ULCER

Carl J. Pfeiffer (1) and Stefano Evangelista (2)

(1) Department of Biomedical Sciences
Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg VA, USA
(2) Pharmacology Department,
A. Menarini Pharmaceuticals
Via Sette Santi 3, Florence, Italy

INTRODUCTION

In recent years considerable interest has been demonstrated in defining the role and interactions of sensory nerves and neuropeptides in the gastrointestinal tract, where such afferent nerves are present and where some identical peptides occur in endocrine cells and neuronal tissues. Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the irritant principle of the Capsicum genus peppers, has been proven to be a valuable pharmacologic

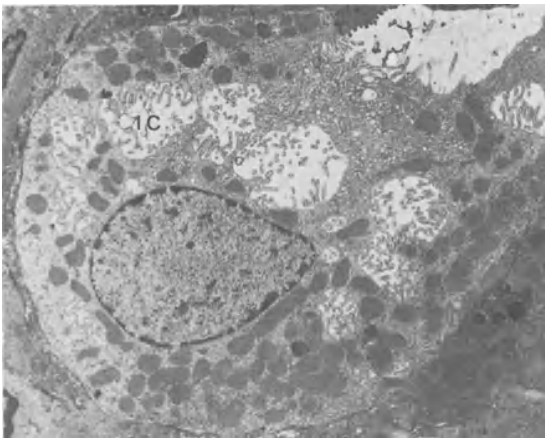


Fig. 1. This parietal cell was in the stomach of a capsaicin pretreated (desensitized) rat subsequently acutely exposed to ethanol. This typical electron micrograph illustrates a normal, healthy cell in active secretory state, with dilated intracellular canaliculi (IC) and numerous mitochondria. X = 7,600.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

probe of high but not absolute selectivity for its actions on primary afferent neurons in the gastrointestinal tract and elsewhere (Maggi and Meli, 1988). Exogenous administration of capsaicin to animals selectively damages sensory afferents in the gut in a manner dependent upon dose, age of animal, etc. The damage occurs in gastric neurons containing tachykinins and calcitonin gene-related peptide, in substance P-containing afferents in anorectal mucosa, and other neurons. In the nervous system of the rat, somatostatin, substance P, and other neuropeptides are also depleted by systemic capsaicin desensitization (Gamse et al., 1984; Sternini and Brecha, 1985; Skofitsch and Jacobowitz, 1985).

Ulcer disease, whether of spontaneous or experimentally-induced origin, is a complex and multifactorial syndrome in which mucosal defense mechanisms are known to play a major role although they remain poorly understood (Robert, 1979 Pfeiffer, 1981; Szabo and Pfeiffer, 1989). Capsaicin-sensitive sensory neurons in the stomach and small intestine have been considered as a factor in the natural defense mechanisms of the gut mucosa, responsive to stimuli which may induce ulcerogenesis (Szolcsányi and Barthó, 1981; Maggi et al., 1987). Consequently, experimental ulcers have been investigated in capsaicin-desensitized rats. Systemic administration of capsaicin, especially when given at high doses to neonatal rats, renders the rats more susceptible to experimental gastric ulcers induced by pylorus ligation, by acid distension of the stomach, and by administration of indomethacin, concentrated ethanol, or acetylsalicylic acid (Szolcsányi and Barthó, 1981; Evangelista et al., 1986; Evangelista et al., 1988). Further, jejunal and ileal ulcers induced by indomethacin (Evangelista et al., 1987a), duodenal ulcers induced by cysteamine or dulcerozine (Maggi et al., 1987), and colonic ulcers induced by trinitrobenzene sulfonic acid (Evangelista and Meli, 1989) are aggravated by capsaicin

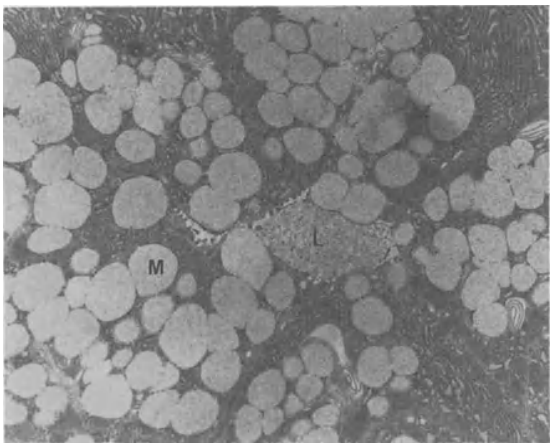


Fig. 2. Section through neck of gastric gland of capsaicin pretreated rat not subjected to the ethanol ulcerogenic procedure. Note that in this typical electron micrograph the sensory afferent denervation did not alter the normal morphology of mucous neck cells. The central lumen (gastric pit) is indicated by (L), and mucous granules by (M). X = 9,300.

pretreatment in rats. Conversely, intragastric administration of capsaicin to rats in close time sequence to the ulcerogenic procedure, or subcutaneous administration 5-7 days before the procedure may reduce ulcer severity (Szolcsányi and Mózsik, 1984; Holzer and Lippe, 1988; Holzer et al., 1989).

The above gastrointestinal actions have been explored physiologically, but little attention has been paid to ultrastructural effects on the gastrointestinal tract following capsaicin administration, notwithstanding a few earlier investigations on central and peripheral nervous system fine structural changes (Joo et al., 1969; Szolcsányi et al., 1971; Jancsó et al., 1977; Jancsó, 1978; Scadding, 1980). Accordingly, the present investigation was undertaken to help elucidate the actions of neonatal capsaicin desensitization alone, and in conjunction with acute ulcerogenesis, on gastrointestinal cell and organelle morphology.

MATERIAL AND METHODS

Animals and Denervation

Male, albino Sprague-Dawley Nossan strain rats aged 2-3 months (180-210 g) were used in this study. They were maintained under constant temperature (21 ± 1°C), humidity (60%), and 12 hour light-dark conditions. Prior to testing and on the second day after birth, rats were subjected to capsaicin desensitization, i.e., permanent degeneration of unmyelinated afferent neurons, by administration of 50 mg capsaicin/kg, Sc, in a volume of 10 ul/g body weight according to standard procedure (Holzer and Lippe, 1988). Alternatively, at the same neonatal stage, control animals were injected with an identical volume of vehicle (10% ethanol, 10% Tween 80, and 80% saline, V/V/V). The injections were performed under ether anesthesia. The effective-

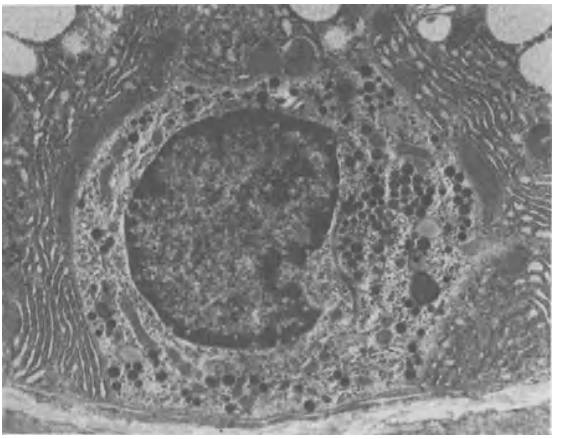


Fig. 3. Normal appearing endocrine cell in ethanol-treated rat.This illustration depicts the normal appearance of peptide granules. As this cell was located within the oxyntic region, the granules are not likely gastrin, but may be somatostatin or another peptide. Note the typical location of the endocrine cell at the peripheral aspect of the gastric gland. X = 19,000.

ness of capsaicin treatment was assessed one day prior to experiments by counting the wiping movements of the eye in rats to which one drop of 0.33 mM capsaicin solution was instilled into one eye. Capsaicin pretreated rats that showed any wiping movements, i.e., incomplete sensory denervation, were excluded from this study, according to the recommendation of Lippe et al., 1989.

Ulcer Induction

At the age of 2-3 months the rats were divided into four groups for testing, including: a) group of normal rats subjected to induction of acute gastric ulcers by ethanol administration, b) capsaicin desensitized rats also subjected to the ethanol treatment, c) group of normal control rats subjected to induction of intestinal lesions by indomethacin, and d) capsaicin desensitized rats also subjected to the indomethacin treatment. The gastric lesions were induced in 24 hour fasted rats by gavage of absolute alcohol (1.25 ml/kg/rat). This dose was selected to induce a sub-maximal degree of gastric ulcers at the sacrifice time of 60 minutes post-administration. Control rats received a comparable volume of saline. Intestinal lesions were induced by administering indomethacin in aqueous suspension (0.9% NaCl plus 0.5% carboxymethylcellulose plus 0.5% Tween 80 in distilled water) to non-fasted rats at a dose of 8 mg/kg in a volume of 5 ml/kg. This dose was selected to induce a submaximal degree of intestinal ulceration. Rats were killed 72 hours after indomethacin treatment.

Electron Microscopy

Tissue samples were taken for ultrastructural analysis at time of sacrifice from the gastric oxyntic region in ethanol-treated and control rats and from the jejunum (20 cm distal to pylorus) in indomethacin-treated and control rats. Specimens were prepared for electron microscopy in accordance with our

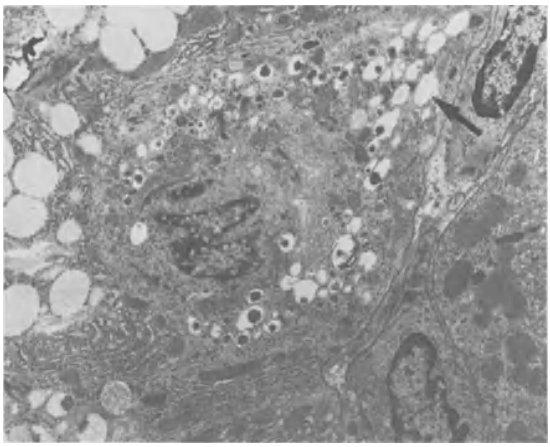


Fig. 4. Gastric endocrine cell of rat pre-treated with capsaicin but not subjected to ethanol exposure. The peptide granules are largely replaced by vacuoles (arrow), but surrounding cells, such as parietal cell on the right side and chief cells on the left side appear normal. X = 11,600.

procedures for gastrointestinal tissue (Pfeiffer et al., 1974; Pfeiffer et al., 1987b, c). Samples were placed immediately in cold 5% glutaraldehyde/3% formaldehyde fixative in 0.1 M Na cacodylate buffer at pH 7.4. Specimens were subsequently washed in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide in 0.1 M cacodylate for 1 hour, washed in buffer again, and dehydrated in a series of alcohols. Semi-thin sections (1 μ) were cut from tissue embedded in Poly/Bed 812 (Polysciences, Inc.), and were stained with 1% toluidine blue in 1% sodium borate for 30 sec, followed by 0.5% safranin in 0.5% sodium borate for 10 seconds, and were studied by light microscopy. Thin sections were doubly stained with lead citrate and uranyl acetate and were studied with a JEOL 100 CX-II transmission electron microscope operating at 80 KV.

RESULTS

Gross ulcerative lesions of moderate severity were observed on the gastric body and fundic mucosal surface in alcohol- and indomethacin-treated rats, and on the jejunal mucosa of indomethacin-treated animals. These responses will not be described here as they have been described elsewhere in dose-response experiments (Kent et al, 1969; Brodie et al., 1970; Okabe et al., 1982; Holzer and Sametz, 1986; Kasuya et al., 1989; Tarnawski and Hollander, 1989).

The stomachs of alcohol-treated rats showed by light microscopy typical localized areas of superficial erosion, but differences between capsaicin-treated and non-capsaicin-treated rats were not noted. Similarly, light microscopy of indomethacin-treated rats showed typical damage to the jejunal surface including vacuolization and enhanced sloughing of surface epithelial cells on or near apices of villi, and lymphatic dilatation, but capsaicin did not induce any marked effects. Detailed histology will not be described here as it is not novel.

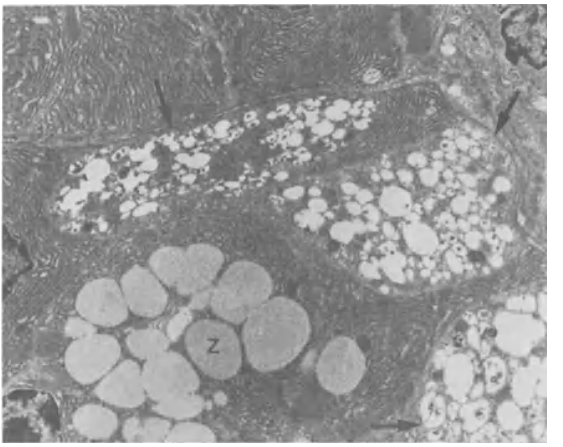


Fig. 5. This electron micrograph clearly illustrates the cell-specific changes induced by capsaicin-pretreatment in this rat which was also subjected to ethanol treatment. Note that three different endocrine cells (arrows) have become markedly degranulated, but adjacent chief cells appear quite normal. Zymogen granules (Z) are shown in the chief cell at the bottom of the figure. X = 7,500.

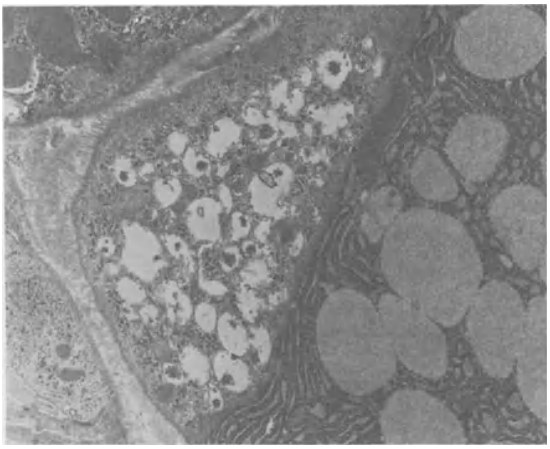


Fig. 6. Another example of a degranulated gastric endocrine cell in a capsaicin-treated rat, not subjected to ethanol. The vacuoles are membrane-bound, with slight evidence of residual peptide. A portion of a normal chief cell is shown on the right. X = 1,600.

Ultrastructural analysis of the gastric mucosa revealed both specific effects and some lack of effects which were of interest. In general, capsaicin desensitization did not alter the cell morphology of most cell types. Specifically, parietal cells were not altered and showed a normal, healthy appearance (Fig. 1). Likewise, mucous neck cells (Fig. 2) and surface epithelial cells (SEC) containing mucus were not altered by capsaicin desensitization, although the SEC were damaged in localized regions by the ethanol treatment, irrespective of capsaicin treatment. Chief cells were normal, and mucosal mast cells were not degranulated in capsaicin-treated rats. However, electron microscopy revealed a striking change in gastric mucosal endocrine cells which occurred in both ethanol and non-ethanol-treated animals. These changes were not seen in rat gastric endocrine cells of normal rats. Normally, the endocrine cells contain numerous electron-dense cytoplasmic (peptide) granules of small to moderate size (Fig. 3), but in capsaicin-treated rats these granules were swollen and void of their peptide content (Fig. 4). This marked response was observed in most, but not all gastric endocrine cells even though other adjacent different cell types appeared normal (Fig. 4-6). The peptide content in this endocrine cell reaction was either mostly (Fig. 4) or entirely (Fig. 5) absent, with respect to morphologic criteria. There was some limited evidence of ultrastructural changes in gastric mucosa nerve fibers in capsaicin-pretreated rats (exposed or non-exposed to ethanol). Specifically, intracellular edema (Fig. 7) was present in some interglandular nerve fibers, but otherwise these fibers appeared intact, with retention of normal neurotransmitter granules.

Electron microscopy of jejunal tissue samples revealed only a few minor cellular changes which could be ascribed to the pretreatment of capsaicin. These changes included such morphologic alterations as localized disruptions of capillary endothelial cells in the mucosa, and some vacuolization within the cytoplasm of surface absorption cells and smooth muscle cells found within the lamina propria of the villi. Other changes, such as local-

ized sites of in situ epithelial cell degeneration, including vacuolization and microvillar degeneration (Fig. 8), were observed, but cannot be proven to be exclusively due to any drug treatment. A limited degree of in situ epithelial cell degeneration occurs in normal gut tissue. Interestingly, the marked endocrine cell changes observed in the stomachs of capsaicin-pretreated rats was not seen in the jejunal endocrine cells.

DISCUSSION

The present data are amongst the first in which electron microscopic investigations have been directed toward examining capsaicin-induced sensory afferent neuron degeneration in the gastric and jejunal mucosa. A limited number of other ultrastructural studies on this phenomenon have been focused on central and other peripheral neural changes (Joo et al., 1969; Szolcsányi et al., 1971; Szolcsányi et al., 1975; Jancsó et al., 1977; Jancsó, 1978; Scadding, 1980). Some of the cytopathologic changes observed in the present study, not observed in capsaicin-treated control rats, were part of the sequence of the ethanol- or indomethacin-induced ulcerogenic process, and were of the expected type of morphologic epithelial changes seen with such ulcerogens. The specific morphologic actions of capsaicin pretreatment were of greater interest. In this regard, it was notable that cytopathologic changes were absent in most mucosal cell types, including those cell types which are known to be highly sensitive to noxious agents, such as parietal cells and chief cells (Pfeiffer, 1975). Parietal cells, perhaps because of their high metabolic activity, undergo some rather drug-specific ultrastructural changes in rats and ferrets in response to caffeine, corticoid, and acetylsalicylic administration (Pfeiffer and Stephens, 1968; Pfeiffer and Roth, 1970; Pfeiffer and Weibel, 1973). Chief cells, in contrast, are sensitive to

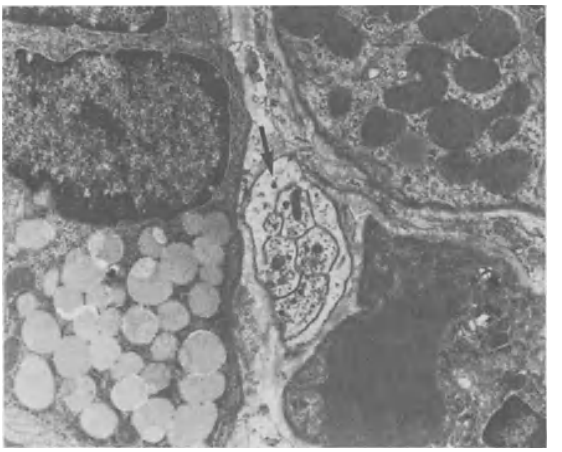


Fig. 7. Electron micrograph of gastric mucosa of capsaicin pretreated rat not exposed to ethanol. In this figure the portions of chief cell (left side) and parietal cell (upper right) shown are normal. The small mucosal neuron between these cells shows some peripheral intracellular edema (arrow) and a normal content of electron dense and clear neurotransmitter granules. X = 16,700.

ulcerogenic agents but tend to undergo non-specific changes such as swelling of rough endoplasmic reticula and nuclear degeneration, following most all ulcerogenic agents which have been studied at the ultrastructural level. (Pfeiffer, 1975). One specific cell type of the gastric mucosa which has been shown in earlier studies of ulcerogenic drugs to be relatively resistant to induced cytomorphologic alteration is the endocrine cell (Pfeiffer, 1975). Hence, it is of particular interest in the present experiment that this is the cell type which was most responsive to capsaicin pretreatment. Accordingly, an interaction between these gastric endocrine cells and capsaicin-sensitive sensory neurons is suggested. Assuming such an interaction, the cause and effect components remain unclear. The major part of gastrointestinal tract tissue levels of sensory neuropeptides has been shown to remain subsequent to systemic capsaicin desensitization owing to the non-destroyed intrinsic neurons which contain these neuropeptides (Holzer et al., 1980; Maggi and Meli, 1988). However, neuropeptides are released from capsaicin-sensitive nerve fibers (Szolcsányi and Barthó, 1981) and the present morphologic evidence indicates a depletion of related peptides in gastric endocrine cells after systemic capsaicin. Such peptides may play a protective role in the mucosal defense mechanism. Somatostatin has long been known to afford protection against ulcer, and duodenal ulcers have been associated with depletion of calcitonin gene-related peptide (CGRP) in rats (Evangelista et al., 1989). Rat gastric ulcers can also be inhibited by exogenous administration of CGRP, bombesin, cholecystokinin-8 and epidermal growth factor (Maggi et al., 1987; Evangelista et al., 1987b). It will be important in future studies to identify specifically by immunochemical methods which peptides are present in the ultrastructurally affected gastric endocrine cells, as

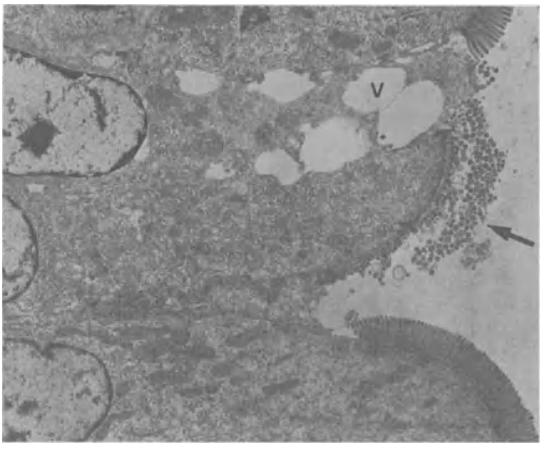


Fig. 8. Capsaicin-related changes in the jejunal epithelium were not noted. This electron micrograph does depict, however, surface epithelial cell degeneration of two cells between normal, intact epithelial cells. Vacuolization (V) and degenerate microvilli (arrow) are evident. This animal was not desensitized by capsaicin but was treated with the ulcerogenic agent, indomethacin. However, this damage could also depict normal <u>in situ</u> cellular degeneration which occurs in intestinal villi, but can by enhanced by noxious drugs. X = 6,600.

many peptides are found in the stomach in both nerve fibers (Ekblad et al., 1985) and endocrine cells (Helmstaedter et al., 1977). Degranulation of gut endocrine cells has also been observed in mice infected with Schistosoma mansoni (Farag et al., 1989).

The ultrastructural changes we observed in small gastric nerve fibers were somewhat similar to those reported by Szolcsanyi and co-workers (1975) in capsaicin desensitized rats, in corneal tissue, except that we did not note mitochondrial damage. Normal nerve fibers in the stomach do not show the edema found in the present investigation (Faussone-Pellegrini et al., 1989). However, the function characterization of the type of affected nerve fibers remains to be undertaken, as they are not proven to be sensory afferent fibers.

References

Brodie, D.A., Cook, P.G., Bauer, B.J., and Dagle, G.E., 1970, Indomethacin-induced intestinal lesions in the rat, **Toxicol. Appl. Pharmacol.** 17:615.

Ekblad, E., Ekelund, M., Graffner, H., Hakanson, R., and Sundler, F., 1985, Peptide-containing nerve fibers in the stomach wall of rat and mouse, **Gastroenterology** 89:73.

Evangelista, S. and Meli, A., 1989, Influence of capsaicin-sensitive fibers on experimentally-induced colitis in rats, **J. Pharm. Pharmacol.** 41:574.

Evangelista, S., Maggi, C.A., Giuliani, S., and Meli, A., 1988, Further studies on the role of the adrenals in the capsaicin-sensitive "gastric defense mechanism", **Int. J. Tiss. Reac.** X:253.

Evangelista, S., Maggi, C.A., and Meli, A., 1986, Evidence for a role of the adrenals in the capsaicin-sensitive "gastric defense mechanism", **Proc. Soc. Exp. Biol. Med.** 182:568.

Evangelista, S., Maggi, C.A., and Meli, A., 1987a, Involvement of capsaicin-sensitive mechanism(s) in the antiulcer defence of intestinal mucosa in rats, **Proc. Soc. Exp. Biol. Med.** 184:264.

Evangelista, S., Maggi, C.A., and Meli, A., 1987b, Influence of peripherally-administered peptides on ethanol-induced gastric ulcers in the rat, **Gen. Pharmac.** 18:647.

Evangelista, S., Renzi, D., Mantellini, P., Surrenti, C., and Meli, A., 1989, Duodenal ulcers are associated with a depletion of duodenal calcitonin gene-related peptide-like immunoreactivity in rats, **Eur. J. Pharmac.** 164:389.

Farag, H.F., Abou Basha, L.M., Fikry, N., and Girgus, S., 1989, A study of the ultrastructure of the intestine of mice experimentally infected with Schistosoma mansoni and treated with praziquantel. **J. Egypt. Soc. Parasitol.** 19:279.

Faussone-Pellegrini, M.S., Pantalone, D., and Cortesini, C., 1989, An ultrastructural study of the smooth muscle cells and nerve endings of the human stomach. **J. Submicrosc. Cytol. Pathol.** 21:421.

Gamse, R., Leeman, S.E., Holzer, P., and Lembeck, F., 1981, Differential effects of capsaicin on the content of somatostatin, substance P and neurotensin in the nervous system of the rat. **Nauyn-Schmiedeberg's Arch. Pharmacol.** 317:140.

Helmstaedter, V., Feurle, G.E., and Forssmann, W.G., 1977, Relationship of glucagon-somatostatin and gastrin-somatostatin cells in the stomach of the monkey. **Cell Tiss. Res.** 177:29.

Hökfelt, T., Efendic, S., Hellerström, C. et al., 1975, Cellular localization of somatostatain in endocrine-like cells and neurons of the rat with special references to the A_1-cells of the pancreatic islets and to the hypothalamus, **Acta Endocrinol.** 80(Suppl. 200):1.

Holzer, P., and Lippe, I. Th., 1988, Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa, **Neuroscience** 27:981.

Holzer, P., Gamse, R., and Lembeck, F., 1980, Distribution of substance P in the rat gastrointestinal tract--lack of effect of capsaicin pretreatment, **Eur. J. Pharmac.** 61:303.

Holzer, P., Pabst, M.A., and Lippe, I. Th., 1989, Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa. **Gastroenterology** 96:1425.

Holzer, P., and Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons, **Gastroenterology** 91:975.

Jancsó, G., 1978, Selective degeneration of chemosensitive primary sensory neurones induced by capsaicin: glial changes, **Cell Tiss. Res.** 195:145.

Jancsó, G., Király, E., and Jancsó-Gábor, A., 1977, Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. **Nature** 270:741.

Joo, F., Szolcsányi, J., and Jancsó-Gábor, A., 1969, Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin, **Life Sci.** 8:621.

Kasuya, Y., Urushidani, T., and Okabe, S., 1979, Effects of various drugs and vagotomy on indomethacin-induced gastric ulcers in the rat. **Jpn. J. Pharmacol.** 29:670.

Kent, T.H., Cardelli, R.M., and Stamler, F.W., 1969, Small intestinal ulcers and intestinal flora in rats given indomethacin, **Am. J. Pathol.** 54:237.

Lippe, I. Th., Pabst, M.A., and Holzer, P., 1989, Intragastric capsaicin enhances rat gastric acid elimination and mucosal blood flow by afferent nerve stimulation, **Br. J., Pharmacol.** 96:91.

Lundberg, J.M., Brodin, E., Hua, X., and Saria, A., 1984, Vascular permeability changes and smooth muscle contraction in relation to capsaicin-sensitive substance P afferents in the guinea-pig, **Acta. Physiol. Scand.** 120:217.

Maggi, C.A., and Meli, A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons. **Gen. Pharmac.** 19:1.

Maggi, C.A., Evangelista, S., Abelli, L., Somma, V., and Meli, A., 1987a, Capsaicin-sensitive mechanisms and experimentally induced ulcers in rats. **J. Pharm. Pharmacol.** 39:559.

Maggi, C.A., Evangelista, S., Giuliani, S., and Meli, A, 1987b, Anti-ulcer activity of calcitonin gene-related peptide in rats, **Gen. Pharmac.** 18:33.

Okabe, S., Tabata, K., and Ishihara, Y., 1982, Indomethacin: its irritative activity on the gastrointestinal tract of experimental animals, Chpt. 3 in: "Drugs and Peptic Ulcer", Vol. II, C.J. Pfeiffer, ed., CRC Press, Boca Raton.

Pfeiffer, C.J., 1975, Experimental drug-induced gastric ulceration: A study of its ultrastructural component, in: "Experimental Ulcer: Models, Methods and Clinical Validity", Gheorghiu, Th. et al., eds., G. Witzstrock, Baden-Baden.

Pfeiffer, C.J., 1981, Experimental analysis of hydrogen ion dif-

fusion in gastrointestinal mucus glycoprotein, **Am. J. Physiol.** 240:G176.
Pfeiffer, C.J., and Roth, J.L.A., 1970, Studies on the secretory and cytotoxic actions of caffeine on the ferret gastric mucosa. **Exp. Mol. Path.** 13:66.
Pfeiffer, C.J., and Stephens, R.J., 1968, Ultrastructural changes of the parietal cell in the ferret gastric mucosa induced by pylorus-ligation and glucocorticoid administration. **J. Ultrastruct. Res.** 21:524.
Pfeiffer, C.J., and Weibel, J., 1973, the gastric mucosal response to acetylsalicylic acid in the ferret: an ultrastructural study. **Am. J. Digest. Dis.** 18:834.
Pfeiffer, C.J., Keith, J.C.Jr., and April, M., 1987a, Topographic localization of gastric lesions and key role of plasma bicarbonate concentration in dogs with experimentally induced gastric dilatation. **Am. J. Vet. Res.** 48:262.
Pfeiffer, C.J., Murray, M.J., and Fainter, L., 1987b, The equine colonic mucosal granular cell: Identification and X-ray microanalysis of apical granules and nuclear bodies. **Anat. Rec.** 219:258.
Pfeiffer, C.J., Pfeiffer, D.C., and Szabo, S., 1987c, Early ultrastructural changes in rat duodenal mucosa associated with cysteamine-induced ulcer, **Exp. Mol. Pathol.** 46:102.
Pfeiffer, C.J., Rowden G., and Weibel, J., 1974, "Gastrointestinal Ultrastructure," Academic Press, New York. Robert, A., 1979, Cytoprotection by prostaglandins, **Gastroenterology** 77:761.
Scadding, J.W., 1980, The permanent anatomical effects of neonatal capsaicin on somatosensory nerves. **J. Anat.** 131:473.
Skofitsch. G., and Jacobowitz, D., 1985, Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat, **Peptides** 6:747.
Sternini, C., and Brecha, N., 1985, Effect of neurotoxin treatment on calcitonin gene-related peptide-like immunoreactivity in the upper gastrointestinal tract, **Gastroenterology** 88:1600.
Szabo, S. 1984, Pathogenesis of duodenal ulcer disease, **Lab. Invest.** 51:121.
Szabo, S., and Pfeiffer, C.J. (eds.), 1989, "Ulcer Disease: New Aspects of Pathogenesis and Pharmacology", CRC Press, Boca Raton.
Szolcsányi, J., Joo, F, and Jancsó-Gábor, A., 1971, Mitochondrial changes in preoptic neurons after capsaicin desensitization of the hypothalamic thermodetectors in rats. **Nature** 229:116.
Szolcsányi, J., Jancsó-Gábor, A., and Joo, F., 1975, Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin, **Naunyn-Schmiedeberg's Arch. Pharmacol.** 287:157.
Szolcsányi, J., and Barthó, L., 1981, Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat, in: "Gastrointestinal Defense Mechanisms", Vol. 29, Mózsik, G., Hanninen, O., and Jávor, T., eds., Pergamon Press, New York.
Szolcsányi, J., and Mózsik, G., 1984, Effects of capsaicin on the development of gastric mucosal damage by different necrotizing agents and of gastric cytoprotection by PGI_2, atropine and cimetidine on rats, in: "Recent Advances in Gastrointestinal Cytoprotection", Mózsik, G. and Bertelli, A., eds., Akadémiai Kiadó, Budapest.
Tarnawski, A., and Hollander, D., 1989, Sucralfate protection of the gastric mucosa against ethanol injury, Chpt. 26 in:

"Ulcer Disease: New Aspects of Pathogenesis and Pharmacology", Szabo, S., and Pfeiffer, C.J., eds., CRC Press, Boca Raton.

AFFERENT NERVE-MEDIATED CONTROL OF GASTRIC MUCOSAL BLOOD FLOW AND PROTECTION

Peter Holzer

Center for Ulcer Research and Education
University of California at Los Angeles and
Veterans Administration Wadsworth Medical Center
Los Angeles, California, U.S.A., and
Department of Experimental and Clinical Pharmacology
University of Graz
Graz, Austria

Introduction

The gastric mucosa is constantly exposed to chemical hazard, the threats being of both endogenous and exogenous origin. Most tissues would rapidly disintegrate if exposed to the concentrations of hydrochloric acid that bathe but do not harm the gastric surface epithelium. This is because a multitude of mechanisms constituting the gastric mucosal barrier (Davenport, 1972) prevent hydrogen ions from entering the tissues in quantities that would produce cell injury. Yet even a weakening of the barrier will usually not lead to any major lesion formation because emergency or protective mechanisms will take effect, limit the damage and promote repair of the injured tissue.

While a number of protective mechanisms has been identified in the gastric mucosa, it is still not clear how these mechanisms are called into operation and are coordinated in the face of pending injury to the mucosa. A well-studied response to barrier disruption and back-diffusion of acid into the gastric mucosa is a marked rise in mucosal blood flow (Ritchie, 1975; Cheung et al., 1975; Whittle, 1977; Bruggeman et al., 1979; Starlinger et al., 1981). This increase in blood flow is thought to be an important mechanism of protection which, by facilitating the disposal of acid, prevents the build-up of an injurious concentration of hydrogen ions in the tissue. The anatomical and functional organization of the gastric mucosal circulation requires submucosal arterioles to be dilated in order to increase blood flow to the mucosa (Gannon et al., 1982; Guth and Leung, 1987). Thus the message of acid back-diffusion needs to be transmitted over the distance of more than 500 μm which separates the surface of the mucosa from submucosal arterioles in the rat stomach. Considering this distance and the rapidity with which mucosal blood flow is increased following barrier disruption (Bruggeman et al., 1979), it would seem conceivable that

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

neurons mediate the blood flow response to acid back-diffusion. Since atropine, however, does not inhibit the vasodilatation induced by acid back-diffusion (Bruggeman et al., 1979), this idea has not yet attracted much attention.

Apart from autonomic and enteric neurons, the gastric mucosa and submucosa is innervated by capsaicin-sensitive sensory neurons which form a particularly dense plexus of fibers around submucosal blood vessels (Furness et al., 1982; Sharkey et al., 1984; Sternini et al., 1987; Green and Dockray, 1988). These nerve fibers arise from two different sources. One group are "spinal" sensory neurons which originate from cell bodies in the dorsal root ganglia and reach the stomach via the splanchnic and mesenteric nerves, a route whereby they pass through the celiac ganglion (Green and Dockray, 1988). The other group are vagal sensory nerve fibers having their cell bodies in the nodose ganglion.

The idea that capsaicin-sensitive sensory neurons in the gastric mucosa and submucosa play a role in gastric mucosal protection originated from the evidence that these neurons are involved in inflammatory reactions of the skin, eye and respiratory tract (see Holzer, 1988; Maggi and Meli, 1988). They are particularly sensitive to chemical noxious stimuli and induce a number of responses that are interpreted as protecting or as promoting repair of damaged tissue. Vagal sensory fibers innervating the stomach have been found to be sensitive to acid and other noxious chemicals including ethanol (Clarke and Davison, 1978), and there is functional evidence that capsaicin-sensitive sensory neurons also are sensitive to hydrogen ions (Cervero and McRitchie, 1982; Martling and Lundberg, 1988; Bevan and Yeats, 1989). These observations clearly suggest that capsaicin-sensitive sensory neurons might subserve a protective role in the gastric mucosa by sensing pending injury due to acid or other endogenous or exogenous factors and signalling for appropriate measures of defense and repair. To test this overall hypothesis we set out (1) to examine whether defunctionalization of capsaicin-sensitive neurons would weaken, and stimulation of these neurons would strengthen, the ability of the gastric mucosa to resist experimentally imposed injury, and (2) if so, which protective mechanisms are under the control of capsaicin-sensitive sensory neurons. This review gives a brief account of the major findings of these studies and the conclusions that have been drawn from them.

Defunctionalization of Sensory Neurons Exacerbates Gastric Injury

Defunctionalization of sensory neurons, by treating rats with a high dose of capsaicin (≥ 50 mg/kg) at least a week before the experiments, provided clear evidence that capsaicin-sensitive sensory neurons control certain mechanisms of gastric mucosal protection. Table 1 summarizes the findings which are available to date in this respect. Ablation of sensory neurons does not cause gastric mucosal damage by itself (Holzer and Sametz, 1986; Esplugues and Whittle, 1990) but leads to exacerbation of mucosal lesion formation in response to a variety of exogenous and endogenous factors including acid, cysteamine, indomethacin, ethanol, aspirin, platelet-activating factor, and thromboxane (for references see Table 1). Injury induced by cold and restraint stress, however, was found to be unchanged after

TABLE 1. Effect of ablation of capsaicin-sensitive sensory neurons on experimental lesion formation in the rat gastric mucosa

Injurious factor	Effect of nerve ablation on lesion formation	References
Pylorus ligation	Aggravation	A
Acid distension	Aggravation	A
Acid back-diffusion	Aggravation	B
Cysteamine	Aggravation	C
Indomethacin	Aggravation	C,D
Ethanol	Aggravation	C,E,F,G
Acetylsalicylic acid	Aggravation	H
Platelet-activating factor	Aggravation	I
Thromboxane mimetic	Aggravation	K
Cold restraint stress	No change	L

A: Szolcsányi and Barthó (1981); B: Holzer et al. (1990); C: Holzer and Sametz (1986); D: Evangelista et al. (1986); E: Evangelista et al. (1987); F: Esplugues and Whittle (1990); G: Yonei et al. (1990); H: Evangelista et al. (1988a); I: Esplugues et al. (1989); K: Whittle and Esplugues (1989); L: Dugani and Glavin (1986).

ablation of sensory neurons (Dugani and Glavin, 1986). These data suggest that capsaicin-sensitive nerves either are not involved in the protection from stress-induced ulceration or their protective role is overridden by certain stress-induced processes.

Stimulation of Sensory Neurons Prevents Gastric Injury

Szolcsányi and Barthó (1981) were the first to propose a protective function of capsaicin-sensitive sensory neurons in the gastric mucosa when they observed that intragastric administration of small quantities of capsaicin, to stimulate sensory nerve endings, protected from injury following pylorus ligation. In later experiments it was found that intragastric capsaicin also prevented injury produced by ethanol (Holzer and Lippe, 1988; Holzer et al., 1990b) or acidified aspirin (Holzer et al., 1989). Figure 1 shows that gross injury of the rat gastric mucosa caused by acidified aspirin is reduced by intragastric capsaicin in a dose-related manner (Holzer et al., 1989). Histologically, the depth of the aspirin- and ethanol-induced mucosal erosions which involved up to two thirds of the mucosal depth was significantly reduced (Holzer et al., 1989, 1990b). Ethanol-induced superficial injury to the mucosa was not prevented by capsaicin as revealed by light and scanning electron microscopy (Holzer et al., 1990b) and by measurement of the transmucosal potential difference (Holzer and Lippe 1988). Aspirin-induced surface damage, however, was attenuated by capsaicin (Holzer et al., 1989).

Pathways and Mediators of Sensory Nerve-Mediated Gastric Mucosal Protection

The hypothesis that the protective effect of intragastric capsaicin is mediated by sensory neurons was ascertained by the finding that ablation of capsaicin-sensitive sensory neurons

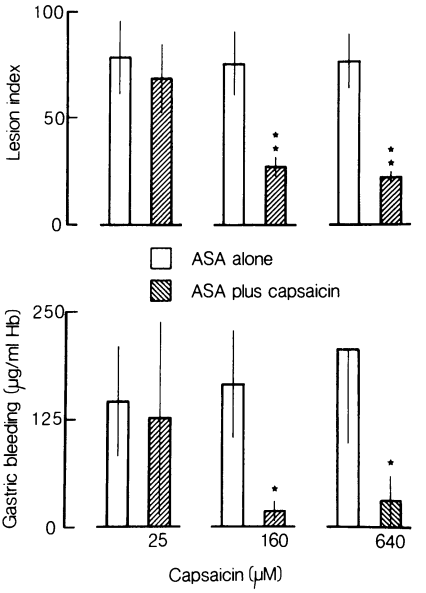


Fig. 1. Effect of various concentrations of capsaicin on gross mucosal damage (lesion index; upper panel) and on gastric bleeding (hemoglobin content of the gastric perfusate; lower panel) as assessed after a 30-min perfusion of the rat stomach with aspirin (ASA; 25 mM) in saline of pH 1.5. Means ± SEM of 7 rats. * $P < 0.05$, ** $P < 0.01$ versus rats given aspirin alone (U test). Taken from Holzer et al. (1989; copyright by American Gastroenterological Association).

("sensory denervation") totally prevented any protective action of capsaicin against ethanol injury (Holzer and Lippe, 1988). According to the neuropharmacology of capsaicin (see Szolcsányi, 1984; Holzer, 1988; Maggi and Meli 1988) this observation indicates that intragastric capsaicin protects the rat gastric mucosa by stimulation of sensory neurons. Further experiments designed to elucidate the neural pathways of sensory nerve-mediated gastric mucosal protection demonstrated that the protective effect of capsaicin remained unchanged after acute surgical or pharmacological blockade of the autonomic nervous system and after acute removal of the adrenal glands (Holzer and Lippe, 1988). These data show that the protective effect of capsaicin is not brought about by a reflex via parasympathetic or sympathetic efferent neurons and hence is due to a local neural mechanism within the gastric wall. In analogy to a local effector function of sensory nerve endings in the skin, eye and respiratory tract (Szolcsányi, 1984; Holzer, 1988; Maggi and Meli, 1988) it may be hypothesized that the protective action of capsaicin involves only sensory neurons and results from a local release of transmitter substances from sensory nerve endings within the gastric wall. This concept, however, does not take account of a possible implication of the enteric nervous system. There is good evidence that stimulation of sensory nerve endings in the guinea-pig small intestine is capable of activating enteric cholinergic neurons (see Barthó and Holzer, 1985).

Inherent in the assumption that capsaicin-induced protection of the gastric mucosa results from a local neural mechanism within the stomach is the question as to what mediator substances are released from capsaicin-sensitive nerve endings that strengthen gastric mucosal resistance. Sensory nerve endings in the stomach arising from dorsal root ganglion neurons contain a number of putative peptide transmitters which, at least in part, coexist in the same nerve fibers (Furness et al., 1982; Sharkey et al., 1984; Sternini et al., 1987; Green and Dockray, 1988). Particularly well established is the presence of calcitonin gene-related peptide (CGRP) and substance P in these neurons. We were able to demonstrate that capsaicin (10 μM) administered intra-arterially to the vascularly perfused rat stomach induces a large increase in the release of CGRP into the venous effluent (Holzer et al., 1990c). Identical data were obtained by Gray et al. (1989). Further experiments showed that close arterial administration of CGRP to the rat stomach prevented gross injury produced by 25% ethanol or acidified aspirin (Lippe et al., 1989a), the two injury models in which capsaicin previously had been found to be protective (for further details see Lippe, this volume). Substance P also is released by capsaicin in the rat and guinea-pig stomach (Renzi et al., 1988), and subcutaneous administration of substance P-related peptides likewise is able to prevent experimentally induced gastric injury (Evangelista et al., 1989). From these data it would appear that CGRP and tachykinins are among the mediators of capsaicin-induced mucosal protection.

Eicosanoids have been ruled out as mediators of sensory nerve-mediated gastric mucosal protection. Two lines of evidence support this contention. First, indomethacin administered at doses shown to inhibit gastric prostaglandin synthesis failed to alter the protective action of intragastric capsaicin against ethanol injury (Holzer et al., 1990b). Second, capsaicin given intragastrically at a dose known to prevent ethanol-induced injury did not affect the ex vivo formation of prostaglandin E_2, 6-oxo-prostaglandin F_{1a} and leukotriene C_4 (Holzer et al., 1990b).

Mechanisms of Sensory Nerve-Mediated Gastric Mucosal Protection

As outlined above, circumstantial evidence has been put forward that the protective action of sensory neuron stimulation is mediated by a local release of peptides within the gastric wall. Some of the peptides contained in sensory nerve endings including CGRP and substance P possess vasodilator properties (see Holzer, 1988), and CGRP has been shown to be a powerful stimulant of gastric mucosal blood flow (Bauerfeind et al., 1989; Lippe et al., 1989a). These findings suggest that sensory neurons facilitate gastric mucosal protection by way of an increased blood flow through the mucosa. We indeed were able to demonstrate that gastric mucosal blood flow as measured by the aniline (Lippe et al., 1989b) or hydrogen gas (Holzer et al., 1990b) clearance technique was markedly enhanced after intragastric administration of capsaicin. This increase in gastric mucosal blood flow also was seen when capsaicin was administered together with an injurious concentration of ethanol (25%), increased blood flow being associated with a reduction in gross lesion formation (Figure 2.) Since intragastric administration of capsaicin had no effect on blood pressure it can be inferred that the increase in mucosal blood flow resulted from mucosal

vasodilatation. Further experiments established that capsaicin-induced mucosal vasodilatation was, like the capsaicin-induced protection from ethanol injury (Holzer and Lippe, 1988), mediated by sensory neurons (Lippe et al., 1989b).

Capsaicin-induced stimulation of sensory nerve endings in the gastric mucosa failed to affect a number of other functional parameters of the gastric mucosa. The amount of gastric mucus as determined by an Alcian Blue technique remained unaltered by intragastric capsaicin (160 μM; Lippe and Holzer, unpublished). Intragastric administration of capsaicin (160 μM) had no effect on basal gastric acid output when the stomach of urethane-anesthetized rats was perfused with saline of pH 6 (Lippe et al., 1989b). When the stomach was perfused with saline of pH 3 intragastric capsaicin significantly reduced acid output, which has been interpreted as reflecting increased disposal of acid rather than inhibition of acid secretion (Lippe et al., 1989b). It is

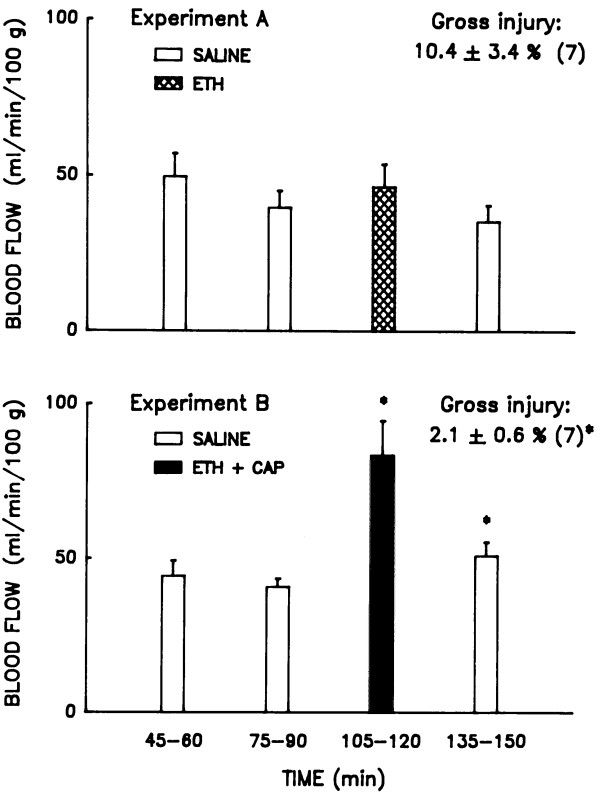


Fig. 2. Effect of intragastric ethanol alone (ETH; 25%; experiment A) or in combination with capsaicin (CAP; 160 μM; experiment B) on rat gastric mucosal blood flow as measured by the hydrogen gas clearance technique and on gross injury. Abscissa: time from completion of the experimental setup, ethanol or ethanol plus capsaicin being administered during the period of 90-120 min. Gross injury is expressed as a percentage of the total area of the glandular mucosa. Means ± SEM, n = 7. **P*<0.05 versus respective values in experiment A (U test). Taken from Holzer et al. (1990b; copyright by American Gastroenterological Association.

likely that this increased elimination of acid is a consequence of the increase in mucosal blood flow produced by capsaicin (Holzer et al., 1990b; Lippe et al., 1989b), all the more since gastric output of bicarbonate remained unchanged under these conditions (Lippe and Holzer, unpublished). Following perfusion of the stomach with saline of pH 2 gastric acid output became negative, i.e. acid was lost from the perfusion medium probably in part due to acid back-diffusion. This acid loss from the gastric lumen was not significantly altered by intragastric capsaicin indicating that capsaicin did not weaken the gastric mucosal barrier (Lippe et al., 1989b). This contention is supported by a number of additional findings. Thus intragastric capsaicin was also devoid of any effect on vascular permeability and transmucosal potential difference (Holzer and Lippe, 1988) and failed to cause any macro- or microscopic damage to the mucosa by itself (Lippe et al., 1989b). It can be ruled out, therefore, that capsaicin increased mucosal blood flow and protected from injury by virtue of a slight irritant action on the mucosa. This conclusion is consistent with the findings that the process of "adaptive cytoprotection" which is initiated by mild gastric irritants is independent of the sensory innervation of the stomach (Evangelista et al., 1988b; Miller et al., 1989).

Sensory Neurons Signal for an Increase in Gastric Mucosal Blood Flow in the Face of Pending Acid Injury

The data described in the previous section of this review clearly indicate that stimulation of sensory neurons in the stomach strengthens the resistance of the gastric mucosa against injury by way of an increase in gastric mucosal blood flow. This hypothesis is in keeping with the concept that maintenance or facilitation of mucosal blood flow is an important mechanism of gastric mucosal protection (Cheung et al., 1975; Ritchie, 1975; Whittle, 1977; Gannon et al., 1982; Pihan et al., 1986; Guth and Leung, 1987). While sensory neuron stimulation by intragastric administration of capsaicin has been instrumental in unravelling the mechanisms that are under the control of sensory neurons, the physiologic or pathophysiologic conditions calling these neurons into operation remained unknown. Since there is evidence to suggest that capsaicin-sensitive neurons are sensitive to acid (Cervero and McRitchie, 1982; Martling and Lundberg, 1988; Bevan and Yeats, 1989) it appeared conceivable that acid back-diffusion is a condition under which sensory neurons are activated and lead to the well-documented increase in gastric mucosal blood flow (Cheung et al., 1975; Ritchie, 1975; Whittle, 1977; Bruggeman et al., 1979). This hypothesis was tested by examining whether ablation of capsaicin-sensitive neurons inhibits the blood flow increase in response to acid back-diffusion. Gastric mucosal blood flow was measured by the hydrogen gas clearance technique using a needle electrode inserted at the interface between gastric mucosa and submucosa. Perfusion of the stomach with 0.15 N HCl resulted in the loss of some acid (about 20 μEqu/15 min) from the gastric perfusion medium but did not change blood flow as compared to that seen under perfusion with saline alone (Holzer et al., 1990a). However, disruption of the gastric mucosal barrier by adding ethanol (15%) to the acid perfusion medium markedly increased mucosal blood flow and enhanced acid loss from the gastric perfusion medium by a factor of 5 indicating acid back-diffusion. Sensory denervation by a subcutaneous pretreatment of rats with a high dose of capsaicin significantly inhibited the blood flow increase caused by barrier disruption

in the presence of acid (Holzer et al., 1990a). Statistical analysis (Quade test) revealed that after defunctionalization of sensory neurons there was no significant rise in blood flow. Associated with this inhibition of the blood flow response was a significant exacerbation of gross lesion formation and a significant increase in the mucosal depth of the lesions. Mean arterial blood pressure, basal mucosal blood flow and acid loss from the gastric perfusion medium were not altered by ablation of sensory neurons.

Additional proof that the blood flow response to acid back-diffusion is mediated by neurons came from experiments in which the drug tetrodotoxin (60 ng/min), a blocker of nerve conduction, was infused close arterially to the stomach (Holzer et al., 1990a). When Krebs buffer as the vehicle was administered, disruption of the mucosal barrier in the presence of acid was followed by the usual increase in gastric mucosal blood flow. Tetrodotoxin suppressed this blood flow increase and aggravated gross damage in the gastric mucosa. The mean arterial blood pressure was not significantly affected by this dose of tetrodotoxin (Holzer et al., 1990a). As reported previously (Bruggeman et al., 1979), atropine failed to change the gastric blood flow response to acid back-diffusion indicating that postganglionic parasympathetic neurons or cholinergic vasodilator neurons of the enteric nervous system are not implicated. Also unlikely is an involvement of the sympathetic nervous system since stimulation of sympathetic efferent neurons causes mucosal vasoconstriction (see Guth and Leung, 1987). However, the inhibitory effect of tetrodotoxin indicates that the rise in blood flow due to acid back-diffusion relies on nerve conduction. It would seem, therefore, that acid back-diffusion is sensed by capsaicin-sensitive sensory nerve endings which in turn signal for an increase in blood flow to the mucosa by way of a reflex mechanism. This process may be brought about by sensory neurons only, the vasodilator response being the result of an "axon reflex" (Holzer, 1988), or involve effector neurons of the enteric and/or autonomic nervous system that do not utilize norepinephrine or acetylcholine, acting via muscarinic receptors, as their transmitters.

Summary and Perspectives

The findings reviewed here put a new perspective on how mechanisms of gastric mucosal resistance to injury are called into operation and coordinated by the nervous system. Certain chemosensitive neurons in the stomach play a physiologic role in monitoring the presence of potentially or actually injurious factors in the superficial mucosa. Once activated they strengthen gastric mucosal defense against deep injury, a key process in this respect being an increase in blood flow through the gastric mucosa. Facilitated blood flow is likely to facilitate a variety of gastric protective mechanisms. On the one hand, increased blood flow will add to the removal of injurious factors from the mucosa. On the other hand, enhanced blood flow will promote or support a wide range of processes that either reduce the vulnerability, or aid the repair, of the gastric mucosa (Gannon et al., 1982; Guth and Leung, 1987; Silen, 1987). Facilitation of mucosal blood flow thus can be seen as a central governing mechanism of gastric mucosal protection. This mechanism is under the control of sensory neurons which are on the watch for injurious factors such as acid.

Our findings in the stomach are consistent with the evidence that capsaicin-sensitive sensory neurons regulate local blood flow in various regions of the gastrointestinal system (Rózsa et al., 1988; Rózsa and Jacobson, 1989; Thiefin et al., 1989). Although blood flow appears to be a major target that is under the control of sensory neurons, a number of other processes that have a bearing on gastric mucosal integrity also may be regulated by these neurons. There is increasing evidence that sensory neurons and their neuropeptide mediators subserve a trophic role in certain cells or tissues (see Holzer, 1988). Furthermore, sensory nerve endings are in close contact with components of the immune system (Fink and Weihe, 1988; Popper et al., 1988) suggesting that functions of the immune system are regulated by sensory neurons (see Payan et al., 1984; Stead et al., 1987; Holzer, 1988). It would be worth examining whether these functional aspects of sensory neurons are relevant for restitution and healing of damaged gastric mucosa. A completely new perspective opens up if one considers that the long-term integrity of the gastric mucosa may be under the subtle control of the nervous system and that, vice versa, improper functioning of these neural control mechanisms may predispose to gastric ulcer disease.

Acknowledgements

The research performed by the author was supported by the Max Kade Foundation, the National Institute of Health, Veterans Administration Research Funds, the Austrian Scientific Research Funds and the Franz Lanyar Foundation of the Medical Faculty of the University of Graz.

References

Barthó, L., Holzer, P., 1985, Search for a physiological role of substance P in gastrointestinal motility, **Neuroscience** 16:1.

Bauerfeind, P., Hof, R., Hof, A., Cucala, M., Siegrist, S., von Ritter, Ch., Fischer J.A., Blum, A.L., 1989, Effects of hCGRP I and II on gastric blood flow and acid secretion in anesthetized rabbits, **Am. J. Physiol.**, 256:G145.

Bevan, S., Yeats, J.C., 1989, Protons activate a sustained inward current in a subpopulation of rat isolated dorsal root ganglion (DRG) neurones, **J. Physiol. (London)**, 417:81P.

Bruggeman, T.M., Wood, J.G., Davenport, H.W., 1979, Local control of blood flow in the dog's stomach: vasodilatation caused by acid back-diffusion following topical application of salicylic acid, **Gastroenterology**, 77:736.

Cervero, F., McRitchie, H.A., 1982, Neonatal capsaicin does not affect unmyelinated efferent fibers of the autonomic nervous system: functional evidence, **Brain Res.**, 239:283.

Cheung, L.Y., Moody, F.G., Reese, R.S., 1975, Effect of aspirin, bile salt and ethanol on canine gastric mucosal blood flow, **Surgery**, 77:786.

Clarke, G.D., Davison, J.S., 1978, Mucosal receptors in the gastric antrum and small intestine of the rat with afferent fibres in the cervical vagus, **J. Physiol. (London)**, 284:55.

Davenport, H.W., 1972, The gastric mucosal barrier, **Digestion**, 5:162.

Dugani, A.M, Glavin, G.B., 1986, Capsaicin effects on stress pathology and gastric acid secretion in rats, **Life Sci.**, 39:1531.

Esplugues, J.V., Whittle, B.J.R., 1990, Morphine potentiation of ethanol-induced gastric mucosal damage in the rat. Role of local sensory afferent neurons, **Gastroenterology**, 98:82.
Esplugues, J.V., Whittle, B.J.R., Moncada S., 1989, Local opioid-sensitive afferent sensory neurones in the modulation of gastric damage induced by Paf, **Br. J. Pharmacol.**, 97:579.
Evangelista, S., Lippe, I.T., Rovero, P., Maggi, C.A., Meli, A., 1989, Tachykinins protect against ethanol-induced gastric lesions in rats, **Peptides**, 10:79.
Evangelista, S., Maggi, C.A., Giuliani, S., Meli, A., 1988a, Further studies on the role of the adrenals in the capsaicin-sensitive "gastric defence mechanism", **Int. J. Tiss. React.**, 10:253.
Evangelista, S., Maggi, C.A., Meli, A., 1986, Evidence for a role of adrenals in the capsaicin-sensitive "gastric defence mechanism" in rats, **Proc. Soc. Exp. Biol. Med.**, 182:568.
Evangelista, S., Maggi, C.A., Meli, A., 1987, Influence of peripherally-administered peptides on ethanol-induced gastric ulcers in the rat, **Gen. Pharmacol.**, 18:647.
Evangelista, S., Maggi, C.A., Meli, A., 1988b, Lack of influence of capsaicin-sensitive sensory fibers on adaptive cytoprotection in rat stomach, **Digest Dis. Sci.**, 33:1050.
Fink, T., Weihe, E., 1988, Multiple neuropeptides in nerves supplying mammalian lymph nodes: messenger candidates for sensory and autonomic neuroimmunomodulation? **Neurosci. Lett.**, 90:39.
Furness, J.B., Papka, R.E., Della N.G., Costa, M., Eskay, R.L., 1982, Substance P-like immunoreactivity in nerves associated with the vascular system of guinea-pigs, **Neuroscience**, 7:447.
Gannon, B., Browning, J., O'Brien, P., 1982, The microvascular architecture of the glandular mucosa of rat stomach, **J. Anat.**, 135:667.
Gray, J.L., Bunnett, N.W., Mulvihill, S.J., Debas, H.T., 1989, Capsaicin stimulates release of calcitonin gene-related peptide (CGRP) from the isolated perfused rat stomach, **Gastroenterology**, 96:A181.
Green, T., Dockray, G.J., 1988, Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig, **Neuroscience**, 25:181.
Guth, P.H., Leung, F.W., 1987, Physiology of the gastric circulation, in: "Physiology of the Gastrointestinal Tract", L.R. Johnson, ed., Raven Press, New York.
Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.
Holzer, P., Lippe, I.T., 1988, Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa, **Neuroscience**, 27:981.
Holzer, P., Livingston, E.H. Guth, P.H., 1990A, Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury, **Gastroenterology**, submitted.
Holzer, P., Pabst, M.A., Lippe, I.T., 1989, Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa, **Gastroenterology**, 96:1425.
Holzer, P., Pabst, M.A., Lippe, I.T., Peskar, B.M., Peskar,

B.A., Livingston, E.H., Guth, P.H., 1990b, Afferent nerve-mediated protection against deep mucosal damage in the rat stomach, **Gastroenterology**, in press.
Holzer, P., Peskar, B.M., Peskar, B.A., Amann, R., 1990c, Release of calcitonin gene-related peptide induced by capsaicin in the vascularly perfused rat stomach, **Neurosci. Lett.**, 108:195.
Holzer, P., Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons, **Gastroenterology**, 91:975.
Lippe, I.T., Lorbach, M., Holzer, P., 1989a, Close arterial infusion of calcitonin gene-related peptide into the rat stomach inhibits aspirin- and ethanol-induced hemorrhagic damage, **Regul. Pept.**, 26:35.
Lippe, I.T., Pabst, M.A., Holzer, P., 1989b, Intragastric capsaicin enhances rat gastric acid elimination and mucosal blood flow by afferent nerve stimulation, **Br. J. Pharmacol.**, 96:91.
Maggi, C.A., Meli, A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, **Gen. Pharmacol.**, 19:1.
Martling, C.-R., Lundberg, J.M., 1988, Capsaicin-sensitive afferents contribute to the acute airway edema following tracheal instillation of hydrochloric acid or gastric juice in the rat, **Anesthesiology**, 68:350.
Miller, T.A., Smith, G.S., Stanislawska, M., Phan, T.M., Henagan, J.M., 1989, Role of vagal innervation in adaptive cytoprotection, **Digest Dis. Sci.**, 34:1318.
Payan, D.G., Levine, J.D., Goetzl, E.J., 1984, Modulation of immunity and hypersensitivity by sensory neuropeptides, **J. Immunol.**, 132:1601.
Pihan, G., Majzoubi, D., Haudenschild, C., Trier, J.S., Szabo, S., 1986, Early microcirculatory stasis in acute gastric mucosal injury in the rat and prevention by 16,16-dimethyl prostaglandin E_2 or sodium thiosulfate, **Gastroenterology**, 91:1415.
Popper, P., Mantyh, C.R., Vigna, S.R., Maggio J.E., Mantyh, P.W., 1988, The localization of sensory nerve fibers and receptor binding sites for sensory neuropeptides in canine mesenteric lymph nodes, **Peptides**, 9:257.
Renzi, D., Santicioli, P., Maggi, C.A., Surrenti, C., Pradelles, P., Meli, A., 1988, Capsaicin-induced release of substance P-like immunoreactivity from the guinea pig stomach in vitro and in vivo, **Neurosci. Lett.**, 92:257.
Ritchie, W.P., 1975, Acute gastric mucosal damage induced by bile salts, acid and ischemia, **Gastroenterology**, 68:699.
Rózsa, Z., Jacobson, E.D., 1989, Capsaicin-sensitive nerves are involved in bile-oleate induced intestinal hyperemia, **Am. J. Physiol.**, 256:G476.
Rózsa, Z., Mattila, J., Jacobson, E.D., 1988, Substance P mediates a gastrointestinal thermoreflex in rats, **Gastroenterology**, 95:265.
Sharkey, K.A., Williams, R.G., Dockray, G.J., 1984, Sensory substance P innervation of the stomach and pancreas. Demonstration of capsaicin-sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing, **Gastroenterology**, 87:914.
Silen, W., 1987, Gastric mucosal defense and repair, in: "Physiology of the Gastrointestinal Tract", L.R. Johnson, ed., Raven Press, New York.
Starlinger, M., Schiessel, R., Hung, C.R., Silen, W., 1981, H^+ back diffusion stimulating gastric mucosal blood flow in the rabbit fundus, **Surgery**, 89:232.

Stead, R.M., Bienenstock, J., Stanisz, A.M., 1987, Neuropeptide regulation of mucosal immunity, **Immunol. Rev.**, 100:333.
Sternini, C., Reeve, J.R., Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.
Szolcsányi, J., 1984, Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function, in: "Antidromic Vasodilatation and Neurogenic Inflammation", L.A. Chahl, J. Szolcsányi, F. Lembeck, eds., Akadémiai Kiadó, Budapest.
Szolcsányi, J., Barthó, L., 1981, Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat, in: "Gastrointestinal Defense Mechanisms", G. Mózsik, O. Hänninen, T. Jávor, eds., Pergamon Press and Akadémiai Kiadó, Oxford and Budapest.
Thiefin, G., Raybould, H.E., Leung, F.W., Taché, Y., Guth, P.H., 1989, Role of capsaicin-sensitive vagal afferent fibers in increased gastric mucosal blood flow induced by electrical vagal stimulation in the rat. **Gastroenterology**, 96:A509.
Whittle, B.J.R., 1977, Mechanism underlying gastric mucosal damage induced by indomethacin and bile salts, and the actions of prostaglandins, **Br. J. Pharmacol.**, 60:455.
Whittle, B.J.R., Esplugues, J.V., 1989, Gastric mucosal damage induced by an intravascular thromboxane mimetic is potentiated by the neurotoxin tetrodotoxin and capsaicin treatment, **Gastroenterology**, 96:A544.
Yonei, Y., Holzer, P., Guth, P.H., 1990, Laparotomy-induced gastric protection against ethanol injury is mediated by capsaicin-sensitive sensory neurons, **Gastroenterology**, in press.

VAGAL AFFERENT INNERVATION AND REGULATION OF GASTRIC FUNCTION

Helen E. Raybould, Peter Holzer, Gerard Thiefin,
Helge H. Holzer, Masashi Yoneda, and Yvette F. Tache

Center for Ulcer Research and Education
VA West Los Angeles
Department of Medicine and Brain Research Institute
University of California, Los Angeles, USA

It has been recognized since the beginning of the century that many gastric functions are independent of the extrinsic nervous innervation but can be modulated through these nerves by mechanisms involving afferent fibers. The visceral afferent innervation may regulate gastric function in at least two ways: first, by initiating reflexes mediated through the CNS, vago-vagal reflexes and secondly, producing changes in function through local effector mechanisms. In the following article, experimental evidence will be presented to suggest that vagal afferents regulate several gastric functions, including the gastric phase of acid secretion and mucosal blood flow, the central modulation of vago-vagal reflexes and postprandial gastric motor function.

Functional Ablation of Vagal Afferents by Perineural Capsaicin

Many previous studies have investigated the role of afferent fibers in the regulation of gastric function by sectioning the vagus; as it is a mixed sensory and motor nerve, interpretation of the data from these studies is difficult. The apparent absence of a reflex after nerve section could simply be due to the removal of vagal efferent "tone". In the present studies, the technique of applying the sensory neurotoxin, capsaicin, to the vagus nerve has been used to functionally ablate vagal afferents.

Local application of capsaicin directly onto the nerve reproduces many of the functional and morphological effects of adult systemic treatment, but has the advantage of defining the nerve pathway of interest and avoids central nervous system effects. Jansco et al. (1980) observed that both systemic administration and local application of capsaicin to the saphenous nerve prevents neurogenic inflammation in response to electrical stimulation of the nerve. Capsaicin application to the sciatic nerve in rats results in a depletion of peptides and other markers for afferent C-fibers in central and peripheral terminal fields (Ainsworth et al., 1981; Buck and Burks, 1986) and reduces postsynaptic responses in the spinal cord in response to activation of C-fibers (Wall and Fitzgerald, 1981). Perineural

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

application of capsaicin to the saphenous nerve reduces the C-fiber compound action potential from seven days for up to 3-4 months after treatment (Lynn, 1987). There is evidence for similar functional and morphological changes following application of capsaicin to visceral nerves, such as the vagus. Perineural application of capsaicin results in a marked loss of C-fibers at the site of capsaicin application and 1 cm proximal to the site (Dinn and Ritter, 1985). Perineural application of capsaicin to the vagus nerve inhibits axonal transport of peptides including substance P and somatostatin (Gamse et al., 1984) and greatly reduces the cardiovascular and respiratory reflex responses to intravenous injection of capsaicin (Jansco and Such, 1983).

We have used perineural application of capsaicin to the cervical vagus nerves to functionally ablate the vagal afferent pathway to the upper gastrointestinal tract. A 1% capsaicin solution or its vehicle (10% Tween 80 in olive oil) was applied to both cervical vagi for 30 minutes 7-20 days before experiments. Perineural treatment of the vagus nerves will result in depletion of peptides in the peripheral terminal fields; 80-90% of the calcitonin gene-related peptide (CGRP) and substance P (SP) in the lungs is of extrinsic vagal sensory origin and is reduced by perineural capsaicin application (Lundberg and Saria, 1987). However, in the stomach, combined immunohistochemical and retrograde tracing techniques have demonstrated that only 2-5% of the vagal afferents to the stomach contain the sensory peptides CGRP and SP (Green and Dockray, 1988; Sternini et al., 1987). Thus measurement of these peptides in gastric tissue after perineural capsaicin treatment does not provide any information on the effectiveness of the lesion produced by capsaicin. The changes in gastric motility and acid secretion in response to electrical stimulation of the peripheral vagus are not modified by perivagal capsaicin pretreatment (Raybould and Tache, 1988; Thiefin et al., 1990). In addition, the bradycardia induced by electrical stimulation of the cervical vagus is unaltered by perivagal capsaicin treatment (Yoneda and Raybould, unpublished results). These observations suggest that the vagal efferent pathway is functionally unaltered by the perineural application of capsaicin.

VAGAL AFFERENT FIBERS AND GASTRIC MUCOSAL FUNCTION

Vago-Vagal Reflexes - Peripheral Modulation

The gastric phase of the gastric acid response to a meal is mediated partly through a distension-sensitive mechanism and partly through the presence of nutrients (Debas, 1987). The secretory response to gastric distension induced by isotonic saline or an air-filled balloon is markedly reduced by vagotomy (Schiller et al., 1980; Feldman et al., 1979). Distension of the intact stomach in dogs produces a vagally-dependent increase in gastric acid secretion in the absence of alterations in serum gastrin concentration (Debas et al., 1974). Since the absence of tonic vagal efferent discharge following vagotomy alters the response of the parietal cell to other hormonal and paracrine factors, we examined the role of vagal afferent innervation in the gastric acid secretory response to gastric distension in rats in which the vagal afferents were functionally ablated by perineural application of capsaicin (Raybould and Tache, 1989).

Capsaicin treatment had no effect on basal gastric acid secretion measured in urethane-anesthetized rats with acute gastric fistulas 7-21 days after treatment of the vagus nerves with capsaicin compared to rats in which the vagi were treated with the vehicle alone. Distension of the stomach with 5 ml of saline for 6 minutes in vehicle-treated rats produced a marked stimulation of gastric acid secretion to 6.2 times basal gastric acid secretion; this response lasted for 10 min. The secretory response following gastric distension was significantly reduced by 40% in capsaicin-treated rats (Figure 1). Gastric distension repeated after bilateral cervical vagotomy produced a small, but significant, increase in gastric acid secretion; however, there was no significant difference between the vehicle and capsaicin-treated groups suggesting that the vagally-mediated portion of the response was capsaicin-sensitive. In order to establish that the decrease in gastric acid secretion induced by gastric distension was not due to a non-specific alteration of the mucosa to secrete acid, the effects of capsaicin pretreatment on the secretory response to secretagogues was also studied. In the same experimental paradigm, the secretory response to administration of a cholinergic agonist, bethanechol (0.5 mg/kg sub-

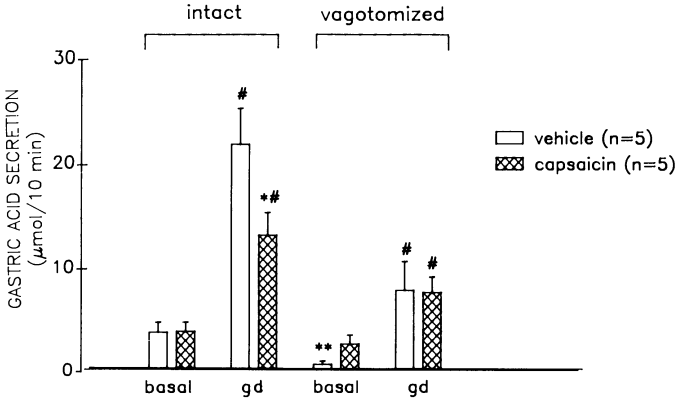


Fig. 1. The effect of perivagal capsaicin pretreatment on the gastric acid secretory response to gastric distension (gd). Capsaicin or vehicle was applied to both cervical vagi under pentobarbitone anesthesia 7-14 days prior to experiments. The secretory response to gastric distension was measured before and again after bilateral vagotomy in urethane-anesthetized rats via a double lumen gastric cannula placed in the forestomach and continuous titration of the gastric contents to pH 7.0. Gastric distension (5 ml for 6 min) significantly increased gastric acid secretion in both groups (# $p<0.01$). Capsaicin pretreatment significantly attenuates the response to distension (# $p<0.05$ vehicle vs capsaicin treated groups). The response to gastric distension was significantly reduced by vagotomy in vehicle- but not capsaicin-treated rats; following vagotomy there was no significant difference in the response to distension between the two groups. Basal gastric acid secretion was unaltered by capsaicin treatment; vagotomy decreased basal gastric acid secretion in both groups but this only reached statistical significance in vehicle-treated rat (** $p<0.05$). Each column represents mean ± SE. From Raybould and Tache, 1989.

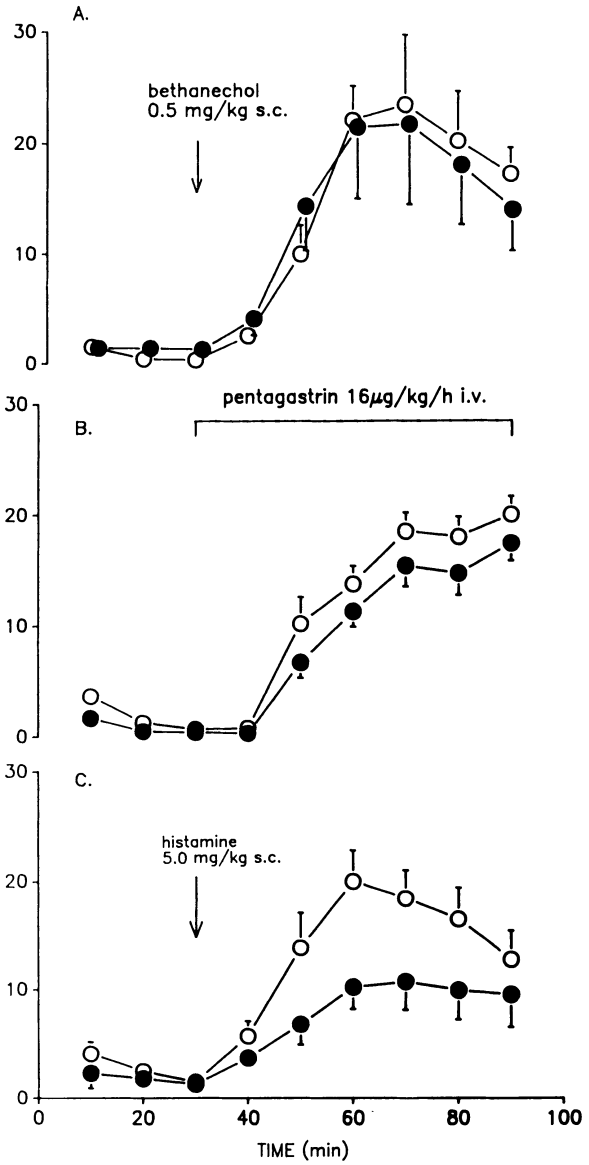


Fig. 2. Effect of perivagal capsaicin pretreatment on the secretory response to peripheral secretagogues. Gastric acid secretion was measured in urethane anesthetized rats by a double lumen catheter in the forestomach; the gastric contents were flushed every 10 min with two 5 ml volumes of saline and acid secretion measured by titration with 0.1 N NaOH. Capsaicin pretreatment significantly reduced the secretory response to histamine (C) but the response to bethanechol (A) and pentagastrin (B) were unaltered. Each point represents mean ± SE, 4-8 rats in each group. From Raybould and Tache, 1989.

cutaneously) or pentagastrin (15 μg/kg per h intravenously) were unaltered by capsaicin pretreatment. However, the secretory response following subcutaneous administration of histamine (5 mg/kg) was reduced by 42% by capsaicin pretreatment (Figure 2).

These results indicate that capsaicin-sensitive vagal afferent fibers partially mediate the increase in gastric acid secretion in response to histamine and to gastric distension. However, the sensitivity to other secretagogues is unaltered, suggesting that functional ablation of vagal afferents does not alter the ability of the parietal cells to secrete acid. These results support the hypothesis that gastric distension increases gastric acid secretion by a vago-vagal reflex, the afferent arm of which is capsaicin-sensitive. The mechanism by which capsaicin-sensitive vagal afferent fibers participate in the secretory response to histamine is at present unclear. This result confirms that of Alfoldi et al. (1986) who demonstrated that the secretory response to histamine, but not pentagastrin or bethanechol, in awake gastric fistula rats was markedly reduced by systemic capsaicin pretreatment. Histamine receptors have been identified electrophysiologically on nodose ganglion cells of the rabbit (Higashi, 1986). Thus histamine may stimulate vagal afferents and increase gastric acid secretion in part by a vago-vagal reflex, similar to that for gastric distension.

Vago-Vagal Reflexes - Central Modulation

Gastric acid secretion in response to intravenous injection of 2-deoxy-D-glucose, which acts via activation of vagal reflex pathways, is reduced by systemic capsaicin treatment (Evangelista et al., 1989). It is well-established that injection of thyrotropin releasing hormone (TRH) or its stable analog, RX77368, into the cerebrospinal fluid or specific medullary nuclei increases gastric acid secretion, motor function, emptying, mucosal blood flow, gastric erosions and release of luminal serotonin and histamine via activation of a vagal cholinergic pathway (Tache et al., 1989). The action of TRH to stimulate gastric acid secretion may involve central modulation of vago-vagal reflexes (McCann et al., 1989; Rogers and McCann, 1989). Therefore, we investigated the effect of vagal sensory denervation u sing capsaicin on the gastric responses following intracisternal administration of the stable TRH analog, RX77368 (Raybould et al., 1990).

Gastric acid secretion was measured in urethane-anesthetized rats with an acute gastric fistula. Perineural capsaicin treatment of the vagus significantly reduced the secretory response to a submaximal dose of RX77368 (30 ng). The secretory response to intracisternal (i.c.) injection of RX77368 was reduced by 60% by capsaicin pretreatment (Figure 3). In experiments where gastric acid secretion and motor function were measured concomitantly, it was found that perivagal capsaicin treatment had no significant effect on the amplitude or frequency of basal gastric contractions and had no effect on the increase in gastric motor function produced by central administration of RX77368 (Table 1, Figure 3). In contrast, the gastric acid secretory response at all effective doses of RX77368 (3-30 ng) was reduced in capsaicin-treated compared with vehicle-treated rats (Figure 3). The degree of inhibition was 56%, 21%, and 51% for doses of 3, 10 and 30 ng respectively. We have shown previously that intracisternal TRH-induced increase in the rate of gastric

TABLE 1

Effect of intracisternal injection of RX77368, a stable TRH analog, on gastric motor function in vehicle- and capsaicin-treated rats

Treatment[a]	Dose (ng)	Motility Index[b] Vehicle	Capsaicin
Basal		6.7± 2.7	6.9± 1.0
RX77368	1	9.0± 2.6	8.1± 1.3
RX77368	3	21.1±11.5*	12.6± 3.4*
RX77368	10	28.1± 8.8*	48.7±13.3*

a: Fasted rats were anesthetized with urethane and implanted with a manometric catheter into the gastric corpus through the pylorus; a further double lumen cannula was placed in the stomach through the rumen for simultaneous measurement of gastric acid secretion. RX77368 was injected intracisternally in step increments (1-10 ng) in 5 rats.
b: Motility index calculated as area under the curve of the trace of intraluminal pressure. Mean ± SE. *$p<0.01$ significantly different from basal.

emptying of liquids in conscious rats is unaltered by perivagal capsaicin treatment (Raybould and Tache, 1988). Thus, capsaicin-sensitive vagal afferent fibers are involved in the acid secretory response to central administration of TRH analog, but not in the changes in gastric motor function.

Central administration of TRH increases gastric mucosal blood flow (Thiefin et al., 1989). Since increases in gastric acid secretion are often accompanied by increases in mucosal blood flow (Guth and Leung, 1989), we studied the effect of perineural capsaicin treatment on the increase in gastric mucosal blood flow induced by central administration of TRH analog. Gastric mucosal blood flow (GMBF) was measured in urethane-anesthetized rats using the hydrogen gas clearance technique (Leung et al., 1984). There was no significant difference in the basal GMBF between vehicle and capsaicin pretreated rats. Injection of RX77368 (30 ng i.c.) in vehicle-treated rats increased GMBF and this increase was significantly reduced in capsaicin-treated rats by 65% (Figure 4). The gastric acid secretory response to RX77368 i.c. measured concomitantly with GMBF was also reduced by 65% in capsaicin-treated rats.

These results show that the gastric acid secretory response and increase in gastric mucosal blood flow in response to a stable TRH analog, RX77368, injected at a submaximal dose (30 ng) in urethane-anesthetized rats is decreased by 61-65% and 65% respectively by lesion of capsaicin-sensitive vagal afferent fibers. In contrast, the stimulatory effect of intracisternal RX77368 on gastric motor activity is not modified by perineural capsaicin treatment. Therefore, capsaicin-sensitive vagal afferent fibers are necessary for full expression of the secretory and vascular responses to TRH, but not in the changes in gastric motor function.

Recent electrophysiological data has shown that the discharge of around 50% of neurons in the nucleus of the solitary tract, the region where vagal afferents terminate, that received

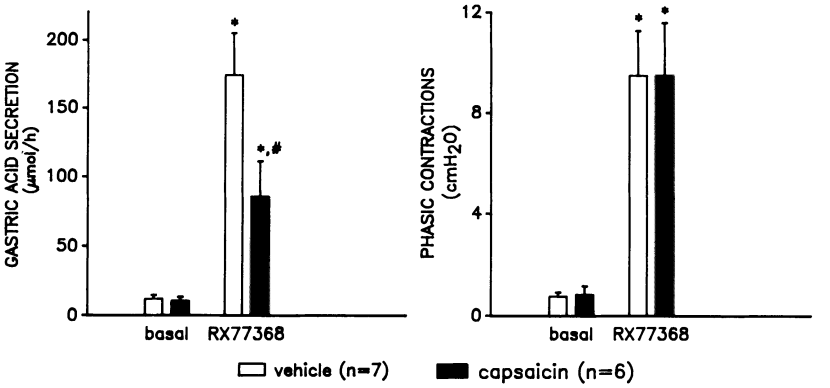


Fig. 3. The effect of intracisternal (i.c.) administration of the stable TRH analog, RX77368, on gastric secretory and motor function in urethane-anesthetized rats. Gastric motor function was measured manometrically simultaneously with gastric acid secretion. Injection of RX77368 increased gastric acid output and the amplitude of phasic gastric contractions (*$p<0.01$ RX77368-stimulated vs basal). Perivagal capsaicin treatment had no effect on basal secretory or motor function but significantly reduced the gastric secretory response to RX77368 (# $p<0.01$ vehicle- vs capsaicin-treated groups). In contrast, the increase in amplitude of phasic gastric contractions induced by RX7768 was unaltered by capsaicin pretreatment. Each point represents mean ± SE. From Raybould et al., 1990.

an input from the stomach, was inhibited by microinjection of TRH, regardless of whether the discharge of the neuron was inhibited or excited by gastric distension (Rogers and McCann, 1989). In addition, the neuronal response to gastric distension was abolished by microinjection of TRH. These observations suggest that TRH acts to stimulate gastric function by increasing the discharge of vagal preganglionic neurons and also by modulating the sensory arm of vago-vagal reflexes. Thus, perivagal capsaicin may remove the vagal afferent pathway upon which TRH acts and thus results in a diminution of the gastric response.

It is also possible that the reduction in the gastric acid secretory response to TRH by lesion of capsaicin-sensitive vagal afferents is the result of some alteration in the peripheral rather than central mechanisms involved in stimulation of gastric acid secretion and gastric mucosal blood flow. It is well established that the stimulatory action of TRH on gastric acid secretion, motility and blood flow are all cholinergic (Taché et al., 1989). Recent evidence indicates that TRH releases histamine into the gastric lumen and portal blood (Yanigasawa et al., 1990); the gastric acid secretory response to i.c.TRH is reduced by around 62% following administration of the specific H_2 antagonist cimetidine suggesting that histamine may play a role in the gastric acid response to central TRH. Therefore, it is possible that the reduction in the secretory and vascular responses following administration of the TRH analog may be explained by a reduction in the response to histamine. Alternatively, the

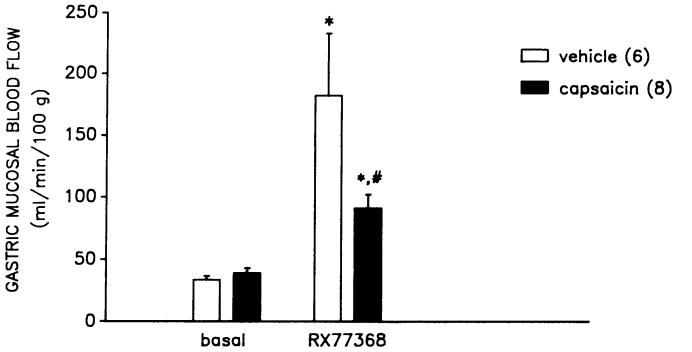


Fig. 4. The effect of perivagal capsaicin treatment on the increase in gastric mucosal blood flow (GMBF) in response to intracisternal injection of the stable TRH analog RX77368 (30 ng). GMBF was measured in urethane-anesthetized rats by the hydrogen gas clearance technique with the needle electrode inserted into the basal portion of the gastric mucosa. Gastric acid secretion was measured concomitantly. Blood flow and acid secretion were determined for a 15 min period before and again after administration of RX77368. There was no significant difference in basal GMBF in vehicle- and capsaicin-pretreated rats. Intracisternal administration of RX77368 increased gastric GMBF (*P<0.01) in both groups. However, the response to RX77368 was reduced by 65% in the capsaicin-pretreated group (#p<0.05 vehicle- vs capsaicin-treated group). In these same rats, the gastric acid response to RX77368 was also reduced by 65%. Results expressed as mean ± SE. From Raybould et al., 1990.

decreased vascular response to central administration of RX77368 by perineural capsaicin treatment may limit the magnitude of the secretory response. Previous studies have shown an increase in blood flow in association with increases in gastric acid secretion, presumably to meet the higher metabolic requirements of the tissue (Guth and Leung, 1989).

Vagal Afferents and Local Effector Mechanisms

Evidence is accumulating that in addition to transmission of sensory information, afferent nerve endings may also subserve a local effector role (Holzer, 1988); the evidence is best for their role in the regulation of the vasculature. Antidromic activation of sensory nerve fibers induces vasodilation in various tissues including skin, tooth pulp and nose (Holzer, 1988). The release of neuropeptides from peripheral sensory endings may produce these vascular changes. A similar phenomenon has been proposed for sensory nerve endings in the regulation of gastrointestinal blood flow (Holzer, 1988). Electrical stimulation of the peripheral cut end of the vagus nerve increases gastric blood flow in cats (Martinson, 1965), dogs (Peter et al., 1963; Swan and Jacobsen, 1967) and rats (Yano et al., 1983) and dilates gastric submucosal arterioles in rats and cats (Guth and Smith, 1977). The prompt increase in gastric mucosal blood flow (Yano et al., 1983) and gastric submucosal arteriolar diameter

(Guth and Smith, 1977) after the onset of vagal stimulation suggests a direct vasodilator effect of vagal activation, not secondary to augmented acid secretion. The vascular response to electrical stimulation of high-threshold vagal fibers partly involves a non-cholinergic pathway (Martinson, 1965; Morishito and Guth, 1986; Vagne et al., 1982). We investigated a possible contribution of peptidergic vagal afferent fibers in the gastric vascular response to electrical vagal stimulation.

Gastric mucosal blood flow (GMBF) was measured by hydrogen gas clearance in urethane-anesthetized rats pretreated with either capsaicin or vehicle to the vagus nerves. Electrical stimulation of the peripheral cut end of the subdiaphragmatic vagus nerve significantly increased GMBF and gastric acid secretion in both capsaicin- and vehicle-pretreated rats (Figure 5). This increase in GMBF in response to vagal stimulation was significantly reduced by 48% in capsaicin-pretreated rats. Gastric acid secretion was significantly increased in both groups but there was no significant difference in the acid response between the two groups of animals. Electrical vagal stimulation in rats treated with atropine to block vagal efferent pathway GMBF was significantly increased in the vehicle group; in contrast, there was a small but not significant increase in GMBF in the capsaicin-treated group. In both groups, atropine completely abolished the secretory response to electrical vagal stimulation. These results demonstrate that the non-cholinergic increase in GMBF induced by electrical vagal stimulation, which represented around 25% of the vascular response, is mediated through activation of capsaicin-sensitive vagal afferent fibers. However, these fibers do not contribute to the gastric secretory response to electrical vagal stimulation.

The mechanism by which capsaicin-sensitive vagal afferent fibers mediate part of the vascular response to electrical vagal stimulation may be through antidromic stimulation resulting in release of mediator substances from nerve terminals. Neuropeptides can be released from sensory nerve endings and are proposed to be mediators of this vascular effect (Holzer, 1988: Rozsa et al., 1985). Candidate substances that may mediate the non-cholinergic increase in GMBF induced by electrical vagal stimulation include calcitonin gene-related peptide (CGRP), substance P and vasoactive intestinal peptide (VIP). These peptides are present in 2-5% of vagal afferent fibers (Green and Dockray, 1988; Sternini et al, 1987) and both VIP and substance P are released from the stomach in response to electrical stimulation of the vagus nerve (Pederson et al., 1981). Recently it has been demonstrated that CGRP can be released from the vascularly perfused stomach by intra-arterial administration of capsaicin (Holzer et al., 1990). These peptides exert a vasodilator effect in different vascular beds and in particular, intra-arterial injection of α-CGRP and VIP, unlike substance P and neurokinin A, increases gastric mucosal blood flow (Bauerfeind et al., 1989; Holzer et al., 1990).

VAGAL AFFERENT FIBERS AND GASTRIC MOTOR FUNCTION

The emptying of meals from the stomach is regulated by chemoreceptive mechanisms in the intestine. These mechanisms are capable of producing selective responses to different meal components. Intraduodenal infusion of acid, fatty acids, fats, carbohydrates, amino acids or proteins have all been shown to alter

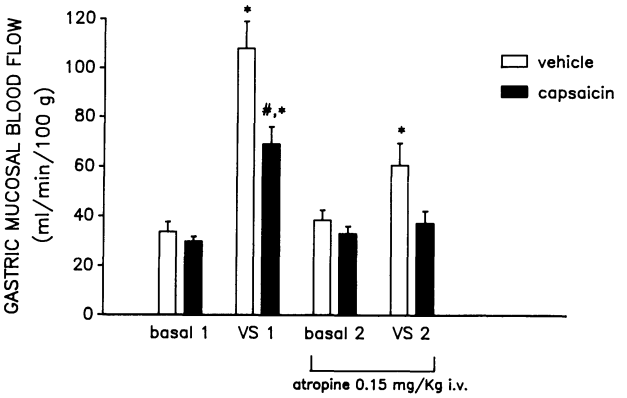


Fig. 5. Effect of electrical stimulation of the vagus nerve on gastric acid secretion and gastric mucosal blood flow (GMBF) in capsaicin-treated rats. GMBF was measured using the hydrogen gas clearance technique. Each point or bar represents mean ± SE of 8 rats in each group. The effects of electrical vagal stimulation were studied twice, before (VS1) and again after administration of atropine (VS2). Increased GMBF during VS1 was significantly increased in both vehicle- and capsaicin-treated rats (*$p<0.05$ compared with previous basal value in the same group); however, the response was significantly reduced by capsaicin pretreatment (# $p<0.05$). Following atropine, in the vehicle-treated groups VS2 significantly increases GMBF (**$p<0.05$). VS2 significantly lower during VS2 than VS1. In contrast, there is no significant increase in GMBF in the capsaicin treated group. From Thiefin et al., 1990.

gastroduodenal motor function and delay gastric emptying. The action of different nutrients to delay gastric emptying may be mediated by both neural and hormonal pathways.

Neural Mechanisms

Malagelada has provided evidence for reflex control of gastric motility from the duodenum in conscious dogs (De Ponti and Malagelada, 1987; Azpiroz and Malagelada, 1986). Duodenal acid perfusion or distension decreases proximal gastric motility and there is good evidence that in the dog this is mediated through a vagal non-adrenergic, non-cholinergic pathway. Duodenal infusion of different components of a meal also produce changes in gastric motility. In conscious dogs, intestinal perfusion with an isotonic mixture of protein, fat and carbohydrate decreased gastric tone by a vago-vagal non-adrenergic, non-muscarinic pathway (Azpiroz and Malagelada, 1986). In these studies, vagal efferent tone was replaced by continuous infusion of cholinergic agonist.

The nature of the afferent signals that may constitute the afferent arm of these reflex alterations in gastroduodenal motility have been studied electrophysiologically in single fiber studies or extracellular recordings from nodose ganglion cells (for reviews see Grundy and Scratcherd, 1989; Mei, 1985). Two

types of mechanoreceptor have been identified in the wall of the gastrointestinal tract, in the muscle and mucosa. The receptors localized in the muscle respond to distension and active contraction of the muscle. Due to their ability to respond to both isometric and isotonic contractions they are considered to be in series tension receptors. Specific chemoreceptors have been identified in the intestine of the cat, including glucoreceptors, amino acid receptors, lipid and acid receptors. However, many of these chemoreceptors also exhibit mechanical sensitivity. It has been proposed that the response of these afferents to chemical stimulants is a result of local changes in muscle tension produced by the stimuli and that the afferent fiber discharge is a result of local mechanical changes rather than chemical sensitivity.

Hormonal Mechanisms

In addition to neural pathways involved in the intestinal feedback inhibition of gastric function, there is evidence for a role of CCK in the regulation of postprandial gastric motor function. CCK is a potent inhibitor of gastric emptying of both liquids and solids in several species including man. In man, reproduction of postprandial plasma levels of CCK by infusion of

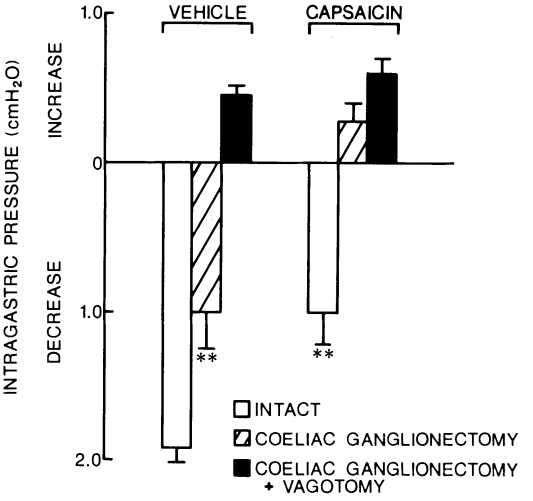


Fig. 6. Effect of celiac-superior mesenteric ganglionectomy and vagotomy on the decrease in intraluminal pressure induced by intravenous administration of CCK-8 (33 pmol). Each column represents mean ± SE of 6 rats in each group. Gastric motility was measured manometrically in urethane-anesthetized rats in which the vagus nerves had been treated with either vehicle or capsaicin 7-14 days before experiments. Intravenous injection of CCK decreases intraluminal pressure; removal of the celiac-superior mesenteric ganglion in vehicle-treated rats decreased the response to CCK (** $p<0.01$); subsequent vagal section abolishes the response. In contrast, in capsaicin-treated rats, the response is significantly reduced compared to vehicle treated controls (** $p<0.01$); in addition, removal of the celiac-superior mesenteric ganglion alone abolishes the response to CCK. From Raybould and Tache, 1988.

the exogenous peptide delayed gastric emptying (Liddle et al., 1986). In the dog, the dose of CCK-8 required to inhibit gastric emptying was the same as the ED_{50} for stimulation of pancreatic secretion and gallbladder contraction (Debas et al., 1975). These observations are consistent with a physiological role for CCK in the regulation of post-prandial gastric emptying. Recently, this hypothesis has been directly tested with the use of specific and potent CCK antagonists. Administration of L364,718, a potent CCK receptor antagonist for the peripheral type receptor (the "A" receptor), reverses the inhibition of gastric emptying following intragastric protein in the rat (Green et al., 1989). The effects of a mixed nutrient or glucose meal on gastric emptying was reversed by administration of the CCK antagonist loxiglumide in man (Fried et al., 1989).

Vagal Afferent Fibers and Hormonal Mechanisms

Recently, we have obtained evidence that CCK released postprandially may act via stimulation of a vagal afferent pathway to modify gastric function and that this may be an important mechanism in intestinal feedback regulation of gastric emptying (Raybould, 1990). CCK decreases proximal gastric motility (Raybould et al., 1987) but enhances antral and pyloric contractions (Murphy et al., 1987). In dogs, both of these effects seem to be important for CCK to inhibit gastric emptying (Yamagishi and Debas, 1978). Pyloroplasty and antrectomy inhibit the action of low doses of CCK but higher doses are still effective and abolished only by vagotomy. However, in the rat, the action of CCK on gastric emptying was unimpaired by pyloric resection or pyloroplasty (Smith et al., 1988). Until recently, the mechanism by which CCK inhibits proximal gastric tone was unclear although there is evidence that it is partially dependent on the integrity of the vagus nerve (Okike and Kelly, 1977). Exogenous administration of sulphated cholecystokinin octapeptide (CCK-8) in urethane-anesthetized rats decreases intragastric pressure in the proximal stomach by pathways involving both the vagal and splanchnic nerves; the vagal pathway is non-adrenergic and hexamethonium-sensitive suggesting CCK is acting via a vago-vagal reflex (Raybould et al., 1987).

In urethane-anesthetized rats pretreated with perivagal application of capsaicin, the decrease in intraluminal pressure produced by CCK (0.1-100 pmol i.v.) was reduced by 44-69% (Raybould and Taché, 1988). In these rats, sympathetic denervation completely abolished the residual response to CCK, suggesting that perivagal capsaicin treatment removed the vagal reflex pathway by which CCK decreases proximal gastric motility (Figure 6). The role of the vagal afferent pathway in CCK-induced delay in gastric emptying of a non nutrient liquid meal was also studied. In normal rats and in rats in which the vagus had been pretreated with vehicle, intravenous administration of CCK (300 pmol) inhibited the emptying of liquid meal by 55%, 20 minutes after orogastric administration of the liquid (Figure 7). Perivagal capsaicin treatment completely abolished the CCK-induced delay in gastric emptying. These results suggest that CCK acts via a vagal afferent pathway to decrease gastric motility and that this pathway is important in mediating the CCK-induced delay in gastric emptying. Perivagal capsaicin treatment alone significantly increased the rate of gastric emptying; this suggests a tonic action of the vagal afferent fibers to delay gastric emptying. The action of capsaicin was specific for CCK

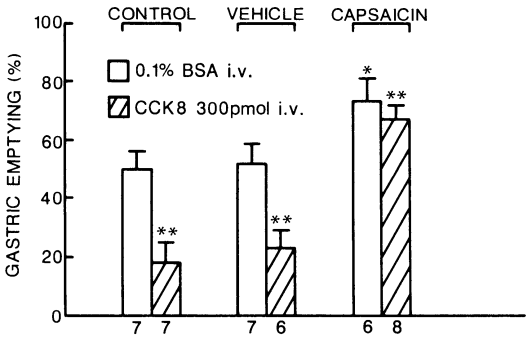


Fig. 7. Effect of perineural treatment of vagus nerves with capsaicin on the CCK-induced delay in gastric emptying in conscious rats. CCK-8 was administered intravenously under light ether anesthesia 5 min before administration of the liquid meal and gastric emptying measured after 20 min. CCK-8 (300 pmol) significantly reduced the rate of gastric emptying in both control and vehicle-treated rats compared to administration of BA (bovine serum albumin) (**$p<0.002$). Perivagal capsaicin treatment 7-14 days before experiments abolished the delay in gastric emptying induced by CCK (*$p<0.002$); in addition, this treatment alone significantly increased gastric emptying (*$p<0.05$). Each column represents mean ± SE of the number of rats indicated at the base. From Raybould and Tache, 1988.

and had no effect on atropine- or CRF-induced delay of gastric emptying (Raybould and Taché, 1988; Raybould et al., 1990).

Electrophysiological Evidence for Sensitivity of Vagal Afferents to CCK

Candidate CCK receptor populations for the mediation of changes in gastric motility and emptying have been identified on the vagus nerve. CCK binding sites have been demonstrated on the vagus nerve and to be transported towards the periphery (Zarbin et al., 1981). More importantly, these binding sites occur on all subdiaphragmatic branches of the vagus (Moran et al., 1987). These receptor sites are of the peripheral subtype (A receptors) as binding with CCK-8(s) is not displaced by the desulfated peptide (Moran et al., 1987). CCK binding sites are relatively specific to the vagus nerve since no sites are found on the rat sciatic nerve. High affinity binding sites for CCK have been demonstrated in the caudal subnucleus of the NTS, the site of central termination of vagal afferent fibers (Ladenheim et al., 1988). Unilateral nodose ganglionectomy produced a marked reduction in the density of CCK binding sites in the medial NTS on the ipsilateral side. This suggests that these putative CCK receptors are of vagal origin and that they may be located on vagal afferent fibers. Since the inhibition of gastric emptying by CCK is not abolished by resection of the pylorus but is abolished by perivagal capsaicin, it is suggested that CCK binding sites on vagal afferent fibers are important for mediating its action on gastric motor function. In the intestine it appears, at least in ferret and sheep, that the action of CCK on polymo-

dal receptive endings (i.e. endings responding to both mechanical and chemical stimuli) is secondary to changes in smooth muscle tone (Cottrell and Iggo, 1986). Increased afferent fiber discharge in response to CCK in an in vitro preparation of the sheep intestine is abolished when smooth muscle contraction is inhibited by hexamethonium or adrenergic agonists (Cottrell and Iggo, 1986). However, rat gastric mechanoreceptors seem to be directly stimulated by exogenous CCK (Davison and Clarke, 1988; Raybould and Davison, 1989). Intravenous administration of CCK (2-4 nmol/kg) stimulated the discharge of gastric mechanoreceptor despite a decrease in intraluminal pressure. However, in vagal capsaicin-treated rats, the units still responded to gastric distension but there was no response to CCK (Raybould and Davison, 1989).

Capsaicin-Sensitive Vagal Afferents and Duodenal Nutrients

Physiological saline, then nutrients, were infused at 37°C at 0.05 ml/min into the duodenum immediately distal to the pylorus and drained 4-5 cm distal to the pylorus. Intraduodenal infusion with peptone, casein, acid, glucose or soya bean trypsin inhibitor (SBTI) decreased baseline gastric intraluminal pressure and the height but not the frequency of phasic contractions of the gastric corpus. We examined the afferent pathway that mediates the effects of intraduodenal nutrients by using perineural application of capsaicin. Perivagal application of capsaicin significantly reduced the effects of peptone, glucose and SBTI on gastric motility by 42%, 68% and 99% respectively (Figure 8). Thus it seems that intraduodenal peptone and glucose inhibit gastric motility by a capsaicin-sensitive vagal afferent pathway.

The decrease in proximal gastric motility in the urethane-anesthetized rat is mediated via an action of CCK at an "A" type

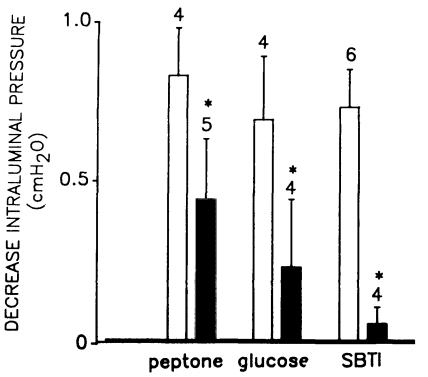


Fig. 8. Effect of perivagal capsaicin treatment on the decrease in gastric motility induced by introduodenal infusion of nutrient. Gastric motor function was measured manometrically in urethane-anesthetized rats and cannulas were placed in the duodenum 0.5 and 4-5 cm distal to the pylorus to infuse and drain infusates respectively. Infusion of saline (0.1 ml/min at 37°C was replaced by peptone (9%), glucose (10%) or soya bean trypsin inhibitor (0.02%, SBTI). Perivagal capsaicin treatment decreased the nutrient induced decrease in gastric motility. Each column represents mean ± SE of 6-8 rats.

receptor site since administration of the CCK "A" receptor antagonist, L364,718 abolished the action of low doses of CCK and markedly reduced that of higher doses. The effect of exogenous administration of CCK on gastric emptying is also abolished by CCK "A" receptor antagonists (Green et al., 1988; Lotti et al., 1987; Pendleton, 1987). The effect of release of endogenous CCK from endocrine cells and subsequent increases in plasma levels of CCK on proximal gastric motility was studied using intraduodenal infusion of nutrients. We infused nutrients that should increase plasma levels of CCK, that is, casein (18%), peptone (9%) and SBTI (0.2%) and nutrients that do not: glucose (10%) and acid (0.1N HCl) (Liddle et al., 1986). The response to duodenal peptone, casein, SBTI were inhibited by 82%, 73% and 74% respectively following administration of the CCK "A" receptor antagonist L364,718 (0.1 mg/kg intraperitoneally). However, the response to glucose and acid were unaltered by administration of the antagonist (decrease in gastric motility reduced by 25% and 5%). Thus nutrients shown to increase plasma levels in the rat (protein and SBTI) act to decrease gastric motility via a CCK-sensitive mechanism.

SUMMARY

In this article, we have presented evidence that vagal capsaicin-sensitive afferent fibers are involved in the regulation of gastric mucosal and motor function. Gastric acid secretion stimulated by gastric distension, histamine and central injection of TRH analog are all partly dependent on vagal capsaicin-sensitive afferent mechanisms. It is possible that as vagal efferent activity releases histamine, the common final pathway is the reduction in the response to histamine. At present, it is unclear as to the mechanism by which capsaicin-sensitive afferents are involved in the secretory response to histamine. With regard to the gastric acid and mucosal blood flow responses to TRH, it is not clear whether the sensory neurons represent a component of the efferent pathway that is activated by TRH or whether their role is to set the sensitivity of, or exert feedback control on this efferent pathway. As perineural capsaicin application decreases peptide content in the peripheral terminal fields of sensory neurons and these peptides may produce local effector functions within the tissue, it is possible that alterations in the gastric responses to TRH result from a decrease in the local effector functions of vagal neurons. From the experiments on electrical stimulation of the vagus nerve, it is evident that antidromic stimulation of vagal afferents can stimulate gastric mucosal blood flow, although under these experimental conditions there was no evidence for a capsaicin-sensitive stimulation of gastric acid secretion. The physiological relevance of this stimulation of gastric mucosal blood flow is at present unclear, but it is possible that physiological stimuli, such as distension or nutrients, may stimulate afferents and signal for an increase in gastric mucosal blood flow. In addition, pathophysiological or noxious stimulation of vagal afferents may also signal for an increase in gastric mucosal blood flow and may play a role in the response of the mucosa to injury.

Acknowledgements

This work was supported by NIH Grant DK 41004. The authors would

like to thank Nigel Bunnett for his critical reading of the manuscript

References

Ainsworth, A., Hall, P., Wall, P.D., Allt, G., MacKenzie, M.L., Gibson, S., and Polak, J.M., 1981, Effects of capsaicin applied locally to adult peripheral nerve. II. Anatomy and enzyme and peptide chemistry of peripheral nerve and spinal cord, **Pain**, 11:379.

Alfoldi, P., Obal, F., Toth, E., and Hideg, J., 1986, Capsaicin pretreatment reduces the gastric acid secretion elicited by histamine but does not affect the responses to carbachol and pentagastrin, **Europ. J. Pharmacol.**, 123:321.

Azpiroz, F., and Malagelada, J.R., 1986, Vagally mediated gastric relaxation induced by intestinal nutrients in the dog, **Am. J. Physiol.**, 215:G727.

Bauerfeind, P., Hof, R., Hof, A., Cucula, M., Siegrist, S., von Ritter, C.H., Fischer, J.A., and Blum, A.L., 1989, Effects of hCGRP I and II on gastric blood flow and acid secretion in anesthetized rabbits, **Am. J. Physiol.**, 256:G145.

Buck, S.H., and Burks, T.F., 1986, The neuropharmacology of capsaicin: a review of some recent observations, **Pharmacol. Rev.**, 38:179.

Cottrell, D.F., and Iggo, A., 1986, The responses of duodenal tension receptors in sheep to pentagastrin, cholecystokinin and some other drugs, **J. Physiol.**, 354:477.

Davison, J.S., and Clarke, G.D., 1988, Mechanical properties and sensitivity to CCK of vagal gastric slowly adapting mechanoreceptor, **Am. J. Physiol.**, 255:G55.

Debas, H.T., 1987, Peripheral regulation of gastric acid secretion, in: "Physiology of the Gastrointestinal Tract," L.R. Johnson, ed., Raven Press, New York.

Debas, H.T., Konturek, S.J., Walsh, J.H., and Grossman, M.I., 1974, Proof of a pyloro-oxyntic reflex for stimulation of acid secretion, **Gastroenterology**, 66:526.

Debas, H.T., Farooq, O., and Grossman, M.I., 1975, Inhibition of gastric emptying is a physiological action of cholecystokinin, **Gastroenterology**, 68:1211.

De Ponti, F., Azpiroz, F., Malagelada, H.R., 1987, Reflex gastric relaxation in response to distension of the duodenum, **Am. J. Physiol.**, 252:G595.

Dinn, T.T., and Ritter, S., 1985, Capsaicin induces neuronal degeneration in the brain and spinal cord of adult rats, **Soc. Neurosci.**, 11:349.

Evangelista, S., Santicioli, P., Maggi, C.A., and Meli, A., 1989, Increase in gastric secretion induced by 2-deoxy-D-glucose is impaired in capsaicin pretreated rats, **Brit. J. Pharmacol.**, 98:35.

Feldmen, M., Dickerson, R.M., McClelland, R.N., Cooper, K.A., Walsh, J.H., and Richardson, C.T., 1979, Effect of selective proximal vagotomy on food-stimulated gastric acid secretion and gastrin release in patients with duodenal ulcer, **Gastroenterology**, 76:926.

Fried, M., Lochner, C., Erlacher, U., Beglinger, C., Jansen, J., Lamers, J., and Stadler, G.A., 1989, Role of CCK in the regulation of gastric emptying and pancreatic secretion in man, **Gastroenterology**, 96:A159.

Gamse, R., Petsche, U., Lembeck, F., and Jansco, G., 1982, Capsaicin applied to peripheral nerve inhibits axoplasmic

transport of substance P and somatostatin, **Brain Res.**, 239:447.
Green, T., and Dockray, G.J., 1988, Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea pig, **Neurosci.**, 25:181.
Green, T., Dimaline, R., and Dockray, G.J., 1989, Neuroendocrine control mechanisms of gastric emptying in the rat, in: "Nerves and the Gastrointestinal Tract," M.V. Singer and H. Goebell, eds., Kluwar, Netherlands.
Green, T., Dimaline, R., Peikin, S., and Dockray, G.J., 1988, The action of the cholecystokinin antagonist L364,718 on gastric emptying of liquid meals in the conscious rat, **Am. J. Physiol.**, 255:G685.
Grundy, D., and Scratcherd, T., 1989, Sensory afferents from the gastrointestinal tract, in: "Handbook of Physiology," Volume 6, Chapter 10.
Guth, P.H., and Leung, F.W., 1987, Physiology of the gastric circulation, in: "Physiology of the Gastrointestinal Tract," L.R. Johnson, ed., Raven Press, New York.
Guth, P.H., and Smith, E., 1977, Nervous regulation of the gastric microcirculation, in: "Nerves and the Gut," F.P. Brooks and P.W. Ewers, eds., C.B. Stack, New York.
Higashi, H., 1986, Pharmacological aspects of visceral sensory receptors, **Progress in Brain Research**, 67:149.
Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neurosci.**, 24:739.
Holzer, P., Taché, Y. and Guth, P.H., 1990, The vasodilator neuropeptides α-CGRP and VIP, but not substance P and neurokinin A, increase rat gastric mucosal blood flow, **Gastroenterology**, 98:A175.
Holzer, P., Peskar, B.M., Peskar, B.A., and Amman, R., 1990, Release of calcitonin gene-related peptide induced by capsaicin in the vascularly perfused rat stomach, **Neurosci. Lett.**, 109:195.
Jansco, G., Kiraly, E., and Jansco-Gabor, A., 1980, Direct evidence for an axonal site of action of capsaicin, **N.S. Arch. Pharmacol.**, 313:91.
Jansco, G., and Such, G., 1983, Effects of capsaicin applied perineurally to the vagus nerve on cardiovascular and respiratory functions in the cat, **J. Physiol. Lond.**, 341:359.
Ladenheim, E.E., Speth, R.C., and Ritter, R.C., 1988, Reduction of CCK-8 binding in the nucleus of the solitary tract in unilaterally nodosectomized rats, **Brain Res.**, 474:125.
Leung, R.V., Guth, P.H., Scremin, O.U., Golanska, E.M., and Kauffman, G.L., Jr., 1984, Regional gastric mucosal blood flow measurements by hydrogen gas clearance in the anesthetized rat and rabbit, **Gastroenterology**, 87:28.
Liddle, R.A., Green, G.M., Conrad, C.K., and Williams, J.A., 1986, Proteins but not amino acids, carbohydrates or fats stimulate cholecystokinin secretion in the rat, **Am. J. Physiol.**, 251:G243.
Liddle, R.A., Morita, E.T., Conrad, C.K., and Williams, J.A., 1986, Regulation of gastric emptying in humans by cholecystokinin, **J. Clin. Invest.**, 77:992.
Lotti, V.J., Pendleton, R.G., Gould, R.J., Hanson, H.M., Chang, R.S., and Clineschmidt, B.V., 1987, In vivo pharmacology of L-364,718, a new potent nonpeptide cholecystokinin antagonist, **J. Pharmacol. Exp. Ther.**, 241:103.
Lundberg, J.M., and Saria, A., 1987, Polypeptide-containing neu-

rons in airway smooth muscle, **Ann. Rev. Physiol.**, 49:557.
Lynn, B., 1987, The immediate and long-term effects of applying capsaicin to cutaneous nerves, **Acta Physiol. Hung.**, 69:287.
Martinson, J., 1964, The effect of graded stimulating of efferent vagal nerve fibers on gastric motility, **Acta Physiol. Scand.**, 62:256.
McCann, M.J., Hermann, G.E., and Rogers, R.C., 1989, Thyrotropin-releasing hormone: effects on identified neurons of the dorsal vagal complex, **J. Auton. Nerv. System.**, 26:107.
Mei, N., 1985, Intestinal chemosensitivity, **Physiol. Rev.**, 65:211.
Moran, T.H., Smith, G.P., Hostetler, A.M., and McHugh, P.R., 1987, Transport of cholecystokinin (CCK) binding sites in subdiaphragmatic vagal branches, **Brain Res.**, 415:149.
Morishito, T., and Guth, P.H., 1986, Vagal nerve stimulation causes noncholinergic dilatation of gastric arterioles, **Am. J. Physiol.**, 250:G660.
Murphy, R.B., Smith, G.P., and Gibbs, J., 1987, Pharmacological examination of cholecystokinin (CCK-8)-induced contractile activity in the rat isolated pylorus, **Peptides**, 8:127.
Okike, N., and Kelly, K.A., 1977, Vagotomy impairs pentagastrin-induced relaxation of canine gastric fundus, **Am. J. Physiol.**, 232:E504.
Pederson, R., O'Dorisio, T., Howe, T., MacIntosh, B., Mueller, M., Brown, J., and Cataland, S., 1981, vagal release of IR-VIP and IR-gastrin from the isolated perfused rat stomach, **Mol. Cell. Endocrinol.**, 23:225.
Pendleton, R.G., Bendesky, R.J., Schaffer, L., Nolan, T.E., Gould, R.J., and Clineschmidt, B.V., 1987, Roles of endogenous cholecystokinin in biliary, pancreatic and gastric function: studies with L-364,718, a specific cholecystokinin receptor antagonist, **J. Pharmacol. Exp. Ther.**, 241:110.
Peter, E.T., Nicoloff, D.M., Leonard, A.S., Walder, A.L., and Wangensteen, O.H., 1963, Effect of vagal and sympathetic stimulation and ablation on gastric blood flow, **JAMA**, 183:1003.
Raybould, H.E. and Davison, J.S., 1989, Perivagal application of capsaicin abolishes the response of vagal gastric mechanoreceptor to cholecystokinin, **Soc. Neurosci.**, 15:973.
Raybould, H.E., Holzer, P., Reddy, S.N., Yang, H. and Tache, Y., 1990, Capsaicin-sensitive vagal afferents contribute to gastric acid and vascular responses to intracisternal TRH analog, **Peptides**, in press.
Raybould, H.E., Koelbel, C.B., Mayer, E.A. and Taché, Y., 1990, Inhibition of gastric motor function by circulating corticotropin-releasing factor in anaesthetized rats, **J. Gastrointest. Motility**, in press.
Raybould, H.E., Roberts, M.E. and Dockray, G.J., 1987, Reflex decreases in intragastric pressure in response to cholecystokinin in rats, **Am. J. Physiol.**, 253:G165.
Raybould, H.E., and Tache, Y., 1988, Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats, **Am. J. Physiol.**, 255:G242.
Raybould, H.E., and Tache, Y., 1989, Gastric distension and histamine stimulate gastric acid secretion by capsaicin-sensitive vagal afferent pathway in the anesthetized rat, **Eur. J. Pharmacol.**, 167:237.
Rogers, R.C., and McCann, M.J., 1989, Effects of TRH on the activity of gastric inflation-related neurons in the solitary nucleus in the rat, **Neurosci. Lett.**, 104:71.
Rozsa, Z., Varro, A., and Jansco, G., 1985, Use of immunoblock-

ade to study the involvement of peptidergic afferent nerves in the intestinal vasodilatory response to capsaicin in the dog, **Europ. J. Pharmacol.**, 115:59.
Schiller, L.R., Walsh, J.H., and Feldman, M., 1980, Distension-induced gastrin release: effects of luminal acidification and intravenous atropine, **Gastroenterology**, 78:912.
Smith, G.P., Falasco, J., Moran, T.H., Joyner, K.M.S., and Gibbs, J., 1988, CCK-8 decreases food intake and gastric emptying after pylorectomy or pyloroplasty, **Am. J. Physiol.**, 255:R113.
Sternini, C., Reeve, J.R., and Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.
Swan, K.G., and Jacobson, E.D., 1967, Gastric mucosal blood flow and secretion in conscious dogs, **Am. J. Physiol.**, 212:891.
Tache, Y., Stephens, R.L., and Ishikawa, T., 1989, Central nervous system action of TRH to influence gastrointestinal function and ulceration, **Ann. N.Y. Acad. Sci.**, 553:169.
Thiefin, G., Tache, Y., Leung, F.W., and Guth, P.H., 1989, CNS actions of thyrotropin-releasing hormone to increase gastric mucosal blood flow in the rat, **Gastroenterology**, 97:405.
Thiefin, G., Raybould, H., Leung, F.W., Tache, Y., and Guth, P.H., 1990, Capsaicin-sensitive vagal afferent fibers contribute to gastric mucosal blood flow response to electrical stimulation, **Am. J. Physiol.**, in press.
Vagne, M., Konturek, S.J., and Chayvialle, J.A., 1982, Effect of vasoactive intestinal peptide on gastric secretion in the cat, **Gastroenterology**, 83:250.
Wall, P.D., and Fitzgerald, M., 1981, Effects of capsaicin applied locally to adult peripheral nerve. I. Physiology of peripheral nerve and spinal cord, **Pain**, 11:363.
Yamagishi, T., and Debas, H.T., 1978, Cholecystokinin inhibits gastric emptying by acting on both proximal stomach and pylorus, **Am. J. Physiol.**, 234:E375.
Yanigasawa, K., Yang, H., Walsh, J.H., and Tache, Y., 1990, Role of acetylcholine, histamine and gastrin in the acid response to intracisternal injection of TRH analog, RX77368, in the rat, **Regul. Peptides**, 27:161.
Yanigasawa, Y. and Tache, Y., 1990, Central vagal stimulation by intracisternal injection of TRH analog, RX77368 increases gastric histamine secretion in the rat, **Gastroenterology**, 98:A151.
Yano, S.A., Fujiwara, A., Ozaki, Y., and Harada, M., 1983, Gastric blood flow responses to autonomic nerve stimulation and related pharmacological studies in rats, **J. Pharm. Pharmacol.**, 35:641.
Zarbin, M.A., Wamsley, J.K., Innis, R.B., and Kuhar, M.J., 1981, Cholecystokinin receptors: presence and axonal flow in the rat vagus nerve, **Life Sciences**, 29:696.

DECREASE OF DUODENAL CALCITONIN GENE-RELATED PEPTIDE- AND SUBSTANCE P-LIKE IMMUNOREACTIVITY IN RAT DUODENAL ULCERS

Daniela Renzi, Stefano Evangelista*, Paola Mantellini and Calogero Surrenti

Gastroenterology Unit, Department of Pathophysiology
University of Florence, Viale Morgagni 85
50100 Firenze, Italy
*Pharmacology Department, Menarini Pharmaceuticals
Via Sette Santi 3, 50131 Firenze, Italy

SUMMARY

We have investigated the endogenous levels of duodenal calcitonin gene-related peptide- (CGRP) and Substance P- (SP) like immunoreactivity (li) following the induction of duodenal ulcers in rats.

Using three duodenal ulcerogens, namely cysteamine, dulcerozine or mepirizole given in a single oral dose, a decrease of duodenal CGRP-li and SP-li was observed. Time-relationship studies of this phenomenon show that CGRP-li and SP-li were decreased concomitantly to the formation of gastroduodenal ulcers after the administration of cysteamine (900 mg/kg p.o.).

Pretreatment with the selective sensory neurotoxin capsaicin induced CGPR-li decrease in the duodenum, which was not further decreased by an ulcerogenic dose of cysteamine, indicating that cysteamine induced a release of CGRP-li of capsaicin-sensitive origin. Otherwise duodenal SP-li was not sensitive to capsaicin pretreatment and its duodenal content decreased by cysteamine originates from an intrinsic source.

Our observations indicate that CGRP and SP may play an important local role in duodenal ulcerogenesis.

INTRODUCTION

Calcitonin gene-related peptide (CGRP) and Substance P (SP) are the major components of the afferent peptidergic innervation of the upper gastrointestinal tract (Dockray, 1988). Approximately 80% of the peptides contained in the afferent nerve fibers sensitive to neurotoxin capsaicin are transported to the periphery (Dockray, 1988) and high levels of both peptides have been found in the rat stomach and duodenum (Holzer et al., 1980;

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

Sternini et al., 1987). As yet the physiological role of these peptides is almost unknown.

Recently it has been reported that this afferent innervation is involved in the defence mechanism(s) against gastroduodenal ulcers (Holzer, 1988). Thus rats treated as newborns with capsaicin are more prone than controls to develop gastric lesions in response to a variety of ulcerogenic stimuli (Holzer and Sametz, 1986; Evangelista et al., 1988) and duodenal ulcers induced by cysteamine and dulcerozine (Maggi et al., 1987a). Moreover, exogenous administration of some peptides has been shown to afford protection against duodenal ulcer in rats (Evangelista et al., 1990a; Maggi et al., 1987b).

In view of the above, we have investigated the relationships between duodenal ulcers induced by several ulcerogens and endogenous levels of CGRP and SP. Similar studies were carried out in rats treated as newborns with the sensory neurotoxin capsaicin to assess the origin of CGRP-li and SP-li released by cysteamine. Changes in gastric CGRP-li were also examined in view of the causative role of gastric acid hypersecretion in the duodenal ulcerogenesis (Szabo, 1987) and the potent antisecretory effect exerted by CGRP (Taché et al, 1984.)

MATERIALS AND METHODS

General. Female albino rats, Sprague-Dawley Nossan strain, weighing 180-210 g, were housed at constant room temperature (21±1°C), relative humidity (60%) and with 12 h light-dark cycle (light on at 6.00 a.m.)

Induction of gastric and duodenal ulcers. Duodenal ulcers were induced in 24 h fasted rats by gavage administration of dulcerozine (300 mg/kg). Controls received saline. The animals were sacrificed 0.5, 6, 16 or 24 h after cysteamine challenge and their duodenal ulcers scored by an observer unaware of the treatments, according to an arbitrary scale between 0 and 4: 0=no ulcers, 0.5=redness, 1 or 1.5=superficial mucosal erosion covering a small or large duodenal area, 2 or 2.5=deep ulcer usually with a small or large transmural necrosis, 3 or 3.5=small or large perforated ulcer, 4=death (Evangelista et al., 1989). Gastric ulcers were scored as described above according to an arbitrary scale between 0 and 3 in relation to size (Evangelista et al., 1988). In other experiments, histamine (40 mg/kg s.c.) was given three times (at 2.5 h intervals) and the animals sacrificed 7.5 h after the first treatment.

Gastric and duodenal CGRP-li determination. Gastric mucosal samples were scraped from the oxyntic region, immediately frozen and weighed. Duodenal samples (the first 2 cm caudal to the pylorus) were excised, blotted in filter paper, weighed and immediately frozen at -20°C.

All samples were extracted with 2 N acetic acid (1/10, w/v) at 95°C, freeze-dried and reconstituted in phosphate buffer (0.1 M, pH 7.4) for CGRP-li-radioimmunoassay (Evangelista et al., 1989) and SP-li (Renzi et al., 1988).

Sensory denervation. On the second day of life, rats received 50 mg/kg s.c. of capsaicin in a volume of 10 µl/g body

weight. This treatment has been reported to cause a permanent degeneration of unmyelinated afferent neurons (Holzer and Lippe, 1988). Controls received equal volumes of vehicle (10% ethanol, 10% Tween 80 and 80% saline v/v/v). All injections were performed under ether anesthesia. The rats were then grown to adulthood and used for the experiments at the age of 2-3 months. In order to check the effectiveness of the treatment, one day before the experiments a drop of 0.33 mM solution of capsaicin was instilled into one eye of the rats and the wiping movements were counted. Capsaicin-pretreated rats that showed any wiping movements were excluded from the study (Holzer and Lippe, 1988).

Statistical analysis. Statistical analysis was performed by means of Student's t test for unpaired data and regression analysis.

RESULTS AND DISCUSSION

Our results show that experimentally-induced duodenal ulcers are coupled with decrease in CGRP-li and SP-li (Table 1). The degree of the duodenal ulcers induced by cysteamine, dulcerozine or mepirizole is inversely correlated with the levels of duodenal CGRP-li (Evangelista et al., 1989). Similar relationships were found for SP-li and duodenal ulcer formation induced by cysteamine (Evangelista et al., 1990b), but not in those induced by mepirizole and dulcerozine.

The differences observed with ulcerogens acting through similar mechanisms (Scremin et al., 1989; Szabo, 1987) and the observation that other neuropeptides such as galanin, NKA, NPY and VIP were not decreased by cysteamine (Theodorsson, Evangelista and Renzi, unpublished data) indicate that duodenal CGRP-li and SP-li decrease is not only the consequence of tissue damage, but they are selectively decreased in duodenal ulcers.

Temporal relationship studies show that although (Fig. 1) gastric (Panel A) and duodenal (Panel B) ulcers were present 16 h after cysteamine challenge, a significant decrease in either gastric or duodenal CGRP-li and SP-li was observed only 24 h after cysteamine administration.

A prompt (1-6 h) increase in gastric acid secretion is a known causative factor in duodenal ulcers (Szabo, 1987). This phenomenon is concomitant to or followed by a series of local effects which contribute to the pathogenesis of ulcer such as decrease in bicarbonate secretion, alterations in duodenal motility and blood flow and delayed gastroduodenal emptying (Szabo, 1987).

Endogenous CGRP-li is not affected during earlier phases of cysteamine-induced increase of gastric acid secretion (4-6 h after a single cysteamine dose). This is substantiated by the observation that repeated hypersecretory doses of histamine (Takeuchi et al., 1986) were unable to produce lesions in the duodenum and did not affect gastric and duodenal CGRP-li up to 8 h after the first histamine administration (data not shown).

Dissociation between acid load and blood flow in the duodenum has been recently shown following the administration of mepirizole in rats (Scremin et al., 1989). The hypothesis was

Table 1

Effect of dulcerozine, mepirizole and cysteamine on duodenal ulcers and CGRP- or SP-like immunoreactivity (li) at 24 h after the ulcerogen.

Treatments:	pathologic score (mean ± S.E.)	duodenal CGRP-li (pmol/g tissue)	duodenal SP-li (pmol/g tissue)
Saline -		55.8±3.2	7.33±0.73
Dulcerozine (300 mg/kg p.o.)	2.71±0.21	23.2±3.5**	5.45±0.46*
Mepirizole (200 mg(kg p.o.)	1.90±0.42	40.3±2.9*	5.56±0.46**
Cysteamine (900 mg/kg p.o.)	1.43±0.38	26.0±2.7**	4.78±0.53**

* and ** = P < 0.05 and 0.01 as compared to group treated with saline. n = 8 for each group.
(Modified from Evangelista, S., Renzi, D., Mantellini, P., Surrenti C., and Meli, A., 1989, **Eur. J. Pharmacol.**, 164:389.

advanced that decline in blood flow, in spite of sustained hypersecretion at the time of the development of duodenal ulcers, could be related to a transmitter depletion after a prolonged stimulation of blood flow (Scremin et al., 1989). In this case, CGRP and SP may be among the likely candidates since both peptides have been reported to increase duodenal blood flow (DiPette et al., 1987; Gronstad et al., 1983).

Gastroduodenal CGRP and SP are contained in capsaicin-sensitive afferent fibers, which are widely distributed around blood vessels (Sharkey et al., 1984; Sternini et al., 1987). Systemic administration of high doses of capsaicin in newborn rats produced a marked depletion in duodenal CGRP-li (78% as compared to controls), which was not further decreased by an ulcerogenic dose of cysteamine (Table 2), indicating that cysteamine induced a CGRP-li release from a capsaicin-sensitive pool.

On the other hand, the almost completely intrinsic origin of SP neurons in the rat duodenum (Table 2) suggests that SP may have a local role in duodenal ulcers. Moreover, local release of SP-li in the small intestine has been reported in a hypersecretory situation such as after vagal nerve stimulation (Gronstad et al., 1985). Peripheral release of CGRP and other sensory

Table 2

Effect of cysteamine (900 mg/kg p.o., sacrifice 24 h later) and neonatal pretreatment with capsaicin (50 mg/kg s.c.) or both on duodenal ulcers and SP- and CGRP-like immunoreactivity (li).

Pretreatments:	Treatments:	duodenal SP-li (pmol/g tissue)	duodenal CGRP-li (pmol/g tissue)
Vehicle	Saline	14.8±1.0	41.8±0.8
Vehicle	Cysteamine	10.1±0.8*	20.5±2.7**
Capsaicin	Saline	14.0±0.5	9.2±1.0**
Capsaicin	Cysteamine	11.4±1.6*	14.6±1.6**

* and ** = P < 0.05 and 0.01 as compared to vehicle+saline group. n = 8 for each group.

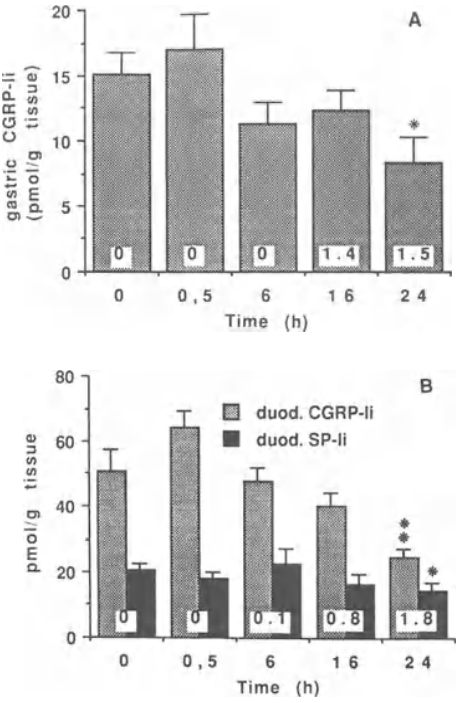


Fig. 1. Gastric CGRP-li (panel A) and duodenal CGRP-li and SP-li (panel B) at various times following the administration of a single dose of cysteamine (900 mg/kg p.o.). The mean score of gastric (scale 0-3) and duodenal (scale 0-4) ulcers is shown inside the columns.Statistical significance from the control group (time 0) is shown as * = $P < 0.05$, ** = $P < 0.01$. n = 8-10 for each group.

peptides has been hypothesized to participate in a capsaicin-sensitive "defence mechanism" against the ulcerogenic stimuli (Holzer and Lippe, 1988; Renzi et al., 1988).

In fact, in capsaicin pretreated rats there is a worsening of gastric ulcers induced by several stimuli (Evangelista et al., 1988) and duodenal ulcers induced by cysteamine and dulcerozine (Maggi et al., 1987a). The lack of antiulcer peptides contained in these afferents has been proposed to play a pivotal role in this phenomenon (Evangelista et al., 1988).

In addition, intragastric administration of capsaicin affords gastric protection against gastric damage, and this effect seems brought about by a local release of these peptides from afferent nerve endings acting also through local changes in mucosal blood flow (Holzer et al., 1989; Holzer and Lippe, 1988).

In conclusion, duodenal CGRP-li and SP-li are selectively affected by several duodenal ulcerogens during the late phases of ulcer formation and they might be among the local mediators which afford protection against the ulcerogenic stimuli.

References

DiPette, D.J., Schwarzenberger, K., Kerr, N., and Holland, B.O., 1987, Systemic regional hemodynamic effects of calcitonin gene-related peptide, **Hypertension** (suppl. III) 9:142.

Dockray, G.J., 1988, Regulatory peptides in the neuroendocrinology of gut-brain relations, **Quat. J. Exp. Physiol.**, 73:703.

Evangelista, S., Maggi, C.A., Giuliani, S., and Meli, A., 1988, Further studies on the role of the adrenals in the capsaicin-sensitive "gastric defence mechanism", **Int. J. Tiss. Reac.**, 10:253.

Evangelista, S., Maggi, C.A., Rovero, P., Patacchini, R., Giuliani, S., and Giachetti, A., 1990a, Analogs of neurokin A (4-10) afford protection against gastroduodenal ulcers in rats. **Peptides** 11:293.

Evangelista, S., Renzi, D., Mantellini, P., Surrenti, C., and Meli, A., 1989. Duodenal ulcers are associated with a depletion of duodenal calcitonin gene-related peptide-like immunoreactivity in rats, **Eur. J. Pharmacol.**,164:389.

Evangelista, S., Renzi, D., Mantellini, P., Surrenti, C., and Meli, A., 1990b, Duodenal SP-like immunoreactivity is decreased in experimentally-induced duodenal ulcers, **Neurosci. Lett.**, 112:352.

Gronstad, K.O., De Magistris, L., Dahlstron, A., Nilsson, O., Price, B., Zinner, M.J., Jaffe, B.M., and Ahlman, H., 1985, The effects of vagal nerve stimulation on endoluminal release of serotonin and substance P into the feline small intestine. **Scand. J. Gastroenterol.**, 20:163.

Gronstad, K.O., Yeo, C.J., Ahlman, H. Jaffe, B.M., and Zinner M.J., 1983, The effects of endoluminal SP on jejunal blood flow, in: "Substance P", P. Skrabanek and D. Powell, eds., Boole Press, Dublin.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.

Holzer, P., Gamse, R., and Lembeck, F., 1980, Distribution of substance P in the rat gastrointestinal tract: lack of effect of capsaicin pretreatment, **Eur. J. Pharmacol.**, 61:303.

Holzer P., and Lippe, I. Th., 1988, Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa, **Neuroscience**, 27:981.

Holzer, P., Pabst, M.A., and Lippe, I.Th., 1989, Intragastric capsaicin protects against aspirin-induced lesion formation bleeding in the rat gastric mucosa, **Gastroenterology**, 96:1425.

Holzer, P., and Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurones, **Gastroenterology**, 91:975.

Maggi, C.A., Evangelista, S., Abelli, L., and Meli, A., 1987a, Capsaicin-sensitive mechanisms experimentally induced duodenal ulcers in rats, **J. Pharm. Pharmacol.** 39:559.

Maggi, C.A., Evangelista, S., Giuliani, S., and Meli, A., 1987b, Anti-ulcer activity of calcitonin gene-related peptide in rats, **Gen. Pharmacol.**, 18:33.

Renzi, D., Santicioli, P., Maggi, C.A., Surrenti, C., Pradelles, P., and Meli, A., 1988, Capsaicin-induced release of substance P-like immunoreactivity from guinea-pig stomach in vitro and in vivo, **Neurosci. Lett.**, 92:254.

Scremin, O.U., Leung, F.W., and Guth, P.H., 1989, Pathogenesis

of duodenal ulcer in the rat: dissociation of acid load and blood flow, **Am. J. Physiol.**, 256:G1058.
Sharkey, K.A., Williams, R.G., and Dockray, G.J., 1984, Sensory Substance P innervation of the stomach and pancreas, **Gastroenterology**, 87:914.
Sternini, C., Reeve, J.R., and Brecha, N., 1987, Distribution characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal capsaicin-treated rats, **Gastroenterology**, 93:852.
Szabo, S., 1987, Mechanisms of mucosal injury in the stomach duodenum: time-sequence analysis of morphologic, functional, biochemical histochemical studies, **Sc. J. Gastroenterol.**, 22(suppl,127):21.
Taché, Y., Pappas, T., Lauffenburger, M., Goto, Y., Walsh, J.H., and Debas, H., 1984, Calcitonin gene-related peptide: potent peripheral inhibitor of gastric acid secretion in rats and dogs, **Gastroenterology**, 87:344.
Takeuchi, K., Furukawa, O., Tanaka, H., and Okabe, S., 1986, A new model of duodenal ulcers induced in rats by indomethacin plus histamine, **Gastroenterology**, 90:636.

OPIOID-SENSITIVE PERIPHERAL NEURONAL ACTIVITY IN THE MODULATION OF GASTRIC MUCOSAL INJURY

J.V. Esplugues and B.J.R. Whittle*

Departments of Pharmacology
University of Valencia, Spain
and *Wellcome Research Laboratories
Beckenham, Kent, U.K.

INTRODUCTION

There is growing evidence that capsaicin-sensitive afferent neurones participate in the protective mechanisms of the gastric mucosa against damage. Animals pretreated systemically with capsaicin, at doses that lead to the ablation of capsaicin-sensitive afferent neurons, show an increase in the level of macroscopically apparent mucosal damage in different experimental models of ulceration (Szolcsányi and Barthó, 1981; Holzer and Sametz, 1986). Furthermore, acute stimulation with capsaicin of afferent nerve endings located in the gastric mucosa protects against different ulcerogenic mechanisms (Szolcsányi and Barthó, 1981; Holzer and Lippe, 1988; Holzer et al., 1989).

Morphine and other opioids have been shown to potentiate gastric mucosal injury in various experimental models (Selye, 1935; Gyires et al., 1985; Till et al., 1988), although the mechanisms responsible for such action remain unclear. Following early observations on the regulatory effects of opioids and enkephalins on neuropeptide-release by sensory nerve endings (Konishi et al., 1980; Brodin et al., 1983), studies on vascular permeability and blood flow in the skin have suggested that opioids can inhibit the activity of primary afferent neurons (Barthó and Szolcsányi, 1981; Smith et al., 1982; Lembeck and Donnerer, 1985; Gamse and Saria, 1987). Furthermore, analgesia studies have indicated that morphine-like opioids can modulate afferent sensory neurons by an action on peripheral sites (Ferreira and Nakamura, 1979; Bentley et al., 1981; Russel et al., 1987).

In the present paper, we have therefore examined the possibility that opioids could influence the ability of the mucosa to withstand damage by exerting a peripheral action on local capsaicin-sensitive neuronal mechanisms. To evaluate this relationship, we have investigated, both macroscopically and histologically, the effects of primary afferent neuronal desensitization following systemic capsaicin pretreatment, and have compared this with the actions of morphine and other opioids on the gastric damage elicited by two injurious agents. In the first

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

series of studies, gastric mucosal injury was induced by the local intra-arterial infusion of the endogenous phospholipid Paf (platelet activating factor, PAF-acether). Intravascular Paf is one of the most potent ulcerogens so far described (Rosam et al., 1986), and in low doses induces extensive mucosal damage which may result from changes in the mucosal microcirculation (Whittle et al., 1986; Esplugues and Whittle, 1989a). In contrast, ethanol, the second ulcerogen employed in this study, can exert a direct irritant effect on the luminal side of the mucosa when administered intragastrically.

GASTRIC DAMAGE INDUCED BY INTRAVASCULAR PAF

To administer substances by close-arterial infusion, the left gastric artery of the pentobarbitone-anesthetized rat was cannulated as previously described (Esplugues and Whittle, 1988). The local intra-arterial infusion of Paf (50 ng kg^{-1} min^{-1}) for 10 min induced extensive damage to the rat gastric mucosa, macroscopically characterized as extensive hyperemia and vasocongestion, with areas of hemorrhagic damage. On histological evaluation, microvascular engorgement and congestion was the predominant feature, with extravasation of red blood cells being apparent, as well as epithelial and glandular destruction, with focal areas of necrosis that extended throughout the depth of the mucosal tissue.

The neuronal mechanisms that may modulate the damaging actions of Paf were then investigated. Paf-induced gastric damage was not affected by vagotomy or by pretreatment with atropine, ganglion blockade with hexamethonium or by α and β adrenoceptor blockade, but was greatly augmented in rats receiving a local infusion of tetrodotoxin (TTX) as shown in Fig. 1. This neurotoxin specifically binds to and blocks axonal fast sodium channels and thus prevents nervous conduction and mediator release from nerve terminals (Kao, 1966). Administration of TTX alone did not lead to any macroscopically detectable mucosal damage (Fig. 1).

These observations thus suggested that a neuronal mechanism was involved in modulating the damaging effect of Paf which was local and non-adrenergic non-cholinergic in nature. Furthermore, inactivation of such mucosal neuronal mechanism did not directly disrupt the mucosa, at least under these acute conditions.

Effects of Opioids on Paf-Induced Gastric Mucosal Damage

Pretreatment with morphine (0.75 - 3 mg kg^{-1} i.v.) induced a significant and dose-dependent potentiation in the extent of gastric mucosal damage induced by the local infusion of Paf. Macroscopically, the nature of the injury was comparable to that appearing after Paf infusion in rats pretreated with TTX, and was characterized by extensive hyperemia and hemorrhage of diffuse location. Pretreatment with morphine alone did not induce any macroscopically detectable damage to the gastric mucosa.

When analyzed histologically, pretreatment with morphine also augmented the degree of Paf-induced damage that could be observed in the wax-embedded sections of the corpus mucosa. The nature of the damage was characterized by the same histological features that were observed with Paf alone, but with a signifi-

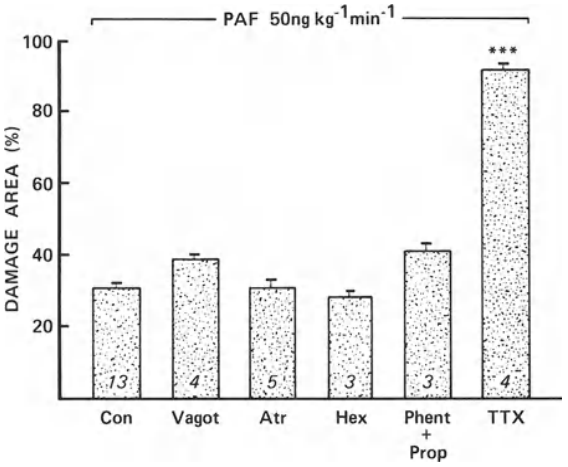


Fig. 1. Effects of vagotomy or pretreatment with atropine (5 mg kg^{-1}, i.v.), hexamethonium (5 mg kg^{-1} i.v.), the combination of phentolamine and propanolol (10 mg kg^{-1} and 1 mg kg^{-1}, i.v.) or tetrodotoxin (0.25 μg kg^{-1} min^{-1} infused locally throughout the experiment with 3 ml of a 20 μM solution of tetrodotoxin instilled into the gastric lumen) on the level of gastric damage induced by the local intra-arterial infusion of Paf (50 ng kg^{-1} min^{-1} for 10 min). Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage, are the mean ± SEM of (n) experiments in each group. Significant difference from the control intra-arterial infusion of Paf is given as ***$p<0.001$.

cantly greater degree of vasocongestion throughout the whole mucosa, with extensive subepithelial oedema being also observed.

The infusion of Paf induced a consistent fall in systemic arterial blood pressure. Administration of morphine at the doses used in our experiments (0.75 - 3 mg kg^{-1}) did not significantly increase the hypotensive effects of Paf (Esplugues et al., 1989), therefore obviating any non-specific augmentation of damage by this mechanism. Any involvement of a change in luminal acidity appears unlikely, since antisecretory doses of cimetidine did not influence the gastric damage induced by this combination, while other studies have shown that intragastric acid did not augment Paf-induced gastric damage (Rosam et al., 1986).

The enhancing effect of morphine on gastric damage induced by Paf appeared to be mediated through a specific interaction with classical opiate receptors since the opioid antagonist naloxone, which can act on both central and peripheral opioid receptors, abolished such potentiation (Fig. 2). Furthermore, the opioid receptors involved appear to be located peripherally since N-methyl nalorphine, a peripherally-acting quaternary opioid receptor antagonist which does not penetrate the blood brain barrier (Smith et al., 1982) equally inhibited the potentiating action of morphine (Fig. 2). In addition, administration of the peripherally-acting morphine analogue N-methyl morphine (Smith et al., 1982), which itself does not induce any macrosco-

pic damage to the gastric mucosa, significantly augmented the level of mucosal damage appearing after the local infusion of Paf (Fig. 3), further reinforcing the idea of a peripheral site of action of opioids in this model.

Effects of Capsaicin Pretreatment on Paf-Induced Gastric Mucosal Damage

To deplete sensory neuropeptides, adult rats (190 - 210g) were treated under halothane anesthesia with increasing doses of capsaicin (20, 30 and 50 mg kg^{-1}, s.c.) for three consecutive days and the animals were used 2 weeks later, as described previously in detail (Esplugues et al., 1989). As with TTX and morphine, capsaicin-pretreatment itself did not induce any macroscopic damage to the rat gastric mucosa when analyzed two weeks later. Furthermore, in capsaicin-pretreated rats, the local intra-arterial infusion of saline did not cause any macroscopic damage to the rat gastric mucosa. However, animals pretreated with capsaicin showed a significant enhancement in the extent of gastric mucosal damage induced by the local intra-arterial infusion of Paf (Fig. 3). The mucosal damage was hemorrhagic and diffuse, affecting both corpus and antral regions.

When studied histologically, animals pretreated with capsaicin also had a significantly higher degree of damage than those receiving Paf alone. The features of this augmented damage were similar to those present in animals receiving TTX or morphine pretreatment, being characterized by an enhanced vasocongestion throughout the mucosa, with extensive subepithelial oedema (Esplugues et al., 1989).

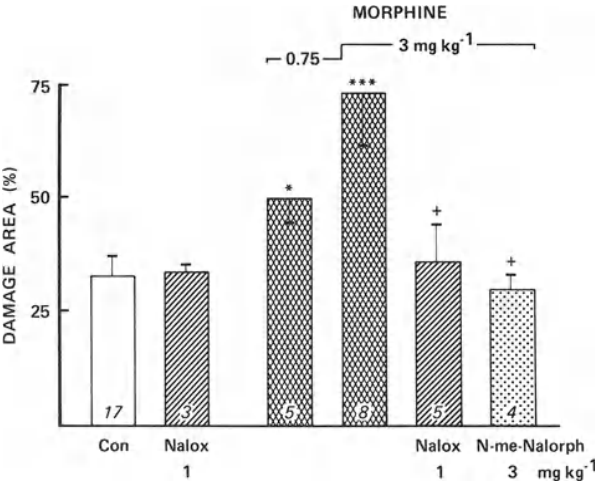


Fig. 2. Effects of the opiate antagonist naloxone (1 mg kg^{-1}, i.v.) and N-methyl nalorphine (3 mg kg^{-1}, i.v.) on the potentiation by morphine (0.75 and 3 mg kg^{-1}, i.v.) of the gastric damage induced by the local intra-arterial infusion of Paf (50 ng kg^{-1} min^{-1} for 10 min). Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage, are the mean ± SEM of (n) experiments in each group. Significant difference from the control intra-arterial infusion of Paf is given as *$p<0.05$ and ***$p<0.001$, and from the combination of morphine and Paf by +<p 0.05.

Effects of Morphine Pretreatment on Ethanol-Induced Gastric Damage

The extent of gastric mucosal damage elicited by the intragastric administration of 1 ml of ethanol at various concentrations (25%, 50% and 100%), was significantly increased by 15 min pretreatment with morphine (3 - 9 mg kg^{-1} i.v.) as shown in Fig. 4. Macroscopically, the potentiation by morphine of mucosal damage following challenge with ethanol was characterized as a significant increase in the area of the lesions affecting the corpus region. Furthermore, morphine induced the appearance of hemorrhagic lesions in the antral mucosa, an area not exhibiting distinct macroscopic damage following challenge with ethanol alone.

The potentiating effect of morphine on ethanol-induced gastric damage cannot be attributed to variations in the rate of gastric emptying since intragastric volume determined after challenge with ethanol was not different in control or morphine pretreated rats. Furthermore, intragastric concentration of the nonabsorbable marker phenol red, administered simultaneously with ethanol to control and morphine-pretreated rats, was likewise not significantly different in the two groups, indicating

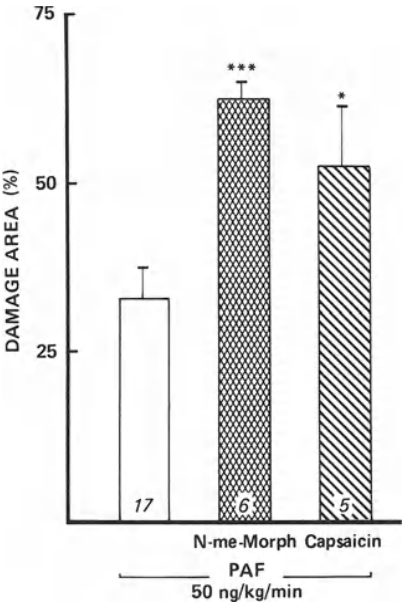


Fig. 3. Potentiation by pretreatment with N-methyl morphine (15 mg kg^{-1}, i.v., 10 min previously) or capsaicin (2 weeks before), of the gastric mucosal damage induced by the local intra-arterial infusion of Paf (50 ng kg^{-1} min^{-1} for 10 min). Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage, are the mean ± SEM of (n) experiments in each group. Significant difference from the control intra-arterial infusion of Paf is given as *$p<0.05$ and ***$p<0.001$.

no difference in the rate of gastric emptying (Esplugues and Whittle, 1990).

The potentiation by morphine of ethanol-induced gastric damage was abolished by low doses of the opioid antagonists naloxone and N-methyl nalorphine. In the absence of morphine pretreatment, neither naloxone nor N-methyl nalorphine significantly modified the level of mucosal damage induced by ethanol alone (Esplugues and Whittle, 1990). Although the opioid receptors involved in this potentiating effect have not been fully characterized, the specificity of morphine, naloxone and N-methyl nalorphine seems to indicate the involvement of μ receptors in this mechanism.

Effects of Capsaicin Pretreatment on Ethanol-Induced Gastric Mucosal Damage

Animals systemically pretreated with capsaicin showed an increase in the levels of macroscopically apparent gastric mucosal damage observed after the intragastric administration (1 ml) of a range of ethanol concentrations (25%, 50% and 100%) as shown in Fig. 4. Macroscopically, there was an increase in the area of hemorrhagic damage to the corpus mucosa, with the appearance of distinct hemorrhagic lesions in the antral mucosa to an extent comparable to that observed following morphine administration.

Histological Evaluation of the Potentiation by Morphine and Capsaicin-Pretreatment of Ethanol-Induced Gastric Damage

Histological evaluation of the effects of morphine and capsaicin pre-treatment following ethanol challenge was conducted on the mucosal sections obtained from the groups receiving

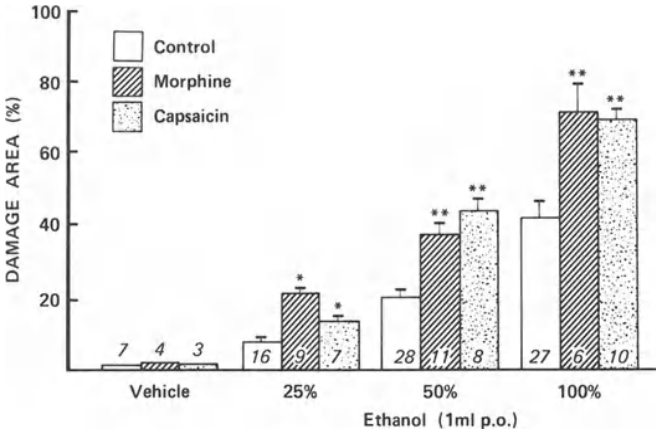


Fig. 4. Potentiation by pretreatment with morphine (9 mg kg^{-1} i.v., 15 min) or capsaicin (2 weeks earlier) of the rat gastric mucosal damage induced by various intragastric concentrations of ethanol (25% - 100%). Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage 5 min after challenge, are the mean ± SEM of (n) experiments in each group. Significant difference from the control ethanol-alone groups is given as *p<0.05, **p<0.01 and***p<0.001..pa

50% ethanol. As shown in Fig. 5, this concentration of ethanol alone induced damage to the epithelial cell layer (Type I damage) involving nearly two thirds of the total length evaluated. However, only moderate disruption of the subepithelial glandular structure (Type II damage) was noted, with less than 10% of the length of the section exhibiting deep necrotic and hemorrhagic damage (Type III damage).

Pretreatment with capsaicin, or administration of morphine (9 mg kg^{-1} i.v.) before challenge with 50% ethanol caused a small, but significant, enhancement of Type I damage, while inducing a twofold increase in the length of section exhibiting Type II damage and a fivefold increase in the length of section with Type III damage. There were no significant differences in the degree of any type of histologically assessed damage after ethanol between animals pretreated either with capsaicin or morphine. The potentiation by morphine of all types of histologically assessed damage following challenge with 50% ethanol, was abolished by pretreatment with either naloxone or N-methyl nalorphine. Neither capsaicin pretreatment nor administration of morphine alone did significantly modified type I, II or III damage compared with the vehicle control group in the absence of ethanol challenge.

Effects of Capsaicin Pretreatment on Morphine Potentiation of Ethanol-Induced Gastric Mucosal Damage

The similarities of the effects of morphine to those of capsaicin pretreatment in potentiating both Paf- and ethanol-induced gastric damage suggest a common mechanism of action. To

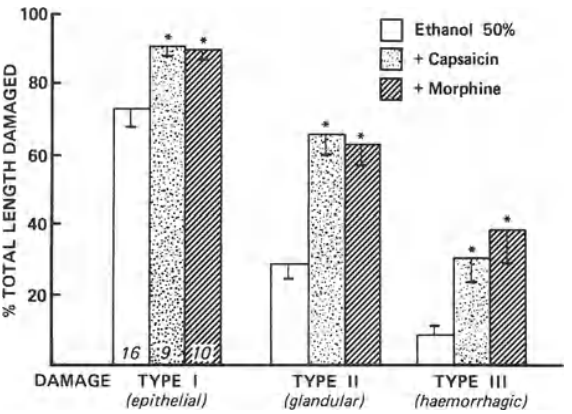


Fig. 5. Histological evaluation of the rat gastric corpus mucosa following a 5-min intragastric challenge with 50% ethanol (1 ml) and the effects of systemic capsaicin desensitization (2 weeks earlier) or pretreatment with morphine (9 mg kg^{-1} i.v., 15 min before). Data are shown as the length of section exhibiting damage of varying degrees, type I (epithelial cell damage), type II (glandular disruption in the mid to upper mucosa) and type III (deeper hemorrhage and necrosis), expressed as percent of total section length. Results are given as mean ± SEM of (n) values, and statistically significant difference from the ethanol-alone group is given as *$p<0.05$.

study such possibility, in a further series of experiments, gastric damage was assessed after oral administration of ethanol (50%) in rats that had received a submaximal dose of morphine (6 mg kg^{-1} i.v.), capsaicin pretreatment or both. As described before, the level of damage induced by ethanol was increased in animals that had been treated with either morphine or capsaicin. However, administration of morphine to capsaicin-pretreated rats does not induce any further significant enhancement in the submaximal level of damage induced by ethanol, as assessed by both macroscopic and histological techniques (Esplugues and Whittle, 1990).

CONCLUSIONS

These results indicate that opioids enhance the degree of gastric mucosal damage induced by two agents that act through different ulcerogenic mechanisms, topical ethanol and intravascular Paf. This effect appears to be brought about by interaction at classical opiate receptors, located in the periphery, as demonstrated by the effects of peripherally acting agonists and antagonists.

Ablation of primary afferent neurons, following capsaicin pretreatment in adult rats, augmented the damaging effects of Paf and ethanol on the gastric mucosa to an extent similar to that observed after morphine administration. When compared macroscopically and histologically, the major features of the increased damage appearing after both treatments were also similar suggesting, therefore, a common mechanism of action. The finding that morphine can not further increase the gastric mucosal damage elicited by submaximal concentrations of ethanol in capsaicin-pretreated rats implies that an intact sensory neuronal system in the gastric mucosa is needed for morphine to induce this peripheral action. This concept would be in keeping with the idea, demonstrated in other tissues, that opioids modulate the activity of primary afferent neurons.

Recent studies have demonstrated that the fall in gastric mucosal blood flow induced by Paf infusion is significantly enhanced by capsaicin pretreatment or the administration of morphine (Pique et al., 1990). These findings suggest that primary afferent neurones may modulate blood flow in the gastric microcirculation, perhaps by the release of vasodilator sensory neuropeptides, and that such endogenous neuropeptides may thus protect against mucosal injury by a local vascular mechanism.

Treatment with the neurotoxin tetrodotoxin, acute morphine administration or capsaicin pretreatment did not induce any detectable mucosal damage when administered alone to non-challenge stomachs. These observations suggest that peripheral sensory neurons play a protective modulatory role in the gastric mucosa only under pathological situations such as those following challenge by noxious stimuli. The lack of effect of the opioid-antagonists used in our experiments to influence the degree of Paf or ethanol mucosal damage when administered alone, implies that in these models of acute gastric damage, endogenous enkephalins do not modulate the activity of peripheral sensory neurons. However, this finding does not rule out the possibility that, during certain stress situations, the release of high levels of enkephalins could inhibit the activity of such neurones and, therefore, aggravate gastric mucosal damage.

Acknowledgements

The preparation of this manuscript has been supported by grants from Fondo de Investigaciones Sanitarias (FIS) and CAYCIT, Spain

References

Barthó, L., and Szolcsányi, J., 1981, Opiate agonists inhibit neurogenic plasma extravasation in the rat, **Eur. J. Pharmacol.**, 73:101.

Bentley, G.A., Newton, S.H., and Starr, J., 1981, Evidence for an action of morphine and the enkephalins on sensory nerve endings in the mouse peritoneum, **Br. J. Pharmacol.**, 73:325.

Brodin, E., Gazelius, B., Panopoulos, P., and Olgart, L., 1983, Morphine inhibits substance P release from peripheral sensory nerve endings, **Acta Physiol. Scand.**, 117:567.

Esplugues, J.V., and Whittle, B.J.R., 1988, Gastric mucosal damage by local intra-arterial administration of Paf in the rat, **Br. J. Pharmacol.**, 93:222.

Esplugues, J.V., and Whittle, B.J.R., 1989a, Gastric effects of Paf, **Meth. Find. Exp. Clin. Pharmacol.**, 11(Suppl.1):61.

Esplugues, J.V., and Whittle, B.J.R., 1989b, Mechanisms contributing to gastric motility changes induced by PAF-acether and endotoxin in rats, **Am. J. Physiol.**, 256:G275.

Esplugues, J.V., Whittle, B.J.R., and Moncada, S., 1989, Local opioid-sensitive afferent sensory neurones in the modulation of gastric damage induced by Paf, **Br. J. Pharmacol.**, 97:579.

Esplugues, J.V., and Whittle, B.J.R., 1990, Morphine potentiation of ethanol-induced gastric mucosal damage in the rat: Role of local sensory afferent neurons, **Gastroenterology**, 98:82.

Ferreira, S.H., and Nakamura, M., 1979, II-Prostaglandin hyperalgesia. The peripheral analgesic activity of morphine, enkephalins and opioid antagonists, **Prostaglandins**, 18:191.

Gamse, R., and Saria, A., 1987, Antidromic vasodilatation in the rat hindpaw measured by laser Doppler flowmetry: pharmacological modulation, **J. Auton. Nerv. Syst.**, 19:105.

Gyires, K., Furst, S., Farczadi, E., and Marton, A., 1985, Morphine potentiates the gastroulcerogenic effect of indomethacin, **Pharmacology**, 30:25.

Holzer, P., and Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons, **Gastroenterology**, 91:97.

Holzer, P., and Lippe, I. Th., 1988, Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of the gastric mucosa, **Neuroscience**, 27:981.

Holzer, P., Pabst, M.A., and Lippe, I. Th., 1989, Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa, **Gastroenterology**, 96:1425.

Kao, C.Y., 1966, Tetrodotoxin, saxitonin and their significance in the study of excitation phenomena, **Pharmacol. Rev.**, 18:997.

Konishi, S., Tsunoo, A., Yanaihara, N., and Otsuka, M., 1980, Peptidergic excitatory and inhibitory synapses in mammalian sympathetic ganglia: roles of substance P and enkephalin, **Biomed. Res.**, 1:528.

Lembeck, F., and Donnerer, J., 1985, Opioid control of the function of primary afferent substance P fibres, **Eur. J. Pharmacol.**, 114:241.
Pique, J.M., Esplugues, J.V., and Whittle, B.J.R., 1990, Influence of morphine or capsaicin pretreatment on rat gastric microcirculatory response to PAF, **Am. J. Physiol.**, in press.
Rosam, A.C., Wallace, J.L., and Whittle, B.J.R., 1986, Potent ulcerogenic actions of platelet-activating factor on the stomach, **Nature**, 319:54.
Russell, N.J.W., Schaible, H.-G., and Schmidt, R.F., 1987, Opiates inhibit the discharges of fine afferent units from inflamed knee joint of the cat, **Neurosci. Lett.**, 76:107.
Selye, M., 1935, A syndrome produced by diverse nocuous agents, **Nature**, 138:32.
Smith, T.W., Buchan, P., Parsons, D.N., and Wilkinson, S., 1982, Peripheral antinoceptive effects of N-methylmorphine, **Life Sci.**, 31:1205.
Szolcsányi, J., and Barthó, L., 1981, Impaired defense mechanisms to peptic ulcer in the capsaicin-desensitized rat, in: "Gastrointestinal Defense Mechanisms", Gy. Mozsik, O. Hanninen, and T. Javor, eds., Pergamon Press and Akademiai Kiado, Oxford and Budapest.
Till, M., Gati, T., Rabai, K., Szombath, D., and Szekeley, J.I., 1988, Effect of [D-Met^2-Pro^5] enkephalinamide on gastric ulceration and transmucosal potential difference, **Eur. J. Pharmacol.**, 150:325.
Whittle, B.J.R., Morishita, T., Ohya, Y., Leung, F.W., and Guth, P.H., 1986, Microvascular actions of platelet-activating factor on rat gastric mucosa and submucosa, **Am. J. Physiol.**, 251:G77.

RELATIONSHIP BETWEEN SENSORY NEUROPEPTIDES AND OTHER LOCAL VASOACTIVE MEDIATORS IN MODULATING GASTRIC MUCOSAL INTEGRITY

Brendan J.R. Whittle

Department of Pharmacology
Wellcome Research Laboratories
Langley Court, Beckenham, Kent. BRS 3BS U.K.

INTRODUCTION

The local release of sensory neuropeptides such as substance P and calcitonin gene-related factor (CGRP) from primary afferent neurones is considered to play a protective function in the gastric mucosa (see Holzer, 1988). Evidence for such a role is based predominantly on the findings in the rat that capsaicin pretreatment, which induces functional derangement of primary sensory neurones, substantially augments the gastric mucosal injury induced by a number of procedures including pylorus ligation and acid distension (Szolcsányi and Barthó, 1981) and administration of ulcerogens including ethanol and indomethacin (Holzer and Sametz, 1986; Esplugues and Whittle, 1990). Furthermore, acute exposure of the gastric mucosa to intraluminal capsaicin, which is considered to cause an initial stimulation of these afferent neurons and hence induce the release of sensory neuropeptides, has been shown to inhibit the mucosal damage induced by ethanol (Holzer and Lippe, 1988).

Recently, the gastric damage induced by PAF (Paf-acether; platelet-activating factor) has been demonstrated to be potentiated by capsaicin-pretreatment, the administration of capsaicin being either to neonatal rats or two weeks before study in adult rats (Esplugues, Whittle and Moncada, 1989; Pique, Esplugues and Whittle, 1990). Capsaicin-pretreatment significantly enhanced the fall in gastric mucosal blood flow observed following PAF-administration, suggesting that removal of vasodilator neuropeptides was involved in the mechanisms underlying the augmented damage (Pique et al., 1990). Furthermore, acute capsaicin instillation into the gastric lumen to stimulate afferent neurones increased mucosal blood flow, also suggesting a potential vasodilator role of the released neuropeptides (Lippe, Pabst and Holzer, 1989). To explore further the interactions of sensory neuropeptides with pro-ulcerogenic vasoactive mediators, the effects of capsaicin-pretreatment on the gastric damage induced by local intra-arterial infusion of the thromboxane mimetic U-46619 (Esplugues and Whittle, 1988) was investigated.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

Other endogenous vasodilator mediators, such as prostacyclin, also appear to play a local protective function within the mucosa (Whittle, 1977, 1986). In addition to prostacyclin, the endothelium can also elaborate a labile moiety originally described as endothelium-derived relaxing factor or EDRF (Furchgott and Zawadski, 1980; Furchgott, 1983). Nitric oxide (NO), biosynthesized by vascular endothelial cells from the amino-acid precursor, L-arginine, has now been shown to account for the biological activity of EDRF (Palmer et al., 1987, 1988a). The endogenous formation of NO can be selectively inhibited by the L-arginine analogue, N^G-monomethyl-L-arginine (L-NMMA), but not by its enantiomer D-NMMA (Palmer et al., 1988b; Rees et al. 1989). Thus, the interactions between this putative endogenous vasodilator in the gastric microcirculation and the sensory neuropeptides in the regulation of mucosal integrity have also been investigated.

METHODS

Male Wistar rats (230-260g body weight) were anesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and the trachea was cannulated to facilitate respiration. Rectal temperature was maintained at 37°C by thermistor-controlled radiant heat. Systemic arterial blood pressure was recorded from a cannula inserted into a carotid artery. A femoral vein was cannulated, or a 25 gauge butterfly needle was inserted into tail vein, for the administration of drugs.

For the close-arterial administration (10μl min^{-1}) of the thromboxane mimetic U-46619 (11α, 9α epoxy-methano-PGH_2; Caymen Chemicals), the stomach of the anesthetized rat was exposed by a mid-line incision and the left gastric artery was cannulated under a stereomicroscope with a 23 gauge teflon cannula. Stomachs were removed 20 min following termination of the infusion, opened and assessed macroscopically for the area of mucosal damage, as described below.

For further studies on gastric damage, 2ml of acid saline (100 mM HCl) was instilled into the gastric lumen, followed by bolus intravenous administration of L-NMMA (Dept. Medicinal Chemistry, Wellcome Research Labs). Forty-five minutes later, the stomachs were opened along the greater curvature, pinned to a wax block, immersed in neutral buffered formalin and photographed. The percentage of the total gastric mucosa showing macroscopically visible damage was determined via computerized planimetry in a randomized manner, its severity assessed on a 1-3 scale, and the resulting product of area x severity was divided by three to yield a damage index (the maximum thus being 100). The nature of the mucosal injury was assessed histologically in 4μm wax-embedded sections excised from the corpus mucosa and processed using routine techniques.

To deplete sensory neuropeptides, adult rats (190 - 210g) were treated under halothane anesthesia with capsaicin (Fluka Chemic AG) prepared as a 50mg ml^{-1} solution in absolute ethanol, tween 80 and saline (10:10:80 v/v/v). Capsaicin was administered in increasing doses (20, 30 and 50mg kg^{-1}, s.c.) for three consecutive days and the animals were used 2 weeks later, as described previously (Esplugues et al., 1989). To counteract respiratory impairment associated with capsaicin administration,

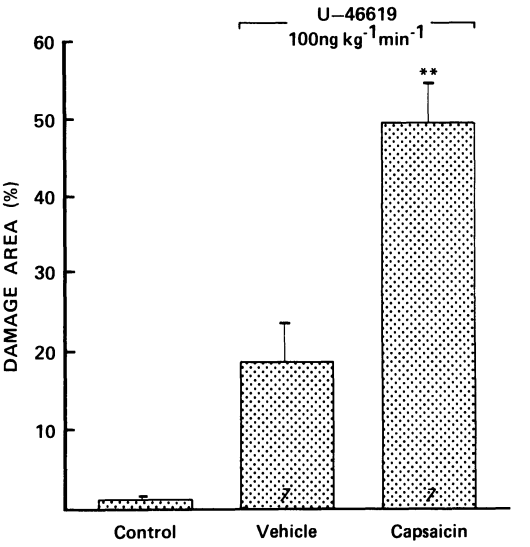


Fig. 1. Gastric mucosal damage induced by close-arterial infusion of the thromboxane mimetic, U-46619 (100 ng kg^{-1} min^{-1} for 10 min) in vehicle-pretreated or capsaicin-pretreated rats. In control studies, local infusion of isotonic saline (10 μl min^{-1} i.a.) did not induce mucosal damage. Results, shown as the % of the total mucosal area exhibiting macroscopic damage, are the mean ± s.e. mean of (n) studies, where significant difference from the vehicle group is given as ** $P<0.01$.

rats were pretreated with terbutaline (0.1 mg kg^{-1} i.m.) and aminophylline (10 mg kg^{-1} i.m.).

All data are expressed as mean ± s.e. mean. Comparisons between groups of parametric data were made by Student's t-test for unpaired data. Comparisons between groups of non-parametric data (damage index) were made by Mann-Whitney U-test, where P values of less than 0.05 were taken as significant.

RESULTS

Effects of Capsaicin Pretreatment on U-46619 Mucosal Damage

Local intra-arterial infusion of U-46619 (100ng kg^{-1} min^{-1}) for 10 min, with the stomach being removed 20 min later, induced distinct macroscopically-observed damage to the gastric mucosa of vehicle-pretreated rats. This damage was significantly ($P<0.01$) augmented in rats that had been pretreated with capsaicin, two weeks earlier (Figure 1). Capsaicin pretreatment alone had no detectable effect on the macroscopic or histological appearance of the gastric mucosa (n=5).

Effects of L-NMMA on Resting BP and on Substance P CGRP-Induced Hypotension

Intravenous bolus administration of L-NMMA (12.5 - 100 mg kg^{-1}) dose-dependently increased systemic BP from its resting

value of 119 ± 1 mmHg, (n = 36) as shown in Figure 2. The increase in BP reached peak values within 10 min and was maintained over the 45 min observation period. Concomitant intravenous administration of L-arginine (300 mg kg^{-1}) significantly reduced the increase in BP produced by L-NMMA (Figure 2). The enantiomer, D-NMMA (100 mg kg^{-1}) did not significantly elevate BP (Figure 2).

Bolus intravenous administration of substance P (3.75 - 300 pmol kg^{-1}) or rat α - CGRP (42 - 336 pmol kg^{-1}), both obtained from Cambridge Research Chemicals (stored frozen in distilled water and diluted freshly in isotonic saline) induced dose-dependent falls in BP (Figure 3).

Following administration of L-NMMA (50mg kg^{-1} i.v.), the hypotensive actions of substance P (7.5 - 30 pmol kg^{-1} i.v.) were significantly attenuated, as shown in Figure 3. Likewise, L-NMMA significantly reduced the fall in BP induced by CGRP (42-168 pmol kg^{-1} i.v.) although the hypotensive response to the higher dose of CGRP (336 pmol kg^{-1} i.v.) was not significantly inhibited (Figure 3).

Effects of L-NMMA on the Gastric Mucosa: Interactions with Capsaicin Pretreatment

Intravenous administration of L-NMMA (25 - 100 mg kg^{-1}) did not induce any detectable damage to the gastric mucosa over the 45 min observation period, as determined by macroscopic observation (Figure 4) and confirmed by histological assessment.

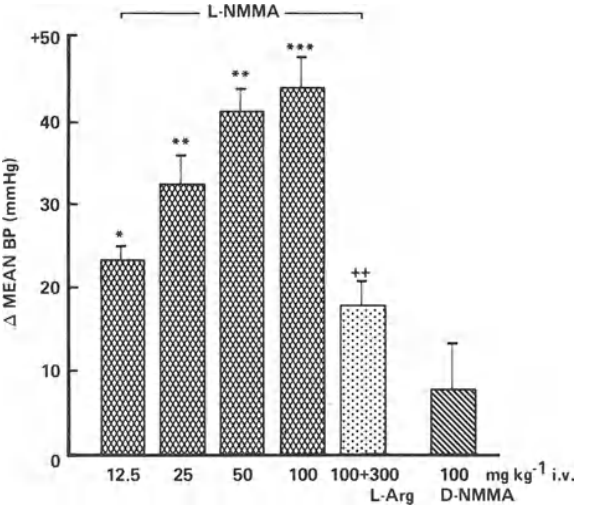


Fig. 2. Effects of bolus intravenous administration of L-NMMA (12.5-100 mg kg^{-1}) on systemic arterial blood pressure (BP) in the anesthetized rat, and its reversal by concurrent administration of L-arginine (300 mg kg^{-1} i.v.). Results, shown as changes in BP (mmHg), are the mean ± s.e. mean of 5-8 experiments in each group, where significant vasodepressor effects from resting levels are shown as * $P<0.05$, ** $P<0.01$, *** $P<0.01$, and significant difference from the corresponding L-NMMA-alone group as $^{++}$ $P<0.01$.

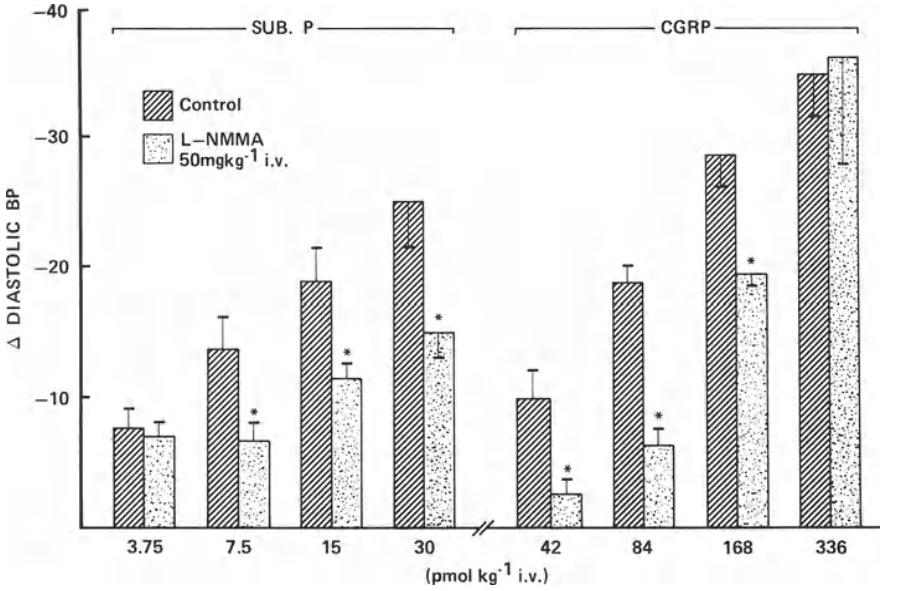


Fig.3. Effects of L-NMMA (50 mg kg^{-1} i.v.) on the vasodepressor actions of substance P (3.75 - 30 pmol kg^{-1} i.v.) and calcitonin gene-related peptide (42 - 336 pmol kg^{-1} i.v.). Results, shown as the change in diastolic BP (mmHg) are mean ± s.e. mean of 3-6 experiments in each group, where significant difference from the corresponding control group is shown as * $P<0.05$.

Pretreatment with capsaicin, two weeks before the study, did not induce any detectable damage to the gastric mucosa following intragastric instillation of acid saline over this observation period (Figure 4). Under these conditions, however, L-NMMA (25, 50 and 100 mg kg^{-1} i.v.) induced a dose-related increase in the macroscopically-observed area of damage, involving 33 ± 7% (n = 5), 39 ± 7% (n = 4) and 50 ± 3% (n = 5) respectively of the total mucosal area. This increase in the area and severity of damage induced by L-NMMA, shown in Figure 4 as the damage index, was characterized macroscopically as surface cell sloughing, vasocongestion and hemorrhage, which was confirmed by histological assessment. Concomitant administration of L-arginine (300 mg kg^{-1} i.v.) significantly reduced the extent of damage induced by L-NMMA (100 mg kg^{-1}) as shown in Figure 4, while administration of D-NMMA (100 mg kg^{-1} i.v.) in capsaicin-pretreated rats did not induce any gastric mucosal damage (n = 3).

DISCUSSION

The present observations indicate that the gastric mucosal damage induced by local intra-arterial infusion of the thromboxane mimetic, U-46619 (Esplugues and Whittle, 1988) can be potentiated by pretreatment with capsaicin to deplete sensory neuropeptides. This thromboxane mimetic, like endogenous thromboxane A_2 derived predominantly from platelets and inflammatory cells, induces vasoconstriction in the gastric mucosal microcirculation with marked focal effects on the submucosal vessels (Whittle et

al., 1981, 1985) and such actions may underlie its ability to induce mucosal damage. Recent studies have also demonstrated that the mucosal injury induced by the endothelium-derived vasoconstrictor peptide, endothelin-1 (Whittle and Esplugues, 1988) was substantially augmented by capsaicin-pretreatment (Whittle and Lopez-Belmonte, 1990). Furthermore, gastric damage induced by endothelin-1 was inhibited by concurrent local intra-arterial administration of CGRP (Whittle and Lopez-Belmonte, 1990). Inhibition of aspirin- and ethanol-induced mucosal injury has also been reported to be inhibited by local administration of CGRP (Lippe et al., 1989). Since capsaicin-sensitive neurones containing neuropeptides including CGRP have been located in association with the submucosal microvasculature (Sharkey et al., 1984; Ekblad et al., 1985; Sternini et al., 1987; Su et al., 1987; Green and Dockray, 1988), these observations could support a functional interaction between such vasoconstrictor mediators and the endogenous vasodilator sensory neuropeptides in the regulation of mucosal blood flow.

Sensory neuropeptides may interact with other local vasodilator mediators in the gastric microcirculation. Furthermore, the vasodilatation induced by substance P and CGRP is dependent on the presence of an intact endothelium, suggesting mediation by the release of an endothelium-derived relaxing factor (Furchgott, 1983; Brain et al., 1985). The involvement of NO in the hypotensive responses to these neuropeptides in vivo was therefore investigated by the use of L-NMMA to inhibit its biosynthesis from L-arginine. Intravenous administration of L-NMMA, which increased systemic BP as previously reported (Rees et al., 1989; Whittle et al., 1989), attenuated the hypotensive actions of submaximal doses of substance P and CGRP. However, L-NMMA failed to inhibit the hypotensive effects of high doses of CGRP, supporting the competitive nature of the actions of L-NMMA.

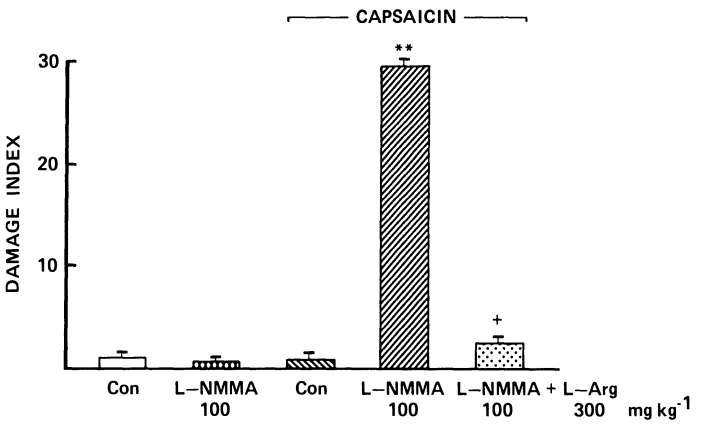


Fig.4. Potentiation of gastric mucosal injury induced by L-NMMA (100mg kg^{-1} i.v.), and its reversal by concomitant administration of L-arginine (300 mg^{-1} kg^{-1} i.v.) in rats pretreated with capsaicin two weeks earlier. Results, expressed as the macroscopic damage index, are the mean ± s.e. mean of (n) experiments, where significant difference from the control (intragastric 100 mM HCl) is shown as ** $P<0.01$ and from the L-NMMA group as $^{+}P<0.05$.

Previous studies with L-NMMA have demonstrated inhibition of the hypotensive actions of other endothelium-dependent vasodilators such as acetylcholine and bradykinin, but not those of the endothelium-independent vasodilators, prostacyclin and glyceryl trinitrate (Rees et al., 1989; Whittle et al., 1989). The inhibitory actions of L-NMMA on the responses to the neuropeptides, like its effect on resting BP, were reversed by concomitant administration of the precursor for NO biosynthesis, L-arginine, while the specificity of the actions of the inhibitor were confirmed by the failure of the enantiomer, D-NMMA, which does not inhibit NO formation, to have any such effect (Whittle, et al., 1989). Thus, the release of NO may contribute to the hypotensive actions of these neuropeptides, and to their potential local vasodilator effect on the gastric microcirculation.

Our recent studies have demonstrated that L-NMMA, in doses comparable to those in the current studies, reduces gastric mucosal blood flow, indicating a role of endogenous NO in the regulation of the gastric microcirculation (Pique, Whittle and Esplugues, 1989). Previous studies have shown that vasodilatation induced by acetylcholine in the rat gastric mucosa can be attenuated by chemical disruption of the gastric endothelial cells (Kitagawa, Takeda and Kohei, 1987) which is likely to reflect the inhibition of NO release in the gastric microcirculation. The local release of NO could also contribute to the functional vasodilation in the mucosal microvasculature following stimulation of acid secretion by cholinergic agents, as well as that induced by other secretagogues including gastrin or histamine.

Administration of L-NMMA did not alone induce acute damage to the gastric mucosa. However, in capsaicin-pretreated rats, L-NMMA induced distinct mucosal injury, as assessed by both macroscopic and histological observation. Thus, concurrent inhibition of NO formation and depletion of sensory neuropeptides can compromise mucosal integrity. Since L-NMMA itself would be expected to reduce the endothelium-dependent vasodilator activity of endogenous CGRP or substance P in the gastric microcirculation, the nature of this interaction would appear complex. However, L-NMMA in these doses did not abolish the cardiovascular responses to the neuropeptides. Moreover, the inhibitory activity of L-NMMA on the hypotensive responses was overcome by high doses of CGRP. This could reflect the situation in the microvasculature where high local endogenous levels of these neuropeptides may be achieved and hence offset the inhibitory actions of L-NMMA. It may therefore be necessary for capsaicin-pretreatment to deplete these neuropeptides, and for L-NMMA to inhibit NO formation and thus attenuate the activity of endothelium-dependent vasodilators other than the neuropeptides in the gastric microcirculation, before mucosal injury is initiated. Indeed, capsaicin-pretreatment alone, like L-NMMA alone, failed to induce mucosal injury. It is not yet known whether endogenous neuropeptides, released from sensory neurones, can modulate the release of NO or other products such as prostacyclin from endothelial cells in the gastric mucosa or other microvascular beds.

Endogenous NO and the sensory neuropeptides may therefore interact to modulate the gastric microcirculation, particularly under conditions of challenge when an adequate blood flow is essential for tissue integrity. Furthermore, other recent studies have demonstrated that the cyclo-oxygenase inhibitor, indo-

methacin, greatly augments the damage induced by L-NMMA following capsaicin-pretreatment, suggesting close interactions of NO and sensory neuropeptides with a local prostanoid, probably prostacyclin (Whittle et al., 1990). These local vasodilator mediators may not only regulate microvascular perfusion, but also enhance the resistance of the endothelium and other mucosal cells against challenge by endogenous vasoactive mediators released under pathological conditions, including thromboxane A_2 and endothelin-1.

I am most grateful to Mr. Juan Lopez-Belmonte for excellent technical support.

References

Brain, S.D., Williams, T.J., Tipping, J.R., Morris, H.R., and MacIntyre, I., 1985, Calcitonin gene-related peptide is a potent vasodilator, **Nature**, 313:54.

Ekblad, E., Ekelund, M., Graffner, H., Hakanson, R., and Sundler, F., 1985, Peptide-containing nerve fibres in the stomach wall of rat and mouse, **Gastroenterology**, 89:73.

Esplugues, J.V., and Whittle, B.J.R., 1988, Close-arterial administration of the thromboxane mimetic U-46619 induces damage to the rat gastric mucosa, **Prostaglandins**, 35:137.

Esplugues, J.V., and Whittle, B.J.R., 1990, Morphine potentiation ethanol-induced gastric mucosal damage in the rat: Role of local sensory afferent neurons, **Gastroenterology**, 98:82.

Esplugues, J.V., Whittle, B.J.R., and Moncada, S., 1989, Local opioid-sensitive afferent sensory neurones in the modulation of gastric damage induced by PAF, **Br. J. Pharmacol.**, 97:579.

Furchgott, R.F., 1983, Role of endothelium in responses of vascular smooth muscle, **Circ. Res.**, 53:557.

Furchgott, R.F., and Zawadzki, J.V., 1980, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, **Nature**, 288:373.

Green, T., and Dockray, G.J., 1988, Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig, **Neuroscience**, 25:181.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.

Holzer, P., and Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurones, **Gastroenterology**, 91:975.

Kitagawa, H., Takeda, F., and Kohei, H., 1987, Endothelium-dependent increases in rat gastric mucosal hemodynamics induced by acetylcholine and vagal stimulation, **Eur. J. Pharmacol**,, 133:57.

Lippe, I.T., Lorbach, M., and Holzer, P., 1989, Close arterial infusion of calcitonin gene-related peptide into the rat stomach inhibits aspirin- and ethanol-induced hemorrhagic damage, **Regul. Peptides**, 26:35.

Lippe, I.T., Pabst, M.A., and Holzer, P., 1989, Intragastric capsaicin enhances rat gastric acid elimination and mucosal blood flow by afferent nerve stimulation, **Br. J. Pharmacol.**, 96:81.

Palmer, R.M.J., Ferrige, A.G., and Moncada, S., 1987, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor, **Nature**, 327:524.

Palmer, R.M.J., Ashton, D.S., and Moncada, S., 1988a, Vascular endothelial cells synthesize nitric oxide from L-arginine, **Nature**, 333:664.
Palmer, R.M.J., Rees, D.D., Ashton, D.S., and Moncada, S., 1988b, L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation, **Biochem. Biophys. Res. Commun.**, 153:1251.
Pique, J.M., Whittle, B.J.R., and Esplugues, J.V., 1989, The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation, **Eur. J. Pharmacol.**, 171:293.
Pique, J.M., Esplugues, J.V., and Whittle, B.J.R., 1990, Influence of morphine or capsaicin-pretreatment on rat gastric microcirculatory response to PAF, **Am. J. Physiol.**, 258.
Rees, D.D., Palmer, R.M.J., and Moncada, S., 1989, Role of endothelium-derived nitric oxide in the regulation of blood pressure, **Proc. Natl. Acad. Sci. USA**, 86:3375.
Sharkey, K.A., Williams, R.G., and Dockray, G.J., 1984, Sensory substance P innervation of the stomach and pancreas. Demonstration of capsaicin-sensitive sensory neurones in the rat by combined immunohistochemistry and retrograde tracing, **Gastroenterology**, 87:914.
Sternini, C., Reeve, J.R., and Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.
Su, H.C., Bishop, A.E., Power, R.F., Hamada, Y., and Polak, J.M., 1987, Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas, **J. Neurosci.**, 7:2674.
Szolcsányi, J., and Barthó, L., 1981, Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat, in: "Gastrointestinal Defense Mechanisms", Gy. Mozsik, O. Hanninen and T. Javor, eds., Pergamon Press and Akadémiai Kiadó, Oxford and Budapest.
Whittle, B.J.R., 1977, Mechanisms underlying gastric mucosal damage induced by indomethacin and bile salts and the actions of prostaglandins, **Br. J. Pharmac.**, 60:455.
Whittle, B.J.R., 1986, The mechanisms of gastric damage by non-steroid anti-inflammatory drugs, in: "Biological Protection with Prostaglandins", M. Cohen, ed., C.R.C. Press, Florida.
Whittle, B.J.R., and Esplugues, J.V., 1988, Induction of rat gastric damage by the endothelium-derived peptide, endothelin, **Br. J. Pharmacol.**, 95:1011.
Whittle, B.J.R., and Lopez-Belmonte, J., 1990, Inhibition of endothelin-1 induced rat gastric damage by calcitonin gene-related peptide, **Br. J. Pharmac.**, 99:153.
Whittle, B.J.R., Kauffman, G.L., and Moncada, S., 1981, Vasoconstriction with thromboxane A_2 induces ulceration of the gastric mucosa, **Nature**, 292:472.
Whittle, B.J.R., Lopez-Belmonte, J., and Moncada, S., 1990, Regulation of gastric mucosal integrity by endogenous nitric oxide: Interactions with prostanoids and sensory neuropeptides in the rat, **Br. J. Pharmac.**, 99:607.
Whittle, B.J.R., Lopez-Belmonte, J., and Rees, D.D., 1989, Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation, **Br. J. Pharmacol.**, 98:646.
Whittle, B.J.R., Oren-Wolman, N., and Guth, P.H., 1985, Gastric

vasoconstrictor actions of leukotriene C_4, $PGF_{2\alpha}$ and thromboxane mimetic U-46619 on rat submucosal microcirculation *in vivo*, **Am. J. Physiol.**, 248:G580.

EFFECT OF SUBSTANCE P AND RELATED NEUROKININS ON GASTRIC ACID SECRETION

G. Coruzzi, M. Adami, G. Bertaccini

Institute of Pharmacology
University of Parma
43100 Parma, Italy

SUMMARY

Substance P and a series of related neurokinins having various degrees of selectivity for tachykinin receptors have been studied for their effects on gastric acid secretion both "in vitro" and "in vivo". In the isolated gastric fundus from immature rats, substance P, the C-terminal heptapeptide of neurokinin A, NKA(4-10), [Arg]NKB and two synthetic analogues of NKA(4-10), namely [β-Ala^8]NKA(4-10) and [Ala^5]NKA(4-10) (compounds marked Men 10210 and Men 10209, respectively) had no effect on spontaneous secretion but enhanced the secretory response to histamine. All the different neurokinins were effective in the range of concentrations 10^{-7} - 10^{-6} M. In the conscious cat with gastric fistula, substance P dose-dependently increased basal acid secretion, whereas Men 10210 was absolutely ineffective. Men 10209 caused a slight increase in acid output which, however, was only 10% of that induced by dimaprit or pentagastrin. The secretory effect of dimaprit and pentagastrin was not affected by the different neurokinins, conversely the response to 2-Deoxy-D-glucose was slightly reduced by Men 10210 (10 nmol/kg/h). The above data suggest that the natural and synthetic neurokinins studied have negligible effects on gastric acid secretion, thus the gastroprotective effect observed in some experimental conditions is unlikely to be related to an antisecretory effect of these compounds.

INTRODUCTION

Tachykinins (TKs) are a family of natural peptides, originally isolated by amphibian skin, which share the common C-terminal aminoacid sequence Phe-X-Gly-Leu-Met.NH_2 (for review see Bertaccini, 1976). Among natural TKs, substance P (SP) was identified in mammalian tissues including gastrointestinal tract, together with two other analogs, neurokinin A (NKA) and neurokinin B (NKB). Pharmacological receptors for the different neurokinins (NKs) were subsequently characterized by the use of neurokinin fragments and more recently with selective synthetic

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

derivatives. The terms NK1, NK2 and NK3 receptors were recently proposed according to the "Montreal nomenclature" to indicate receptors for substance P, NKA, and NKB, respectively (Regoli et al., 1989).

Among the peripheral effects of neurokinins (NKs), a gastroprotective effect has been recently reported in rats in which gastric lesions were experimentally induced by 95% ethanol (Evangelista et al., 1989a). This effect has been related by the same Authors to specific activation of NK2 receptors, since the greatest degree of protection was afforded by two synthetic analogs, namely [β-Ala^8]NKA(4-10) and [Ala^5]NKA(4-10), which possess higher selectivity for this receptor subtype (Rovero et al., 1989; Evangelista et al., 1990). In addition chronic capsaicin treatment, which depletes sensory neurons of neuropeptides including tachykinins (Hua et al., 1985), has been reported to aggravate ethanol-induced gastric lesions (Holzer et al., 1986), to induce erosive gastritis in rats (Mann, 1977) and to aggravate ulcers of the upper intestinal tract induced by various agents (Evangelista et al., 1987; Maggi et al., 1987). This seems to suggest that neuropeptides released by capsaicin-sensitive neurons are involved in gastrointestinal defence mechanisms.

In order to investigate the possible role of NKs on the gastric secretory mechanisms, SP and some synthetic NKs were tested both "in vitro" and "in vivo" in different animal species. The compounds selected for this study were SP, the C-terminal heptapeptide of NKA, NKA(4-10), two synthetic analogs of NKA(4-10) synthesized by Menarini Laboratories, namely [β-Ala^8]NKA(4-10) (Men 10210) and [Ala^5]NKA(4-10) (Men 10209) and [Arg]NKB. The aminoacid sequence of the different peptides is shown in Fig. 1.

1	2	3	4	5	6	7	8	9	10	
His	Lys	Thr	Asp	Ser	Phe	Val	Gly	Leu	Met.NH_2	Neurokinin A
			-	-	-	-	-	-	-	NKA (4-10)
			-	-	-	-	β-Ala	-	-	[β-Ala^8]NKA (4-10) (Men 10210)
			-	Ala	-	-	-	-	-	[Ala^5]NKA (4-10) (Men 10209)
Asp	Met	His	Asp	Phe	Phe	Val	Gly	Leu	Met.NH_2	Neurokinin B
H-Arg	-	-	-	-	-	-	-	-	-	[Arg]NKB
Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu-Met.NH_2	Substance P

Fig. 1. Aminoacid sequence of some natural and synthetic neurokinins.

MATERIALS AND METHODS

The effect of NKs was investigated on conscious cats with gastric fistula and on the isolated gastric fundus from the immature rat.

"In vivo" Experiments

Five female cats weighing approximately 3.5 kg were used. They were equipped with a permanent gastric fistula drained by a gastric cannula (Emas et al.,1967) at least 6 weeks before the study. They were fasted for 18 hr before the test, with water "ad libitum", and used once a week.

Drugs were administered through a permanent needle into a leg vein by bolus injection or by continuous infusion in 0.15 M NaCl (30 ml/h). Gastric juice was continuously collected throughout the experiment at 10 min intervals; volume was measured and the acid concentration determined by automatic titration to pH 7.0 with NaOH 0.1 M.

The effect of neurokinins was evaluated in basal conditions and on the hypersecretion induced by submaximal doses of dimaprit (1 μmol/kg/h), pentagastrin (0.005 μmol/kg/h) and 2-deoxy-D-glucose (2-DOG) (200 mg/kg i.v.). The different neurokinins were administered as i.v. bolus at the plateau of acid secretion when dimaprit was used as a stimulant; conversely, since both pentagastrin and 2-DOG did not induce stable levels of acid secretions, neurokinins were infused i.v. for 2h starting 30 min before the stimulant.

Acid secretory responses were reported as mean values ± SEM in mEq H^+ per 10 min.

"In vitro" Experiments

The technique described by Coruzzi et al., (1984) was essentially followed. Fed immature male rats (30-45 g) were used. The gastric fundus was set up at 34°C in a perspex tube with the mucosa facing into the lumen, bathed by 5 ml of oxygenated (100%

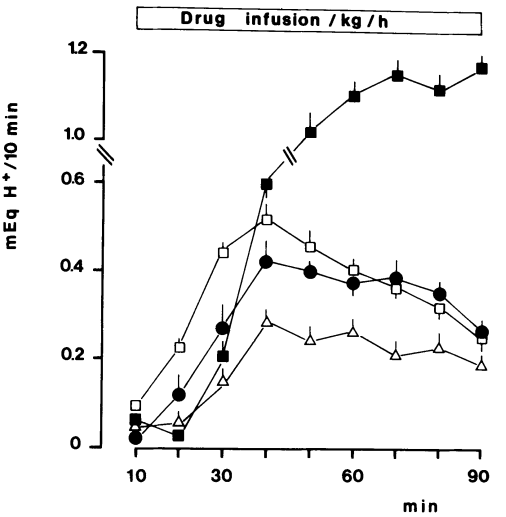


Fig. 2. Conscious gastric fistula cat. Effect of substance P, administered in nmol/kg/h, on basal acid secretion in comparison with dimaprit (■) (1 μmol/kg/h); △ = SP 1; ● = SP 3 and □ =SP 10. Mean values ± SEM from 5 cats.

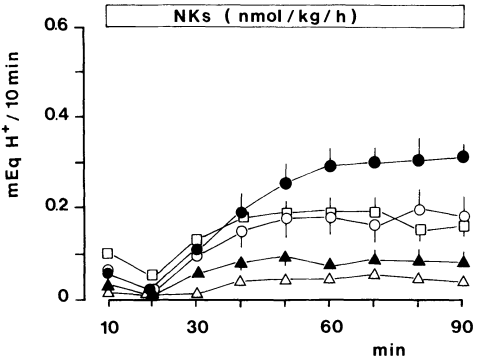


Fig. 3. Conscious gastric fistula cat.
Effect of neurokinins (NKs) on basal acid secretion.
Compounds administered were: [β-Ala^8]NKA (4-10)
10 (△); NKA (4-10) 30 (▲). [Ala^5]NKA (4-10)
10 (□); [Arg]NKB 10 (○) and 30 (●).
Mean values ± SEM from 5 cats.

O_2) unbuffered solution, whereas the serosal surface was bathed by 30 ml of buffered solution gassed with 95% O_2 and 5% CO_2. The composition of the serosal solution was (mM): NaCl 110; $NaHCO_3$ 26; KCl 5; $CaCl_2$ 2.4; $MgCl_2$ 2.4; glucose 16.7 and the mucosal solution contained (mM): NaCl 136; KCl 5; $CaCl_2$ 2.4; $MgCl_2$ 2.4; glucose 16.7.

The acid output was measured by titrating mucosal samples (taken at 15 min intervals) to pH 7 with 10 mM NaOH.

After an equilibration time of about 120 min, the effect of NKs was tested in separate experiments on spontaneous acid secretion or before addition of histamine ($4x10^{-5}$ M), which gave the most reproducible response in this test preparation.

Results were expressed as mean values ± SEM in uEq HCl cm^{-2} h^{-1} and were considered as differences between peak effects and basal pre-drug levels.

Drugs

Compounds used were: histamine, pentagastrin and 2-deoxy-D-glucose (Sigma); dimaprit was synthesized by Dr. Schiavone (De Angeli); substance P, NKA(4-10), [β-Ala^8]NKA(4-10) (Men 10210), [Ala^5]NKA(4-10) (Men 10209) and [Arg]NKB were a generous gift from Menarini Laboratories, Florence, Italy.

RESULTS

Gastric Fistula Cat

Basal acid secretion in this experimental model is very low (about 0.1 mEq/10 min) and tended to disappear within 10-20 min. SP (0.3-3 nmol/kg/h) infused for 90 min caused a dose-related increase in acid output, being however definitely less efficacious than dimaprit (Fig. 2) or pentagastrin (data not shown). Compound Men 10210 was completely ineffective up to 30 nmol/kg/h, whereas both compound Men 10209 and [Arg]NKB caused a

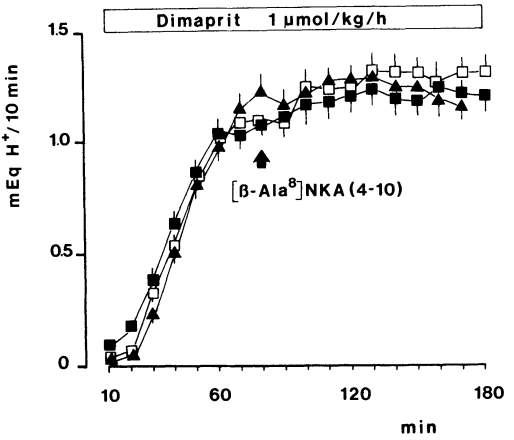


Fig. 4. Conscious gastric fistula cat. Effect of [β-Ala^8]NKA (4-10) on dimaprit-induced acid secretion (■). □ = 10 nmol/kg/h and ▲ = 30 nmol/kg/h. Mean values ± SEM from 5 cats.

slight increase in basal acid output, which however was not dose-related (Fig. 3). None of the compounds examined influenced the hypersecretion induced by dimaprit nor did they inhibit pentagastrin-induced acid secretion; an example of the ineffectiveness of Men 10210 on dimaprit- and pentagastrin-induced acid secretion is shown in Fig. 4 and Fig. 5. Conversely, a moderate but significant inhibitory effect was observed against 2-DOG, after administration of Men 10210 at 10 nmol/kg/h, however the effect was not dose-related and reached a maximum inhibition of about 25% inhibition (Fig. 6).

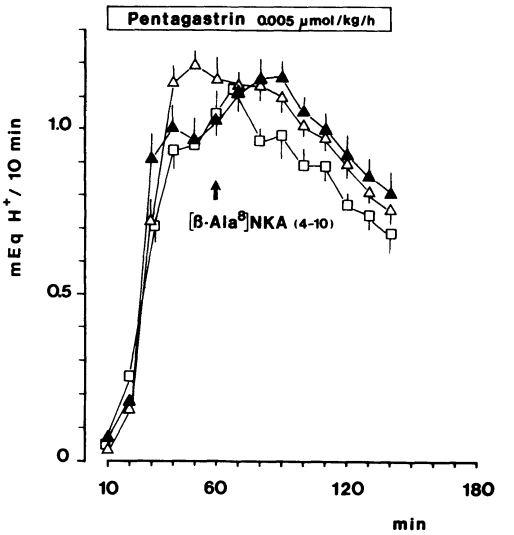


Fig. 5. Conscious gastric fistula cat. Effect of [β-Ala^8]NKA (4-10 3 (□) and 30 (△) nmol/kg/h on pentagastrin induced secretion (▲). Mean values ± SEM from 5 cats.

SP, [Arg]NKB, NKA(4-10) and the synthetic analogs Men 10209 and Men 10210 were all ineffective in modifying basal acid secretion up to 10^{-5} M. Conversely they caused a significant enhancement of the response to a sub-maximal concentration of histamine (Fig. 7 and 8).

DISCUSSION

Data obtained in the present study showed that both natural and synthetic neurokinins have a negligible effect on gastric acid secretion. The slight stimulatory effect observed after SP administration in the conscious cat confirmed previous results obtained in the atlantic cod, in which SP and eledoisin-related peptides tended to stimulate, rather than inhibit, acid secretion and only high doses of physalaemin caused an inhibitory effect on both basal and histamine-induced secretion (Holstein and Cederberg, 1986). Data concerning the effect of neurokinin A and B on acid secretion are not available at present; results concerning SP, the most investigated neurokinin, are rather confusing: in the dog a significant reduction in the response to pentagastrin (Martensson et al., 1984) and peptone meal (Konturek et al., 1981) was observed after SP in both the vagally innervated and denervated stomach. The effect was evident at doses which do not influence blood pressure, thus suggesting that the effect is not related to changes in the blood flow to the stomach. Elevation of plasma SP-like immunoreactivity following a protein meal or bombesin infusion was demonstrated in dogs (Jaffe et al., 1982; Martensson et al., 1984), thus SP may be involved in the postprandial control of gastric secretion. However, these results were not confirmed in rats: according to some Authors (Goto et al., 1984) SP inhibited the response to bethanechol but not that to pentagastrin or histamine, whereas in other studies (Yokotani et al., 1985) SP had no effect on bethanechol but inhibited vagally-induced acid secretion.

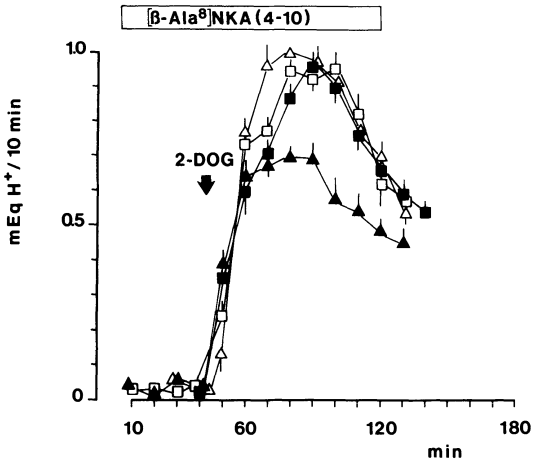


Fig. 6. Conscious gastric fistula cat. Effect of [β-Ala^8]NKA (4-10 3 (□), 10 (▲) and 30 (△) nmol/kg/h on the response to 2-DOG (2-deoxy-D-glucose) 200 mg/kg i.v. (■). Mean values ± SEM from 5 cats.

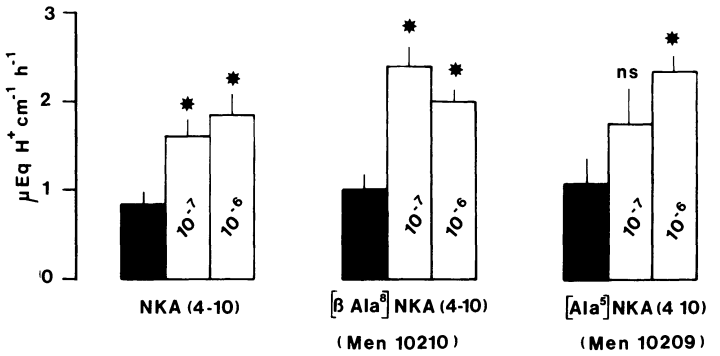


Fig. 7. Isolated gastric fundus from the immature rat. Enhancing effect of neurokinins (□) on histamine-induced acid secretion (■) ($4x10^{-5}$ M). Compounds were administered in molar concentration. * = Significantly different ($P<0.05$) from controls (■). Mean values ± SEM from 6-8 observations.

On the other hand, controversial results have been obtained concerning the effect of sensory denervation by capsaicin on gastric acid secretion (Alfoldi et al., 1986; Dugani and Glavin, 1986; Evangelista et al., 1989b). The effect seems to be dependent on the type of administration (acute or chronic) or on the choice of animal models (anesthetized vs chronic gastric cannula animals).

The ineffectiveness of neurokinin analogs acting on different types of NKs receptors observed in this study, tends to exclude a major role of these peptides in the control of gastric secretory process, thus the anti-ulcer effect reported in previous studies must be related to an interference in defence mechanisms and not to their antisecretory action. Indeed the gastroprotection induced by NKA(4-10) against 95% ethanol-induced lesions was reversed by N-ethyl maleimide and by indomethacin, suggesting a role for endogenous sulphydryl groups (Evangelista et al., 1989a) or prostaglandins (Evangelista et al., 1990).

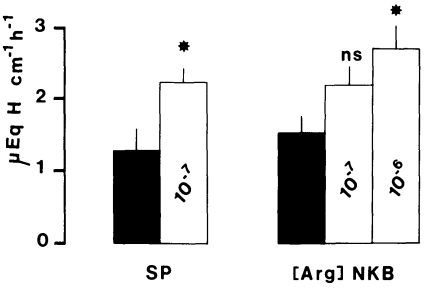


Fig. 8. Isolated gastric fundus from the immature rat. Enhancing effect of Substance P (SP) (□) and [Arg]NKB (□) on the acid response to histamine ($4x10^{-5}$ M) (■). * = Significantly different ($P<0.05$) from controls (■). Mean values ± SEM from 6-8 observations.

References

Alfoldi, P., Obal, F., Toth, E., and Hideg, J., 1986, Capsaicin-pretreatment reduces the gastric acid secretion elicited by histamine but does not affect the responses to carbachol and pentagastrin, **Eur. J. Pharmacol.**, 123:321.

Bertaccini, G., 1976, Active polypeptides of nonmammalian origin, **Pharmacol. Rev.**, 28:127.

Coruzzi, G., Adami, M., and Bertaccini, G, 1984, Action of histamine and of some H2 antagonists on gastric secretion in vitro, **Agents and Actions**, 14:515.

Dugani, A.M., and Glavin, G.B., 1986, Capsaicin effects on stress pathology and gastric acid secretion in rats, **Life Sci.**, 39:1531.

Emas, S., Swan, K.G., and Jacobson, E.D., Methods of studying gastric secretion, in: "Handbook of Physiology", Vol. 2, Section 6, C.F Code, ed., American Physiological Society, Washington, DC.

Evangelista, S., Lippe, I.Th., Rovero, P., Maggi, C.A., and Meli, A., 1989a, Tachykinins protect against ethanol-induced gastric lesions in rats, **Peptides**, 10:79.

Evangelista, S., Maggi, C.A., and Meli, A., 1987, Involvement of capsaicin-sensitive mechanism(s) in the antiulcer defence of intestinal mucosa in rats, **Proc. Soc. Exp. Biol. Med.**, 184:264.

Evangelista, S., Santicioli, P., Maggi, C.A., and Meli, A., 1989b, Increase in gastric secretion induced by 2-deoxy-D-glucose is impaired in capsaicin-treated rats, **Br. J. Pharmacol.**, 98:35.

Evangelista, S., Maggi, C.A., Rovero, P., Patacchini, R., Giuliani, S., Meli, A., and Giachetti, A., 1990, Neurokinin (NK)-2 receptor agonists afford protection against gastroduodenal ulcers in rats, **Peptides**, 11 (in press).

Goto, Y., Garcia, R., and Debas, H.T., 1984, Specificity of substance P (SP) inhibition to neural mechanism of gastric acid secretion, **Gastroenterology**, 86:1094.

Holstein, B., and Cederberg, C., 1986, Effects of tachykinins on gastric acid and pepsin secretion and on gastric outflow in the Atlantic cod, Gadus morhua, **Am. J. Physiol.**, 250:G309.

Holzer, P., and Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons, **Gastroenterology**, 91:975.

Hua, X.Y., Theodorsson-Norheim, E., Brodin, E., Lundberg, J.M., and Hoekfelt, T., 1985, Multiple tachykinins (NKA, NKK and SP) in capsaicin-sensitive sensory neurons in the guinea pig, **Regulatory Peptides**, 13:1.

Jaffe, B.M., Akande, B., Modlin, I.M., Reilly, P., and Albert, D., 1982, Cholinergic modulation of substance P release, **Dig. Dis. Sci.**, 27:28.

Konturek, S.J., Jaworek, J., Tasler, J., Cieszkowski, M., and Pawlik, W., 1981, Effect of substance P and its terminal hexapeptide on gastric and pancreatic secretion in the dog, **Am. J. Physiol.**, 241:G74.

Maggi, C.A., Evangelista, S., Abelli, L., and Meli, A., 1987, Capsaicin-sensitive mechanism(s) and experimentally-induced duodenal ulcers in rats, **J. Pharm. Pharmacol.**, 39:559.

Mann, N., 1977, Capsaicin-induced acute erosive gastritis: its prevention by antacid, metiamide and cimetidine, **J. Kent. Med. Ass.**, 75:71.

Martensson, H.G., Akande, B., Yeo, C., and Jaffe, B.M., 1984,

The role of substance P in the control of gastric acid secretion, **Surgery**, 95:567.
Regoli, D., Drapeau, G., Dion, S., and D'Orleans-Juste, P., 1987, Pharmacological receptors for substance P and neurokinins, **Life Sci.**, 40:109.
Rovero, P., Pestellini, V., Patacchini, R., Giuliani, S., Santicioli, P., Maggi, C.A., Meli, A., and Giachetti, A., 1989, A potent and selective agonist for NK2 tachykinin receptor, **Peptides**, 10:593.
Yokotani, K., and Fujiwara, M., 1985,, Effects of substance P on cholinergically stimulated gastric acid secretion and mucosal blood flow in rats, **J. Pharm. Exp. Ther.**, 232:826.

EFFECT OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) ON ASPIRIN- AND ETHANOL-INDUCED INJURY IN THE RAT STOMACH

Irmgard Theresia Lippe

Harvard Medical School, Dept. of Biological Chemistry and Molecular Pharmacology, Boston, Mass. USA
and Dept. of Exp. and Clin. Pharmacology
University of Graz, Austria

INTRODUCTION

It has been shown that the mammalian stomach, particularly the mucosa and submucosa, is densely innervated by primary afferent neurons containing calcitonin gene-related peptide (CGRP) (Rosenfeld et al., 1983; Sternini et al., 1987; Su et al., 1987; Green and Dockray, 1988).

Gastric CGRP is found mainly in capsaicin-sensitive afferent neurons which have been shown to play an important role in gastric mucosal protection. Chemical ablation of these nerves with a high dose of the neurotoxin capsaicin (0.16mmol/kg) aggravates gastric lesion formation in various experimental ulcer models (Szolcsányi and Barthó, 1981; Holzer and Sametz, 1986; Evangelista et al., 1986). On the other hand stimulation of these nerve endings by perfusing a low concentration of capsaicin (160 μM for 30 min) through the stomach was found to afford protection against ethanol- and aspirin-induced gross mucosal lesion formation in the rat stomach (Holzer and Lippe, 1988; Holzer et al., 1989; Lippe et al., 1989a) as well as against histological damage due to the ulcerogens (Holzer et al., 1989; Holzer et al., 1990a).

Since the antilesion effect of intragastric capsaicin is not affected by either surgical (e.g. subdiaphragmatic vagotomy, coeliac ganglionectomy, bilateral adrenal ligation) or pharmacological (e.g. atropine, guanethidine, phentolamine, propranolol) blockade of the autonomic nervous system (Holzer and Lippe, 1988; Lippe et al., 1989a), it seems conceivable that afferent nerve-mediated protection is brought about by a local release of transmitter substances from sensory nerve endings within the gastric mucosa. The marked increase in gastric mucosal blood flow caused by intragastric capsaicin is likewise independent of the autonomic nervous system (Lippe et al., 1989a). Thus the gastroprotective effect of capsaicin might be mediated via a release of vasodilator substances within the gastric mucosa. CGRP is a very potent vasodilator and was shown to be released from afferent nerve endings in various tissues (for review see Holzer,

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

Holzer, 1988) including the mesenteric vascular bed (Kawasaki et al., 1988) and the vascularly perfused stomach (Holzer et al., 1990b). These observations and the fact that CGRP is contained almost exclusively in capsaicin-sensitive afferent neurons make CGRP a major candidate for mediating both the vasodilator and the protective effect of capsaicin in the gastric mucosa.

The objective of the present study was therefore to investigate the effects of rat alpha-CGRP, the form of CGRP present in afferent nerves (Mulderry et al., 1988), on experimentally induced lesion formation in the stomach of the rat.

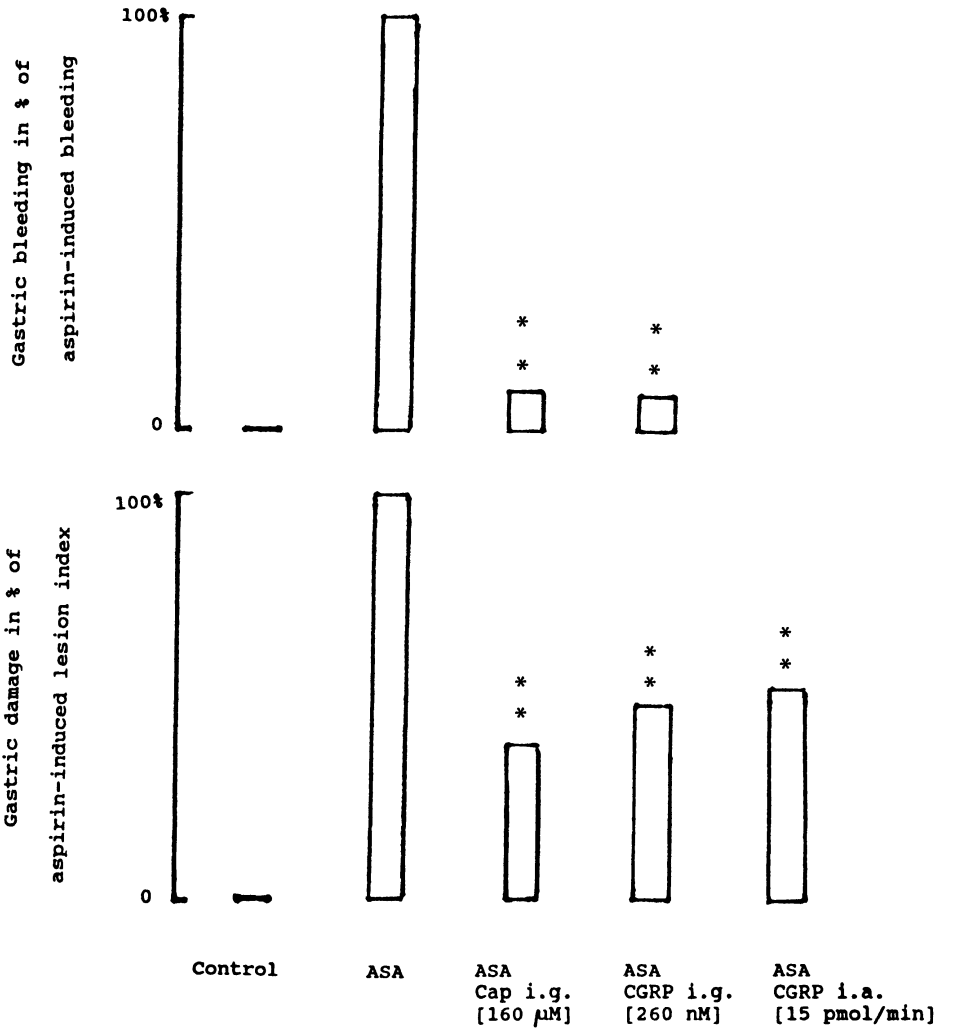


Fig. 1. Effect of CGRP and Capsaicin administered intragastrically and effect of CGRP applied by close arterial infusion to the stomach on aspirin- (25mM, pH 1.5) induced gastric bleeding (upper panel) and aspirin-induced gastric damage (lower panel) compared to controls (saline perfusion only) (n=6-11). ** $p < 0.01$ vs the value obtained by perfusing the rat stomach with aspirin alone.

METHODS

For all experiments adult Sprague-Dawley rats (strain OFA-SD, Forschungsinstitut für Versuchstierzucht, Himberg Austria), of either sex and weighing 280-320g, were used. The rats were deprived of food, but not of water, 20 h prior to experimentation. Urethane (1.5g/kg subcutaneously) served as anaesthetic and the body temperature of the rat was maintained at 37°C. The trachea was cannulated to ensure patent airway, one carotid artery was fitted with a cannula to constantly record mean arterial blood pressure (MAP) and to withdraw blood samples, and one jugular vein was cannulated for intravenous administration of drugs. For close arterial infusion of CGRP to the stomach a cannula was introduced retrogradely until the tip of the cannula lay just above the branching of the celiac artery. The stomach was continuously perfused with bodywarm saline (0.7-0.8 ml/min) via catheters in the esophagus and pylorus. After completion of surgery an equilibration period of 1 h was allowed before the experiments were begun.

Gastric mucosal lesions were induced by perfusing the stomachs with either ethanol (25% W/W in saline) or aspirin (25mM in acidified saline, pH 1.5) for 30 min. Lesions were scored under a dissecting microscope by a naive observer and expressed as severity of lesions (for details see Holzer and Sametz, 1986); CGRP was administered intragastrically concomitantly with the ulcerogens, or intravenously or intraarterially 5 min prior to the ulcerogens.

The clearance of [14C-dimethylamine]-aminopyrine from the blood into the gastric lumen was measured as an index for gastric mucosal blood flow (Tague and Jacobson, 1976).

Gastric acid output (pH) and gastric bicarbonate (pCO_2) output were measured by means of appropriate electrodes and calculated according to Fändricks and Stage (1986) and Forsell and Olbe (1985).

In some experiments gastric bleeding, which is a common feature of aspirin-induced lesions, was determined as hemoglobin content of the gastric output (Holzer and Sametz, 1986; Holzer and Lippe, 1988; Holzer et al., 1989 Lippe et al., 1989a, Lippe et al., 1989b; Holzer et al, 1990a).

RESULTS AND DISCUSSION

Intragastric Administration of CGRP

Rat alpha-CGRP (260 nM) was perfused through the stomach at a rate of 0.7-0.8 ml/min. Locally intragastrically administered CGRP had no effect on MAP, did not exert any change in the gastric-aminopyrine clearance and furthermore did not influence acid or bicarbonate output from the stomach. When CGRP was perfused concomitantly with the ulcerogens, mucosal damage induced by ethanol was not affected by intragastric CGRP, but aspirin-induced hemorrhagic lesions were significantly reduced from 92 ± 10 to 45 ± 8 (n=10, $p < 0.01$, U test). Gastric bleeding was also diminished dramatically (see fig. 1). Maggi et al. (1987) reported previously that subcutaneous injection of CGRP is without effect on ethanol-induced gross mucosal damage but reduces lesions caused by aspirin.

The lack of protection against ethanol-induced lesions was not due to a possible inactivation of the peptide by the presence of ethanol (Lippe et al., 1989b). It seems that intragastrically administered CGRP, unlike the mediator substance released by intragastric capsaicin, does not gain access to the blood vessels and hence fails to affect ethanol-induced injury (fig. 2). If so, this would also mean that the protective effect against aspirin-induced lesions is brought about by a mechanism other than an effect on mucosal blood vessels. If other effects of CGRP, such as release of somatostatin (Dunning and Taborsky, 1987; Zdon et al., 1988) or formation of prostaglandins in vein endothelial cells (Crossman et al., 1987) were to contribute to the protective effect against aspirin-induced lesions, this would still not provide an explanation for the lack of effect against ethanol damage. Furthermore it was found that both somatostatin (Diel and Szabo, 1986) and prostaglandins (Pihan et al., 1986; Robert et al, 1979; Guth et al., 1984) do inhibit ethanol injury in the stomach.

It seems therefore that the route of intragastric administration does not consistently mimic the protective effect of mediators released from afferent nerve endings in the gastric

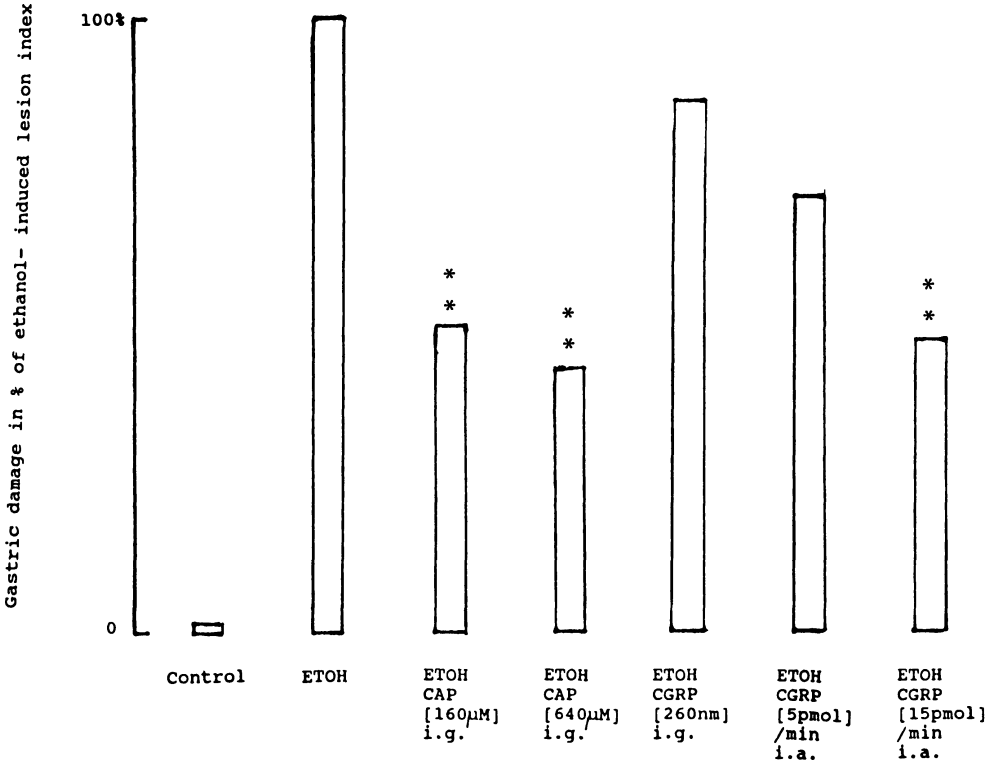


Fig.2. Effect of CGRP and Capsaicin given intragastrically and CGRP given by close arterial infusion on ethanol- (25%) induced gastric damage in the rat stomach compared to controls (saline perfusion) (n=6-11). ** $p < 0.01$ vs the value obtained by perfusing the rat stomach with ethanol alone.

mucosa, probably due to lack of adequate access of the peptide from the gastric lumen.

Intravenous Administration of CGRP

Intravenous infusion of CGRP (16.7 and 50 pmol/min) caused an immediate, significant and dose-dependent fall in MAP (Lippe et al., 1989b). The formation of aspirin-induced lesions was unaffected, whereas the lesions induced by perfusion of the stomach with ethanol were markedly aggravated under CGRP i.v. infusion.

Intravenous CGRP exerted no effect on the output of gastric acid or gastric bicarbonate; however when the gastric acid secretion was stimulated with pentagastrin infusion, CGRP was able to reduce the acid as well as the bicarbonate output.

The effect of intravenously applied CGRP on aminopyrine clearance was dose-dependent and a total intravenous dose of 500 pmol caused a significant increase in gastric mucosal blood flow (e.g. aminopyrine clearance) of about 60%; this is about the same increase brought about by intragastric administration of capsaicin (Lippe et al., 1989a).

Under the experimental conditions the different effects of i.v. CGRP might be due to the overpowering hypotensive action of the peptide. The effect on MAP is due to the vasodilator action on various vessels (Brain et al., 1985; Marshall et al., 1986; Uddman et al., 1986; Bauernfeind et al., 1989). The gastric mucosal blood flow was increased by intravenous CGRP and this increase roughly paralleled the fall in MAP (Lippe et al., 1989b). An increase in mucosal blood flow is generally thought to play a crucial role in the ability of the gastric mucosa to protect itself against injuries (Guth and Leung, 1987; Cheung, 1984; Pihan et al., 1986). The beneficial effect of mucosal vasodilatation might be overcome by the prominent hypotensive effect of intravenously infused CGRP.

Intracerebral as well as intravenous administration of CGRP (Taché, 1988) inhibits stimulated but not basal gastric acid output in anaesthetized rats, whereas in conscious rats the basal acid output is also reduced (Lenz et al., 1985). CGRP given intravenously seems to be more potent in inhibiting gastric acid output than in affecting mucosal blood flow (Leung et al., 1987) and our data indicate that only hypotensive doses of intravenous CGRP can raise mucosal blood flow.

Close Arterial Administration of CGRP

CGRP was infused intraarterially locally to the stomach of the rat and at the doses given (5 pmol/min and 50 pmol/min) exerted no significant effect on MAP. This lack of effect in lowering blood pressure suggests that the peptide is effectively eliminated from the portal circulation. Intraarterially administered CGRP dose-dependently reduced gastric lesion formation due to ethanol, with infusion of 15 pmol/min causing a reduction in the lesion index of 53% (fig. 2.). The same dose of CGRP was also able to significantly (49%) reduce aspirin-induced gastric damage (Lippe et al., 1989b)(fig. 1).

It is likely that this protective effect of intraarterially administered alpha-CGRP results from a local vasodilator effect on the mesenteric blood vessels (Kawasaki et al., 1988; Marshall et al., 1986; Uddman et al., 1986; Bauernfeind et al., 1989) including those supplying the rat stomach. This has been confirmed by measurements of gastric mucosal blood flow by means of the hydrogen gas-clearance technique as close arterial infusion of CGRP increased gastric mucosal blood flow significantly (P. Holzer, personal communication).

Taken together, these results strongly support the concept that increase or maintenance of mucosal blood flow plays an important role in gastric protection against injury (Guth and Leung, 1987; Cheung, 1984; Pihan et al., 1986); consistent with this hypothesis are reports, (Pihan et al., 1986; Whittle and Esplugues, 1988; Lopez-Belmonte and Whittle, 1990) that close arterial infusion of vasoconstrictors weakens gastric mucosal defence.

CONCLUSION

The protective effect of close intraarterial infusion of the vasodilator peptide alpha-CGRP very closely resembles the stimulation of sensory afferent nerve endings by intragastric capsaicin, which likewise protects against aspirin- and ethanol-induced lesion formation in the rat stomach (Holzer and Lippe, 1988; Holzer et al., 1989; Lippe et al., 1989a; Holzer et al., 1990a). In the rat stomach CGRP is located exclusively in primary afferent neurons (Green and Dockray, 1988) and from the present study it appears that CGRP may really be amongst the key mediators which, when released locally from afferent nerve endings in the stomach, strengthen gastric mucosal defence.

References

Bauernfeind, P., Hof, R., Hof, A., Cucala, M., Siegrist, S., von Ritter, C., Fischer, J.A., and Blum, A.L., 1989, Effects of hCGRP I and II on gastric blood flow and acid secretion in anesthetized rabbits, **Am. J. Physiol.**, 256:G145.

Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R., and MacIntyre, I., 1985, Calcitonin gene-related peptide is a potent vasodilator, **Nature (London)**, 313:54.

Cheung, L.Y., 1984, Gastric mucosal blood flow: its measurement and importance in mucosal defense mechanisms, **J. Surg. Res.**, 36:282.

Crossman, D.I., McEwan, J., MacDermot, J., MacIntyre, I., and Dollery, C.T., 1987, Human calcitonin gene-related peptide activates adenylate cyclase and releases prostacyclin from human umbilical vein endothelial cells, **Br. J. Pharmacol.**, 92:695.

Diel, F., and Szabo, S., 1986, Dose-dependent effects of linear and cyclic somatostatin on ethanol-induced gastric erosions: the role of mast cells and increased vascular permeability, **Regulatory Peptides**, 13:235.

Dunning, B.E., and Taborsky, G.J., 1987, Calcitonin gene- related peptide: a potent and selective stimulator of gastrointestinal somatostatin secretion, **Endocrinology**, 120:1774.

Evangelista, S., Maggi, C.A., and Meli, A., 1986, Evidence for role of adrenals in the capsaicin-sensitive "gastric defense mechanism" in rats, **Proc. Soc. Exp. Biol. Med.**, 182:568.

Fändriks, L., and Stage, L., 1986, Simultaneous measurements of gastric motility and acid-bicarbonate secretions in the anesthetized cat, **Acta Physiol. Scand.**, 128:563.
Forssell, H., and Olbe, L., 1985, Continuous computerized determination of gastric bicarbonate secretion in man, **Scand. J. Gastroenterol.**, 20:767.
Green, T., and Dockray, G.J., 1988, Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig, **Neuroscience**, 25:181.
Guth, P.H., and Leung, F.W., 1987, Physiology of the gastric circulation, in: "Physiology of the Gastrointestinal Tract", L.R. Johnson, ed., Raven Press, New York.
Guth, P.H., Paulsen, G., and Nagata, H., 1984, Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat: effect of prostaglandin cytoprotection, **Gastroenterology**, 87:1083.
Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.
Holzer P., and Lippe, I.Th., 1988, Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa, **Neuroscience**, 27:981.
Holzer, P., Pabst, M.A. and Lippe, I. Th., 1989, Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa, **Gastroenterology**, 96:1425.
Holzer, P., Pabst, M.A., Lippe, I.Th., Peskar, B.A., Peskar, E.H., Livingston, E.H., and Guth, P.H., 1990a, Afferent nerve-mediated protection against deep mucosal damage in the rat stomach related to increased mucosal blood flow but not eicosanoid formation, **Gastroenterology**, in press.
Holzer, P., Peskar, B.A., Peskar, E.H., and Amann, R.,1990b, Release of calcitonin gene-related peptide induced by capsaicin in the vascularly perfused rat stomach, **Neuroscience Letters**, in press.
Holzer, P., and Sametz, W., 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons, **Gastroenterology**, 91:975.
Kawasaki, H., Takasaki, K., Saito, A., and Goto, K., 1988, Calcitonin gene-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance vessels of the rat, **Nature (Lond.)**, 335:164.
Lenz, H.J., Mortrud, M.T., Rivier, J.E., and Brown, M.R., 1985, Calcitonin gene-related peptide inhibits basal, pentagastrin, histamine and bethanechol stimulated gastric acid secretion, **Gut**, 26:550.
Leung, F.W., Tallos, E.G., Taché, Y.F., and Guth, P.H., 1987, Calcitonin gene-related peptide inhibits acid secretion without modifying blood flow, **Am. J. Physiol.**, 252:G215.
Lippe, I.Th., Lorbach, M., and Holzer, P., 1989a, Close arterial infusion of calcitonin gene-related peptide into the rat stomach inhibits aspirin- and ethanol-induced hemorrhagic damage, **Regulatory Peptides**, 26:35.
Lippe, I.Th., Pabst, M.A., and Holzer, ,P., 1989b, Intragastric capsaicin enhances rat gastric acid elimination and mucosal blood flow by afferent nerve stimulation, **Br. J. Pharmacol.**, 96:91.
Lopez-Belmonte, J., and Whittle, B.J.R., 1990, Inhibition of endothelin-1-induced rat gastric damage by calcitonin gene-related peptide, **Br. J. Pharmacol.**, 99:153.

Maggi, C.A., Evangelista, S., Giuliani, S., and Meli, A., 1987, Anti-ulcer activity of calcitonin gene-related peptide in rats, **Gen. Pharmacol.**, 18:33.

Marshall, I., Al-Kazwini, S.J., Holman, J.J., and Craig, R.K., 1986, Human and rat alpha-CGRP but not calcitonin cause mesenteric vasodilatation in rats, **Eur. J. Pharmacol.**, 123:217.

Mulderry, P.K., Ghatei, M.A., Spokes, R.A., Jones, P.M., Piersson, A.M. Hamid, Q.A., Kause, S., Amara, S.G., Burrin, J.M., Legon, S., Polak, J.M., and Bloom, R., 1988, Differential expression of alpha-CGRP and beta-CGRP by primary sensory neurons and enteric autonomic neurons of the rat, **Neuroscience**, 25:195.

Pihan, G., Majzoubi, D., Haudenschild, C., Trier, J.S., and Szabo, S., 1986, Early microcirculatory stasis in acute gastric mucosal injury in the rat and prevention by 16,16-dimethyl prostaglandin E_2 or sodium thiosulfate, **Gastroenterology**, 91:1415.

Robert, A., Nezamis, J.E., Lancaster, C., and Hanchar, A.J., 1979, Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury, **Gastroenterology**, 77:433.

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.

Sternini, C., Reeve, J.R., and Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.

Su, H.C., Bishop, A.E., Power, R.F., Hamada, Y., and Polak, J.M., 1987, Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas, **J. Neurosci.**, 7:2674.

Szolcsányi, J., and Barthó, L, 1981, Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat, in: "Gastrointestinal Defense Mechanisms", G. Mózsik, O. Hänninen and T. Jávor, eds., Pergamon Press, Oxford, and Akadémiai Kiadó, Budapest.

Taché, Y., 1988, CNS peptides and regulation of gastric acid secretion, **Ann. Rev. Physiol.**, 50:19.

Tague, L.L., and Jacobson, E.D., 1976, Evaluation of [^{14}C-] aminopyrine clearance for determination of gastric mucosal blood flow, **Proc. Soc. Exp. Biol. Med.**, 151:707.

Uddman, R., Edvinsson, L., Ekblad, E., Håkanson, R., and Sundler, F., 1986, Calcitonin gene-related peptide (CGRP): perivascular distribution and vasodilatory effects, **Regulatory Peptides**, 15:1.

Whittle, B.J.R., and Esplugues, J.V., 1988, Induction of rat gastric damage by the endothelium-derived peptide endothelin, **Br. J. Pharmacol.**, 95:1101.

Zdon, M.J., Adrian, T.E., and Modlin, I.M., 1988, Gastric somatostatin release: evidence for a direct mediation by calcitonin gene-related peptide and vasoactive intestinal peptide, **J. Surg. Res.**, 44:680.

VISCERAL PAIN: PATHOPHYSIOLOGY AND CLINICAL ASPECTS

Paolo Procacci, Marco Maresca, and
Riccardo Maria Cersosimo

Pain Center, University of Florence, Italy

The sensations normally felt in the viscera are generally known as "common sensations" or "coenaesthesia" (from the Greek koiné = common and aìsthesis = sensation). These sensations are many and are not as well defined as somatic sensations. Typical examples are for the stomach a feeling known as appetite and for the rectum and the bladder a feeling of fullness.

A common sensation can become clearly unpleasant but not painful, as in motion sickness or in the sensation of "air hunger". We must note that in the viscera, much more than in somatic tissues, transitional and unpleasant feelings are often felt. When they reach a certain level, they become painful. Keele (1962), in his studies on sensations induced by chemicals on cantharidin blister in the skin, used the term "metaesthesia" to indicate these feelings. The term seems very appropriate for many visceral sensations that are unpleasant but below the level of pain, such as a feeling of disagreeable fullness or acidity of the stomach, a sensation of light cramp in the abdomen, or the desire to micturate when it is not possible. These "metaesthesic sensations" can arise from every viscus, but more often from the hollow viscera, i.e. from the gastrointestinal and urogenital tract. They can remain under the level of pain even for a long time, but they give a strong sense of malaise and anxiety. They can disappear, but they can also precede the onset of visceral pain, such as a typical attack of angina pectoris, a renal or biliary colic, or a strong gastric pain. The metaesthesic sensations are not generally considered in modern textbooks of pain, but we observed that they are extremely important. Not infrequently, a myocardial infarction is defined painless, but a careful clinical history shows that the patient felt a sense of gastric fullness or "indigestion". These sensations are also important in other diseases, such as gastritis or irritable colon.

Visceral pain can arise from a visceral common sensation. Sensations from the bladder are typical examples. The common sensation is the feeling of fullness, accompanied by a desire to micturate. If it is inconvenient to micturate, the desire to do so becomes stronger and unpleasant, but it is still not painful.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

In severe urinary retention, as occurs in acute urinary bladder obstruction, this unpleasant feeling becomes increasingly painful.

The origin of metaesthesic and painful visceral sensations has been discussed in the past and recently; the discussion is still open. High threshold receptors, considered as true nociceptors by some schools, have been identified in the skin (Burgess and Perl, 1967; Bessou and Perl, 1969, Perl, 1985). As regards visceral organs, there is some experimental evidence for the existence of a separate category of high threshold receptors in some organs such as the heart, the lungs, the gallbladder and biliary ducts, the testes and perhaps the uterus (Widdicombe, 1974; Baker et al., 1980; Cervero, 1982; Kumazawa, 1986; Robbins et al., 1987). On the other hand, Iggo (1974), Wall (1978), Malliani et al., (1989) observed that painful stimuli can induce an increased activity of receptors which at low level of activity are involved in regulating reflexes. They suggested that such receptors at high level of activity break through to give the conscious perception of pain and consequently they did not accept the existence of true nociceptors, at least in the viscera. This is the old problem of specificity of sensory receptors, which has been widely debated in the past and in recent times. There are still now two main currents of thought: one assumes that receptors are specific for each type of sensation, the other considers the specificity of sensation as due to a different pattern or processing of information. We have already extensively discussed this problem (Procacci and Maresca, 1984).

It is well known that most internal organs have a dual afferent innervation. Some afferent fibres are joined to sympathetic nerves, other afferent fibres to the parasympathetic nerves. Visceral pain has been considered as only due to impulses transmitted by afferent fibres running in sympathetic nerves; however, afferent fibres running in parasympathetic nerves can also play a role in visceral pain, at least for some particular radiation (a typical example is cardiac pain radiated to the neck and to the jaws).

Some particular aspects are observed in the innervation of the gastrointestinal system. Langley (1921) defined the "enteric nervous system" as a third autonomic division, different from the sympathetic and parasympathetic division for its anatomical and functional properties. Although the central nervous system projects to the enteric nervous system, this input from the central nervous system is quantitatively minor in comparison to the number of intrinsic enteric neurons, which may mediate reflex activity independently of central control. The enteric nervous system has therefore been likened to a mini-"brain" in the bowel (Gershon and Rothman, 1984).

Visceral afferent fibres are few in number in comparison to somatic afferent fibres (about 10% of the total amount of spinal afferent fibres in the cat)(Dounman, 1965; Cervero et al., 1984). But the relatively small number of visceral afferent fibres innervates an area equivalent to at least a quarter of the body surface, with extensive ramifications of fibres and a great overlap between the fields of adjacent dorsal roots (even up to 100%)(Downman, 1965). As a consequence, afferent impulses from a given visceral area enter the central nervous system via many dorsal roots. This "multiple entry" may ensure widespread

evocation of reflexes and spatial summation in the central nervous system of the impulses evoking pain.

As there is a continuum of sensations (from normal common sensations to pain) in human pathophysiology, we can suppose that there is a continuum in physiological mechanisms. The first step is represented by the enteric nervous system and by locally acting substances; the second step is represented by the sensory nerves, with a centrifugal and a centripetal flux of fast and slow acting substances; the third step is represented by the most elementary spinal reflexes, or, perhaps, by a possible reflex action through the sympathetic ganglia; the fourth step is represented by more and more integrated reflexes, up to conditioned reflexes. Every step corresponds to more complex actions of different substances, fast and slow, acting in periphery or in the synapses. The synapse itself is now considered as a very plastic structure. Synaptic receptors can be sensitized or desensitized, can be masked or unmasked. The transmitters and the modulators are many and their interplay can change at every moment. The consequence is that the whole nervous system must be considered a plastic structure. This explains why processing of sensations can be modified in different moments and why pain can appear in the same anatomical but not functional conditions.

The stimuli apt to induce pain in viscera are different from those which induce pain in somatic structures. This explains why in the past the viscera were considered to be insensitive to pain. In fact, it was observed that viscera could be exposed to such stimuli as burning or cutting without evoking pain. This apparent insensitivity of the viscera was due to failure to apply adequate stimuli.

The main factors capable of inducing pain in visceral structures are the following (Ayala, 1937; Procacci et al., 1986):

1) abnormal distension and contraction of the hollow viscera muscle walls;
2) rapid stretching of the capsule of such solid visceral organs as the liver, spleen and pancreas;
3) abrupt anoxemia of visceral muscles;
4) formation and accumulation of pain-producing substances;
5) direct action of chemical stimuli, especially important in the esophagus and stomach;
6) traction or compression of ligaments and vessels;
7) necrosis of some structures (myocardium, pancreas).

Some of these factors may be concomitant and interact in many clinical conditions.

As regards the contraction of the hollow viscera wall, it must be noted that a strong contraction in isometric conditions provokes a more severe pain than in isotonic conditions. This may explain the strong pain of some diseases, such as acute mechanical intestinal obstruction and biliary or ureteral colics.

The different visceral structures show different pain sensitivity. Serous membranes have the lowest pain threshold and are followed, in order of ascending threshold, by the hollow viscera wall and by parenchymatous organs.

Experimental investigations have been carried out in man on algogenic visceral conditions. Esophageal pain has been induced

by many authors, using mechanical, electrical and chemical stimuli. Gastric algogenic conditions were studied by Wolf and Wolff (1947) on a patient with a large gastric stoma. Different kinds of stimuli were applied. No pain could be induced when the healthy mucosa of the fundus of the stomach was squeezed between the blades of a forceps. Electrical stimuli, intense enough to cause pain in the tongue, and chemical stimuli, such as 50 or 90% alcohol, 1.0 N HCl, 0.1 N Na OH and 1:30 suspension of mustard, were also ineffective in evoking pain when applied to healthy gastric mucosa. However, if the mucosa was inflamed, all the above-mentioned procedures induced strong pain. Kinsella (1948) observed that squeezing the inflamed appendix provoked pain.

True visceral pain is deep, dull, not well defined and differently described by the patients. It is difficult to locate this type of pain, which tends to radiate and frequently reaches parts of the body that are far from the affected organ. It is often accompanied by a sense of malaise. It induces strong autonomic reflex phenomena, including diffuse sweating, vasomotor responses, changes of arterial pressure and heart rate, and an intense psychic alarm reaction.

When an algogenic process affecting a viscus recurs frequently or becomes more intense and prolonged, the location becomes more exact and the painful sensation is progressively felt in more superficial structures, sometimes far from the site of origin. This phenomenon is usually called "referred pain". It seems to be caused by two different mechanisms which, at least in part, overlap (Procacci et al., 1986): 1) central convergence of visceral and cutaneous impulses, 2) visceromuscular and viscerocutaneous reflexes which give rise to algogenic conditions in the periphery.

In clinical practice, the distinction between true visceral pain, referred pain and somatic pain is often difficult: these are the so-called "intricate conditions". The concept was first proposed by Froment and Gonin (1956), who observed that angina pectoris can be related to cervical osteoarthritis, esophageal hernia or cholecystitis. A very important clinical problem often arises, i.e. whether a symptomatology that can be defined, according to Heberden's definition, "angina pectoris", is due in different patients to diseases of the esophagus, to ischemia of the heart, to a fibrositis of the chest muscles or to many of these factors. As a matter of fact, we have observed all these possibilities, i.e. that a single algogenic factor gives origin to the pain, or that many factors intermingle.

The intricate conditions, well known for cardiac pain, are even more complex in the gastrointestinal tract. Here, in fact, we must consider the interactions between the nervous system and autacoids in the extensive sense of the word, i.e. peptides and other substances. This micromilieu can vary in every moment and consequently the activity and the relationships of different substances can change both at the receptor level and in the synaptic junction. But in the gastroenteric system we must add a peculiar factor; the action of food on receptors and the interactions between the products of digestion and autacoids on receptors and other structures (pacemakers, etc.). The problem, as stressed many years ago by Keele and Armstrong (1964), is extre-

mely complex. This seems a major problem in gastroenterology and in pathophysiology in general.

Mnemonic traces may play an important role in visceral pain. It has been demonstrated that the mnemonic process is facilitated if the experience to be retained is repeated many times or is accompanied by pleasant or, above all, unpleasant emotions (Benedetti, 1969). It has also been demonstrated that phenomena of learning play an important role in pain experience (Melzack, 1973). Pain experience develops not only in learning avoidance reflexes but also as memory. Nathan (1962) observed that in some subjects different kinds of stimuli could call to mind forgotten painful experiences. We carried out a series of investigations on patients with previous myocardial infarction and on patients with angina pectoris, who appeared normal at a complete exam of sensibility (Procacci et al., 1968, 1972). In these patients we provoked a sensory stimulation in the same metameres of the heart by inducing ischemia of the upper limbs, with the limbs at rest. In normal subjects, ischemia of the limbs at rest induces paraesthesic sensations but never pain. In many patients with previous myocardial infarction, ischemia of the upper limbs provoked the onset of pain similar to that felt during the infarction; in many patients with angina pectoris, ischemia of the upper limbs provoked a pain attack similar to an attack of angina pectoris. These phenomena are probably due to the activation of mnemonic traces. The formation of mnemonic traces is facilitated in myocardial infarction by the strong emotions which accompany pain, in angina pectoris by the repetition of attacks.

Similar mechanisms may also be active in painful diseases of the abdominal organs. We observed that, during the first biliary or renal colic, referred pain followed true visceral pain after a variable interval. In subsequent episodes, referred pain developed promptly and was not preceded by true visceral pain. These phenomena may be considered as due to the activation of mnemonic traces.

In conclusion, the problem of visceral pain appears extremely complicated because many factors, as we have said before, can interplay both at a peripheral and at C.N.S. level. Further studies of biochemistry, neurophysiology and clinics seem opportune.

References

Ayala, M., 1947, Douleur sympathique et douleur viscérale, **Rev. Neurol.**, 68:222.

Baker, D.G., Coleridge, H.M., Coleridge, J.C.G., and Nerdrum, T., 1980, Search for a cardiac nociceptor; stimulation by bradykinin of sympathetic afferent nerve endings in the heart of the cat, **J. Physiol.**, 306:519.

Benedetti, G., 1969, "Neuropsicologia", Feltrinelli, Milano.

Bessou, P., and Perl, E.R., 1969, Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli, **J. Neurophysiol.**, 32:1025.

Burgess, P.R., and Perl, E.R., 1967, Myelinated afferent fibres responding specifically to noxious stimulation of the skin, **J. Physiol. (Lond.)**, 190:541.

Cervero, F., 1982, Afferent activity evoked by natural stimulation of the biliary system in the ferret, **Pain**, 13:137.

Cervero, F., Connell, L.A., and Lawson, S.N., 1984, Somatic and visceral primary afferents in the lower thoracic dorsal root ganglia of the cat, **J. Comp. Neurol.**, 228:422.
Downman, C.B.B., 1965, Viscerotomes and dermatomes: some comparison of the inner and outer surfaces of the body, in: "Studies in Physiology", D.R. Curtis and A.K. Mc Inktyre, eds., Springer, Berlin.
Froment, R., and Gonin, A., 1956, "Les Angors Coronariens Intriqués", Expansion Scientifique Française, Paris.
Gershon, M.D., and Rothman, T.P., 1984, Experimental and genetic approaches to the study of the development of the enteric nervous system, **Trends Neurosci.**, 7:150.
Iggo, A., 1974, Pain receptors, in: "Recent Advances on Pain", J.J. Bonica, P. Procacci and C.A. Pagni, eds., Thomas, Springfield.
Keele, C.A., 1962, Sensations aroused by chemical stimulation of the skin, in: "The Assessment of Pain in Men and Animals", C.A. Keele and R. Smith, eds., Livingstone, Edinburgh.
Keele, C.A., and Armstrong, D., 1964, "Substances Producing Pain and Itch", Arnold, London.
Kinsella, V.J., 1948, "The Mechanism of Abdominal Pain", Australasian Medical Publishing Company, Sidney.
Kumazawa, T., 1986, Sensory innervation of reproductive organs, in: "Progress in Brain Research, Vol. 67: Visceral Sensation", F. Cervero and J.F.B. Morrison, eds., Elsevier, Amsterdam.
Langley, J.N., 1921, "The Autonomic Nervous System", Heffer, Cambridge.
Malliani, A., Pagani, M., and Lombardi, F., 1989, Visceral versus somatic mechanisms, in: "Textbook of Pain", P.D. Wall and R. Melzack, eds., Churchill Livingstone, Edinburgh.
Melzack, R., 1973, "The Puzzle of Pain", Penguin Books, Harmondsworth.
Nathan, P.W., 1962, Pain traces left in the central nervous system, in: "The Assessment of Pain in Man and Animals", P.D. Wall and R. Melzack, eds., Churchill Livingstone, Edinburgh.
Perl, E.R., 1985, Unraveling the story of pain, in: "Advances in Pain Research and Therapy, Vol. 9", H.L. Fields, R. Dubner and F. Cervero, eds., Raven Press, New York.
Procacci, P., and Maresca, M., 1984, Pain concept in Western civilization: a historical review, in: "Advances in Pain Research and Therapy, Vol. 7", C. Benedetti, C.R. Chapman and G. Moricca, eds., Raven Press, New York.
Procacci, P., Buzzelli, G., Voegelin, M.R., and Bozza, G., 1968, Esplorazione della funzione sensitiva degli arti superiori in soggetti con pregresso infarto miocardico, **Rass. Neurol. Veg.**, 22:403.
Procacci, P., Passeri, I., Zoppi, M., Burzagli, L., Voegelin, M.R., and Maresca, M., 1972, Esplorazione della funzione sensitiva degli arti superiori in soggetti con angina pectoris, **Giorn. Ital. Cardiol.**, 2:978.
Procacci, P., Zoppi, M., and Maresca, M., 1986, Clinical approach to visceral sensation, in: "Progress in Brain Research, Vol. 67: Visceral Sensation", F. Cervero and J.F.B. Morrison, eds., Elsevier, Amsterdam.
Robbins, A., Sato., Y., Hotta, H., and Berkley, K.J., 1987, Response of uterine afferent fibers in the rat to sodium ajamide and CO_2., **Pain**, Suppl.4:S20.
Wall, P.D., 1988, Stability and instability of central pain mechanisms, in: "Proceedings of the Vth World Congress on

Pain", R. Dubner, G.F. Gebhart and M.R. Bond, eds., Elsevier, Amsterdam.
Widdicombe, H.G., 1974, Enteroceptors, in: "The Peripheral Nervous System", J.I. Hubbard, ed., Plenum Press, New York.
Wolf S., and Wolff, H.G., 1947, "Human Gastric Function. An Experimental Study of a Man and His Stomach", Oxford University Press, New York.

CENTRAL AND PERIPHERAL ACTIONS OF CALCITONIN GENE-RELATED PEPTIDE ON GASTRIC SECRETORY AND MOTOR FUNCTION

Yvette Taché, Helen Raybould, and Jen Yu Wei

Center for Ulcer Research and Education
VA Wadsworth Medical Center
Department of Medicine and Brain Research Institute
UCLA, Los Angeles, California, USA

Calcitonin gene related peptide (CGRP) was one of the first examples of a biologically active peptide to be identified by recombinant DNA and molecular biological approach (Rosenfeld et al., 1983; Amara et al., 1985). It was also the first demonstration of tissue-specific alternative processing of a gene. In 1983, Rosenfeld et al. initially reported that the RNA transcript from the calcitonin gene is processed in rat central and peripheral neural tissues to a mRNA encoding the precursor to a previously unknown 37-residue peptide called CGRP or α-CGRP (Rosenfeld et al., 1983). In thyroidal cells, the calcitonin gene generates a mRNA which encodes a calcitonin precursor protein expressing the calcium-regulating hormone, calcitonin (Rosenfeld et al., 1983). Subsequently, both intra- and interspecies variants have been discovered. A second calcitonin gene generating a mRNA expressing β-CGRP or CGRP II was identified in rats (Amara et al., 1985). Rat β and α forms differ in only one amino acid residue at position 35. The same mechanism of specific alternate RNA processing was described in human tissue. The calcitonin gene leads to human α-CGRP or CGRP I which differs from rat α-CGRP by four amino-acids in positions 1, 3, 25 and 35 (Steenbergh et al., 1986). The presence of a second human calcitonin gene encoding β-CGRP or CGRP II which differs from human α-CGRP by three amino acids in positions 3, 22 and 25 has also been reported (Steenbergh et al., 1985). Rat α-CGRP was originally identified in the absence of biological activity. However, the first report on its characterization showed the ubiquitous distribution of CGRP-like immunoreactivity in the central nervous system (CNS) with dense representation in forebrain and medullary nuclei, and in pathways modulating ingestive behavior and autonomic outflow (Rosenfeld et al., 1983). In the periphery, CGRP-LI is present in neurons associated with blood vessels and visceral organs including the gastrointestinal tract (Rosenfeld et al., 1983). The neuroanatomical distribution of the peptide prompted us to study the central and peripheral actions of CGRP to influence gastric function. We initially reported that rat α-CGRP exerts potent central and peripheral actions to inhibit gastric acid secretion in rats (Taché et al., 1984b) and in dogs (Taché et al., 1984a). In this chapter, the state of

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

knowledge on CNS and peripheral effects of CGRP on gastric acid secretion and motor functions, and possible physiological relevance will be reviewed.

CENTRAL ACTION OF CGRP TO INHIBIT GASTRIC FUNCTION

Inhibition of Gastric Acid Secretion by Central CGRP

Injection of rat α-CGRP into the cerebrospinal fluid (CSF) either into the lateral ventricle or cisterna magna inhibits gastric acid secretion in awake rats with ligature of the pylorus (Taché et al., 1984b; Hughes et al., 1984; Lenz et al., 1984). The inhibitory effect was further demonstrated in anesthetized rats when acid secretion was stimulated by peripheral injection of histamine, pentagastrin and bethanechol and by central injection of the vagal stimulant, thyrotropin-releasing factor (TRH) (Taché et al., 1984b; Lenz et al., 1985b; Hughes et al., 1984). In awake dogs, the injection of rat or human α-CGRP into the third ventricle also inhibited gastric acid secretion stimulated by pentagastrin, 2-deoxy-D-glucose, and by a peptone meal but not by histamine (Lenz et al., 1986a; Lenz and Brown, 1987; Lenz et al., 1986b). The ED50 for intracisternal or intracerebroventricular CGRP-induced inhibition of pentagastrin-stimulated acid secretion is 10-13 pmol (Figure 1) (Taché et al., 1984b; Lenz et al., 1984; Lenz et al., 1985; Lenz et al., 1986a). α-CGRP is, with bombesin and calcitonin, the most potent central inhibitor of gastric acid secretion in the rat and dog (Taché et al., 1980; Lenz et al., 1989; Lenz et al., 1986b). Structure activity studies showed that the disulfide bridge of the N-terminus is required and that the N- and C-terminal fragments, α-CGRP-14 and α-CGRP-37, are devoid of biological activity (Lenz et al., 1985; Lenz et al., 1986a). Rat and human α-CGRP are equipotent (Lenz et al., 1985; Lenz and Brown, 1987).

The inhibitory effect of CGRP injected into the CSF represents a centrally-mediated action and not leakage of the peptide from the CSF to the periphery. When CGRP is injected at an antisecretory dose into the dog third ventricle, there is no detectable change in CGRP-like immunoreactivity into the circulation (Lenz et al., 1986b). In rats, 8% leakage of CGRP from the lateral brain ventricle to the peripheral circulation has been

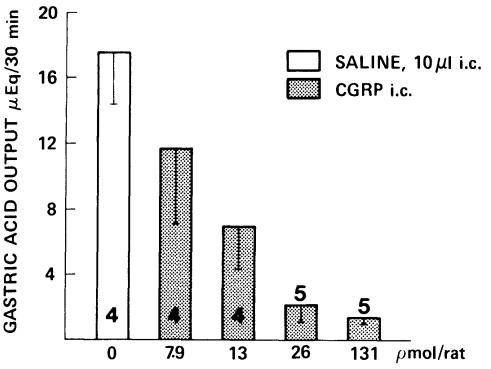


Fig. 1. Dose related inhibition of pentagastrin-stimulated acid secretion by intracisternal injection of rat α-CGRP in urethane-anesthetized rats.

reported (Bauerfeind et al., 1989b). A central action was further ascertained by the demonstration that CGRP antiserum injected intravenously does not alter the inhibitory effect of CGRP injected into the CSF but abolishes that induced by intravenous administration of the peptide (Lenz et al., 1984). Studies using microinjection of rat α-CGRP into specific brain nuclei have localized responsive sites to the dorsal vagal complex and to a lower extent in the lateral hypothalamus whereas the nucleus ambiguus was inactive (Ishikawa and Taché, 1989; Taché et al., 1984b; Ishikawa and Taché, 1988). The influence of α-CGRP on extracellular activity of single neurons in rat forebrain was found to be predominantly inhibitory (Twery and Moss, 1985). Whether such alteration of neuronal activity underlied peptide action on preganglionic vagal neurons needs to be further investigated.

Electrophysiological, surgical and pharmacological evidence in the rat indicates that the action of CGRP from the brain to the stomach is mediated through vagal pathways. We recently demonstrated that intracisternal injection of α-CGRP inhibits unit efferent discharges recorded from the gastric branch of the vagus (Wei and Taché, 1990). The inhibitory effect is dose related in terms of the magnitude and duration (Figure 2). Moreover, the inhibition of pentagastrin-stimulated acid secretion induced by CSF injection of CGRP is abolished by vagotomy (Taché et al., 1984b; Lenz et al., 1985b). Lastly, microinjection of CGRP into the dorsal vagal complex which regulates vagal outflow to the stomach (Shapiro and Miselis, 1985), inhibits gastric acid secretion stimulated by pentagastrin or by the vagal stimulant, baclofen (Figure 3) (Goto et al., 1985a; Ishikawa and Taché, 1989). Although central injection of CGRP was also reported to increase selectively noradrenergic sympathetic activity (Fisher et al., 1983; Lenz and Brown, 1990), this pathway is not involved since neither adrenalectomy alone or combined with chemical sympathectomy with guanethidine nor bretylium pretreatment alter the inhibitory effect of CGRP injected into the CSF (Taché et al., 1984b; Lenz et al., 1985b; Lenz et al. 1984). The lack of sympathetic involvement is further demonstrated by the inactivity of intrathecal (T9 level) injection of CGRP at doses effective intracisternally in conscious or anesthetized rats (Yang et al., 1989). In the dog, by contrast, there is no evidence that α-CGRP injected into the third ventricle acts through vagal pathways since CGRP effect is not blocked by vagotomy or ganglionic blockade by chlorisondamine (Lenz et al., 1986a; Lenz et al., 1986b).

Peripheral mechanisms through which the inhibitory effect of CGRP is expressed are yet to be elucidated in rats and dogs. It does not involve changes in circulating gastrin or gastric mucosal blood flow since these parameters are increased rather than decreased after central injection of CGRP in the rat (Taché et al., 1984b; Bauerfeind et al., 1987; Bauerfeind et al., 1989b). In the dog, vasopressin-, opiate- and gastrin-dependent pathways have also been ruled out (Lenz et al.,1986a; Lenz et al., 1986b).

Inhibition of Gastric Motor Function by Central CGRP

Less data are available on the central action of CGRP to inhibit gastric motor function and most of them have been obtained in the rat. Intracisternal or intracerebroventricular

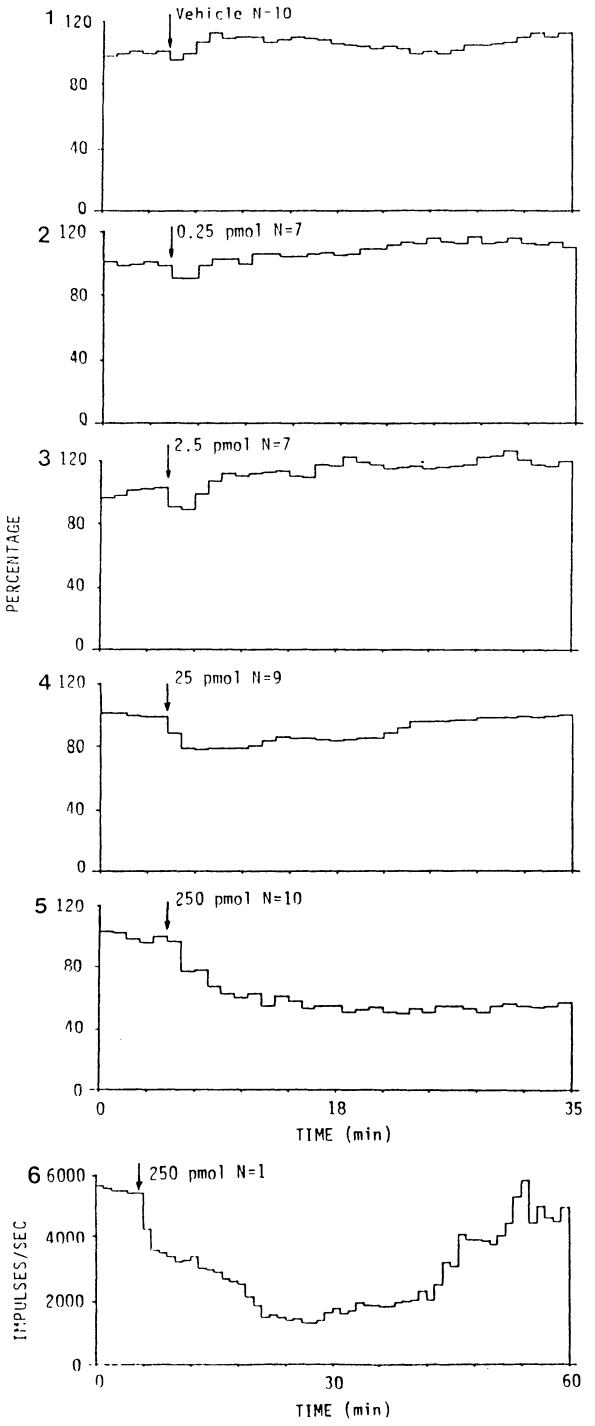


Fig.2

Histogram of dose dependent inhibition of gastric vagal efferent discharges induced by intracisternal injection of rat α- CGRP in urethane-chloralose anesthetized rats. For each experiment, a computer normalizing program takes the mean of 5 min discharge rate (impulses/min) before vehicle or rat α-CGRP injection as 100% and then compares each min impulses count to the mean discharge rate. Results are presented as percentage on the Y axis.

injection of rat α-CGRP delays gastric emptying of a liquid non caloric solution in the awake rat. The ED50 is similar to that inhibiting acid secretion (Raybould et al., 1988; Lenz, 1988). Brain sites of action of CGRP are still unknown. The inhibitory effect is mediated from the brain to the stomach through the autonomic nervous system (Raybould et al., 1988; Lenz, 1988). There is some disagreement concerning the autonomic pathways primarily involved. CGRP action was found completely prevented by adrenalectomy and coeliac ganglionectomy and vagotomy in rats (Raybould et al., 1988). Further pharmacological studies indicate that the adrenergic component of CGRP action involves β-adrenergic receptors (Figure 4) (Raybould et al., 1989). By contrast, in another study, intracerebroventricular injection of α-CGRP-induced delay in gastric emptying of non caloric solution was completely prevented by vagotomy and not by adrenalectomy, noradrenergic blockade or hypophysectomy in the rat (Lenz, 1988).

Intracisternal injection of CGRP at a dose inhibiting gastric emptying is associated with a decrease in tonic and phasic gastric contractions as measured by changes in intraluminal pressure in the fasted anesthetized rat (Figure 5) (Raybould et al., 1989). Consistent with a mediation through the sympathetic nervous system previously observed on gastric emptying (Raybould et al., 1988), the decrease in intraluminal pressure was completely blocked by adrenalectomy combined coeliac and mesenteric ganglionectomy (Figure 6) and partly phentolamine and propranolol (Raybould et al., 1989). In sheep, central injection of CGRP before feeding has an inhibitory effect on gastric contractility (Buéno et al., 1986).

Inhibition of Experimental Gastric Lesions by Central CGRP

Centrally acting peptides which inhibit gastric acid secretion and motor function exert a protective effect against experimental gastric lesions produced by stress but not by necrotizing agents (Taché, 1987b; Taché, 1989; Taché and Ishikawa, 1989). Similarly, intracisternal injection of α-CGRP inhibits gastric erosions induced by exposure to cold restraint for 3 h, or by central injection of the vagal stimulant TRH (Goto and Taché,

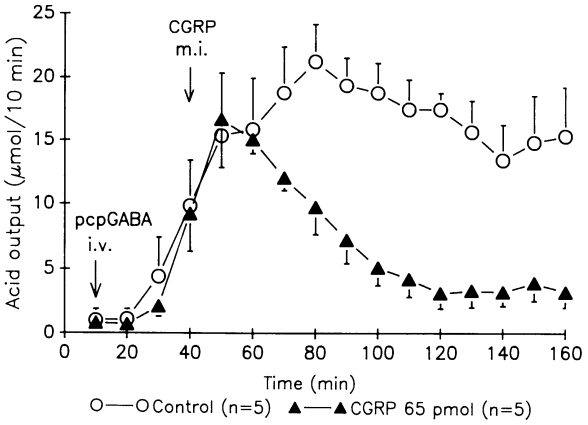


Fig. 3. Inhibition of baclofen (parachlorphenyl GABA, 2 mg/kg, iv)-induced stimulation of acid secretion by rat α-CGRP microinjected into the dorsal vagal complex in urethane-anesthetized rats.

1985b) in fasted rats (Kolve and Taché, 1989). However, unexpectedly, intracisternal injection of α-CGRP also prevents gastric lesions produced by intragastric administration of ethanol (40%, 1 ml) in fasted rats (Kolve and Taché, 1989). This property is unique to CGRP (Figure 7) in as much as intracisternal injection of other centrally acting antisecretory peptides such as bombesin, corticotropin releasing factor (CRF), or calcitonin which inhibited stress ulcer (Gunion et al., 1990); Ishikawa and Taché, 1988; Taché et al., 1979) do not prevent (bombesin, CRF) or even aggravate (calcitonin) ethanol-induced gastric lesions (Taché et al., 1987a; Kolve and Taché, 1989). The mechanisms by which central CGRP induces cytoprotection are not prostaglandin mediated (unpublished observations) and may be related to the increase in gastric mucosal blood flow which occurs following central peptide injection (Bauerfeind et al., 1987; Bauerfeind et al., 1989b). Recent findings support the view that increased gastric mucosal blood flow plays a protective role against ethanol-induced lesions (Holzer et al., 1990c).

Physiological Role of Central CGRP in the Regulation of Gastric Function

The physiological role of CGRP in central gastric control has yet to be defined. However, existing information on CGRP-immunoreactivity and receptor distribution in specific subsets of medullary (dorsal vagal complex, parabranchial nucleus) and forebrain nuclei (hypothalamus, central amygdala) receiving visceral information and influencing autonomic outflow provide anatomical substrate for such a role (Skofitsch and Jacobowitz, 1985; Morishima et al., 1985; Schwaber et al., 1988; Kruger et al., 1988). α- and β-CGRP mRNAs have the same distribution in the brain, however the α-CGRP is the predominant form expressed (Amara et al., 1985). Biochemical evidence indicates that both variants act on the same receptors in the brain. Based on binding assays in rat brain membrane, rat α and β CGRP appear equipotent (Dennis et al., 1990b) whereas human β CGRP displays a relatively higher potency over the α form (Kruger et al., 1988; Dennis et al., 1990b). The recently developed CGRP antagonist, human CGRP-37 (Dennis et al., 1990a), will provide a useful tool to assess physiological relevance of this peptide in relation with central regulation of gastric function.

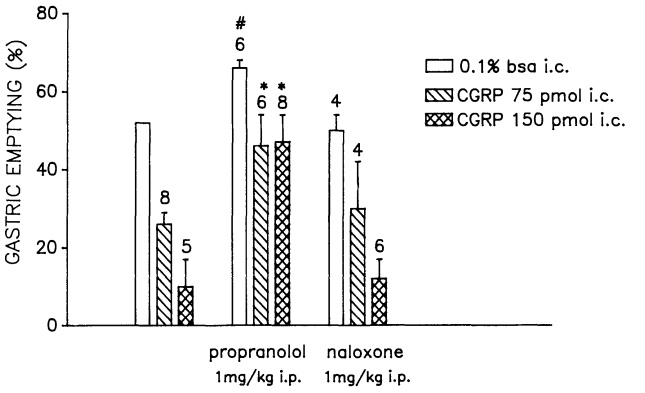


Fig. 4. Effect of propanolol and naloxone treatment on intracisternal CGRP-induced delay in gastric emptying of non caloric solution in conscious rats.

Characteristics of the Inhibitory Response

In assessing whether the inhibitory action of CGRP was specific to the central vs the peripheral route of administration, we found that intravenous injection of the peptide induces a potent suppression of acid secretion in the rat and dog (Taché et al., 1984a). Since then, the inhibitory influence of peripheral administration of CGRP on gastric acid secretion and mechanisms through which the peptide acts have received considerable attention.

In the rat, intravenous injection of rat α-CGRP inhibits acid secretion stimulated by pentagastrin, histamine, bethanechol and by vagal activation induced by intracisternal TRH (Taché et al., 1984a; Leung et al., 1987; Lenz et al., 1985a). Intravenous doses required to produce an inhibitory effect are in the same dose range as intracisternal doses in the rat (Lenz et al., 1984; Lenz et al., 1985; Taché et al., 1984b; Lenz et al., 1985a). Human and rat α-CGRP share the same biological activity whereas the linear molecule without the disulfide bond, the N-terminal fragments, CGRP-14, and C-terminal residues CGRP23-27 do not influence gastric acid secretion (Lenz et al., 1985). In awake dog, intravenous administration of rat or human α-CGRP inhibits gastric acid secretion stimulated by pentagastrin, a meal, sham feeding and bombesin whereas the acid response to histamine and bethanecol are not modified (Taché et al., 1984a; Lenz et al., 1986a; Pappas et al., 1986; Helton et al., 1989). Rat α-CGRP is one of the most potent inhibitors of acid secretion in both rats and dogs (Taché et al., 1984a; Pappas et al., 1986). In rabbits, human β-CGRP inhibits whereas human α-CGRP was reported to increase pentagastrin-stimulated acid secretion while both peptides increased gastric blood flow (Bauerfeind et al., 1989a). Further studies in healthy volunteers established that intravenous infusion of rat α-CGRP and human β-CGRP but not human α-CGRP result in a significant and prolonged inhibition of pentagastrin and bethanechol-stimulated acid secretion (Kraenzlin et al., 1985; Beglinger et al., 1988).

Mechanisms Involved in CGRP-Induced Inhibition of Acid Secretion

In the rat, the mechanisms through which peripheral rat α-CGRP inhibits acid secretion are independent from the vagus and prostaglandin synthesis or gastric mucosal blood flow which was unchanged or increased (Taché et al., 1984a; Leung et al., 1987; Bauerfeind et al., 1987; Bauerfeind et al., 1989b; Holzer et al., 1990b). α-CGRP has been shown to either produce no change in bombesin and gastrin release in the isolated perfused rat

Fig. 5. Recording of changes in gastric intraluminal pressure induced by intracisternal injection of rat α-CGRP in urethane-anesthetized rats.

stomach (Madaus et al., 1989) or to decrease gastrin release from antral mucosa/submucosa tissues (Young et al., 1989). In the dog, the inhibition of bombesin- or meal-stimulated acid secretion induced by intravenous infusion of rat α-CGRP is not related to suppression of gastrin secretion since the increase in plasma levels of gastrin in response to a meal or bombesin was not modified (Pappas et al., 1986). CGRP action is also independent from vagal innervation since the peptide inhibits pentagastrin-stimulated acid secretion in dogs with Heidenhain pouch (Taché et al., 1984a). In humans, intravenous infusion of rat α-CGRP decreases basal levels of stimulatory (gastrin), and inhibitory (enteroglucagon, gastric inhibitory peptide and neurotensin) peptides (Kraenzlin et al., 1985). Other studies in humans showed that the antisecretory effect of human β-CGRP was unrelated to increase in bicarbonate secretion, or changes in splanchnic blood flow (Beglinger et al., 1988).

There is consistent evidence in rats and dogs that CGRP inhibitory effect involves somatostatin release (Helton et al., 1989). Rat α- or β-CGRP is the most potent peptide to stimulate in vitro somatostatin secretion from isolated vascularly perfused rat stomach preparation (Yamatani et al., 1986; Koop et al., 1987; Chiba et al., 1989; Madaus et al., 1989) or from rat antral mucosa/submucosa tissues (Young et al., 1989; Young et al., 1990). The mechanisms by which α-CGRP increases somatostatin secretion in the isolated perfused rat stomach or rat antral mucosa/submucosa do not involve muscarinic or β-adrenergic receptors (Koop et al., 1987) or intramural neurons (Koop et al., 1987; Young et al., 1990). A recent study demonstrates that human α-CGRP induces a dose related release of somatostatin and parallel increase in celular cAMP content in D-cells preparation isolated from fundic canine mucosa (Chiba et al., 1989). Binding studies show the presence of specific CGRP binding sites on D cells (98,000 receptors/D cells) (Chiba et al., 1989). These findings suggest that CGRP acts directly on D-cell secretion through specific receptors linked with cAMP production (Chiba et al., 1989).

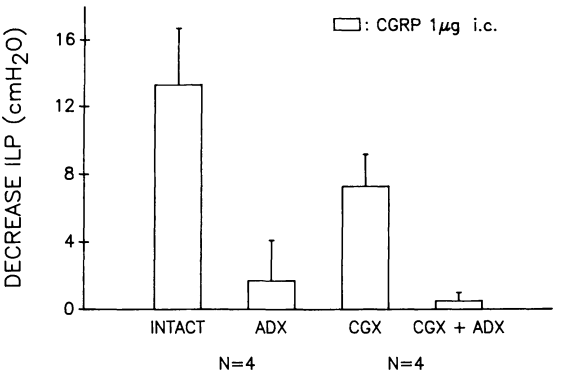


Fig. 6. Inhibitory effect of adrenalectomy (ADX), coeliac and mesenteric ganglionectomy (CGX) performed singly or in combination on the decrease in intraluminal pressure induced by intracisternal injection of rat α-CGRP in urethane-anesthetized rats.

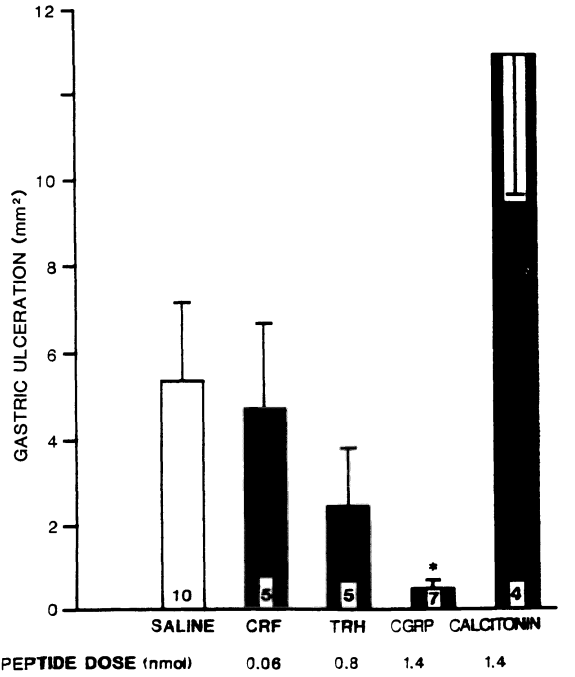


Fig.7. Effect of intracisternal injection of various peptides on gastric lesions induced by oral administration of ethanol in conscious fasted rats.

In conscious and anesthetized dogs, intravenous infusion of human α-CGRP increases intravenous plasma levels of somatostatin-like immunoreactivity and arterial concentrations of somatostatin-28 and somatostatin-14 (Dunning and Taborsky, 1987; Helton et al., 1989; Bunnett et al., 1990; Reasbeck et al., 1988). Human α-CGRP appears to be the most effective stimulant of gut somatostatin currently known in the dog (Dunning and Taborsky, 1987). CGRP action is not secondary to changes in blood pressure (Dunning and Taborsky, 1987). Further studies in pigs demonstrated that intra-arterial infusion of human α-CGRP stimulates the release of prosomatostatin-derived peptides and somatostatin 28 which originates mostly from the corpus but not from the antrum or intestine (Bunnett et al., 1990).

However, other mechanisms in addition to somatostatin are involved in mediating the inhibitory effect of CGRP on acid secretion. This is suggested by the demonstration that CGRP is a more potent inhibitor of acid secretion than somatostatin, and that changes in acid secretion and somatostatin levels are not always parallel, particularly at low doses of CGRP (Pappas et al., 1986; Helton et al., 1989). There are several reports that CGRP alters cholinergic transmission in the enteric nervous system. α-CGRP inhibits basal or K^+ stimulated acetylcholine release from the fundic and antral mucosa/submucosa of rats and this effect is sensitive to pertussus toxin (Young et al., 1989; Young et al., 1990; Orloff et al., 1989).

There is little evidence for an effect of CGRP directly at

the level of the parietal cell. A low number of binding sites (7,200/cells) has been found in the parietal cells (Chiba et al., 1989). Moreover rat α-CGRP did not alter the response to histamine in the isolated rabbit gastric gland (Taché et al., 1984a). In enriched parietal cell preparation from dog fundic mucosa, rat or human α-CGRP did not modify the aminopyrine response to bethanechol, histamine or pentagastrin (Taché et al., 1984a; Chiba et al., 1989). By contrast, in an enriched fraction of parietal cells from guinea-pig, human α-CGRP was reported to induce a dose related inhibition of aminopyrine accumulation stimulated by histamine, carbachol, pentagastrin and DBcAMP (Umeda and Okada, 1987). Whether these different results reflect species differences needs to be further investigated.

Inhibition of Gastric Motor Function by Peripheral CGRP

Intravenous injection of rat α-CGRP inhibits gastric emptying of a non caloric solution in rat (Raybould et al., 1988) and decreases gastric motility. Unlike CCK, the decrease in intraluminal pressure induced by intravenous CGRP is not altered by perivagal capsaicin treatment (unpublished observation). In studies using strips of guinea pig and rat fundus, human and rat α- and β-CGRP induced an equipotent and sustained relaxation of the strips (Katsoulis and Conlon, 1989). The effect is not modified in the presence of tetrodotoxin, adrenergic antagonists, somatostatin or a purinoreceptor antagonist suggesting a direct action on muscle cells (Katsoulis and Conlon, 1989). This has been further confirmed by the demonstration that human or rat α-CGRP are equipotent to inhibit carbachol-induced contraction of dispersed smooth muscle cells of guinea pig stomach. Two classes of specific receptors for CGRP with high and low affinity have been characterized on smooth cells (Maton et al., 1988). The occupation of receptors is linked to cAMP activation and causes cell relaxation (Maton et al., 1988).

Physiological Role of Peripheral CGRP in the Regulation of Gastric Function

The peripheral distribution and release of CGRP along with its potent biological actions on gastric acid secretion and motor activity upon intravenous injection suggest a role of the peptide in the regulation of gastric function. Large amounts of CGRP-like immunoreactivity are located in the stomach with high concentrations in the pylorus and throughout the submucosa and muscular layer (Inui et al., 1989). CGRP in the stomach is primarily located in neuronal processes of capsaicin-sensitive sensory nerve fibers of splanchnic origin (Varro et al., 1988; Green and Dockray, 1987). Recent neuroanatomical studies have revealed that sensory neurons express primarily α-CGRP whereas the intrinsic neurons in the enteric nervous system expressed only β-CGRP (Sternini and Anderson, 1990). CGRP is present in the venous effluent of the isolated and vascularly perfused rat stomach (Holzer et al., 1990a; Inui et al., 1989). Moreover, CGRP secretion is increased by stimulation of gastric sensory afferent pathways by capsaicin or dibutyryl cyclic AMP (Inui et al., 1989; Wimalawansa and MacIntyre, 1988). The release of CGRP at terminals of splanchnic afferent fibers following physiological stimulation of chemo- or mechano-receptors may play a role in a variety of reflex responses to modulate gastric tone and acid secretion. However, such a role needs to be further investigated by the use of specific antibodies and of newly developed

CGRP antagonists acting at the various receptor subtypes (Dennis et al., 1990b; Dennis et al., 1990a).

SUMMARY AND CONCLUSIONS

CGRP exerts a potent central action to inhibit gastric acid secretion in rats and dogs and gastric emptying, contractility and ulcer formation in rats. The site of action to inhibit acid secretion has been localized in the dorsal vagal complex. The inhibition of acid secretion is related primarily to the decrease in vagal efferent activity whereas the inhibition of gastric motor functions involves increases in sympathetic outflow. The central action of CGRP to prevent ethanol-induced lesions is unique to this peptide and not shared by other centrally acting inhibitors of gastric function. It may be related to the increase in gastric mucosal blood induced by central CGRP. The presence of CGRP-like immunoreactivity and receptors in medullary nuclei receiving visceral information and influencing vagal outflow suggests a possible role of the peptide in the vagal regulation of gastric secretion.

Peripheral injection of CGRP also inhibits acid secretion when administered peripherally in rats, dogs, rabbits and humans. Its antisecretory effect is unlikely to be related to a direct action on the parietal cells. It involves specific and marked release of gastric somatostatin through an interaction with CGRP receptors characterized on D cells and coupled with cAMP. In addition, CGRP induces a decrease in acetylcholine transmission in the enteric nervous system which may contribute to the inhibition of acid. Peripheral CGRP inhibits gastric emptying and motility by a direct action on smooth muscles through receptors linked with cAMP. The release of CGRP from spinal afferents innervating the stomach in response to stimulation of capsaicin-sensitive fibers suggests a role of the peptide in the regulation of gastric function.

Acknowledgements

The work was supported by the National Institute of Arthritis, Metabolism and Digestive Disease, Grants AM 30110 and 33061 and the National Institute of Mental Health, Grant MH-0063.

References

Amara, S.G., Arriza, J.L., Leff, S.E., Swanson, L.W., Evans, R.M., Rosenfeld, M.G., 1985, Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide, **Science**, 229:1094.

Bauerfeind, P., Cucala, M., Hof, R.P., Emde, C., Hof, A., Fischer, J., Blum, A.L., 1987, Calcitonin gene-related peptide mediates CNS regulation of gastric secretion and blood flow, **Gastroenterology**, 92:1311.

Bauerfeind, P., Hof, R., Hof, A., Cucula, M., Siegrist, S., Von Ritter, C., Fischer, J.A., Blum, A.L., 1989a, Effects of hCGRP I and II on gastric blood flow and acid secretion in anesthetized rabbits, **Am. J. Physiol.**, 256:G145.

Bauerfeind, P., Koerfer, J., Armstrong, D., Hof, R., Fischer, J.A., Blum, A.L., 1989b, Intravenous anti-CGRP inhibits the increase of gastric blood flow caused by centrally admini-

stered CGRP, **Gastroenterology**, 96:A34.
Beglinger, C., Born, W., Hildebrand, P., Ensinck, J.W., Burkhardt, F., Fischer, J.A., Gyr, K., 1988, Calcitonin gene-related peptides I and II and calcitonin: distinct effects on gastric acid secretion in humans, **Gastroenterology**, 95:958.
Buéno, L., Fargeas, M.J., Julie, P., 1986, Effects of calcitonin and CGRP alone or in combination on food intake and forestomach (reticulum) motility in sheep, **Physiol. Behav.**, 36:907.
Bunnett, N.W., Helton, W.S., Debas, H.T., Ensinck, J.W., 1990, CGRP stimulates the release of pro-somatostatin-derived peptides from the gastric fundus, **Am. J. Physiol.**, 258:G316.
Chiba, T., Park, J., Yamada, T., 1989, Calcitonin gene related peptide (CGRP) stimulates somatostatin secretion from isolated gastric D-cells via cyclic AMP dependent mechanisms, **Gastroenterology**, 96:A86.
Dennis, T., Fournier, A., Cadieux, A., Pomerleau, F., Jolicoeur, F.B., St. Pierre, S., Quirion, R., 1990a, hCGRP8-37, a calcitonin gene-related peptide antagonist revealing CGRP receptor heterogeneity in brain and periphery, **J. Pharmacol. Exp. Ther.**, in press.
Dennis, T., Fournier, A., St. Pierre, S., Quirion, R., 1990b, Structure-activity profile of calcitonin gene-related peptide in peripheral and brain tissue, **J. Pharmacol. Exp. Ther.**, 251:in press.
Dunning, B.E., Taborsky, G.J.Jr., 1987, Calcitonin gene-related peptide: a potent and selective stimulator of gastrointestinal somatostatin secretion, **Endocrinology**, 120:1774.
Fisher, L.A., Kikkawa, D.O., Rivier, J.E., Amara, S.G., Evans, R.M., Rosenfeld, M.G., Vale, W.W., Brown, M.R., 1983, Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide, **Nature**, 305:534.
Goto, Y., Taché, Y., Debas, H., Novin, D., 1985a, Gastric and vagus nerve response to GABA agonist baclofen, **Life Sci.**, 36:2471.
Goto, Y., Taché, Y., 1985b, Gastric erosions induced by intracisternal thyrotropin-releasing hormone (TRH) in rats, **Peptides**, 6:153.
Green, T., Dockray, G.J., 1987, Calcitonin gene-related peptide and substance P in afferents to the upper gastrointestinal tract in the rat, **Neurosci. Lett.**, 76:151.
Gunion, M.W., Kauffman, G.L., Taché, Y., 1990, Intrahypothalamic microinfusion of corticotropin-releasing factor elevates gastric bicarbonate secretion and protects against cold-stress ulceration in rats, **Am. J. Physiol.**, 258:G152.
Helton, W.S., Mulholland, M.M., Bunnett, N.W., Debas, H.T., 1989, Inhibition of gastric and pancreatic secretion in dogs by CGRP: role of somatostatin, **Am. J. Physiol.**, 256:G715.
Holzer, P., Peskar, B.M., Peskar, B.A., Amann, R., 1990a, Release of calcitonin gene-related peptide induced by capsaicin in the vascularly perfused rat stomach, **Neurosci. Lett.**, 108:195.
Holzer, P., Taché, Y., Guth, P., 1990b, The vasodilator neuropeptides α-CGRP and VIP, but not substance P and neurokinin A, increase rat gastric mucosal blood flow, **Gastroenterology**, 98:A175.
Holzer, P., Livingston, E.H., Guth, P.H., 1990c, Sensory neurons signal for an increase in rat gastric mucosal blood flow in response to acid back-diffusion, **Gastroenterology**, 98:A175.
Hughes, J.J., Levine, A.S., Morley, J.E., Gosnell, B.A., Silvis,

S.E., 1984, Intraventricular calcitonin gene-related peptide inhibits gastric acid secretion, **Peptides**, 5:665.
Inui, T., Chiba, T., Okimura, Y., Morishita, T., Nakamura, A., Yamaguchi, A., Yamatani, T., Kadowaki, S., Chihara, K., Fujita, T., 1989, Presence and release of calcitonin gene-related peptide in rat stomach, **Life Sci.**, 45:1199.
Ishikawa, T., Taché, Y., 1988, Intrahypothalamic microinjection of calcitonin prevents stress-induced gastric lesions in rats, **Brain Res. Bull.**, 20:415.
Ishikawa, T., Taché, Y., 1989, Inhibitory effect of α-CGRP microinjected into the dorsal vagal complex on gastric acid secretion in the rat, **Dig. Dis. Sci.**, 34:979.
Katsoulis, S., Conlon, J.M., 1989, Calcitonin gene-related peptides relax guinea pig and rat gastric smooth muscle, **Eur. J. Pharmacol.**, 161:129.
Kolve, E., Taché, Y., 1989, Intracisternal α-CGRP prevents gastric ulcer formation in the rat, **Gastroenterology**, 96:A266.
Koop, H., Eissele, R., Kühlkamp, V., Bothe, E., Dionysius, J., Arnold, R., 1987, Calcitonin gene-related peptide stimulates rat gastric somatostatin release in vitro, **Life Sci.**, 40:541.
Kraenzlin, M.E., Ch'ng, J.L.C., Mulderry, P.K., Ghatei, M.A., Bloom, S.R., 1985, Infusion of a novel peptide, calcitonin gene-related peptide (CGRP) in man. Pharmacokinetics and effects on gastric acid secretion and on gastrointestinal hormones, **Regul. Pept.**, 10:189.
Kruger, L., Mantyh, P.W., Sternini, C., Brecha, N.C., Mantyh, C.R., 1988, Calcitonin gene-related peptide (CGRP) in the rat central nervous system: patterns of immunoreactivity and receptor binding sites, **Brain Res.**, 463:223.
Lenz, H.J., 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.
Lenz, H.J., Hester, S.E., Saik, R.P., Brown, M.R., 1986a, CNS actions of calcitonin gene-related peptide on gastric acid secretion in conscious dogs, **Am. J. Physiol.**, 250:G742.
Lenz, H.J., Klapdor, R., Hester, S.E., Webb, V.J., Galyean, R.F., Rivier, J.E., Brown, M.R., 1986b, Inhibition of gastric acid secretion by brain peptides in the dog. Role of the autonomic nervous system and gastrin, **Gastroenterology**, 91:905.
Lenz, H.J., 1988, Calcitonin and CGRP inhibit gastrointestinal transit via distinct neuronal pathways, **Am. J. Physiol.**, 254:G920.
Lenz, H.J., Rivier, J.E., Brown, M.R., 1985, Biological actions of human and rat calcitonin and calcitonin gene-related peptide, **Regul. Pept.**, 12:81.
Lenz, H.J., Brown, M.R., 1990, Cerebroventricular calcitonin-related peptide inhibits rat duodenal bicarbonate secretion by release of norepinephrine and vasopressin, **J. Clin. Invest.**, 85:25.
Lenz, H.J., Mortrud, M.T., Vale, W.W., Rivier, J.E., Brown, M.R., 1984, Calcitonin gene-related peptide acts within the central nervous system to inhibit gastric acid secretion, **Regul. Pept.**, 9:271.
Lenz, H.J., Mortrud, M.T., Rivier, J.E., Brown, M.R., 1985a, Calcitonin gene related peptide inhibits basal, pentagastrin, histamine and bethanecol stimulated gastric acid secretion, **Gut**, 26:550.
Lenz, H.J., Mortrud, M.T., Rivier, J.E., Brown, M.R., 1985b, Cen-

tral nervous system actions of calcitonin gene-related peptide on gastric acid secretion in the rat, **Gastroenterology**, 88:539.
Lenz, J.H., Forquignon, I., Druge, G., Greten, H., 1989, Effects of neuropeptides on gastric acid and duodenal bicarbonate secretions in freely moving rats, **Regul. Pept.**, 24:293.
Leung, F.W., Tallos, E.G., Taché, Y.F., Guth, P.H., 1987, Calcitonin gene-related peptide inhibits acid secretion without modifying blood flow, **Am. J. Physiol.**, 252:G215.
Madaus, S., Schusdziarra, V., Seufferlein, M., Classen, M., 1989, Comparison of α- and β-CGRP on gastrin (G), somatostatin (SLI) and bombesin-like immunoreactivity (BLI) from the isolated perfused rat stomach, **Gastroenterology**, 96:A3112.
Maton, P.N., Sutliff, V.E., Zhou, Z.-C., Collins, S.M., Gardner, J.D., Jensen, R.T., 1988, Characterization of receptors for calcitonin gene-related peptide on gastric smooth muscle cells, **Am. J. Physiol.**, 254:G789.
Morishima, K., Takagi, H., Akai, F., Tohyama, M., Emson, P.C., Hillyard, C.J., Girgis, S.I., MacIntyre, I., 1985, Light and electron microscopic studies of calcitonin gene-related peptide immunoreactive neurons and axon terminals of the nucleus of the tractus solitarius of the rat, **Brain Res.**, 344:191.
Orloff, S.L., Bunnett, N.W., Debas, H.T., 1989, Calcitonin gene-related peptide (CGRP) differentially regulates acetylcholine release from gastric mucosa and musculature, **Gastroenterology**, 96:A378.
Pappas, T., Debas, H.T., Walsh, J.H., Rivier, J., Taché, Y., 1986, Calcitonin gene-related peptide-induced selective inhibition of gastric acid secretion in dogs, **Am. J. Physiol.**, 250:G127.
Raybould, H.E., Kolve, E., Taché, Y., 1988, Central nervous system action of calcitonin gene-related peptide to inhibit gastric emptying in the conscious rat, **Peptides**, 9:735.
Raybould, H.E., Kolve, E., Taché, Y., 1989, Central nervous system action of calcitonin gene-related peptide (CGRP) to inhibit gastric motor function by adrenergic mechanisms in the rat, **Gastroenterology**, 96:A410.
Reasbeck, P.G., Burns, S.M., Shulkes, A., 1988, Calcitonin gene-related peptide: enteric and cardiovascular effects in the dog, **Gastroenterology**, 95:966.
Rosenfeld, M.G., Mermod, J.-J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W., Evans, R.M., 1983, Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing, **Nature**, 304:129.
Schwaber, J.S., Sternini, C., Brecha, N.C., Rogers, W.T., Card, J.P., 1988, Neurons containing calcitonin gene-related peptide in the parabrachial nucleus project to the central nucleus of the amygdala, **J. Comp. Neurol.**, 270:416.
Shapiro, R.E., Miselis, R.R., 1985, The central organization of the vagus nerve innervating the stomach of the rat, **J. Comp. Neurol.**, 238:473.
Skofitsch, G., Jacobowitz, D.M., 1985, Autoradiographic distribution of 125I calcitonin gene-related peptide binding sites in the rat central nervous system, **Peptides**, 4:975.
Steenbergh, P.H., Höppener, J.W.M., Zandberg, J., Lips, C.J.M., Jansz, H.S., 1985, A second human calcitonin/CGRP gene, **FEBS**, 183:403.
Steenbergh, P.H., Höppener, J.W.M., Zandberg, J., Visser, A., Lips, C.J.M., Jansz, H.S., 1986, Structure and expression of the human calcitonin/CGRP gene, **FEBS Lett.**, 209:97.

Sternini, C., Anderson, K., 1990, Differential distribution of α- and β-calcitonin gene-related peptide in enteric and sensory neurons, **Gastroenterology**, 98:A526.
Taché, Y., Simard, P., Collu, R., 1979, Prevention by bombesin of cold-restraint stress induced hemorrhagic lesions in rats, **Life Sci.**, 24:1719.
Taché, Y., Ishikawa, T., 1989, Role of brain peptides in the ulcerogenic response to stress, in: "Hans Selye Symposia on Neuroendocrinology and Stress: Neuropeptides and Stress," Y. Taché, J.E. Morley and M.R. Brown, eds., Springer-Verlag, New York.
Taché, Y., Vale, W., Rivier, J., Brown, M., 1980, Brain regulation of gastric secretion: influence of neuropeptides, **Proc. Natl. Acad. Sci. USA**, 77:5515.
Taché, Y., Pappas, T., Lauffenburger, M., Goto, Y., Walsh, J.H., Debas, H., 1984a, Calcitonin gene-related peptide: potent peripheral inhibitor of gastric acid secretion in rats and dogs, **Gastroenterology**, 87:344.
Taché, Y., Gunion, M., Lauffenburger, M., Goto, Y., 1984b, Inhibition of gastric acid secretion by intracerebral injection of calcitonin gene-related peptide in rats, **Life Sci.**, 35:871.
Taché, Y., Kolve, E., Maeda-Hagiwara, M., Kauffman, G., 1987a, CNS action of calcitonin to alter experimental gastric ulcers in rats, **Gastroenterology**, 41:651.
Taché, Y., 1987b, The peptidergic brain-gut axis: Influence on gastric ulcer formation, **Chronobiol. Int.**, 4:11.
Taché, Y., 1989, Central nervous system action of neuropeptides to influence or prevent experimental gastroduodenal ulceration, in: "Ulcer Disease: New Aspects of Pathogenesis and Pharmacology," S. Szabo and C.J. Pfeiffer, eds., CRC Press, Inc., Boca Raton.
Twery, M.J., Moss, R.L., 1985, Calcitonin and calcitonin gene-related peptide alter the excitability of neurons in rat forebrain, **Peptides**, 6:373.
Umeda, Y., 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.
Varro, A., Green, T., Holmes, S., Dockray, G.J., 1988, Calcitonin gene-related peptide in visceral afferent nerve fibres: quantification by radioimmunoassay and determination of axonal transport rates, **Neuroscience**, 26:927.
Wei, J.Y., Taché, Y., 1990, Alterations of efferent discharges of the gastric branch of the vagus nerve by intracisternal injection of peptides influencing gastric function in rats, **Gastroenterology**, 98:A531 (Abstract).
Wimalawansa, S.J., MacIntyre, I., 1988, Heterogeneity of plasma calcitonin gene-related peptide: partial characterization of immunoreactive forms, **Peptides**, 9:407.
Yamatani, T., Kadowaki, S., Chiba, T., Abe, H., Chihara, K., Fukase, M., Fujita, T., 1986, Calcitonin gene-related peptide stimulates somatostatin release from isolated perfused rat stomach, **Endocrinology**, 118:2144.
Yang, H., Cuttitta, F., Raybould, H., Taché, Y., 1989, Intrathecal injection of bombesin inhibits gastric acid secretion in the rat, **Gastroenterology**, 96:1403.
Young, R.L., Rings, M.C., Lassiter, D.C., Harty, R.F., 1989, Calcitonin gene-related peptide: effects on somatostatin, gastrin, and acetylcholine release from rat antral fragments, **Gastroenterology**, 96:A562.

Young, R.L., Ren, J., Lassiter, D.C., Harty, R.F., 1990, Calcitonin gene-related peptide: mechanisms of modulation of rat antral endocrine cells and cholinergic neurons, **Gastroenterology**, 98:A534.

SENSORY NERVES OF THE INTESTINES: ROLE IN CONTROL OF PYLORIC REGION OF DOGS

Gervais Tougas, Hans-D. Allescher, John Dent* and Edwin E. Daniel

McMaster University Medical Centre
Hamilton, Ontario, Canada

*The Royal Adelaide Hospital
Adelaide, South Australia, Australia

GROSS AND MICROSCOPIC ANATOMY OF PYLORUS

The pyloric region, as described by Torgersen (1) in 1942, consists of a circular thickening of the innermost circular muscle layer found at the junction of the stomach and duodenum. Schulze-Delrieu et al. (2) confirmed the presence of this ring in all mammalian species studied, albeit with some variation in size. This was termed the distal pyloric muscle loop. A second thickened muscular ring, the proximal pyloric muscle loop, has been identified in a number of species, and is connected to the distal pyloric muscle loop by a fan-like arrangement of oblique muscle fibres forming a loop over the distal antrum, running from the greater to the lesser curvature. These two circular muscle loops and their connecting fibres meet at a ridge, or torus, located on the lesser curvature aspect of the stomach. Figure 1 illustrates schematically the muscular anatomy of the human pyloric area.

The antral circular muscle is continuous with the pyloric muscle loops and torus. The longitudinal muscle layer joins the distal pyloric muscle loop along with the muscularis mucosae, in the absence of a true submucosa in this region (2). The resulting apposition of mucosae and muscularis mucosae results in a tight attachment of the former with the distal loop, causing the bulging of proximal and distal mucosa that are only loosely attached to muscle upon contraction of the muscle ring. The mucosa bulging can therefore act as a luminal plug.

Contractions of the upper and lower pyloric muscle loops will markedly alter the size of the luminal opening. Based on radiological observations, Keet (3) has described the pyloric segment at rest as being partially closed by the tonic activity of the distal loop and by the bulging of the torus from the lesser curve. During contractions, the pyloric segment is obliterated by contractions of the proximal muscle loop, the torus and the longitudinal muscle fibres ending on the distal muscle loop. No direct observation of the independent contraction of those

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

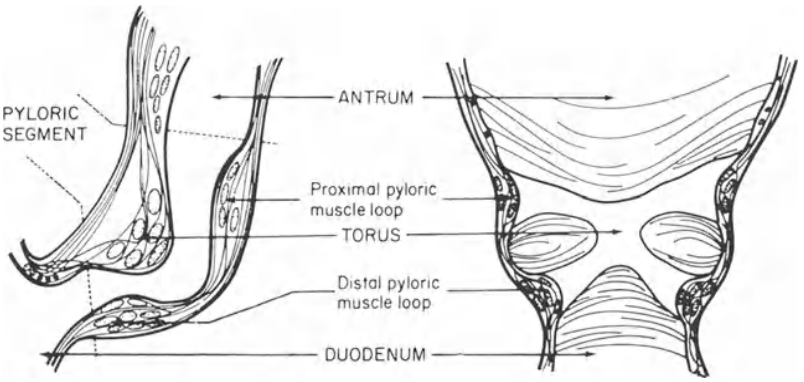


Fig. 1. Muscular anatomy of the human antropyloroduodenal segment. Left: Longitudinal section, Right: Mucosal view following section of the segment along the greater curvature. Between the two muscle loops is the pyloric groove bulging outward at the beginning of the pyloric contraction resulting in the pyloric pseudodiverticulum. In the canine pylorus, the distal pyloric loop is more obliquely oriented and the proximal loop is less prominent and difficult to recognize. In the cat, the pyloric segment is very narrow even when the muscle is paralysed. (Reproduced with permission from Schulze-Delrieu et al., 1984 (4)).

muscle loops and of the torus have been made but if this description is accurate, these would provide a basis for the redundancy of control of pyloric activity; i.e. removal or damage to the distal loop would not necessarily damage or prevent luminal closure by any of the other elements.

NEURAL ORGANIZATION

A myenteric plexus separates the antral longitudinal and circular muscle layers in the pyloric segment and is continuous with the duodenal plexus, as is the longitudinal muscle layer, albeit reduced in size by the loss of fibres to the distal pyloric loop (4). However the duodenal circular muscle layer is not continuous with the pyloric and antral circular muscle but rather clearly separated (5). A deep muscular plexus is found within duodenal but not pyloric circular muscle (4). As previously mentioned, the submucous plexus is absent in the pyloric segment.

MICROSCOPIC ANATOMY

The myenteric plexus of the pyloroduodenal area is ganglionated with large ganglia containing immunoreactive nerve cells for enkephalins (ENK), substance P, VIP, neuropeptide Y (NPY) and galanin (GAL) (Daniel, unpublished). The density of those immunoreactive fibres is markedly higher at the pyloric sphincter than in the overlying antral circular muscle or duodenal circular muscle. This high immunoreactivity in the pyloric sphincter plexus

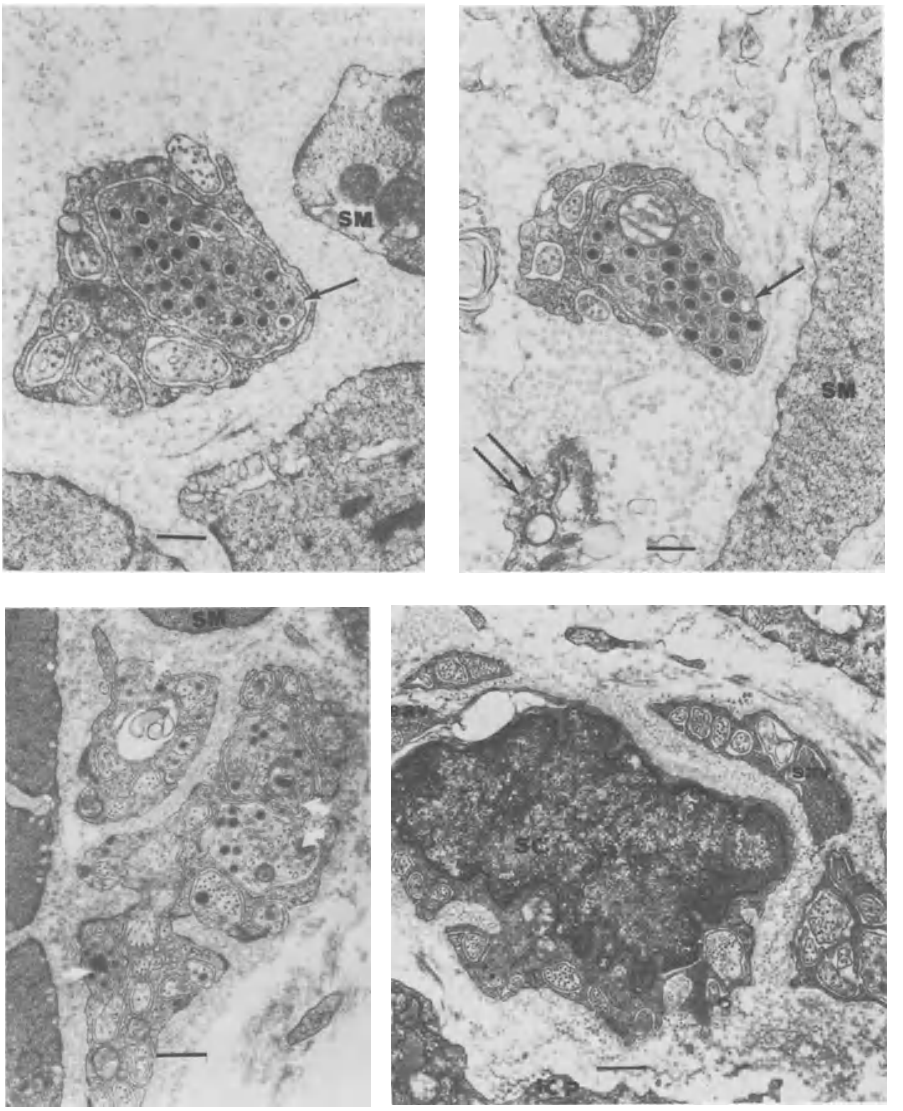


Fig. 2. Micrographs of nerves in canine pyloric sphincter. Top: Small nerve bundles near muscle cells (SM), showing profiles of varicosities (arrows) with large granular vesicles (LGV), one with (left) and the other without (right) glial cell covering part of an ICC cell profile is present (double arrow). Bottom: A larger nerve bundle containing (left) profiles of nerve varicosities with a mixture of small agranular vesicles (SAV) and LGV (curved arrows) as one with LGV (straight arrow) and (right) profiles of varicosities containing SAV. Many axons are present in all four cases. A putative ICC or fibroblast was at bottom in the latter. The calibration bars show about 292 nm.

layer has been documented in the dog, human and cat (6,7,8,9) and is illustrated in Figure 2. Using histochemical methods, Allescher et al. (10) have confirmed the previous report of Costa et al. (11) showing the presence of adrenergic nerve fibres in the sphincteric region. It is probable that a dense innervation of cholinergic nerves might be present as well but no studies using methods specific for these nerves have been published yet.

Only two studies of the ultrastructure of the pyloric region have been published to date. In the guinea pig, Cai and Gabella (12) demonstrated a high density of nerve profiles in this region where most of the varicose nerves contained a mixture of large granular synaptic vesicles and of small agranular synaptic vesicles rather than the usual predominance of the latter type. Large granular vesicles have been shown in a number of immunocytochemical studies to contain neuropeptides but the content of small agranular vesicles, often considered to be acetylcholine, is not certain at present. We have recently completed an ultrastructural study of the canine pylorus with several striking findings: a very high density of nerves close to muscle, 3-5 times higher than in antral circular muscle (4), an extremely high proportion (> 50%) of nerves with LGV, in fact the highest reported anywhere, equal to the rat anococcygeus (Figure 3). It is likely that these LGV contain neuropeptides. Finally, there was a lower density of gap junctions than in the antral circular muscle, a finding similar to previous reports from other sphincteric areas such as the LES which has a lower density of gap junctions than the circular muscle of the oesophageal body (13).

The distribution of interstitial cells of Cajal in the pyloric region was examined in our laboratory for the first time (4). In general two groups of ICC can be defined based on ultrastructural appearance, possibly relevant for function in the GI tract. These cells may be involved in the pacemaking activity of GI smooth muscle and possibly play a role in non-adrenergic, non-cholinergic inhibitory neurotransmission (14,15). The ICC found in the canine pylorus were mostly similar to those of the circular muscle, not closely related to nerves, unlike the relationship noted in the LES region (Figure 3). However the second type of ICC, those associated with myenteric plexus cells, were also seen. The pyloric ICC or their processes were in contact through gap junctions with one another or with smooth muscle cells. This would support the notion that these ICC are designed to provide pacemaking activity to muscle rather than acting as a pathway for neurotransmission. The canine pylorus has been shown to possess TTX insensitive tonic and phasic activity, independent of antral or duodenal activity (16). We suggest that ICC of one or the other type may be necessary for such an action.

An interesting finding of these ultrastructural studies of the canine pylorus was the absence of close proximity between varicose nerve endings and either ICC or smooth muscle cells. This makes the observation (see below) that the pylorus could follow single vagal stimuli or single pulses of field stimulation up to frequencies of 0.5 to 0.7 Hz difficult to understand in anatomical terms.

SUMMARY

In brief, the canine pyloric sphincter appears to be very dense-

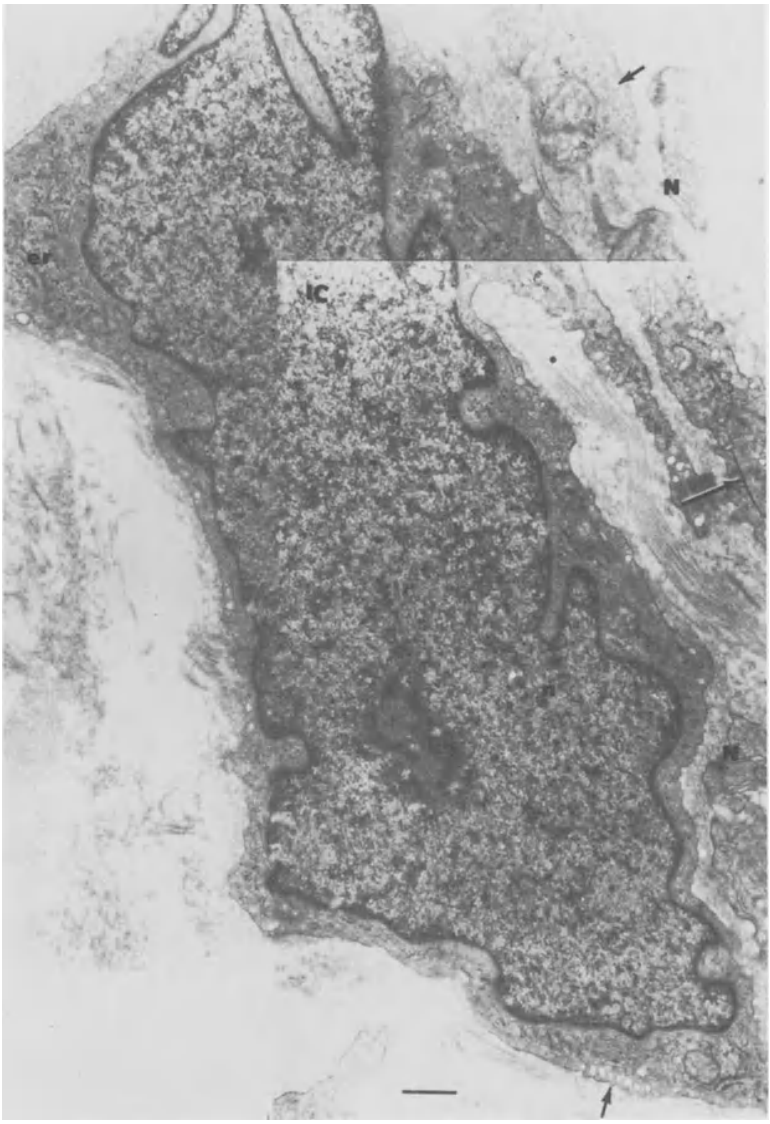


Fig. 3. Montage of Interstitial Cell of Cajal (IC) of the circular muscle type in the canine pyloric sphincter. Note the lobulated nucleus (n), the many plasmalemmal caveolae (short arrow) to another ICC and the associated but not close nerve profiles (N). Length bar = 511 nm.

ly innervated by nerves, many of which contain neuropeptides, compared to antral muscle. It also contains ICC with gap junctions connecting them with one another and to smooth muscle. However unlike other tissues, many ICC are not associated with nerves and no close nerve varicosities-smooth muscle or -ICC contacts were observed. This leaves some aspects of neural control difficult to explain in structural terms.

NEURAL PATHWAYS INVOLVED IN GASTRODUODENAL COORDINATION

The control of pyloric activity is accomplished by a complex, integrated hierarchy of extrinsic and intrinsic enteric neural pathways, associated with a number of humoral mechanisms. Extrinsic neural pathways include both vagal and splanchnic nerves. This complex system allows for a high degree of plasticity in the response since compensation for the loss of a particular control can be made by other mechanisms (17). Both intrinsic and extrinsic systems are involved in the basal and postprandial motility of this area, and the coordination of antral pyloric and duodenal motility (17). Using the canine pyloric responses to a number of stimuli in vivo we have examined the sensory and motor neural pathways involved in the control of pyloric sphincter activity in the dog (10,18). The overall system is highly integrated and, as will be seen, involves both local enteric reflexes and central, vagally mediated pathways.

The model we have been using has been described in detail elsewhere (10). Briefly, under chloralose and urethane

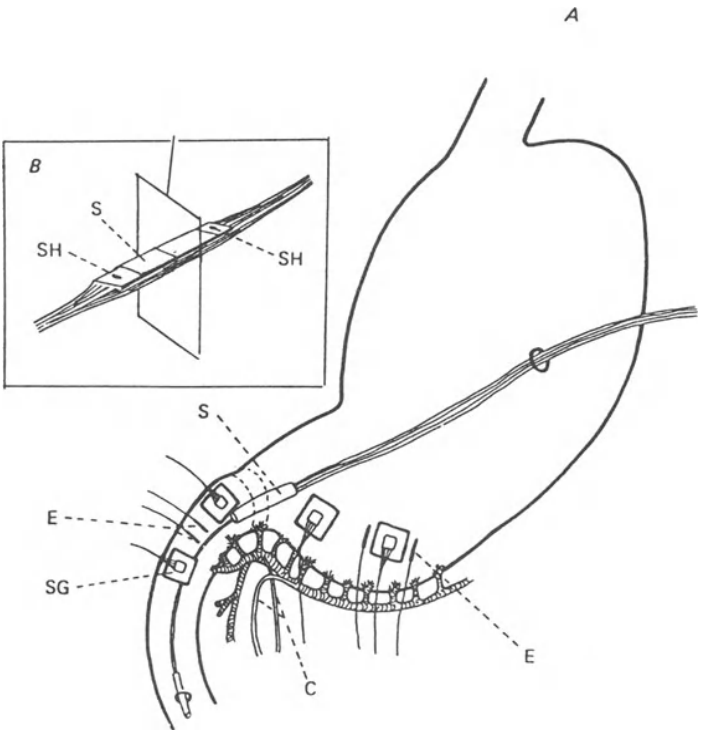


Fig. 4. Schema of the canine model used for pyloric motility and the recording devices used. The manometric assembly consists of a multi-lumen side-hole catheter and a sleeve sensor (S), and is inserted through a gastrostomy. Strain gauges (SG) and silver chloride wire electrodes (E) are sutured in the antrum and duodenum. Intraarterial cannulae (C) are used for close intraarterial injections to the antrum, pylorus and duodenum. The inset demonstrates the sleeve sensor (S) and the antral and duodenal side holes (SH).

anaesthesia, in healthy mongrel dogs, selective intraarterial cannulations of the gastroepiploic artery allowed selective arterial infusions either to the pylorus and proximal duodenum or to the distal antrum. Strain gauges were sutured to the antral and proximal duodenal serosa, oriented so that circular muscle activity be recorded. A manometric assembly incorporating a sleeve sensor was inserted through a small fundic gastrostomy and positioned so that the sleeve sensor was straddling the pylorus. This system allows for simultaneous intraluminal manometry and circular muscle activity recording and is shown in Fig. 4.

Using such a model, the basal activity of the pylorus was found to consist of both a resting pyloric pressure (approximately 10 mmHg) and spontaneous phasic contractions in the absence of antral or duodenal activity. They are therefore isolated pyloric pressure waves (IPPW) (10). This activity was markedly reduced by atropine but the local administration of TTX (50-100 μg I.A.) to the pylorus caused an increase in basal pyloric motor activity. The administration of TTX nevertheless blocked all pyloric motor responses following vagal stimulation and and antral and duodenal field stimulation (see below). Vagal transection had different effects on basal pyloric motility depending on whether the motor activity of the pylorus was normal (increased basal activity) or basal activity was high (where vagal transection did not alter or only slightly decreased activity). Excitation of the vagal stump resulted in pyloric excitation when low frequencies were used (.1-.5 Hz) but inhibition was seen with higher frequencies (1-5 Hz). The excitatory effect was blocked by atropine and hexamethonium but the inhibitory effect of high frequency stimulation was unchanged by atropine, hexamethonium, propranolol and phentolamine. Only TTX abolished this effect.

Field stimulation of the duodenum at frequencies of .5 to 1 Hz caused an immediate pyloric activation. This activation was abolished by atropine or hexamethonium but not by propranolol or phentolamine. Therefore this response to duodenal field stimulation apparently involves an orally projecting chain of cholinergic neurones with nicotinic synapses.

Antral field stimulation, on the other hand, inhibited pyloric phasic and tonic activity as well as inhibiting pyloric activation due to duodenal field stimulation (Figure 5). This inhibitory effect of antral field stimulation was not affected by atropine, hexamethonium or propranolol, but was blocked by TTX.

The effect of antral and duodenal field stimulation was abolished by antral and duodenal transection respectively, but this transection had no effect on the action of vagal stimulation on pyloric motility.

The evidence would therefore be indicative of a number of levels of control of pyloric activity: first, the presence of basal phasic and tonic pyloric activity even in the presence of TTX supports a myogenic control present in the sphincter area; secondly, vagal stimulation at low frequencies excites the pylorus via a neural pathway involving muscarinic and nicotinic synapses; thirdly, the excitatory response of the pylorus after duodenal field stimulation is mediated by a chain of orally

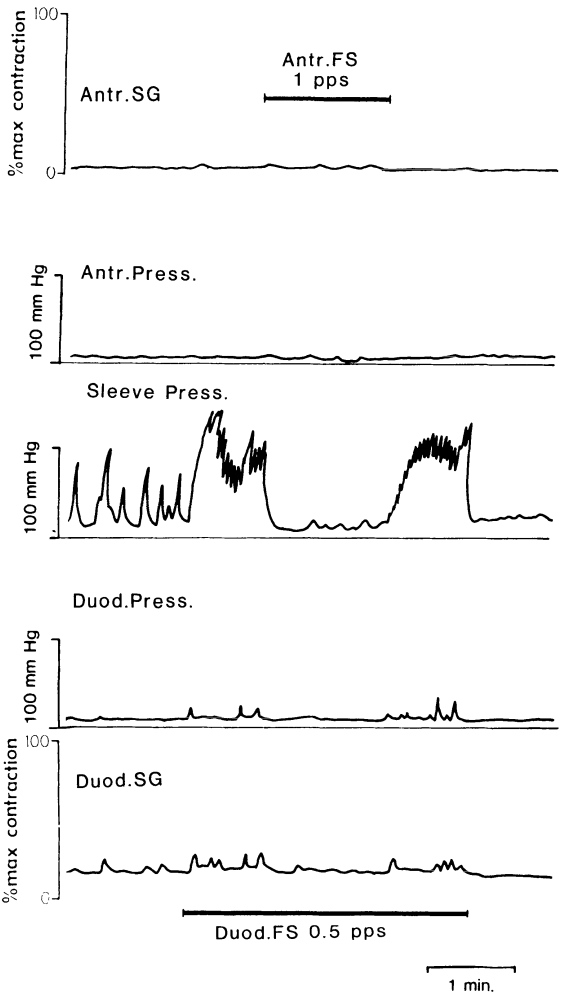


Fig. 5. Inhibitory effect of antral field stimulation. Manometric tracing showing the effect of antral field stimulation (1 Hz, .5ms, 40 V) on the pyloric motor excitation resulting from duodenal field stimulation.

projecting cholinergic nerves with nicotinic and muscarinic synapses; finally, antral field stimulation is a potent inhibitor of pyloric motility through non-adrenergic, non-cholinergic mechanisms.

We subsequently studied the response of the pylorus to intraduodenal hydrochloric acid infusion (.1 N HCl, 1 cc/min) and examined the neural reflexes involved (18). This slow infusion of acid elicited a strong phasic and tonic pyloric contraction, with phasic duodenal activity and inhibition of antral activity (Fig. 6). This response was not affected by prior vagotomy but was markedly diminished by atropine and hexamethonium. Naloxone, phentolamine, propranolol, and the CCK antagonist CR-1392 had no effect on this response. It therefore was concluded that this response of the pylorus to intraduodenal acidification was

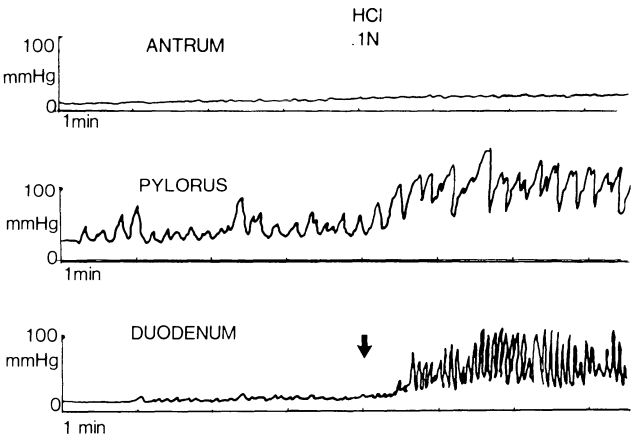
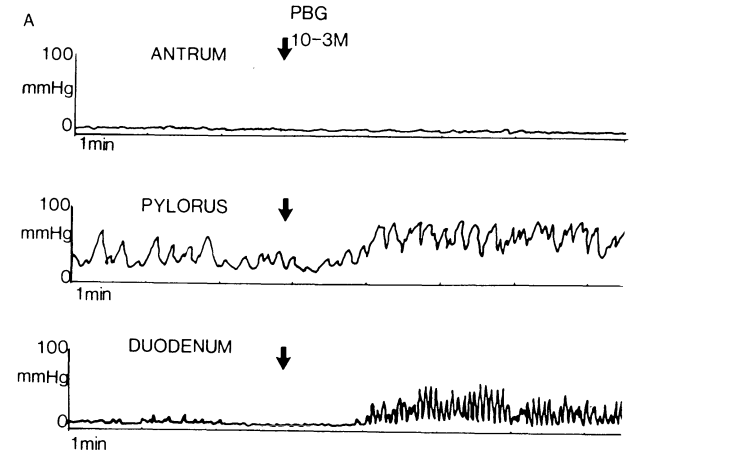


Fig. 6. Manometric tracing of the response of the canine pylorus following duodenal acidification (HCl .1N, .92 cc/min). The response of the pylorus is independent of any antral activity and is accompanied by duodenal propagated activity resulting in a rapid clearance of the acid load.

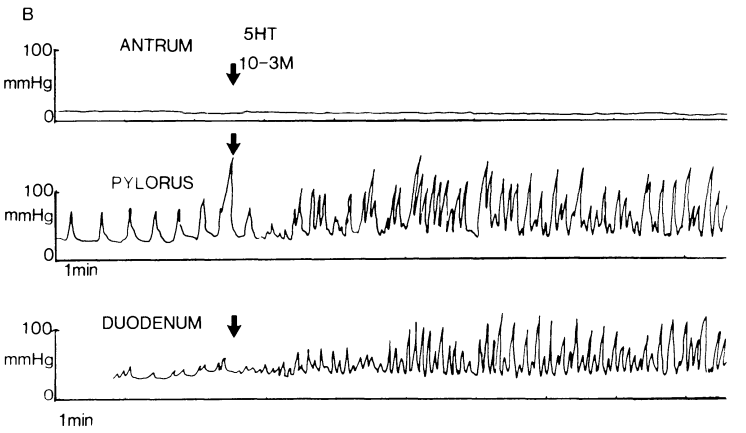
mediated through an orally projecting chain of cholinergic nerves (18).

Vagal input did not appear to be necessary as evidenced by the lack of effect of vagotomy on this response. Note that this observation does not exclude the involvement of vagal sensory nerves in the response. This response was inhibited by antral field stimulation, antral contractions and vagal stimulation, as was the pyloric response to duodenal field stimulation. Therefore a similar chain of muscarinic and nicotinic cholinergic nerves appeared involved. However the prior administration of 2% xylocaine to the duodenal mucosa prevented the response from occurring. Intraluminal infusion of serotonin (10-5 M 1 cc/min) and of the 5HT3 agonist phenylbiguanide (10-3M 1 cc/min) mimicked the pyloric response to acid and was similarly blocked by prior xylocaine treatment and by atropine and hexamethonium (18, Tougas G., unpublished) (Figure 7a,b).

Since the response to intraluminal serotonin and phenylbiguanide is blocked by the same agent as the response to duodenal field stimulation, it is reasonable to assume that the same pathway of orally projecting neurones is involved. To further characterize the type of serotoninergic receptors involved, we then administered selective 5HT-2 and 5HT-3 antagonists and observed their effect in this pyloric response to acid, 5HT and phenylbiguanide (Tougas G., unpublished). Whereas the selective 5HT-2 antagonist ketanserin (10-7 to 10-5 M) had no effect on the pyloric response to any of these 3 agonists or on the effect of duodenal field stimulation, the selective 5HT-3 antagonist zacopride administered either intraluminally or intraarterially effectively blocked the pyloric response to intraduodenal hydrochloric acid, as well as the response to intraluminal serotonin and phenylbiguanide (Figure 8). Furthermore zacopride failed to



EFFECT OF LUMINAL PHENYLBIGUANIDE INFUSION

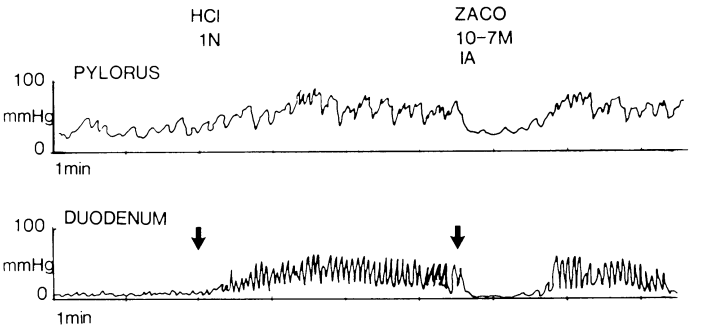


EFFECT OF LUMINAL 5HT INFUSION

Fig. 7. Motor response of the antropyloroduodenal segment following duodenal infusion of Serotonin (5-HT, 10 micromolar, .92 cc/min) (Fig. 7a) and the $5HT_3$ agonist phenylbiguanide (PBG, .1 millimolar, .92 cc/min) (Fig. 7b).

have any action on the response to intraduodenal field stimulation suggesting that its effect is on sensory nerves rather than on the chain of cholinergic nerves that is involved in this latter response.

The exact source of the serotonin mediating the response to acid remains uncertain but it must be in close proximity to the mucosa as evidenced by the potent effect of the intraluminal administration of 5HT-3 agonists and antagonists on this response. Two possible sources seem more likely: 1) The presence of serotonin-containing nerve cells is likely and these nerve cells could have nerve endings that are sensitive to duodenal luminal



EFFECT OF ZACOPRIDE IA ON ACID RESPONSE

Fig. 8. Effect of an intraluminal infusion of the 5-HT_3 antagonist zacopride (ZAC, .1 micromolar, .92 cc/min, acidified to pH 1.2) on the response to intraduodenal acidification. The delay in the response is due to the dead space of the catheter used for the infusion. The infusate was directed at the proximal duodenum, 1 cm distal to the pyloric sphincter. A similar response was obtained with 5-HT and PBG.

acidity resulting in excitation of these nerves through release of serotonin and activation of the orally projecting chain of nerves responsible for this response. 2) Alternatively, the serotonin that is released by duodenal acidification may originate from the abundant enterochromaffin cell population present in the proximal duodenal area of the canine intestine. We propose that its release is due to a direct action of hydrochloric acid on those cells instead of an effect on neuronal endings with subsequent excitation of these nerves and secondary release of serotonin by the EC cells. A few factors militate in favour of an enterochromaffin cell origin of the 5-HT: the relative paucity of 5-HT containing nerve endings in the mucosa of the canine intestine (Furness J.B., personal communication), the documented release of large amounts of serotonin following duodenal acidification (19), in amounts that are difficult to conceive being released by neuronal cells but could easily be released by the EC cells.

SUMMARY

The canine pylorus is the target for a large array of extrinsic and intrinsic nerve-produced effects. Some of them have intermediate nicotinic synapses. In all cases observed so far the final nerve terminals mediating excitation are cholinergic, but those mediating inhibition are both adrenergic and non-adrenergic non-cholinergic. The role of the dense peptidergic innervation is unclear especially in view of the observation that this muscle and its nerves contain receptors to all of those studied (VIP, PHI, GAL, SP, NKA, NKB, CCK, and others. The pylorus is also the target of an important reflex initiated by acid in the duodenal lumen. Our preliminary work suggests that this reflex is initiated by acid induced release of 5HT from the EC cells to act on 5HT receptors on sensory nerves which acti-

vate the ascending chain of cholinergic nerves in the myenteric plexus. This same stimulus may also activate other vagal sensory nerves.

Acknowledgements

The work of Drs. Tougas and Daniel was supported by the Medical Research Council of Canada. The work of Dr. Allescher was supported by a research grant from Deutsche Forschungsgemeinschaft DFG Al/245 1-1. Dr. Dent's work was supported by a visiting scientist award from MRC Canada.

References

1. Torgersen, J., 1942, The muscular build and movements of the stomach and duodenal bulb, **Acta Radiol.**, 45(suppl):1.
2. Shulze-Delrieu, K. and Wall, J.P., 1984, Determinants of flow across isolated gastroduodenal junctions of cats and rabbits, **Am. J. Physiol.**, 245:G357.
3. Keet, A.D.Jr., 1957, The prepyloric contractions in the normal stomach, **Acta Radiologica**, 48:413.
4. Daniel, E.E., Berezin, I., Allescher, H.D., Manaka, H. and Posey-Danier, V.P., 1990, Morphology of the canine pyloric sphincter in relation to function, **Can. J. Physiol. Pharmacol.**, in press.
5. Code, C.F., 1970, The mystique of the gastroduodenal junction, **Pendic. Rev. Gastroenterol.**, 2:20.
6. Gonda, T., Daniel, E.E., McDonald, T.J. Fox, J.E.T., Brooks, B.D., Oki, M., 1990, The distribution of galanin-immunoreactive nerves and possible functions of a new neuropeptide in dog gastrointestinal tract. Comparison to VIP, **Am. J. Physiol.**, 256:G884.
7. Wattchow, D.A., Cass, P.J., Furness, J.B., 1987, Abnormalities of peptide-containing nerve fibers in infantile hypertensive pyloric stenosis, **Gastroenterology**, 92:443.
8. Edin, R., 1980, The vagal control of the pyloric motor function: a physiological and immunohistochemical study in cat and man, **Acta Physiol. Scand.**, 485(suppl):1.
9. Lidberg, P., Edin, R., Lundberg, J.M., The involvement of substance P in the vagal control of the feline pylorus, **Acta Physiol. Scand.**, 114:307.
10. Allescher, H.-D., Daniel, E. E., Dent, J., Fox, J. E. T., Kostolanska, F., 1988, Extrinsic and intrinsic neural control of pyloric sphincter pressure in the dog, **J. Physiol. (Lond.)**, 401:17.
11. Costa, M., Gabella, G., 1971, Adrenergic innervation of the alimentary canal, **Z. Zellforch**, 122:357.
12. Cai, W.Q., Gabella, G., 1984, Structure and innervation of the musculature at the gastroduodenal junction in the guinea pig, **J. Anat.**, 139:93.
13. Daniel, E.E., Posey-Daniel, V.P., 1984, Effect of scorpion venom on structure and function of lower esophageal sphincter (LES) and body circular muscle (BCM) from oppossum, **Can. J. Physiol. Pharmacol.**, 62:360.
14. Barajas-Lopes, C., Berezin, I., Daniel, E.E., Huizinga, J.D., 1990, Role of the interstitial cells of Cajal in colon pace-maker generation, **Am. J. Physiol.**, 257:C830.

15. Daniel, E.E., Posey-Daniel, V.P., 1984, Neuromuscular structures in oppossum esophagus: role of interstitial cells of Cajal, **Am. J. Physiol.**, 9:G305.
16. Allescher, H.-D., Daniel, E.E., Fox, J.E.T., Kostolanska, F., 1988, Extrinsic and intrinsic neural control of pyloric sphincter pressure in the dog, **J. Physiol. (Lond.)**, 401:17.
17. Grundy, D., 1988, Vagal control of gastrointestinal function, **Bailliere's Clin. Gastroenterol.**, 2:23.
18. Allescher, H.-D., Daniel, E. E, Dent, J., Fox, J. E. T., Kostolanska, F., 1989, Neural reflex of the canine pylorus to intraduodenal acid infusion, **Gastroenterology**, 96:18.
19. Ormsbee, H.S. III, Fondacaro, J.D., 1985, Action of serotonin on the gastrointestinal tract, **Proc. Soc. Exp. Biol. Med.**, 178:333.

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) AND THE SPECIFIC MOTOR RESPONSE TO CAPSAICIN OF THE HUMAN ISOLATED ILEUM

Carlo Alberto Maggi, Sandro Giuliani, Paolo Santicioli, Riccardo Patacchini, Elvar Theodorsson *, Damiano Turini **, Gabriele Barbanti **, Antonio Giachetti and Alberto Meli

Pharmacology Department, Smooth Muscle Division, Research Laboratories, A. Menarini Pharmaceuticals, Via Sette Santi 3 Florence, 50131 Italy,
* Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden and
** Dept. of Surgical Pathology and Urologic Clinic, University of Ferrara, Italy

INTRODUCTION

Capsaicin, the pungent ingredient of many red peppers, has been shown to possess a selective stimulant action on certain neuropeptide-containing primary afferents which are widely distributed in the gastrointestinal tract of several species (Maggi and Meli 1988 and Holzer 1988 for reviews). On isolated gut segments from rats or guinea pigs, capsaicin exerts a variety of motor responses (contraction, relaxation or both) which depend upon the release from sensory nerves of certain neuropeptides, such as tachykinins or calcitonin gene-related peptide (CGRP) (see as examples Barthó et al., 1987; Maggi et al., 1986; 1988a). A specific feature of the action of capsaicin on sensory nerves is represented by desensitization, which means that after a single application of a maximally effective concentration of capsaicin the sensory fibers become unresponsive to subsequent applications of this drug.

We have investigated (Maggi et al., 1988b; 1988a) the effect of capsaicin on the longitudinal muscle of the human ileum attempting to assess whether endogenous peptides such as tachykinins, CGRP or VIP might be held responsible for the motor action of capsaicin at this level.

METHODS

Experiments were performed on ileal strips from patients undergoing abdominal surgery for carcinoma of the bladder base (enterocystoplasty). Pre-anesthetic medication and anesthesia were made as described previously (Maggi et al., 1988b; 1989a,b). All specimens appeared macroscopically normal with no

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

signs of tumor or inflammation. The tissues were placed in ice-cold Krebs solution within 2-3 min from surgical removal. The specimens were pinned flat on a Petri dish containing Krebs solution and the mucosa was carefully dissected out. Small strips (0.5 - 0.8 cm long, 2-3 mm wide) of muscle were cut along the longitudinal axis from which tension was recorded isometrically as described previously (Maggi et al., 1988b). Some strips were studied within 3 h from surgery ("fresh" strips). The remainder were stored overnight in ice-cold (4°C), and continuously oxygenated (96% O_2, 4% CO_2) Krebs solution, e.g. functional experiments started 16-20 h after surgery ("stored" strips).

RESULTS

Electrical field stimulation (10 Hz, 60 V, 0.5 ms pulse width, trains of 5 s every 90 s) of longitudinal strips from the human ileum evoked phasic contractions which were abolished or strongly inhibited by atropine or tetrodotoxin (1 μM each case). Capsaicin (1 μM), invariably inhibited the nerve-mediated contractions (n=15) but in some strips it also produced a delayed increase in spontaneous motility (Maggi et al., 1988b) and in some other strips an increase in tone, evident for a few min from its application.

On the spontaneous activity of the longitudinal strips capsaicin (1 μM) was also inhibitory although the effect was not consistently found in all strips tested (n=6).

The inhibitory effect of 1 μM capsaicin on the electrically-evoked contractions was also studied in the presence of 1 μM tetrodotoxin. Tetrodotoxin produced an almost complete (>95%) inhibition of the nerve-mediated contractions. When this inhibitory effect had fully developed, the parameters of stimulation were increased (pulse width from 0.5 to 5-10 ms, voltage from 60 to 80-100 V) in order to obtain phasic contractions having an amplitude of at least 60% of those recorded before the addition of tetrodotoxin. The inhibitory effect of capsaicin on the longitudinal muscle was not observed in the presence of tetrodotoxin.

Peptides displayed a variety of effects on human ileum. Tachykinins (substance P and neurokinin A) invariably produced a contraction (see Giuliani et al., this book). CGRP evoked some inhibitory effect on the nerve-mediated contractions but this effect was not consistently observed in all strips tested (Maggi et al., 1988b).

VIP produced a concentration-dependent (10 nM -1 μM) inhibition of the nerve-mediated contractions of longitudinal strips. VIP was also tested on spontaneous activity of the longitudinal strips but had a weak and inconsistent inhibitory effect.

The concentration-response curve to VIP (obtained in presence of 1/25 control rabbit serum) for the inhibitory action toward the nerve-mediated contractions was shifted to the right in presence of a highly specific anti VIP serum (#2, raised in a rabbit using porcine VIP, 1/25 for 60 min). Tetrodotoxin pretreatment prevented the inhibitory effect of VIP.

The anti VIP serum (1/25 final dilution for 60 min) signifi-

cantly reduced the inhibitory effect of capsaicin on nerve-mediated contractions by about 70%. By contrast, the R8 anti CGRP serum at a concentration (1/25 for 60 min), shown previously to be active in blocking the specific motor responses to capsaicin in other smooth muscles as well as the motor responses to exogenous CGRP (Maggi et al., 1988c), did not significantly inhibit the effect of capsaicin.

DISCUSSION

Capsaicin produces a variety of local motor responses in isolated preparations from the mammalian gut, ascribable to release of tachykinins and CGRP from sensory nerves of extrinsic origin. However the human small intestine differs from other species, since neither tachykinins nor CGRP seem to contribute to the inhibitory effect of capsaicin. The lack of mimicry by exogenously administered peptides supports this conclusion. In fact, while tachykinins produced a consistent contraction of the human small intestine (Maggi et al., 1989b), CGRP produced an inconsistent inhibitory effect. Moreover, as far as the human small intestine is concerned, capsaicin was found inactive in promoting CGRP release (Maggi et al., 1989a) and the inhibitory effect of capsaicin was insensitive to immunoblockade by anti CGRP serum. On the other hand the possible involvement of tachykinins in the delayed increase in motility or in the immediate small tonic contraction observed in a minority of cases in response to capsaicin cannot be excluded at this stage.

Overall, our findings suggest that endogenous VIP is responsible, at least in part, for the specific motor response to capsaicin of the human isolated small intestine. Evidence supporting this statement may be summarized as follows: a) exogenous VIP closely mimicked the effect of capsaicin; b) the functional response to capsaicin was significantly reduced by VIP antiserum, at a concentration antagonizing the response to exogenous VIP and c) a significant release of VIP-like material by capsaicin was detected (Maggi et al., 1989a).

VIP is an established enteric transmitter in various species (Angel et al., 1983; Grider et al., 1985). VIP-LI has been detected in the human small intestine by radioimmunoassay where it is confined to the nonepithelial layers (Ferri et al., 1983). Furthermore nerve fibers containing VIP-LI have been found in both longitudinal and circular muscle of the human small intestine (Wattchow et al., 1988). A role for VIP as an enteric inhibitory transmitter released from intrinsic neurons in the human intestine might be proposed, in much the same way as it was proposed to act in the animal intestine.

Present findings raise the possibility that at least part of the VIP content of the human small intestine might be stored in capsaicin-sensitive nerves and, by analogy with animal data, that these nerves are of sensory origin. It is interesting to note that capsaicin-sensitive nerves containing VIP-LI have been recently described in the rat lower gut (Chery-Croze et al., 1988). A second possibility is that some VIP neurons of the human gut are capsaicin-sensitive. Kirchgessner et al. (1988) reported that certain neurons in the submucosal plexus of the rat jejunum which share some lactoseries antigens with primary afferents in dorsal root ganglia are somehow affected by neonatal

capsaicin desensitization. These elements, which may be intrinsic sensory neurons of the gut, also contain VIP- and neuropeptide Y-like immunoreactivity. Finally, a third possibility may be entertained: capsaicin determines the release of a yet unidentified transmitter from primary afferents in the human gut which, in turn, acts on VIPergic elements of intrinsic origin.

The action of exogenous VIP, as well as that of capsaicin (which presumably acted by releasing endogenous VIP) was prevented in presence of tetrodotoxin. The tetrodotoxin-resistant contractions were most likely dependent from direct activation of muscle cells and these findings suggest that the action of VIP in the longitudinal muscle of the human ileum was exerted via prejunctional inhibition of transmitter release, although further studies are needed to firmly assess this point.

In conclusion, the present findings provide a functional link between the specific relaxant response to capsaicin and release of VIP in the human small intestine. Further studies are needed to assess the nature of the VIP-related peptide released by capsaicin in the human gut as well as to ascertain or exclude that other neuropeptides, such as tachykinins, might play some role in the functional response to capsaicin.

References

Angel, F., Go, V.L.W., Schmalz, P. F., Szurszewski, J. H., 1983, VIP: a putative transmitter in the canine gastric muscularis mucosa, **J.Physiol. (London)** 341:641.

Barthó, L., Pethó, G., Antal A., Holzer, P., and Szolcsányi, J., 1987, Two types of relaxation due to capsaicin in the guinea-pig isolated ileum, **Neurosci. Letters.** 81:146.

Chery-Croze, S., Bosshard, A., Martin, H., Cuber, J.C., Charnay, Y., and Chayvialle J.A., 1988, Peptide immunocytochemistry in afferent neurons from lower gut in rats. **Peptides** 9:873.

Ferri, G.L., Adrian, T.E., Ghatei, M.A., O'Shaughnessy, D.J., Probert, L., Lee, Y.C., Buchan, A.M.J., Polak, J.M., Bloom, S.R., 1983, Tissue localization and relative distribution of regulatory peptides in separated layers from the human bowel, **Gastroenterology** 84:777.

Grider, J.R., Cable, M.B., Bitar, K.N., Said, S.I., and Makhlouf G.M., 1985, Vasoactive intestinal peptide: relaxant transmitter in tenia coli of the guinea-pig, **Gastroenterology** 89:36.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, CGRP and other neuropeptides, **Neuroscience** 24:739.

Kirchgessner, A.L., Dodd, J., and Gershon, M.D., 1988, Markers shared between dorsal root and enteric ganglia, **J. Comp. Neurol.** 276:607.

Maggi, C.A., Manzini, S., Giuliani, S., Santicioli, P., and Meli, A., 1986, Extrinsic origin of the capsaicin-sensitive innervation of the rat duodenum: possible involvement of calcitonin gene-related peptide (CGRP) in the capsaicin-induced activation of intramural nonadrenergic noncholinergic neurons, **Naunyn. Schmied. Arch. Pharmacol.** 334:172.

Maggi, C.A., and Meli A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, **Gen. Pharmacol.** 19:1.

Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Turini, D., Barbanti, G., Beneforti, D., Misuri, D., Meli, A., 1988b, Specific motor effects of capsaicin on human jejunum, **Eur. J. Pharmacol.** 149:393.

Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Turini, D., Barbanti, G., Beneforti, D., Misuri, D., Meli, A., 1989b, Human isolated small intestine: motor responses of the longitudinal muscle to field stimulation and exogenous neuropeptides, **Naun. Schmied. Arch. Pharmacol.** 339:415.

Maggi, C.A., Patacchini, R., Santicioli, P., Theodorsson, E., and Meli, A., 1988a, Several neuropeptides determine the visceromotor response to capsaicin in the guinea-pig isolated ileal longitudinal muscle, **Eur. J. Pharmacol.** 148:43.

Maggi, C.A., Santicioli, P., Del Bianco, E., Geppetti, P., Barbanti, G., Turini, D., and Meli, A., 1989a, Release of VIP but not CGRP-like immunoreactivity by capsaicin from the human isolated small intestine, **Neurosci. Letters** 98:317.

Maggi, C.A., Santicioli, P., Patacchini, R., Geppetti, P., Giuliani, S., Astolfi, M., Baldi, E., Parlani, M., Theodorsson, E., Fusco, M., and Meli, A., 1988c, Regional differences in the motor response to capsaicin in the guinea-pig urinary bladder: relative role of pre- and postjunctional factors related to neuropeptide-containing sensory nerves, **Neuroscience** 27:675.

Wattchow, D.A., Furness, J.B., Costa, M., 1988, Distribution and coexistence of peptides in nerve fibers of the external muscle of human gastrointestinal tract, **Gastroenterology** 95:32.

STUDIES ON SECRETOMOTOR EFFECTS OF GALANIN ON VARIOUS "IN VIVO" OR "IN VITRO" PREPARATIONS

G. Soldani, C. Belloli (1), P. Ormas (1) G. Coruzzi (2) D.R. Brown (3) and C. Beretta (1)

Laboratory of Pharmacology, Faculty of Veterinary Medicine, University of Pisa
(1) Institutes of Veterinary Pharmacology University of Milan and Bari
(2) Institute of Pharmacology University of Parma, Italy
(3) Department of Veterinary Biology University of Minnesota, MN, U.S.A.

INTRODUCTION

Galanin (GAL) is a sensory neuropeptide of 29 amino acids possessing an N-terminal glycine and an amidated C-terminal alanine (Tatemoto et al., 1983).

GAL-like immunoreactivity, originally found in the porcine intestine, has been detected in myenteric and submucosal neurons which project to the intestinal smooth muscle and mucosa, respectively (Melander et al., 1985; Bauer et al, 1986a; Bishop et al., 1986) was subsequently demonstrated in the central nervous system, particularly in the paraventricular nucleus (Skofitsch and Jacobowitz, 1985) and in respiratory and genitourinary tract of other mammalian species (Cheung et al., 1985; Bauer et al., 1986b).

Previous studies demonstrated that administration of synthetic porcine GAL may affect feeding behaviour (Kyrkouli et al., 1986), gastric secretion (Soldani et al., 1987), intestinal motility (Ferrè et al., 1988) and hormone release (Dunning et al., 1986) in experimental animals, although results obtained were sometimes contradictory. The discrepancy may be attributable to differences in neuromuscular circuitry in various animal species, differences in the amino acid composition among the porcine, rat and bovine GAL (Crawley and Wenk, 1989) or different experimental models.

The present experiments were carried out to characterize the effects of synthetic porcine GAL on food intake and secretomotor functions in animal species of veterinary interest and to compare results with those obtained in experimental animals. In addition, experiments with a 1-10 GAL fragment were also carried out.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

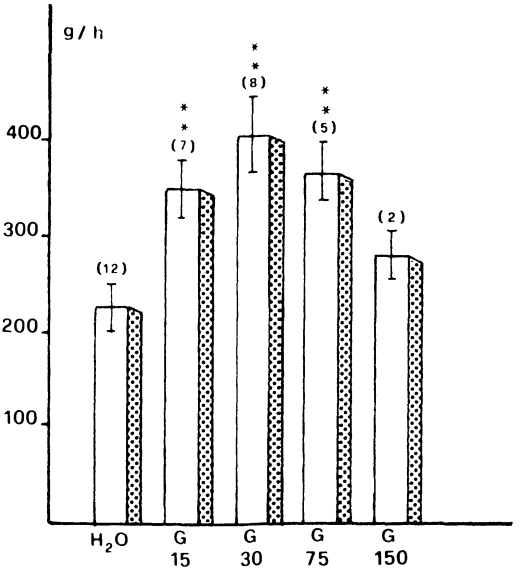


Fig. 1. Orexogenic effects of i.c.v. slow infusions of galanin (G)(ng/kg/min for 30 min) during the 1st h after dosing ( )=number of replications **=P<0.01.

METHODS

Food Intake and Digestive Motility in Sheep

Seven shorn female sheep (Bergamasca breed, 49-64 kg b.w.) equipped with stainless steel cannulas (22 mm in length and 2 mm in diameter) directed towards, but not entering, the lateral cerebral ventricle (Bueno et al., 1983), were implanted with chronic Nickel-Chrome (80:20) electrodes within the muscular layers of their reticulum, abomasum (5-7 cm from pylorus), duodenal bulb (3-4 cm after pylorus), transverse duodenum and colon (Ruckebusch, 1970). The preparative procedures were always performed under surgical anesthesia (pentobarbitone sodium: 25 mg/kg, i.v.). The animals were then housed in special restraining cages, maintained at constant temperature (22 ± 2°C) and fed with hay, water and mineral salt block. To ensure a synchronized daily meal rhythm and a homogeneous pattern of motility of their gastrointestinal tract, the animals were fasted at constant times (9:30-11:30, 12:30-13:30 and 14:30-15:30) while allowed to drink water and lick a mineral salt block ad libitum. Starting during the first fasting time (at 11:20), GAL, dissolved in bidistilled apyrogenic water, was slowly infused (every 48 h) into the cerebral ventricle (i.c.v) (total volume 2 ml; infusion rate = 0.066 ml/min lasting 30 min). Water and food intake were measured gravimetrically in the morning (8:30 and 9:30) and, after dosing, at the end of each feeding period (11:30-12:30, 13:30-14:30, 15:30-16:30). The gastrointestinal electrical activity was always recorded for two hours (11:00-13:00), with an EEG (Neurograph ERA 9 OTE-Biomedica) at the time constant of 0.01 sec., continuously plotted, at 5 sec. intervals, during a period of 5 days, by a multiple linear integrator circuit connected to a potentiometric recorder. During the first 2 hours following GAL administration, the animals were carefully observed to register eventual behavioural changes.

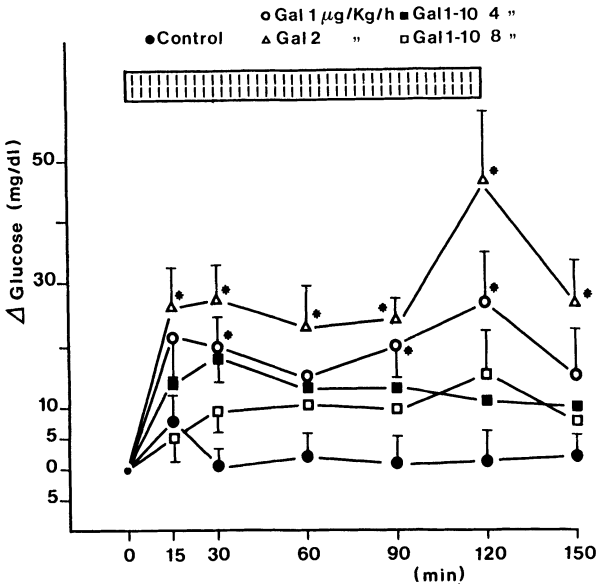


Fig. 2. Effects of GAL or GAL 1-10 infusions ( ) on unstimulated plasma glucose in conscious dogs. Each point represents the mean value of 3-5 dogs ± S.E. (vertical bars). *P<0.05 vs control.

Gastric Acid Secretion and Gastrointestinal Hormones in Dogs

Experiments were performed on 6 adult mongrel dogs (12-17 kg b.w.) chronically implanted with gastric cannulas. After 18 h fast, intravenous catheters were placed in a hindlimb for blood sampling and a forelimb for administration of drugs or control saline infusion. In a first series of experiments, dogs received 120 min infusion of saline (control), GAL (1-2 μg/kg/h) or GAL 1-10 (4-8 μg/kg/h). In a second series of experiments, 2-deoxy-D-glucose (2DG; 100 mg/kg i.v. bolus) was given alone (control) or with simultaneous addition of i.v. infusion of GAL (2-4 μg/kg in 15 min) or GAL 1-10 (4-8 μg/kg in 15 min). Blood samples were obtained at 0, 15, 30, 60, 90 and 120 min after starting the infusion. All samples of 5 ml volume were put into heparinized tubes with the addition of 500 KIU aprotinin (Bayer, Leverkusen, FRG) per ml of blood, centrifuged at 4°C and the plasma stored at -20°C. A small amount of each blood sample was used for determination of plasma glucose, using a glucose-oxidase technique (Sclavo, Siena, Italy). The concentrations of immunoreactive insulin (IRI), glucagon (IRG) or somatostatin (SLI) were determined by RIA. Student's paired t test was used for comparisons between control and treatment means.

Assay of Contractility in Longitudinal Muscle of Pig Proximal Jejunum

The muscularis externa from a 20 cm segment of proximal jejunum was isolated from Yorkshire pigs of either sex, dissected free from epithelium and cut into thin strips (1 cm long and 0.5 cm wide) which were oriented in the longitudinal plane. Muscle strips were suspended isometrically at 1.0g tension in 10 ml

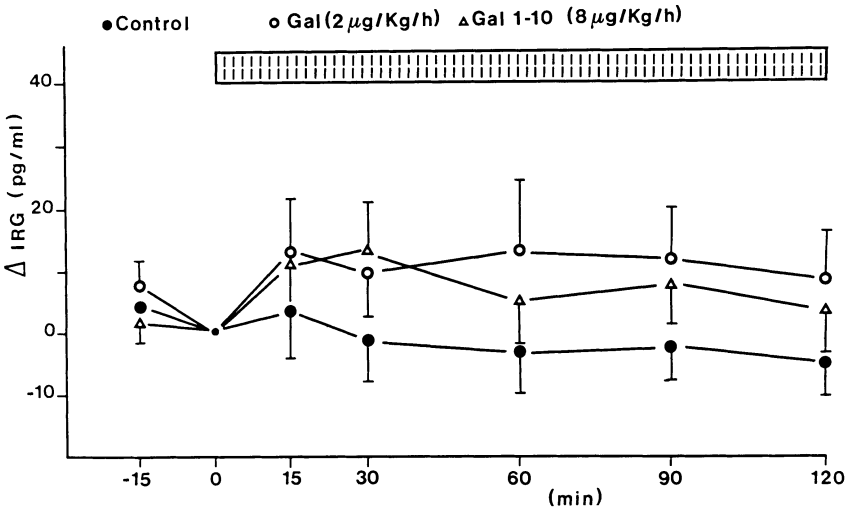


Fig. 3. Effects of GAL or GAL 1-10 infusions ([▦]) on plasma glucagon concentrations (IRG;pg/ml) in conscious dogs. Each point represents the mean value of 3-5 dogs ± S.E. (vertical bars).

capacity silicon-coated organ baths. The strips were bathed in Ringer-HCO_3 buffer, maintained at pH 7.4 and 39°C (porcine core temperature) and oxygenated continuously (5%:95% CO_2/O_2). Muscle contractions were recorded with a force displacement transducer connected to a 4-channel polygraph (Model 79-D, Grass Instruments Co., Quincy, MA). Chart records of muscle contractions were quantified by planimetry and data are expressed in terms of the motor index ratio (MIR); MIR is derived from the following equation: MIR = (A2/A1)-1 where A1 and A2 represent the area of chart pen displacement (in mm^2) per unit time (in sec) in control and GAL treatment periods, respectively.

Assay of Ion Transport in Mucosa-Submucosa of Pig Distal Jejunum

A 20 cm segment of distal jejunum was stripped of its serosa by blunt dissection and the remaining submucosa and mucosa was isolated as described previously (Brown et al., 1990). Mucosal sheets were mounted between two lucite Ussing-type half chambers having an exposed serosal surface area of 2.01 cm^2. Mucosal sheets were bathed in Ringer-HCO_3 buffer under identical conditions to those employed in the smooth muscle bioassay. Bathing media was contained in 10 ml water-jacketed siliconized glass reservoirs. D-Glucose and mannitol were added respectively to the media bathing the contraluminal and luminal sides of each sheet to achieve a final concentration of 10 mM. Short-circuit current (Isc), a bioelectrical measure of active transcellular ion transport, was measured continuously under voltage-clamped conditions as described previously (Brown et al., 1990). In experiments employing electrical transmural stimulation (ES), bipolar pulses of electrical current (300 pulses at 10 Hz, 0.5 msec pulse duration, 2.8 mA/cm^2) were delivered across mucosal sheets by stimulator (Model S-88, Grass Instruments, Quincy, MA) connected to aluminium foil electrodes which were placed diagonally on opposite sides of each tissue. In each experiment, GAL

was administered serosally in cumulative concentrations to one tissue prior to ES; ES was delivered to a second tissue untreated with the peptide which served as a paired control. Peak changes in Isc relative to baseline were determined in response to ES or peptide administration after stabilization of the spontaneous Isc.

Mare Uterine and Bronchial Motility

Tissues were obtained from healthy mares (450-500 kg b.w.) and immediately transferred in oxygenated (95% O_2; 5%CO_2) Krebs-Henseleit solution of the following composition (mM/l): NaCl 113, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.5.

Uterus. Myometrial longitudinal strips (1.5 cm long and 0.3 cm wide) obtained from dioestrus uteri were suspended isometrically under 2.0 g tension at 37°C, according to the technique previously described (Coruzzi et al., 1989). After an equilibration time of about 90 min, the effect of GAL was evaluated on the spontaneous phasic contractions, which were well maintained up to 6-7 hours.

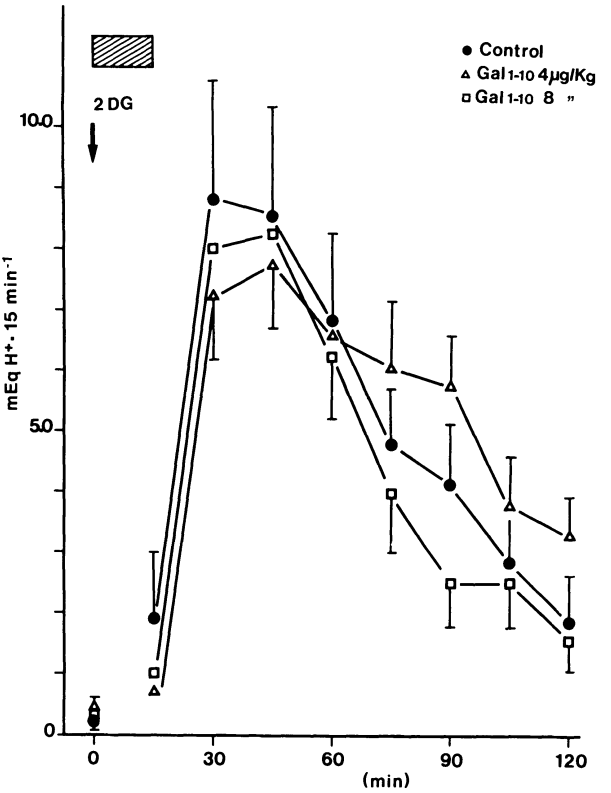


Fig. 4. Effects of GAL 1-10 (infused over a period of 15 min; ▨) on gastric acid secretion stimulated by 2DG (100 mg/kg at the arrow) from gastric fistula dogs. Each point represents the mean value of 4-5 dogs ± S.E. (vertical bars).

Bronchi. Spiral strips (2.0 cm long and 0.3 cm wide) obtained from small bronchi were suspended isometrically at 37°C under 2.0-2.5 g tension. The effect of GAL was evaluated on both the resting tone and the contractions evoked by field stimulation (10 sec trains of pulses, 5 Hz, 0.5 msec, 150-200 mA, delivered at 2 min intervals).

RESULTS

Food Intake and Digestive Motility in Sheep

As shown in Fig. 1, i.c.v. infusion of GAL at doses of 15.0 and 30.0 ng/kg/min for 30 min produced a significant dose-dependent increase of food intake in sheep during the first hour after dosing (+53.01% and +76.66% vs control respectively), but not during the successive periods of observation. At these doses, the peptide never elicited any change in animal behaviour. By contrast, at higher doses (75.0 and 150.0 ng/kg/min for 30 min i.c.v.) GAL produced behavioural changes characterized by restlessness and generic signs of CNS stimulation. The animals appeared to be voracious and packed into their mouths food in such quantity that they were not able to chew or swallow properly. This state resulted in food consumptions lower than those registered with the lower doses of GAL. Moreover, these dose-related behavioural changes lasted about one hour. Both at orexogenic and behaviour-affecting doses, GAL never modified water intake or gastrointestinal motility in sheep.

Gastric Acid Secretion and Gastrointestinal Hormones in Dogs

Basal acid output (0.14 ± 0.06 mEq H^+/15 min; n=9) was not significantly modified by infusion of GAL at the dose of 2 μg/kg/h for 120 min (0.09 ± 0.03 mEq H^+/15min; n=5) nor by infusion of GAL 1-10 at the dose of 8 μg/kg/h for 120 min (0.11 ± 0.06 mEq H^+/15 min;n=4). In these experiments, a rapid onset of hyperglycemia after GAL, but not GAL 1-10, was also observed (Fig. 2). Within 15 min of GAL (2 μg/kg/h), plasma glucose concentrations rose from 92 mg/dl to 119 mg/dl; a maximum was reached at 120 min (140 mg/dl) and concentrations dropped at the

Tab. 1. Changes in plasma insulin concentrations (IRI; μU/ml) at various intervals under basal conditions. N=number of dogs; means ± S.E. * P<0.05 vs control.

	N	IRI basal (μU/ml)	Δ IRI				
			15 min.	30 min.	60 min.	90 min.	120 min.
Control (NaCl 0.9%)	4	16.7± 1.7	-0.1± 1.7	-0.8± 2.0	-1.5± 2.3	-0.2± 1.8	0.1± 1.4
GAL (1 μg/kg/h)	5	12.5± 1.8	-7.1± * 2.3	-9.0± * 1.8	-8.4± * 1.2	-6.5± * 2.6	-5.3± * 1.8
GAL (2 μg/kg/h)	4	14.3± 2.2	-9.5± * 4.2	-11.8± * 1.6	-10.6± * 4.2	-9.7± * 3.3	10.8± * 2.9
GAL 1 - 10 (4 μg/kg/h)	3	18.7± 1.5	-1.6± 2.3	-3.4± 1.7	-1.9± 1.3	0.7± 1.8	-0.3± 2.6
GAL 1 - 10 (8 μg/kg/h)	4	15.0± 1.4	-2.3± 1.6	-1.6± 2.2	2.2± 1.8	1.1± 1.4	-1.6± 2.4

Tab. 2. Changes in plasma somatostatin concentrations (SLI; pg/ml) at various intervals under basal conditions. N=number of dogs; means ± S.E.

	N	SLI basal (pg/ml)	Δ SLI		
			30 min	60 min	90 min
Control (NaCl 0.9%)	5	54.6 ± 7.9	-3.4 ± 5.7	8.8 ± 9.5	-4.2 ± 6.3
GAL (2 μg/Kg/h)	5	49.5 ± 12.6	8.4 ± 6.2	9.5 ± 7.3	-1.5 ± 4.4
GAL 1-10 (8 μg/Kg/h)	4	57.3 ± 11.4	10.9 ± 3.5	4.0 ± 2.2	4.0 ± 3.1

end of the infusion. Reciprocal changes were observed in plasma insulin concentrations (Tab. 1). An immediate decrease in plasma insulin concentrations occurred within 15 min of GAL infusion; by contrast, GAL 1-10 was ineffective (Tab. 1). On these conditions, plasma concentrations of somatostatin (Tab. 2) and glucagon (Fig. 3) were not significantly modified by GAL or GAL 1-10.

GAL, given i.v., dose-dependently inhibited 2DG-stimulated gastric acid secretion: maximal inhibition was obtained with GAL at the dose of 4 μg/kg (2.87 ± 0.74 mEq H^+/15 min vs 8.62 ± 2.54 mEq H^+/15 min at 30 min; $P<0.05$: n=5); by contrast, GAL 1-10 was ineffective (Fig. 4).

The activation of autonomic nerves was produced by neuroglycopenia induced by 2DG and elevated glucose and insulin plasma levels. On these experimental conditions, GAL, but not GAL 1-10, further increased plasma glucose levels and induced a marked but short-lasting inhibition of insulin release (Fig. 5 and 6).

Intestinal Contractility and Ion Transport in Pigs

Under baseline conditions, longitudinal muscle strips from the porcine proximal jejunum displayed irregular phasic contractions with a frequency of approximately 15/min. GAL increased the amplitude of phasic contractions without altering basal muscle tone with an EC_{50} or 13 nM; the maximum contraction produced by GAL was 25% of that produced by 10^{-5} M carbachol. The effects of GAL were unaltered by the neuronal conduction blocker tetrodotoxin (TTX; 10^{-7} M) but were inhibited by omega-conotoxin GVIA (CgTX; Fig. 7). Additional experiments revealed that acetylcholine and substance P do not mediate the contractile actions of GAL.

Under baseline conditions, the distal jejunal mucosa manifests net Na and Cl absorption (Brown et al., 1990). ES produces a transient rise in Isc attributable to the stimulation of active anion secretion; this effect of Es is inhibited by TTX or CgTX indicating that it results from activation of submucosal neurons (Hildebrand and Brown, MS submitted). In 4 out of every 5 tissues examined, the contraluminal addition of 10^{-7} GAL produced a slight (6-10 $\mu A/cm^2$) decrease in basal Isc. Over a wide concentration range, contraluminal GAL attenuated mucosal

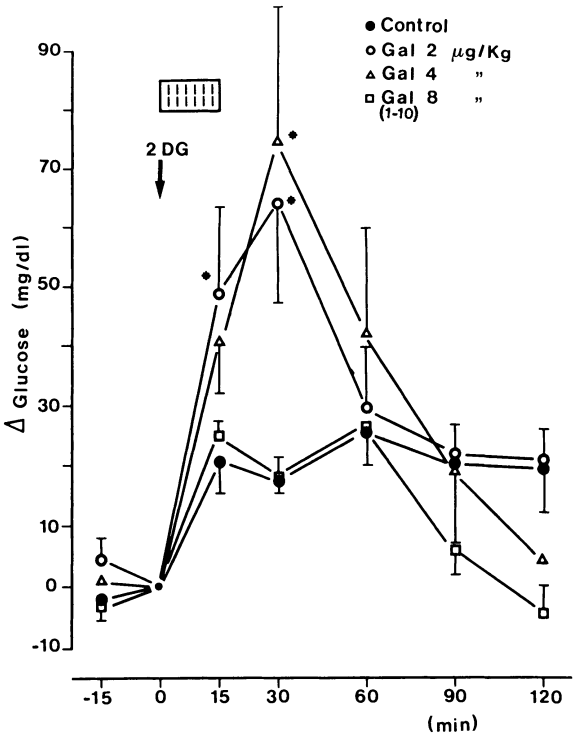


Fig. 5. Effects of GAL or GAL 1-10 (infused over a period of 15 min; ▦) on 2DG-induced hyperglycemia (control) from gastric fistula dogs. Each point represents the mean value of 4-5 dogs ± S.E. (vertical bars). *P<0.05 vs control.

responses to ES in a concentration-dependent manner with an IC_{50} of 13 nM (Fig. 8). The results of additional experiments indicated that GAL effects are not mediated through enteric opioids or norepinephrine.

Mare Uterine and Bronchial Motility

Uterus. GAL (10^{-8} - $5x10^{-7}$ M) did not modify the amplitude and the frequency of spontaneous contractions (Fig. 9), nor did it induce the appearance of phasic motility in quiescent strips.

Bronchi. GAL (10^{-8} - $3x10^{-7}$ M) had no effect on the resting tone of the muscle or the contractile effect of acetylcholine. The peptide was also ineffective in altering the contractile response to field stimulation (Fig. 10).

DISCUSSION

As observed in rats injected with GAL into their paraventricular nucleus (PVN) (Kyrkouli et al., 1986), GAL stimulates food intake also in sheep when injected i.c.v. The orexogenic effect of the peptide is at the lower assayed doses (i.e. 15.0 and 30.0 ng/kg/min for 30 min) but is concealed by other behavioural

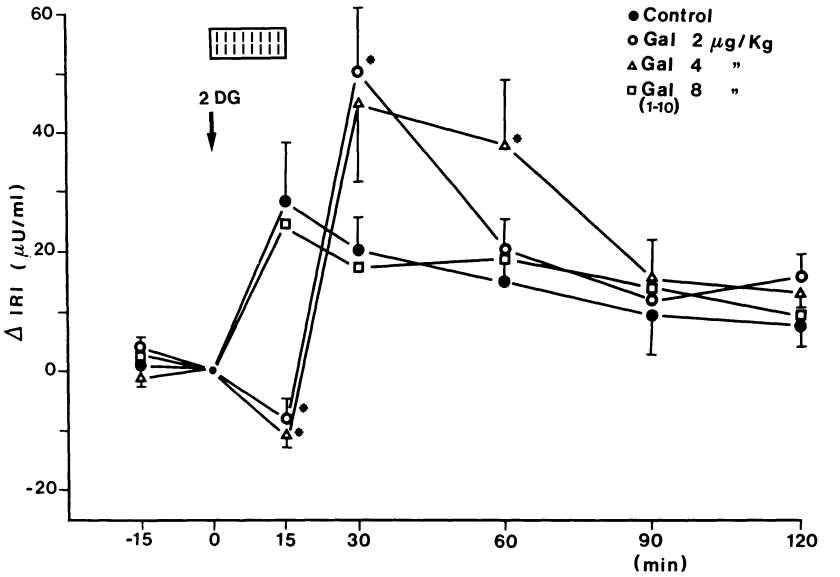


Fig. 6. Effects of GAL or GAL 1-10 ([▦]) on the increase in plasma levels of insulin (IRI; μU/ml) induced by 2DG from gastric fistula dogs. Each point represents the mean value of 4-5 dogs ± S.E. (vertical bars). *P<0.05 vs control.

changes at higher doses (i.e. 75.0 and 150.0 ng/kg/min for 30 min). In any case, however, GAL does not affect water intake or gastrointestinal motility in sheep. These findings suggest a central site of action of GAL administered by the i.c.v. route: a moderate inhibition of the medial hypothalamic area at lower doses and a more intense inhibition of the same area at higher ones. On this basis, GAL could play the role of a short-term signal for hunger in sheep, probably by an involvement of adrenergic pathways. The high concentrations of GAL into PVN (Skofitsch and Jacobovitz, 1985), the colocalization of GAL with norepinephrine in neuronal cells of the locus coeruleus (Rokaeus

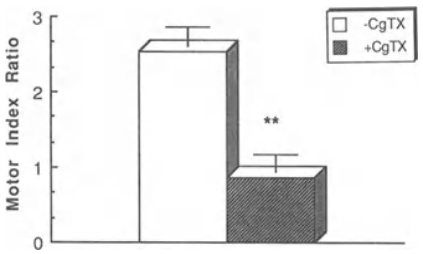


Fig. 7. Histogram depicting the contractile actions of 3 x 10^{-7} M GAL on longitudinal muscle from porcine proximal jejunum in the absence (-CgTX) and presence of 10^{-7} M CgTX (+CgTX). The motor index ratio produced by GAL was reduced by 66% in the presence of CgTX (**P<0.01,n=5 tissues, paired t test).

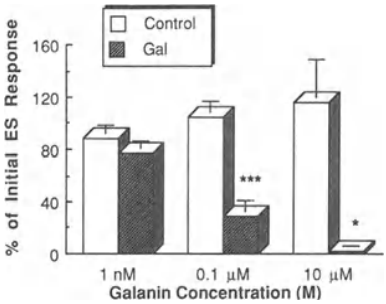


Fig. 8. Inhibition of GAL of mucosal Isc responses to electrical stimulation (ES,10Hz for 30 sec, 0.5 msec pulse duration, 2.8mA/cm^2). Bars represent mean ± S.E. of percentage changes in Isc elevations induced by ES in the absence (Control) and presence (Gal) of GAL at the cumulative concentrations indicated compared to initial tissue response to ES (*P<0.05 and ***P<0.001 vs control mean, n=3-6, unpaired t test). Note that tachyphylaxis did not develop in control tissue responses to repeated ES deliveries.

et al., 1984), the observed release of norepinephrine induced by i.c.v. GAL in the PVN (Melander et al., 1986), the lack of the effect when the peptide is injected in other regions of CNS or peripherally (Kyrkouli et al., 1986) and the central excitation observed at higher doses might explain this hypothesis.

The absence of activity of i.c.v. GAL on sheep gastrointestinal motility may be explained by its inability to cross the blood-brain barrier and reach peripheral gastrointestinal sites (Gonda et al., 1986; Fox et al., 1986). This suggestion seems only partially in disagreement with the inhibition of duodenojejunal and stimulation of colonic motilities (Ferrè et al., 1988) exerted by very high doses of i.c.v. GAL in rats and explained as effects on spinal pathways.

In dogs, GAL had no effect on basal acid secretion, but it significantly decreased acid secretion stimulated by 2DG that activates cholinergic neuronal pathways through a central mechanism. The fact that gastrin release was not significantly affected by GAL indicates that changes in the plasma levels of gastrin are not the major mechanism by which GAL inhibits gastric acid secretion in dogs. It is thus possible that the inhibitory effect of GAL on gastric secretion might be due to an impairment of cholinergic transmission (Soldani et al., 1987). The absence of any significant influence of GAL 1-10 on vagally induced gastric acid secretion indicates that this fragment is unable to impair cholinergic transmission.

Further, the present experiments demonstrate that GAL markedly inhibits not only basal insulin secretion in dogs, which was known before (McDonald et al., 1985), but also insulin secretion induced by 2DG. It has been previously reported that 2DG induces an atropine-sensitive stimulation of the secretion of insulin (Karlsson et al., 1987). Thus GAL, but not GAL 1-10, seems to be a general inhibitor of insulin release in the dog, perhaps by interfering with an essential step in the secretory mechanism of pancreatic B cells. In our experiments, GAL did not

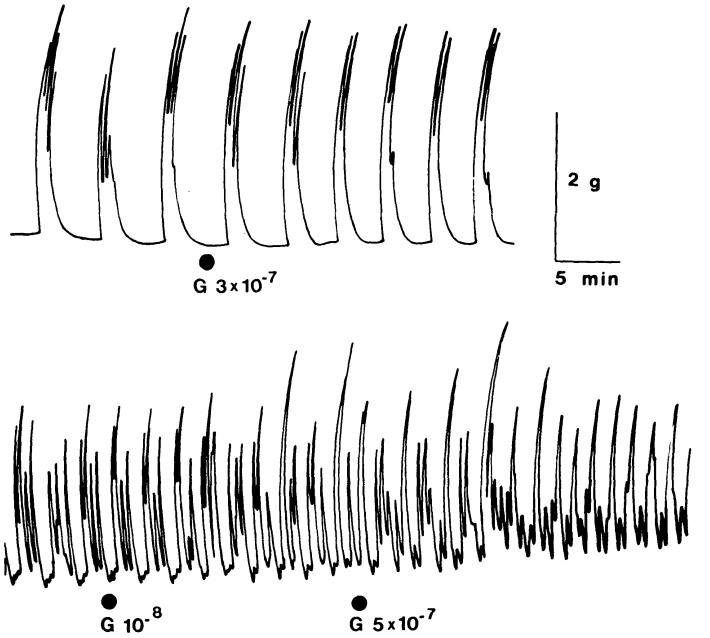


Fig. 9. Isolated mare uterus. Lack of effect of galanin (G) administered in molar concentrations on the spontaneous phasic activity. On the ordinata tension of the transducer in grams. Time in minutes.

affect unstimulated glucagon and somatostatin release, in accordance with McDonald et al. (1985) and Manabe et al. (1986) who described a lack of effect of GAL on peripheral plasma glucagon in conscious dogs. Conversely, Dunning et al. (1986) have reported that, in anesthetized dogs, injection of GAL into the pancreatic artery stimulated glucagon output as measured in the superior pancreatic-duodenal vein. The discrepancies between these results might be due to different experimental conditions (peripheral vs local blood) and suggest the possible existence of differential sensitivity to GAL of the B-, A- and D-pancreatic cells (Hermansen, 1987).

GAL alters both propulsive activity and electrolyte transport in the porcine jejunum. GAL potency in porcine tissues was remarkably similar to that determined in other experiments (Ekblad et al., 1985; Maggi et al., 1989). Although the presynaptic neurotoxin CgTX inhibited the contractile effects of GAL, the neurotransmitters mediating GAL effects have yet to be identified. In addition to its motility action, GAL inhibited basal and ES-stimulated Isc. Its potency in inhibiting mucosal responses to ES was identical to its contractile potency. The ionic bases of GAL actions on the Isc remain to be clearly defined, but probably reflect a GAL-induced reduction of tonic activity in submucosal neurons. GAL has also been reported to inhibit ES-induced elevations in Isc across the guinea-pig distal colon in vitro, although it exhibits considerably lower efficacy in this preparation (McCulloch et al., 1987). Located as it is in myenteric and submucosal neurons of the small intestine, GAL may act in some fashion to coordinate the ongoing activities of

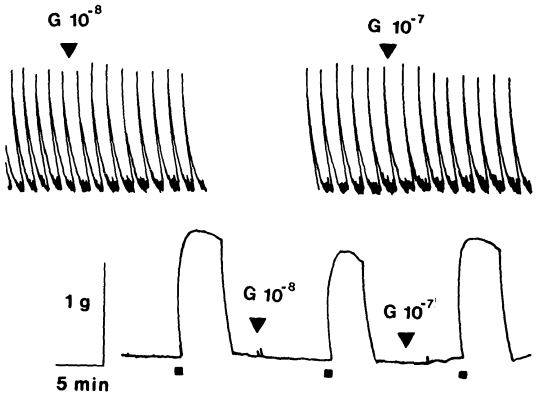


Fig. 10. Isolated mare bronchial muscle. Lack of effect of galanin (G) administered in molar concentrations on electrically-stimulated strips (upper panel) and on unstimulated preparations (lower panel). Galanin did not modify the contractions evoked by acetylcholine (■). On the ordinata tension of the transducer in grams. Time in minutes.

the distinct but neurally-interconnected intestinal epithelium and smooth muscle.

Porcine GAL seems to be without effect on mare uterine and bronchial musculature. The ineffectiveness of this peptide on mare bronchial muscle is in accordance with data obtained in the guinea-pig, in which GAL had no effect on carbachol- or electrically-induced contractions, nor did it have any effect on resting preparations (Ekblad et al., 1985). However, the possibility that the mare muscle would require a specific (equine) GAL, different from porcine GAL used, cannot be discarded. Though immunohistochemical findings demonstrated the presence of GAL-like immunoreactivity in the genital and respiratory tract of different species (Rokaeus, 1987), the functional role of this peptide in these systems remains to be elucidated.

References

Bauer, F.E., Adrian T.E., Christofides, N.D., Ferri, G.L, Yanaihara A.N., Polak, J.M, and Bloom, S.R., 1986a, Distribution and molecular heterogeneity of galanin in human, pig, guinea-pig and rat gastrointestinal tracts, **Gastroenterology**, 91:877.

Bauer, F.E., Christofides, N.D., Hacker, G.W., Blank, M.A., Polak, J.M., and Bloom, S.R., 1986b, Distribution of galanin immunoreactivity in the genitourinary tract of man and rat, **Peptides**, 7:5.

Bishop, A.E., Polak, J.M., Bauer, F.E., Christofides, N.D., Carlei, F., and Bloom, S.R., 1986, Occurrence and distribution of a newly discovered peptide, galanin, in the mammalian enteric nervous system, **Gut**, 27:849.

Brown, D.R., Overend, M.F., and Treder, B.G., 1990, Neurohormonal regulation of ion transport in the porcine distal jejunum. Actions of somatostatin-14 and its natural and synthetic homologs, **J. Pharmacol. Exp. Ther.**, 252:126.

Bueno, L., Duranton, A., and Ruckebusch Y., 1983, Antagonistic effects of naloxone on CCK-octapeptide induced satiety and rumino-reticular hypomotility in sheep, **Life Sci.**, 32:855.
Cheung, A., Polak, J.M., Bauer, F.E., Christofides, N.D., Cadieux, A., Springall, D.R., Bloom, S.R., 1985, The distribution of galanin immunoreactivity in the respiratory tract in pig, guinea-pig, rat and dog, **Torax**, 40:889.
Coruzzi, G., Poli, E., and Bertaccini, G., 1989, Effect of calcium-channel blockers and salbutamol on the isolated mare uterus. Interaction with the calcium agonist Bay K 8644, **J. Vet. Pharmacol. Ther.**, 12:404.
Crawley, J.N., and Wenk, J.L., 1989, Coexistence of galanin and acetylcholine: is galanin involved in memory processes and dementia?, **Trends Neurosci.**, 12:278.
Dunning, B.E., Ahren, B., Veith, R.C., Bottcher, G., Sundler, F., and Taborsky, G.J. Jr., 1986, Galanin: a novel pancreatic neuropeptide, **Am. J. Physiol.**, 251: E127.
Ekblad, E., Hakanson, R., Sundler, F., and Wahlestedt, C., 1985, Galanin: neuromodulatory and direct contractile effects on smooth muscle preparations, **Br. J. Pharmacol.**, 86:241.
Ferrè, J.P., Soldani, G., and Ruckebusch, Y., 1988, Actions of galanin on intestinal and colonic motility in conscious rats and dogs, **Pharmacol. Res. Commun.**, 20 (suppl.1):161.
Fox, J.E.T., McDonald, T.J., Kostolanska, F., and Tatemoto, K., 1986, Galanin: an inhibitory neural peptide of the canine small intestine, **Life Sci.**, 39:103.
Gonda, M., Daniel, E.E., Fox, J.E.T., McDonald, T.J., and Oki, M., 1986, Galanin: locus and mechanism of action of a new peptide in canine gastrointestinal tract, **Gastroenterology**, 90:1432.
Hermansen, K., 1987, Differential sensitivity to galanin of pancreatic insulin, somatostatin and glucagon secretion, **Diabetes**, 36(suppl.1):95A.
Karlsson, S., Bood, M., Ahren, B., 1987, The mechanism of 2-deoxy-glucose-induced insulin secretion in the mouse, **J. Autonom. Pharmacol.**, 7:135.
Kyrkouli, S.E., Stanley, B.G, and Leibowitz, S.F., 1986, Galanin: stimulation of feeding induced by medial hypothalamic injection of this novel peptide, **Eur. J. Pharmacol.**, 122:159.
Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Turini, D., Barbanti, G., Beneforti, P., Misuri, D., and Meli, A., 1989, Human isolated small intestine: motor responses of the longitudinal muscle to field stimulation and exogenous neuropeptides, **Naunyn- Schmiedeberg's Arch. Pharmacol.**, 339:415.
Manabe, T., Yoshimura, T., Kii, E., Tanaka, Y., Ohshio, G., Tobe, J., Akaji, K., Yajima, H., 1986, Galanin-induced hyperglycemia: effect on insulin and glucagon, **Endocr. Res.**, 12:93.
McCulloch, C.R., Kuwahara, A., Condon, C.D., and Cooke, H.J., 1987, Neuropeptide modification of chloride secretion in guinea pig distal colon, **Regul. Peptides**, 19:35.
McDonald, T.J., Duprè, J., Tatemoto, K., Greenberg, G.R., Radziuk, J., and Mutt, V., 1985, Galanin inhibits insulin secretion and induces hyperglycemia: effect on insulin and glucagon, **Endocr. Res.**, 12:93.
Melander, T., Hokfelt, T., Rokaeus, A., Fahrenburg, J., Tatemoto, K., and Mutt, V., 1985, Distribution of galanin-like immunoreactivity in the gastrointestinal tract of several mammalian species, **Cell. Tiss. Res.**, 239:253.
Melander, T., Fuxe, K., Harfstrand, A., Eneroth, P., Andersson, K., and Hokfelt, T., 1986, Effects of intraventricular

injections of galanin on the hypothalamic catecholamine neurons and neuroendocrine regulation, **Neurosci. Lett.**, (suppl.26),S298.
Rokaeus, A., Melander, T., Hokfelt, T., Lundberg, J.M., Tatemoto, K., Carlquist, M., Mutt, V., 1984, A galanin-like peptide in the central nervous system and intestine of the rat, **Neurosci. Lett.**, 47:161.
Rokaeus, A., 1987, Galanin: a new biologically active neuropeptide, **Trends Neurosci.**, 10:158.
Ruckebusch, Y., 1970, The electrical activity of digestive tract of sheep as an indication of the mechanical events in various regions, **J. Physiol.**, 210:857.
Skofitsch, G., and Jacobowitz, D.M., 1985, Immunoistochemical mapping of galanin-like neurons in the rat central nervous system, **Peptides**, 6:509.
Soldani, G., Mengozzi, G., Della Longa, A., Intorre, L., Martelli, F., and Brown, D.R., 1987, An analysis of the effects of galanin on gastric acid secretion and plasma levels of gastrin in the dog, **Eur. J. Pharmacol.**, 154:313.
Tatemoto, K., Rokaeus, A., Jornvall, H., McDonald, T.J., and Mutt, V., 1983, Galanin: a novel biologically active peptide from porcine intestine, **FEBS Lett.**, 164:124.

ASPECTS ON THE ROLE OF TACHYKININS AND VASOACTIVE INTESTINAL POLYPEPTIDE IN CONTROL OF SECRETION, MOTILITY AND BLOOD FLOW IN THE GUT

Elvar Theodorsson[1], Bengt Smedfors[2], Per Hellström[2], Olof Söder[3], Andreas Aly[2], Alexandru Musat[1], Asit B. Panja[2], and Catja Johansson[2]

Departments of Clinical Chemistry[1], Medicine[2], and Pediatric Endocrinology[3], Karolinska Hospital, Stockholm, Sweden

ABSTRACT

Both intrinsic and extrinsic neurons of the gut respond to mechanical and chemical stimuli by the release of neurotransmitters. We summarize here some of our recent work on the role of vasoactive intestinal polypeptide (VIP), substance P (SP) and neurokinin A (NKA) in the secretory, motor and vascular effects of hydrochloric acid stimulation in the isolated rat duodenal loop and electrical nerve stimulation and mechanical stimulation of the cat colon. Isolated duodenal loops of conscious rats were perfused with isotonic saline, and challenged at hourly intervals with brief exposures to increasing concentrations of HCL. The concentrations of bicarbonate and prostaglandin E_2 (PGE_2) released from the duodenal mucosa were significantly augmented already by pH 5.0 whereas VIP was significantly augmented at pH 3.0 and the tachykinins SP and NKA at pH 2.0. Continuous electric stimulation of the pelvic nerve in cats at 4 Hz during 1 s with 10 s rest produced a marked release of NKA-LI and SP-LI from the colon to blood. Reflex activation of the pelvic nervae by mechanical stimulation of the anus or rectal distension produced a less pronounced release of NKA-LI and SP-LI from the colon to blood. There was a simultaneous colonic contraction and vasodilation during each nerve stimulation. Close intraarterial infusions of NKA, neurokinin B, SP, neuropeptide K (NPK), eledoisin and physalemin at doses of 0.1-100 pmol/min induced dose-dependent proximal and distal colonic contractions and vasodilation, NKA being the most potent. The effects of the tachykinins were reduced after tetrodotoxin and atropine, but unchanged after treatment with hexamethonium. Taken together, the present findings indicate that peptides present in primary afferents including VIP, SP, and NKA are involved in physiological regulatory mechanisms including smooth muscle contraction, bicarbonate secretion and vasodilation in the gut.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

INTRODUCTION

The nervous system of the gastrointestinal tract consists of both intrisic and extrinsic neurons. Both types of neurons respond to mechanical and chemical stimuli by release of neurotransmitters. The present paper summarizes some of our recent work on the role of vasoactive intestinal polypeptide and tachykinins in the secretory, motor and vascular effects of hydrochloric acid stimulation in the isolated rat duodenal loop and electrical nerve stimulation and mechanical stimulation of the cat colon.

There are two major sources of vasoactive intestinal polypeptide and tachykinins in the gastrointestinal tract: intrinsic neurons of the submucosal and myenteric plexa and peripheral endings of capsaicin-sensitive sensory neurons. Capsaicin-sensitive neurons are widely distributed in the gastrointestinal tract of several species (Matthews and Cuello, 1984; Sharkey et al., 1984). However, in contrast to the other parts of the body, capsaicin only partially depletes the tissue concentrations of sensory neuropeptides in the gut since they are to a large extent present in intrinsic neurons (Holzer et al., 1986; Ekblad et al., 1987). Neuropeptides in sensory neurons include substance P (SP), neurokinin A (NKA), calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP) which are believed to participate in the regulation of secretion, blood flow, motility and cell growth (Holzer, 1988).

Locally released VIP is a probable regulator of mucosal functions, such as water secretion. Dilution by water secretion is an intestinal defence mechanism against potentially injurious luminal substances. A decade ago Flemström proved the long suspected existence of another protective mechanism, the HCO_3^- secretion by which the gastroduodenal mucosa neutralizes HCL (Flemström, 1977). The duodenum has a supply of VIP and tachykinin containing neurons, and these mediators may participate also in the HCL stimulated duodenal HCO_3^- response. Duodenal acidification releases VIP into the portal blood of pigs, and peripheral blood of man, and HCL stimulated duodenal HCO_3^- secretion can be reduced by a VIP receptor antagonist in rats. Furthermore, exogenous VIP potently stimulates duodenal HCO_3^- secretion in different species, including man.

Stimulation of the pelvic nerve and activating the pelvo-pelvic reflex by distending the rectum or by mechanical stimulation of the anal wall elicits a non-adrenergic non-cholinergic vasodilation and smooth muscle contraction of the colon in the cat. Studies have suggested a role for VIP and kinins in the vasodilation and opioids in the contraction. However the role of tachykinins in this reflex is unknown. In the present work we present evidence that tachykinins may be involved in this response by investigating the presence, molecular forms, release mechanisms and effects of multiple tachykinins in the cat colon.

MATERIALS AND METHODS

Rat Duodenal Loop

A two cm duodenal loop, from the pylorus to a few millimeters proximal to the entrance of the pancreatico-biliary duct

was prepared as described earlier (Isenberg et al., 1985). A polyethylene catheter, placed in the duodenal bulb, was threaded subcutaneously to exit interscapularly. The duodenal segment was drained to the exterior. After recovering from surgery, the rats were trained to accept the test conditions restrained in Bollman cages. The loop was perfused with saline, and effluents were collected in 15 min aliquots at a perfusion rate of 0.5 mL/min. After four 15 min saline perfusion periods, duodenal HCO_3^- secretion was stimulated with either luminal HCL perfusion or i.v. VIP infusion. Three 15 min saline perfusion periods separated the exposures to HCL of increased acidity (0.01, 1, 10, 50, 100 and 150 nmol/L). In some test series, prostaglandin synthesis was inhibited with an intraperitoneal injection of 4 mg/kg indomethacin one hour before exposure to HCL. Perfusate effluents were analyzed for HCO_3^- by backtitration and VIP, SP and NKA by radio-immunoassays and PGE_2 by gas chromatography-mass spectrometry (Aly et al., 1984).

Cat Colon

The experiments were performed on 29 cats of both sexes weighing 3-5 kg. The greater omentum, the spleen and the small intestine were extirpated, leaving the colon intact in position. The volume changes of the proximal and distal colon were measured using volume recording devices.

Extraction of Plasma and Luminal Perfusate

Plasma was extracted by acid ethanol and luminal perfusates using Sep Pack cartridges as described by Theodorsson-Norheim et al., 1987.

Radioimmunoassays

Substance P and neurokinin A were measured using C-terminally directed antisera SP2 (Brodin et al., 1986) and K12 (Theodorsson-Norheim et al., 1985) respectively. Vasoactive intestinal polypeptide was analyzed using a slightly modified method described by Fahrenkrug and Schaffalitzky de Muckatell, 1977).

High Performance Liquid Chromatography (HPLC)

Reverse-phase high performance liquid chromatography was performed with a Supelco^R LC-18-DB, 5 μm, 4.6x250 mm column eluted with a 40 min linear gradient of 20-40% acetonitrile in water containing 0.1% trifluoroacetic acid.

RESULTS

Rat Duodenal Loop

HCO_3^- and PGE_2 were present in all basal effluents whereas VIP, NKA and SP were only detected in basal effluents from a minority of the rats. Brief exposures of the duodenal loop to stepwise, increasing concentrations of HCL (from 0.01 to 150 nmol/L) elevated the alkalinization of the duodenal contents in a concentration-dependent manner. Challenge with 0.01 nmol/L HCL (pH 5) increased duodenal HCO_3^- secretion significantly as compared to both basal saline perfused at pH 6 ($p<0.05$), and a bicarbonate-saline solution perfused at pH 7.6 ($p<0.05$). The duo-

denal HCO_3^- responses to 0.01, 1 and 10 nmol/L HCL were transient and of moderate magnitude, but exposures to higher acidities (50, 100 and 150 nmol/L were followed by sustained increases in secretion rate up to four-fold the basal secretion.

PGE_2 was present in basal duodenal perfusates from all rats. The luminal output of PGE_2 was elevated three-fold from basal levels by as little as 0.01 nmol/L HCL, and was further elevated in a concentration related manner by increasing acidity.

VIP-like immunoreactivity was detected in basal duodenal perfusates from four out of ten rats. Perfusion with 1 nmol/L HCL resulted in a statistically significant ($p<0.05$) elevation of luminal VIP, and subsequent perfusions with stronger solutions of HCL were associated with concentration-related increments. Luminal VIP was present in all effluents from duodenal loops exposed to 100 and 150 nmol/L HCL. Increases of luminal VIP were transient at low acidities. However, exposures to 100 and 150 nmol/L HCL produced sustained elevations. Chromatographic characterization showed the presence of a single component corresponding to intact VIP.

NKA- and SP-LI were detected in basal duodenal contents of three out of nine, and four out of eight rats respectively. Luminal NKA was significantly raised by 10 nmol/L HCL ($p<0.001$), and was present in effluents from all rats exposed to 50 nmol/L or higher concentrations of HCL. Luminal SP was significantly raised by 50 nmol/L HCL ($p<0.05$) and was detected in effluents from all but one rat at 100 nmol/L HCL. In contrast to luminal VIP and PGE_2, elevations of NKA and SP were not sustained at any of the HCL concentrations tested. Chromatographic characterization showed the presence of single components corresponding to intact NKA and SP, respectively.

Indomethacin augmented the luminal output of VIP in response to HCL and also augmented the HCO_3^- response to an intravenous infusion of VIP. However, intravenous infusion of VIP did not change the luminal output of PGE_2, nor did luminal perfusion of PGE_2 (0.1 nmol/L, a dose which potently stimulates HCO_3^-) affect basal concentrations of VIP.

Cat Colon

Occurence and characterization of tachykinins. NKA-LI and SP-LI were found in picomolar amounts in colonic tissues and almost in an order of magnitude higher amounts in vagal, pelvic, splanchnic and lumbar colonic nerves of the cat (Table 1). The plasma concentrations of NKA-LI and SP-LI were higher in the venous effluent (19.0±2.8 pmol/L and 10.6±0.8 pmol/L, respectively) than in arterial plasma (16.5±1.8 pmol/L and 9.2±0.5 pmol/L, respectively) indicating that the peptides were released from the colon under basal conditions. Chromatographic characterization of SP-LI both in extracts of colonic tissues and in the venous effluent showed a major component eluting in the position of SP, and a smaller component eluting in the position of oxidized SP. Chromatographic characterization of NKA-LI in extracts of colonic tissues showed a major component eluting in the position of NKA, a second component in the position of oxidized NKA, and a small component eluting in the position of NKA(3-10)/NKA(4-10) and NPK. NKA-LI in the venous effluent corresponded mainly to NKA.

Table 1. Concentrations of neurokinin A-like immunoreactivity (NKA-LI) and substance P-like immunoreactivity (SP-LI) in acid and water extracts of cat colonic tissues and nerves.

Tissue	NKA-LI (pmol/g)	SP-LI (pmol/g)
Colon		
Proximal	3.5±0.6	18.5±17.1
Distal	5.0±1.6	4.1±1.7
Nerves		
Vagal	39.2±22.2	37.7±19.7
Pelvic	30.4±17.6	29.4±22.6
Splanchnic	37.6±8.4	56.5±29.0
Lumbar colonic	171.2±70.9	194.8±55.3

Stimulation of tachykinin release. Continuous electric stimulation of the pelvic nerve at 4 Hz during 3 min (n=7) released NKA-LI to the colonic venous effluent from 646.8±155.0 to maximally 4994.2±1051.6 fmol/min/100g ($p<0.001$). The release of SP-LI increased from 270±23.0 to maximally 2168±300.6 fmol/min/100g ($p<0.001$). Plasma levels of NKA-LI returned to prestimulation values within 3 and 1 min, respectively, after cessation of nerve stimulation. During nerve stimulation colonic contraction was registered with a decrease in proximal colonic volume from 18.3±3.4 to 0.4±0.1 mL ($p<0.01$), and in distal colonic volume from 19.1 ±2.7 to 0.3±0.1 mL ($p<0.01$) . Simultaneously, colonic blood flow increased from 29.3±6.7 to 60.5±23.3 mL/min/100g ($p<0.01$).

Intermittent electric burst stimulation of the pelvic nerve at 40 Hz for 1 s at 10s intervals during 3 min (n=7) produced a release of NKA-LI to blood from prestimulation levels of 456±95.0 to maximally 2726.8±648.2 fmol/min/100g ($p<0.001$). The release of SP-LI increased from 264.0±24.0 to maximally 2020.6±426.6 fmol/ min/100g ($p<0.001$). The levels of NKA-LI and SP-LI returned to prestimulation values within 3 and 1 min, respectively, after cessation of nerve stimulation. During nerve stimulation the motility and vascular volumes decreased only minimally from 18±3.5 to 15.6 ±0.2 mL, and 18.7±0.9 to 13.6±0.3 mL, respectively, while colonic blood flow increased from 34.4±6.4 to 73.6±17.3 mL/min/100g ($p<0.01$).

Mechanical stimulation of the anus (n=7) produced a release of NKA-LI from prestimulation levels of 647±282.8 to maximally 1650.0± fmol/min/100g ($p<0.01$), and a release of SP-LI from 264.6±14.0 to maximally 887.2±71.4 fmol/min/100g ($p<0.01$). The levels of NKA-LI and SP-LI returned to pre-stimulation levels within 1 min after cessation of nerve stimulation. During stimulation the proximal colonic volumes decreased from 18.6±3.2 to 3.1±1.0 mL ($p<0.01$), and the distal colonic volume from 19.4±2.6 to 2.7±1.1 mL ($p<0.01$). Simultaneously, colonic blood flow increased from 35.7±5.2 to 67.8±12.3 mL/min/100g ($p<0.01$).

Rectal distension (n=7) caused a release of NKA-LI from prestimulation levels of 747.2±282.8 to maximally 1650.0±439.8 fmol/min/100g ($p<0.01$) and a release of SP-LI from 264.6±14.0 to

maximally 887.2±71.4 fmol/min/100g ($p<0.01$). The levels of NKA-LI and SP-LI returned to pre-stimulation values within 1 min after cessation of nerve stimulation. During stimulation, the proximal colonic volume decreased from 18.6±3.2 to 3.1±1.0 mL ($p<0.01$), and the distal colonic volume from 19.4±2.6 to 2.7±1.1 mL ($p<0.01$). Simultaneously, colonic blood flow increased from 32.3±7.4 to 63.1±17.4 mL/min/100g ($p<0.01$).

Effects of tachykinins. NKA, NKB, SP, NPK, eledoisin and physalemin were given as close intraarterial infusions (n=10) at a dose range of 0.1-100 pmol/min. All tachykinins produced a dose-dependent contraction of the proximal and distal colon as well as increased blood flow. The colonic contractions produced by NKA, NKB, SP and NPK, but not eledoisin and physalemin, were reduced after treatment with tetrodotoxin, and atropin also inhibited all but the effects of ELE on the proximal colon. On the other hand, hexamethonium had no effects on the contractile responses. Tetrodotoxin, hexamethonium and atropine did not result in any statistically significant changes in the blood flow.

DISCUSSION

Bicarbonate Secretion in the Rat Duodenum

The duodenal mucosal bicarbonate defence against increasing acidity may involves cooperation of several mediators (Smedfors et al., 1989). PGE_2, present in the basal state and increased even by low acidities may well represent a first line of defence. At higher acidities VIP is likely to start having a role. Thus the concentrations of HCO_3^- and PGE_2 were significantly augmented already by pH 5.0 whereas VIP was significantly augmented at pH 3.0 and the tachykinins SP and NKA at pH 2.0.

Measuring luminal concentrations of mediators, as in the present study, reflects local formation and release. It is likely that a part of the role of the measured mediators is played in such a paracrine fashion even if the site of action of the regulatory peptides is not known. However, a simple overflow of the mediators involved in neurocrine or endocrine processes cannot be excluded in the present model. The prostaglandin synthesis inhibitor indomethacin which markedly inhibits the HCL-stimulated HCO_3^- secretion in the duodenum, augmented the HCL-stimulated luminal output of VIP. This indicates that endogenously formed prostaglandins, besides acting as stimulators of HCO_3^- in response to HCL, also exert an inhibitory tone on VIP release.

Occurrence, Release and Effects of Tachykinins in the Cat Colon

We have shown that NKA-LI and SP-LI occur in the picomolar concentration range in cat colonic tissues and nerves. The NKA-LI/SP-LI ratio was 1:2.6 in colonic gut wall, and 1:1.2 in nervous tissue. HPLC characterization showed that NKA-LI in tissue extracts was heterogeneous and contained components corresponding to NKA, NKA(3-10)/NKA(4-10) and NPK. However, the NKA-LI in plasma corresponded mainly to NKA. The SP-LI both in tissue extracts and plasma corresponded to intact SP. The higher NKA-LI than SP-LI concentrations in the venous effluent probably reflect a more rapid rate of metabolism of SP than for NKA in the circulation (Martling et al., 1987).

NKA-LI and SP-LI can be released from the colon to the circulation upon electrical stimulation of the pelvic nerves. Reflex activation of the pelvic nerves by mechanical stimulation of the anus or rectal distension produced a less pronounced release of NKA-LI and SP-LI from the colon to blood than direct nerve stimulation. There was a simultaneous colonic contraction and vasodilation in response to all modes of nerve stimulation.

On a molar basis, NKA was found to be more potent than the other tachykinins tested to induce colonic contractions and vasodilation.

Taken together, the present findings open the possibility that peptides present in primary afferents are involved in physiological regulatory mechanisms including smooth muscle contractions, bicarbonate secretion and vasodilation in the gut. Furthermore, inflammatory reactions can be elicited by activation of sensory nerves with subsequent local release of local mediators including tachykinins which are also known to influence the immune system and cell proliferation.

References

Aly, A., Gréen, K., and Johansson, C., 1984, Prostaglandin E_2 in basal gastric secretion and during stimulation of muscarinic receptors in man, **Acta Physiol. Scand.**, 122:583.

Brodin, E., Lindefors, N., Dalsgaard, C.J., Theodorsson-Norheim, E., and Rosell, S., 1986, Tachykinin multiplicity in rat CNS as studied using antisera raised against NKA and SP, **Regulatory Peptides**, 13:253.

Fahrenkrug, J., and Schaffalitzky de Muckadell, O.B., 1977, Radioimmunoassay of vasoactive intestinal polypeptide (VIP) in plasma, **J. Lab. Clin. Med.**, 89:1379.

Ekblad, E., Winther, C., Ekman, R., Håkansson, R., and Sundler, F., 1987, Projections of peptide-containing neurons in rat small intestine, **Neuroscience**, 20:169.

Holzer, P., Lippe, I. Th., and Holzer-Petsche, U., 1986, Capsaicin sensitive afferent neurons involved in post-operative and irritation-induced ileus in the rat, **Gastroenterology**, 91:360.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.

Isenberg, J.I., Wallin, B., Johansson, C., Smedfors, B., Mutt, U., Tatemoto, K., Emås, S. 1984, Secretin, VIP and PHI stimulate rat proximal duodenal surface epithelial bicarbonate secretion in vivo, **Regulatory Peptides**, 8:315.

Flemström, G., 1977, Active alkalinization by amphibian gastric fundic mucosa in vitro, **Am. J. Physiol.**, 233:E1.

Martling, C.-R., Theodorsson-Norheim, E., Norheim, I., and Lundberg, J.M., 1987, Bronchoconstrictor and hypotensive effects in relation to pharmacokinetics of tachykinins in the guinea-pig - evidence for extraneuronal cleavage of Neuropeptide K to neurokinin A, **Naunyn. Schmiedeberg's Arch. Pharmacol.**, 336:183.

Matthews, M.R., Cuello, C., 1984, The origin and possible significance of substance P immunoreactive networks in the prevertebral ganglia and related structures in the guinea-pig, **Phil. Trans. R. Soc.**, B306:247.

Sharkey, K.A., Williams, R.G., and Dockray, G.J., 1984, Sensory substance P innervation of the stomach and pancreas: demonstration of capsaicin-sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing, **Gastroenterology**, 87:914.

Smedfors, B., Theodorsson, E., Aly, A., Muscat, A., Pmja, A.B., and Johansson, C., 1989, HCL-induced duodenal HCO_{3-} secretion is associated with concentration related release of local prostaglandin E_2, vasoactive intestinal peptide, neurokinin A and substance P in the rat, **Eur. J. Gastroenterol. Hepatol.**, 1:193.

Theodorsson-Norheim, E., Norheim, I., Öberg, K., Brodin, E., Lundberg, J.M., Tatemoto, K., and Lindgren, P.G., 1985, Neuropeptide K: A major tachykinin in plasma and tumour tissues from carcinoid patients, **Biochem. Biophys. Res. Commun.**, 1131:77.

Theodorsson-Norheim, E., Hemsèn, A., Brodin, E., and Lundberg, J.M., 1987, Sample handling techniques when analyzing regulatory peptides, **Life Sciences**, 41:845.

EFFERENT FUNCTION OF CAPSAICIN-SENSITIVE NERVES AND NEUROGENIC VASODILATION IN RAT MESENTERIC CIRCULATION

Stefano Manzini, Manuela Tramontana*
and Francesca Perretti

Istituto Farmacobiologico Malesci,
Pharmacology Department
Via Porpora 22, 50144 Florence, Italy
*Institute of Internal Medicine
and Clinical Pharmacology
University of Florence, Viale Morgagni 85
50100 Florence, Italy

INTRODUCTION

Sequential or double immunostaining studies revealed the coexistence of substance P and CGRP in perivascular nerve fibers in mesenteric arteries (Uddman et al., 1986) and veins (Warthon et al., 1986). The potential role of capsaicin-sensitive primary afferents in the physiological regulation of dog intestinal blood flow was first suggested by Rozsa et al. (1984, 1985). Later on, it was shown that intraluminal capsaicin administration in isolated perfused rat mesenteric bed elicits remarkable vasodilation which is unaffected by cholinergic or adrenergic blockade, hexamethonium and tetrodotoxin (Manzini and Perretti, 1988). This relaxation was markedly reduced or even abolished after "in vivo" or "in vitro" capsaicin pretreatment suggesting desensitization, a typical feature of a certain subset of primary sensory nerves (Maggi and Meli, 1988). Kawasaki et al. (1988) have presented functional evidence that in mesenteric resistance vessels calcitonin gene-related peptide (CGRP) released from capsaicin-sensitive nonadrenergic-noncholinergic (NANC) fibers may act as a potent endogenous vasodilator. As a whole these findings may indicate that the "efferent" function of these sensory nerves (i.e. local release of sensory neuropeptides) might represent an intramural vascular neuroeffector mechanism for a prompt regulation of mesenteric vascular resistance. This hypothesis is further corroborated by functional and neurochemical data presented in this work.

MATERIALS AND METHODS

Functional Studies

Male albino rats, Wistar Nossan strain, weighing 280-300 g were used. Animals were anesthetized with ether, then abdomen was opened and the superior mesenteric artery isolated and can-

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

nulated with a polyethylene cannula. Preparations were then suspended in a thermostatically controlled (37°C) organ bath (fluid volume, 7 ml, kept constant by drainage) and perfused intra and extraluminally (at a rate of 6 ml/min) with oxygenated (O_2 100%) physiological salt solution (PSS). Drugs were usually added to the reservoir supplying the peristaltic pump. Perfusion pressure, recorded by means of a pressure transducer (Bentley Trantec) attached to the inlet side of the perfusion system, was taken as an indirect measure of the arterial contractile tone. Intraluminal pressure was continuously recorded by means of a poligraph (Basile Unirecord 7050). The preparations were allowed to equilibrate for 30 min and then contracted with norepinephrine (NE) (1 μM) or with a high-K^+ depolarizing medium. Preliminary experiments (n=4) indicated that NE- and high-K^+-induced tonic vasoconstriction usually remained stable for at least 60 min. Those preparations which did not reach a steady vasoconstriction within 20 min were discarded. When a stable tone was reached, capsaicin was administered as single concentration. In each preparation only one concentration of capsaicin was tested. Capsaicin was administered both intra and extraluminally. In

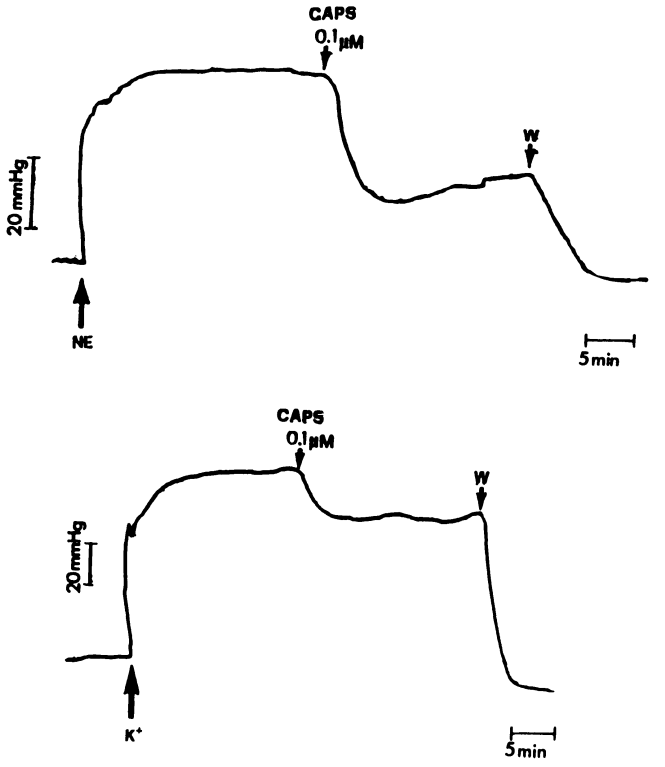


Fig. 1. Typical tracings showing capsaicin-induced relaxation of tonic vasoconstriction elicited by NE (1 μM)(upper tracing) or high-K^+ medium (40 mM)(lower tracing) in isolated perfused rat mesenteric bed. Capsaicin and NE were administered intraluminally, while high K^+ medium was perfused both intra- and extra-luminally. W = washing out.

some experiments, endothelium was chemically removed by intraluminal administration of a bolus (200 µl) of collagenase 0.2%, through a side arm of the perfusing system, just above polyethylene cannula.

Assessment of CGRP-LI and SP-LI Release

Mesenteric arteries and veins were rapidly removed, trimmed, pooled together (at least from 4 animals) and placed in a 2 ml organ bath (at 37°C). Preparations were superfused at a rate of 0.7 ml/min with oxygenated (96% O_2 and 4% CO_2) PSS containing bovine serum albumin (1 g/l) and thiorphan (10 µM). After a 30 min equilibration period, preparations were exposed to capsaicin (1 µM) for 15 min. Superfusates were collected every 4 min in tubes containing enough acetic acid to give a final concentration of 2 N. Capsaicin administration was repeated at a 30 min interval. At the end of the experiment, the tissues were blotted 2-3 times on filter papers and weighed. Superfusates were freeze-dried and stored at -20C° until assay.

CGRP-LI and SP-LI radioimmunoassay was performed as previously described (Manzini et al., 1989). Tissue contents are expressed as pmol/g w/w. Release was measured as Total Evoked Release (pmol/g w/w), which was calculated as the sum of values obtained during 15 min of exposure to capsaicin subtracted by the mean basal values.

Solutions

Throughout the experiments a physiological salt solution of the following composition (mM) was used: NaCl 150, KCl 5.6, CaCl2 2.2, MgCl2 0.5, Glucose 5, NaHCO3 6. Isotonic high-K^+ (20-40 or 60 mM) solutions were obtained by replacement of NaCl with an equimolar amount of KCl.

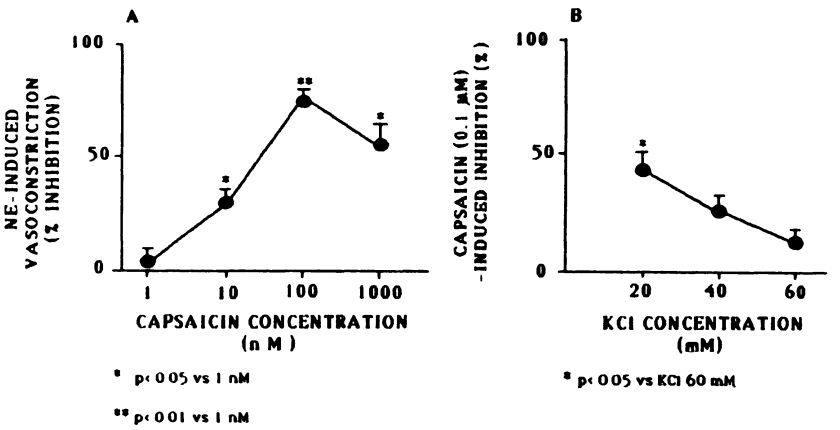


Fig. 2. Concentration-dependent inhibition by capsaicin of NE (1 µM)-induced tonic vasoconstriction in isolated perfused rat mesenteric bed (panel A). Panel B shows the inhibitory effects of capsaicin (0.1 µM) on the tonic vasoconstriction produced by various high K^+ media (20-40 and 60 mM).
Each value is the mean ± S.E. of six experiments.

Statistical Analysis

All data in the text and figures are given as the mean ± S.E. Statistical analysis of the data was performed by means of Student's t test for paired and unpaired data when appropriate.

Drugs

Drugs used were: Albumin (SIGMA-St. Louis U.S.A.), Capsaicin (SERVA-Heidelberg FRG), Carbachol (MERCK-Darmstadt FRG), Collagenase (SERVA-Heidelberg FRG), Norepinephrine L-Tartrate (SERVA-Heidelberg FRG), Papaverine HCl (SIGMA-St. Louis U.S.A.), Thiorphan (PENINSULA-Belmont U.S.A.

RESULTS

Capsaicin-Induced Vasodilation of NE- or High-K^+-Induced Tonic Vasoconstriction in Isolated Perfused Rat Mesenteric Bed

Administration of a high-K^+ medium (20-60 mM) produced a concentration-related tonic vasoconstriction (n=18). The mean amplitudes of these vasoconstrictions were 58 ± 6, 93 ± 9 and 109 ± 7 mm Hg at KCl concentration of 20, 40 and 60 mM, respectively. On these tonic responses capsaicin (0.1 μM) intraluminal administration produced a relaxation whose amplitude was inversely related to potassium concentration being virtually abolished at a KCl concentration of 60 mM (fig. 1 and 2; n=18). On the other hand, intra and extraluminal administration of NE 1 (μM) elicited a tonic vasoconstriction of 82.5 ± 5 mm Hg and on this response capsaicin elicited a prompt, sustained and concentration-dependent relaxation (fig. 1 and 2; n=6).

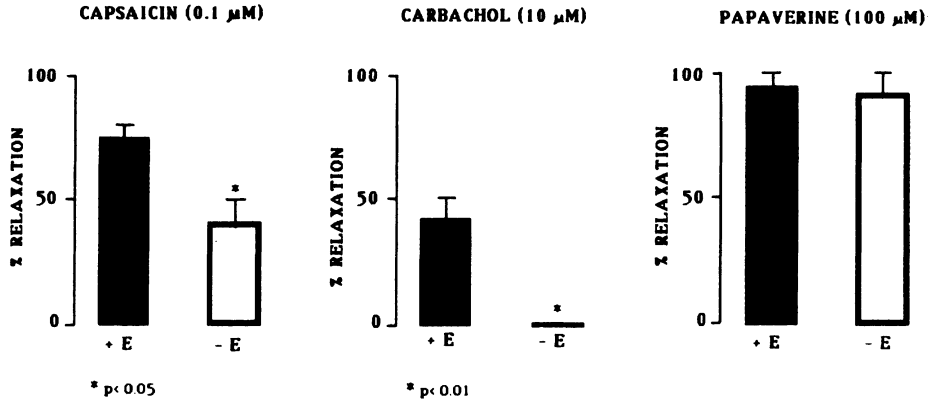


Fig. 3. Effect of collagenase pretreatment (bolus injection of 200μl of a solution 0.2%) on relaxation elicited by capsaicin, carbachol or papaverine on NE (1 μM)-induced tonic vasoconstriction. Filled bars represent relaxation obtained in control preparations, while empty bars refer to vasodilation recorded in collagenase-pretreated mesenteric beds.
Vasodilations were assessed as % of the maximal relaxation taken as abolition of NE-induced tone.
Each bar represents the mean ± S.E. of six experiments.
Each significativity is related to its own control.

In some experiments, preparations were previously challenged with a high-K^+ medium (60 mM) and when a steady tone was reached mesenteric beds were also perfused with NE (1 μM) which determined a further increase in vascular tone (from 74 ± 7 to 106 ± 7 mm Hg). In these experimental conditions capsaicin (0.1 μM) did not elicit any relaxation (n=5).

Effect of Endothelium Removal on Capsaicin-Induced Vasodilation of NE-Induced Vasoconstriction

Furchgott et al. (1987) showed that the intraluminal administration of collagenase might be a suitable method for endothelium removal in perfused tissues. In this set of experiments, we have tried to apply this method to mesenteric bed for assessing the potential role of endothelium in capsaicin responses.

Bolus injection of collagenase (0.2% in 200 μl) did not modify "per se" the amplitude of tonic vasoconstriction elicited by NE (1 μM) (n=6). However, after collagenase challenge, the intraluminal administration of carbachol (10 μM), which in control conditions elicited a relaxation of 40 ± 7 mm Hg, was no longer able to reduce the vascular tone (n=6, fig. 3). On the other hand, the vasodilation produced by a direct smooth muscle relaxant agent such as papaverine (100 μM) was unaffected by collagenase pretreatment (n=6, fig. 3). Collagenase pretreatment significantly reduced the vasodilatory properties of capsaicin (0.1 μM). In fact, amplitude of relaxation was 76 ± 4 and 39 ± 10 mm Hg in control and in endothelium-deprived preparations, respectively (n=6; $p<0.01$, fig. 3).

Capsaicin-Induced SP-LI and CGRP-LI Release from Mesenteric Arteries and Veins

Superfusion of isolated vessels with capsaicin (1 μM) resulted in a prompt and remarkable release of both SP-LI and CGRP-LI

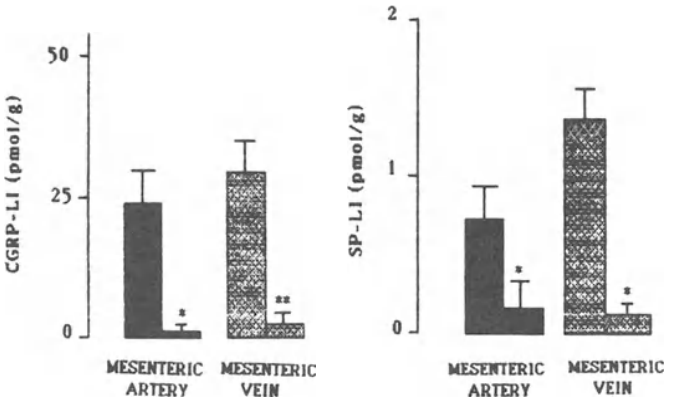


Fig. 4. Capsaicin (1 μM)-induced CGRP-LI (left panel) and SP-LI (right panel) release from mesenteric arteries (black bars) and veins (grey bars). For each tissue the two bars refer to the first challenge (left bar) and the second challenge 30 min later (right bar) with capsaicin.
Each bar represents the mean ± S.E. of five experiments.
* $P<0.01$ as compared to the first challenge
** $P<0.001$ as compared to the first challenge

which can be evoked only once in each preparation, indicating desensitization (n=6) (fig. 4). In both vessels the amounts of CGRP-LI released were significantly greater than those measured for SP-LI ($P<0.01$). On a molar basis, the outflow of SP-LI elicited by capsaicin was 2.9 - 4.6% of the CGRP-LI released, i.e. very close to the SP-LI/CGRP-LI ratio obtained in the same vessels as tissue contents (Geppetti, personal communication).

DISCUSSION

Capsaicin selectively stimulates a certain subgroup of primary afferents leading to a local release of sensory neuropeptides (Holzer, 1988). Desensitization follows this release and therefore these fibers become refractory to further activation by either capsaicin or other stimulatory agents (Maggi and Meli, 1988) Capsaicin-induced vasodilation of mesenteric circulation seems to rely on a similar mechanism since: a) it is unaffected by atropine, guanethidine, hexamethonium or tetrodotoxin (Manzini and Perretti, 1988); b) it undergoes desensitization (Manzini and Perretti, 1988); and c) it is strictly coupled to a release of sensory neuropeptides such as SP-LI and CGRP-LI.

Amplitude of capsaicin-induced relaxation was greater on NE- as compared to high-K^+-induced tonic vasoconstriction, and inversely related to the KCl concentration in the medium. A possible postjunctional reason for this capsaicin selectivity of action could be a preferential ability of locally released neuropeptides to block calcium influx in vascular smooth muscle through receptor- more than potential-operated channel. However, this hypothesis seems unlikely since when vasoconstriction was elicited by simultaneous administration of NE and high-K^+ medium, capsaicin was devoid of relaxant effect. Alternatively, a more suitable prejunctional explanation for this finding might be that since capsaicin stimulates sensory nerves by depolarizing their terminal region through opening a cation non-selective channel (Marsch et al., 1987), its effect will disappear progressively due to a high-K^+ media-induced depolarization of nerve endings.

Experiments with collagenase were performed with the aim of obtaining additional clues for the identity of the putative mediators of capsaicin effects. This procedure seems to be suitable for endothelium removal from isolated perfused mesenteric bed; in fact, functional results indicate a complete loss of carbachol vasodilatory properties, classically linked to the release of an endothelium relaxing factor (Furchgott et al., 1980), while on the other hand the relaxant properties of a drug directly acting on smooth muscle (papaverine) were unaffected. Collagenase treatment determined a reduction to about half of capsaicin-induced relaxation, suggesting that in this response both endothelium-dependent and endothelium-resistant mechanism(s) are involved. Substance P and CGRP are, among other neuropeptides, the most likely candidates for a mediator role of vascular capsaicin effects, and indeed, in this study, we have demonstrated that they are simultaneously released by capsaicin in mesenteric vessels. Both these substances have prominent vasodilatory properties which usually are endothelium-dependent for substance P (Stewart-Lee and Burnstock, 1989) and endothelium-independent for CGRP (Greenberg et al., 1987; Grace et al., 1987). Quite surprisingly, in isolated mesenteric bed substance

P, even at the concentration of 1 μM and in the presence of neutral endopeptidase inhibitor, is completely devoid of relaxing properties (Kawasaki et al., 1988; Perretti et al., 1988). On the other hand, CGRP has been demonstrated to be a potent endogenous vasodilator of mesenteric circulation in various experimental conditions (Marshall et al., 1986; Manzini and Perretti, 1988; Kawasaki et al., 1988). However no cross desensitization was observed between the relaxing action of exogenous CGRP and that of capsaicin (Manzini and Perretti, 1988. As a whole, these data might indicate CGRP as a major candidate for a neurotransmitter role in the local vasodilatatory action of capsaicin-sensitive nerves, however other substance(s) (possibly acting through endothelium) should concur as well. Immunoblockade studies have shown that the intestinal vasodilatory properties of capsaicin in dog mesenteric circulation can be reduced by prior administration of selective antisera toward substance P, cholecystokinin and vasoactive intestinal polypeptide (Rozsa et al., 1985). Furthermore, recent neurochemical studies performed in the human ileum indicate that capsaicin-induced motor effects are strictly coupled to a release of VIP (Maggi et al., 1989). Further studies are necessary to identify if also in rat mesenteric circulation VIP or other neuropeptides are contained in capsaicin-sensitive structures and may therefore concur in capsaicin relaxant effects.

In conclusion, functional and neurochemical evidence indicates that a capsaicin-sensitive neurogenic vasodilating mechanism could play a relevant role in the physiological regulation of rat mesenteric blood flow. This local `efferent' mechanism could be activated `in vivo' through reflexes originating in other intestinal tissues such as duodenum (Rozsa and Jacobsen, 1989), or by local axon reflexes (Kawasaki et al., 1988) and/or by a direct stimulation of the same sensory nerve endings by chemicals or endogenous substances locally produced (Holzer, 1988; Maggi and Meli, 1988). In this respect, it is interesting to note that capsaicin elicited a remarkable release of sensory neuropeptides also in mesenteric vein which, for anatomical reasons, might be extremely suitable to monitor potentially harmful substances absorbed from the intestine and therefore this mechanism might also play a crucial role in local protective responses such as inflammation and oedema.

References

Furchgott, R.F., and Zawadzki J.U., 1980, The obligatory role of endothelial cells in relaxation of arterial smooth muscle by acethylcholine, **Nature**, 288:373.

Furchgott, R.F., Carvalho, M.H., Khan, M.T., and Matsunaga, K. 1987, Evidence for endothelium-dependent vasodilation of resistance vessels by acethylcholine, **Blood Vessels**, 24:145.

Grace, G.C., Dusting, G.J., Kemp, B.E., and Martin, T.J., 1987, Endothelium and the vasodilator action of rat calcitonin gene-related peptide (CGRP), **Br. J. Pharmacol.**, 91:729.

Greenberg, B., Rhoden, K., and Barnes, P., 1987, Calcitonin gene-related peptide (CGRP) is a potent non-endothelium-dependent inhibitor of coronary vasomotor tone, **Br. J. Pharmacol.**, 92:789.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.

Kawasaki, H., Takasaki, K., Saito, A., and Goto, K., 1988, Calcitonin gene-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance vessels of the rat, **Nature**, 335:164.

Maggi, C.A., and Meli, A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, **Gen. Pharmacol.**, 19:1.

Maggi, C.A., Santicioli, P., Del Bianco, E., Geppetti, P., Barbanti, G., Turini, D., and Meli, A., 1989, Release of VIP but not CGRP-LI by capsaicin from the human isolated small intestine, **Neurosci. Lett.**, 98:317.

Manzini, S., and Perretti, F., 1988, Vascular effects of capsaicin in isolated perfused rat mesenteric bed, **Eur. J. Pharmacol.**, 148:153.

Manzini S., Perretti, F., De Benedetti, L., Pradelles, P., Maggi C.A., and Geppetti, P., 1989, A comparison of bradykinin- and capsaicin-induced myocardial and coronary effects in isolated perfused heart of guinea-pig: involvement of substance P and calcitonin gene-related peptide, **Br. J. Pharmacol.**, 97:303.

Marsh, S.J., Stanfeld, C.E., Brown, D.A., Davey, R., and McCarthy, D., 1987, The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro, **Neuroscience**, 23:275.

Marshall, I., Al-Kazwini, S.J., Holman, J.J., and Craig, R.K., 1986, Human and rat a-CGRP but not calcitonin cause mesenteric vasodilatation in rats, **Eur. J. Pharmacol.** 123:217.

Perretti F., Geppetti, P., and Manzini, S., 1988, Stimulation of capsaicin-sensitive sensory fibers induces vasodilation in rat mesenteric bed, **Reg. Peptides** 22:145.

Rozsa, Z., Jancsò, G., and Varrò, V., 1984, Possible involvement of capsaicin-sensitive sensory nerves in the regulation of intestinal blood flow in the dog, **Naunyn-Schmiedeb. Arch. Pharmacol.** 326:352.

Rozsa, Z., Varrò, A., and Jancsò, G., 1985, Use of immunoblockade to study the involvement of peptidergic afferent nerves in the intestinal vasodilatory response to capsaicin in the dog, **Eur. J. Pharmacol.** 115:59.

Rozsa, Z., Sharkey, K.A., Jancsò, G., and Varrò, A., 1985, Evidence for a role of capsaicin-sensitive mucosal afferent nerves in the regulation of mesenteric blood flow in the dog, **Gastroenterology** 90:906.

Rozsa, Z., and Jacobson, E.D., 1989, Capsaicin-sensitive nerves are involved in bile-oleate-induced intestinal hyperemia, **Am. J. Physiol.** 256:G476.

Stewart-Lee, A., and Burnstock, G., 1989, Actions of tachykinins on the rabbit mesenteric artery: substance P and (Glp^6,L-Pro^9) SP_{6-11} are potent agonists for endothelial neurokinin-1 receptors, **Br. J. Pharmacol.** 97:1218

Uddman R., Edvinsson, L., Ekblad, E., Hakanson, R., and Sundler, F., 1986, Calcitonin gene-related peptide (CGRP): perivascular distribution and vasodilatory effects, **Reg. Peptides** 15:1.

Wharton, J., Gulbenkian, S., Mulderry, P.K., Ghatei, M.A., McGregor, G.P., Bloom, S.R., and Polak, J.M., 1986, Capsaicin induces a depletion of calcitonin gene-related peptide (CGRP)-immunoreactive nerves in the cardiovascular system of the guinea pig and rat, **J. Aut. Nerv. Syst.** 16:289.

TACHYKININ RECEPTORS IN THE LONGITUDINAL AND CIRCULAR MUSCLE OF THE HUMAN ILEUM

Sandro Giuliani, Carlo Alberto Maggi, Riccardo Patacchini, Paolo Santicioli, Damiano Turini*, Gabriele Barbanti*, Paolo Rovero**, Antonio Giachetti, and Alberto Meli

Pharmacology Department, Smooth Muscle Division, Research Laboratories, A. Menarini Pharmaceuticals, Via Sette Santi 3 Florence, ITALY
*Dept. of Surgical Pathology and Urologic Clinic University of Ferrara, ITALY
**Chemistry Department, Menarini Pharmaceuticals, Florence ITALY

INTRODUCTION

Tachykinins are a family of peptides which share the common C-terminal sequence Phe - x - Gly - Leu - Met NH_2. Ample evidence, based on pharmacological, physiological, anatomical and neurochemical bases indicates that tachykinins, such as substance P (SP), play a physiological role as excitatory transmitters in the guinea-pig ileum (Costa et al., 1985; see also Barthó & Holzer 1985 for review). In the guinea-pig intestine, SP and other tachykinins, such as neurokinin A (NKA) are mainly stored in certain intrinsic neurons which are thought to play a role as final effectors of atropine-resistant peristalsis (Barthó and Holzer 1985 for review).

The contractile response of the guinea-pig ileum to tachykinins is mediated by specific receptors (Lee et al., 1982). Studies on the longitudinal muscle have shown that at least two distinct tachykinin receptors are present at this level, one of which (NK-1 receptor) mediates the direct response of muscle cells to these peptides while the other (NK-3 receptor) activates intramural effector neurons (Laufer et al., 1986, 1988). Some studies have suggested that also the third type of receptor (NK-2) may mediate the contractile response of the longitudinal muscle of the guinea-pig ileum to these peptides (Dion et al., 1987). In the longitudinal muscle of the rat duodenum, both NK-1 and NK-2 receptors seem to be present (Maggi et al., 1986). Therefore noticeable species-related differences exist with regard to tachykinin receptors mediating the spasmogenic effect of these peptides in the mammalian gut.

As tachykinins are present in the human gastrointestinal tract (Llewellyn-Smith et al., 1984; Wattchow et al., 1984) we

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

dinal and circular muscle of the human ileum and attempted to characterize the receptors involved by means of synthetic, receptor-selective agonists (Maggi et al., 1989;1990).

METHODS

The motor responses to electrical field stimulation and neuropeptides were investigated on 25 ileal strips from 14 patients (age 48-75 years) who underwent abdominal surgery for carcinoma of the bladder base (enterocystoplasty). Pre-anesthetic medication and induction of anesthesia was as described previously (Maggi et al., 1989; 1990). Mucosa-free muscle strips were cut along the longitudinal or circular axis of the ileum and prepared for tension recording in isolated organ baths, as described previously (Maggi et al., 1989; 1990).

Peptides used were: substance P and neurokinin A (NKA) from Peninsula, San Carlos CA, USA. [Pro^9]-SP sulfone and [$MePhe^7$]-neurokinin B, two selective agonists of the NK-1 and NK-3 receptors, respectively (Dion et al., 1987), were kind gifts of Prof. D. Regoli, Dept. of Pharmacology and Physiology, Sherbrooke University, Canada. [βAla^8]-NKA(4-10) a newly developed selective NK-2 receptor agonist was synthesized at Menarini Pharmaceuticals, by conventional solid-phase methods.

RESULTS

Electrical field stimulation produced a frequency-dependent contraction in both longitudinal and circular muscle strips. In the circular strips the contractile response was often preceded by a primary relaxation while this pattern was less frequently observed in the longitudinal muscle (Maggi et al., 1989; 1990). All the motor responses to field stimulation were abolished or greatly reduced by tetrodotoxin (1 μM, n=4). A consistent fraction of the response to field stimulation persisted in the presence of atropine at a concentration (3 μM) which blocked the contractile response to carbachol (0.1 mM).

Both SP and NKA (1 nM- 1 μM) produced a concentration-dependent contraction of the longitudinal and circular muscle, NKA being more potent than SP in both layers 53 and 7 times respectively, Table). The selective NK-1 receptor agonist, [Pro^9]SP

Table 1 EC_{50} and 95% confidence limits for the contractile effect produced by tachykinin or receptor selective tachykinin agonists in the longitudinal or circular muscle of the human ileum.

	EC_{50}	
Peptide	**Longitudinal Muscle**	**Circular Muscle**
SP	278 (124-863)	78 (55-102)
NKA	5.2 (3.8-8.9)	11 (8-18)
[Pro^9]-SP sulfone	>10 μM	84 (65-110)
[βAla^8]-NKA(4-10)	7.6 (5.1-11.5)	15 (9-21)
[$MePhe^7$]-NKB	>10 μM	>10 μM

SP = Substance P; NKA = Neurokinin A; NKB = Neurokinin B

SP = Substance P; NKA = Neurokinin A; NKB = Neurokinin B sulfone was ineffective in the longitudinal strips while it was equipotent to SP in the circular strips (Table). The selective NK-2 receptor agonist, [βAla^8]NKA (4-10) produced a consistent response in both longitudinal and circular strips, its activity being comparable to that of NKA (Table). By contrast, the selective NK-3 receptor agonist [MePhe^7]-NKB was barely effective or ineffective in both longitudinal and circular strips (Table).

DISCUSSION

Tachykinins have been shown to be potent spasmogens in the intestine of several mammalian species. Three distinct receptors, termed NK-1, NK-2 and NK-3 mediate the responses to tachykinins in various mammalian tissues. The present findings, (Maggi et al., 1989; 1990) demonstrate that tachykinins exert a powerful contractile effect on both longitudinal and circular muscles of the human ileum and that NK-2 receptors are the sole mediators of the response in the longitudinal muscle while both NK-1 and NK-2 receptors mediate the response in the circular muscle. Contrary to the guinea-pig ileum, no evidence was found for an involvement of NK-3 receptors in the response of the human ileum to these peptides.

Recently Gates et al. (1989) reported that NK-2 receptors are present on the longitudinal muscle of the human jejunum/ileum while NK-1 and NK-2 receptors are found in the circular muscle. These observations are in good agreement with the results of our functional studies.

These findings raise the possibility that tachykinins are involved as transmitters in the atropine-resistant excitatory response of the human ileum to electrical field stimulation. Preliminary experiments using [Tyr^5,D-Trp^{6,8,9}Arg^{10}]-NKA(4-10) (MEN 10207) a newly developed selective NK-2 receptor antagonist, indicated a selective suppression of the atropine-resistant response in the circular muscle of the human ileum (unpublished data), but further studies are needed for a definitive assessment of the role of these peptides in the regulation of motility of the human intestine.

Acknowledgement

This work was in part supported by the IMI, Rome, Italy Grant # 46287.

References

Barthó, L., and Holzer, P., 1985, Search for a physiological role of substance P in gastrointestinal motility, **Neuroscience**, 16:1.

Costa, M., Furness, J.B., Pullin, D.C., and Bornstein, J., 1985, Substance P enteric neurons mediate non-cholinergic transmission to the circular muscle of the guinea-pig intestine, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 328:446.

Dion, S., D'Orleans-Juste, P., Drapeau, G., Rhaleb, N.E., Rouissi, N., Tousignant, C., and Regoli, D., 1987, Characterization of neurokinin receptors in various isolated organs by the use of selective agonists, **Life Sci.**, 41:2269.

Gates, T.S., Zimmermann, R.P., Mantyh, C.R., Vigna, S.R., Maggio, J.E., Welton, M.L., Passaro, E.P., and Mantyh, P.W.,

the human gastrointestinal tract: localization by autoradiography, **Peptides**, 9:1207.
Laufer, R., Gilon, C., Chorev, M., and Selinger, Z, 1988, Desensitization with a selective agonist discriminates between multiple tachykinin receptors, **J. Pharmacol. Exp. Ther.**, 245:639.
Laufer, R., Wormser, U., Friedman, Z., Gilon, C., Chorev, M., and Selinger, Z., 1986, Neurokinin B is a preferred agonist for a neuronal substance P receptor and its action is antagonized by enkephalin, **Proc. Natl. Acad. Sci. USA**, 82:7444.
Lee, C.M., Iversen, L.L., Hanley, M.R.,and Sandberg, B.E.B., The possible existence of multiple receptors for Substance P, **Naunyn Schmied. Arch, Pharmacol.**, 318:281.
Llewellyn-Smith, I.J., Furness, J.B., Murphy, R., O'Brien, P.E., and Costa, M., 1984, Substance P containing nerves in the human small intestine. Distribution, ultrastructure and characterization of the immunoreactive peptide, **Gastroenterology**, 86:421.
Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Turini, D., Barbanti, G., Giachetti, A., and Meli, A., 1989, Human isolated small intestine: motor responses of the longitudinal muscle to field stimulation and exogenous neuropeptides, **Naunyn Schmiedebergs's Arch. Pharmacol.**, 339:415.
Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Turini, D., Barbanti, G., Giachetti, A., and Meli, A., 1990, Human isolated ileum: motor responses of the circular muscle to electrical field stimulation and exogenous neuropeptides. **Naunyn Schmied.Arch. Pharmacol.**, in press.
Wattchow, D.A., Furness, J.B., and Costa, M., 1988, Distribution and coexistence of peptides in nerve fibers of the external muscle of human gastrointestinal tract, **Gastroenterology**, 95:32.

ALTERATIONS IN RECEPTORS FOR SENSORY NEUROPEPTIDES IN HUMAN INFLAMMATORY BOWEL DISEASE

P. W. Mantyh[1], M. Catton[1], J. E. Maggio[2], and S. R. Vigna[3]

[1]Molecular Neurobiology Lab (151), VA Medical Center, Minneapolis, MN
[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA
[3]Department of Cell Biology, Duke University Medical Center, Durham, N.C.

ABSTRACT

Glutamate and several neuropeptides are synthesized and released by subpopulations of primary afferent neurons. These sensory neurons play a role in regulating the inflammatory and immune responses in peripheral tissues. We have explored what changes occur in the location and concentration of receptor binding sites for sensory neurotransmitters in two human inflammatory diseases, ulcerative colitis and Crohn's disease, using quantitative receptor autoradiography. The sensory neurotransmitter receptors included bombesin, calcitonin gene-related peptide-α, cholecystokinin, galanin, glutamate, somatostatin, neurokinin A (substance K), substance P, and vasoactive intestinal polypeptide. Of the nine receptor binding sites examined only binding sites for substance P and vasoactive intestinal peptide were significantly altered in the inflamed tissue. These data suggest that substance P is involved in regulating the inflammatory and immune responses in human inflammatory diseases and indicate a specificity of efferent action for each sensory neurotransmitter in peripheral tissues.

INTRODUCTION

Neurons that convey sensory information from peripheral tissues to the central nervous system are located in the dorsal root ganglion (DRG); as such they are known as DRG neurons. Recently, several neuropeptides including calcitonin gene-related peptide-α, cholecystokinin, galanin, gastrin releasing peptide (the mammalian analogue of bombesin), somatostatin, neurokinin A (also known as substance K), substance P (SP), and vasoactive intestinal peptide have each been shown to be present in various subpopulations of DRG neurons (35, 45, 83, 107). Several of

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

these neuropeptides have been implicated in the conduction of nociceptive information. For example, intrathecal injection of SP, the best studied of these peptides, produces biting and scratching behavior consistent with a role for SP as a peptide neurotransmitter of some primary afferent nociceptors (38); SP release in the spinal cord is inhibited by opiate analgesics (43); depletion of SP by capsaicin (a neurotoxin relatively selective for unmyelinated sensory neurons (84) including those containing SP) roughly parallels a loss of specific nociceptive response (41); and release of SP in the spinal dorsal horn in response to normally innocuous mechanical stimuli is enhanced in polyarthritic rats (86).

It has become increasingly evident in the last decade that the DRG neurons which convey afferent somatosensory information from peripheral tissues to the spinal cord are also involved in the efferent regulation of the peripheral tissues they innervate. Thus, SP-containing DRG neurons have been implicated in both the afferent central transmission of nociceptive information and in the efferent regulation of inflammation and sensitization of joint sensory endings in a chronic pain state such as arthritis (49,50,51). Support for this concept includes observations that: a) SP is a potent vasodilator (50,93), b) terminals of SP-containing sensory neurons are observed in close apposition to blood vessels (55), and c) electrical stimulation of peripheral nerves at intensities that release SP reproduces many of the physiological changes seen in acute inflammation (26). In rats with experimentally induced arthritis, SP concentrations increase in peripheral nerves innervating the affected joints (49), and if capsaicin is administered to rats before or after the onset of arthritis, the attendant paw swelling and tenderness is diminished (12).

The hypothesis that sensory neurons containing SP and/or other neuropeptides are involved in regulating the inflammatory and immune responses in the peripheral tissues they innervate suggests that, in a pathological state, these neuropeptide-containing sensory neurons may contribute to an altered or abnormal inflammatory response such as that seen in arthritis or inflammatory bowel diseases. In the present report we have used quantitative receptor autoradiography to determine whether receptor binding sites for putative sensory neurotransmitters (neuropeptides and the excitatory amino acid glutamate) are altered in a human inflammatory disease.

The human inflammatory disease we have explored is inflammatory bowel disease (IBD) which is a generic term that refers to chronic inflammatory diseases of the intestine which are of unknown etiology, principally ulcerative colitis and Crohn's disease (100). Ulcerative colitis is an inflammatory, ulcerating process of the colon; Crohn's disease is an inflammation of the intestine characterized by nodular granulomatous inflammatory lesions throughout the entire gut wall that may involve any part of the intestine but primarily attacks the distal small intestine and colon. We chose to explore alterations in receptors for sensory neurotransmitters in these diseases since it has previously been demonstrated that the gastrointestinal tract is innervated by sensory fibers that contain and release neuropeptides including SP (3,22,23,101,104,112,114). In addition, IBD is probably one of the few human inflammatory diseases where surgical removal of inflamed intestinal tissue is used to ameliorate

the symptoms in severe cases, thus making quantitative receptor autoradiographic analysis possible. The rationale for the experiments is that if a particular neurotransmitter is involved in the peripheral inflammatory response, then some alteration in the expression of its receptors may be anticipated. The present report is an exploration of sensory neurotransmitter binding sites in inflamed and normal human gastrointestinal tissue in order to determine whether changes in the location and/or apparent density of these sites are correlated with these diseases.

EXPERIMENTAL PROCEDURES

Human Specimens

Specimens were obtained within 5 minutes after removal, embedded in Tissue-Tek (Miles) and placed in dry ice to minimize post-surgical degradation artifacts. The specimens (Table 1) obtained at the margins of resection for carcinoma (n = 6) were obtained as far from the tumor as possible; specimens obtained from Crohn's disease (n = 4) and ulcerative colitis (n = 4) patients were from areas with the highest degree of inflammation. In all cases the diagnosis was independently determined by a pathologist to be either ulcerative colitis, Crohn's disease, or histologically normal at the site of resection. The tissue was then blocked, placed on a brass microtome chuck, frozen on dry ice and processed for quantitative autoradiography as previously described (65,68,69). The tissue was serially sectioned (30 um), thaw-mounted onto gelatin-coated microscope slides and stored at -70°C over desiccant for no longer than 3 months.

Radioligands

Some of the radioligands used in the present study were ^{125}I-labelled synthetic peptides purified by reverse-phase HPLC to essentially quantitative specific activity (approx 2000 Ci/mmol).

Table 1

Group	No.	Mean age (yr)	Sex M	Sex F	Patient's weight (kg)	Specimen Site ileum	Specimen Site ileum and colon	Specimen Site colon	Patients with No Steriod Treatment
IBD	8	35(15-58)[a]	2	6	63(47-82)[b]	1	3	5	2
Crohn's	4	31(15-44)	1	3	52(47-59)	1	3	1	2
U.C.	4	35(20-58)	1	3	66(54-82)	-	-	4	0
Carcinoma resection	6	68(62-78)	6	0	76(66-82)	-	2	4	6

Details of patients used in the study. [a]Numbers in parentheses are ranges of patients' ages. [b]Numbers in parentheses are ranges of patient's weights. Results from the ileum were the same as the colon (i.e. arterioles, venules and lymph nodules in inflamed tissue contain high concentrations of substance P receptor binding sites) and there was no statistically signficant difference between results from IBD steroid-treated and untreated patients.

Receptor Binding Protocols

Quantitative receptor autoradiography was performed by first bringing the slide-mounted tissue sections to room temperature. The slide-mounted tissue sections were then placed consecutively in a preincubation medium, an incubation medium, a wash solution, and a final dip in distilled water. The preincubations and washes were performed by immersing the entire slide in the appropriate solution whereas the incubation with the radioligand was performed by placing the slides on a flat surface and covering the sections with 1.5 ml of the incubation medium. To determine the non-specific binding, paired serial sections were incubated as described above except that a 1 μM concentration of the appropriate nonradioactive peptide was added to the incubation solution. Each ligand required a unique set of binding conditions which are given below.

Bombesin

Sections were first preincubated in 10 mM HEPES (pH 7.4) for 5 min followed by an incubation in a solution of 10 mM HEPES (pH 7.4), 4.7 mM KCl, 130 mM NaCl, 5 mM $MgCl_2$, 1 mM EGT, 0.1% BSA, 100 mg/ml bacitracin and 100 pM of ^{125}I-(Tyr^4)-bombesin (116a) for 1 hour. After the incubation the sections were washed (4 times, 2 min each) in a solution of 10 mM HEPES (pH 7.4) and 0.01% BSA (76,82,117).

Calcitonin Gene Related Peptide-α

Sections were preincubated in 50 mM Tris-HCl buffer (pH 7.4) for 5 minutes followed by an incubation in a solution of 50 mM Tris-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 2 mM EGTA and 100 pM of ^{125}I-iodohistidyl^{10}-human-CGRP-α (Amersham) for 2 hr. After this the sections were washed (4 times, 3 min each) in a solution of 50 mM Tris-HCl (pH 7.4), 0.1% BSA (70,106,115).

Cholecystokinin

Sections were preincubated for 10 min in 50 mM Tris-HCl buffer (pH 7.7, 20°C) followed by an incubation in the same buffer with 5 mM $MgCl_2$, 0.2% BSA, 0.02% bacitracin, 1 mM dithiothreitol and 100 pM ^{125}I-Bolton-Hunter CCK-8 sulfated (Amersham) for 60 min. After this the sections were washed (4 times, 3 min each) in the same buffer containing 0.1% BSA (27,63,116,120).

Galanin

Sections were preincubated for 10 min in 10 mM HEPES buffer (pH 7.4, 20°C) followed by an incubation in the same HEPES buffer with 100 mM ^{125}I-galanin (mono-iodinated porcine galanin labeled using chloramine-T) added for 1 hr. The sections were then washed (4 times, 3 min each) in the same buffer (80).

Glutamate

Sections were incubated for 45 min at 4°C in 200 nM L-[^{3}H]glutamate (diluted with unlabeled glutamate to a "working" specific activity of 4.5 Ci/mmol, in order to label both the high and low affinity receptor binding sites (31)) in 50 mM Tris-HCl (pH 7.4) containing 2.5 mM $CaCl_2$. Non-specific binding was determined in the presence of 1 mM unlabeled glutamate. After the incubation,

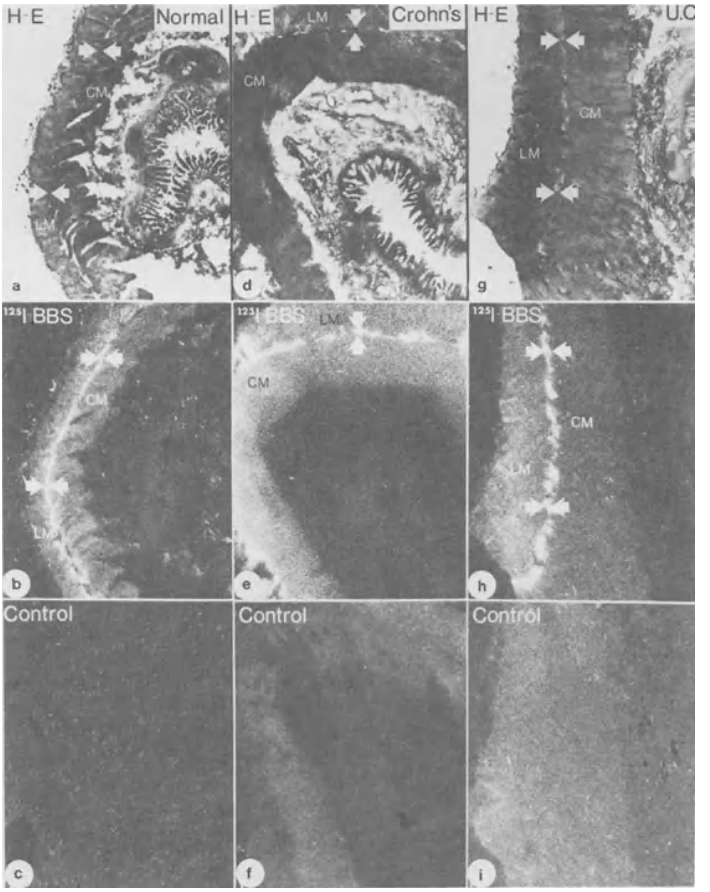


Fig. 1

A series of lightfield and darkfield photomicrographs showing localization of bombesin (BBS) receptor binding sites in transverse sections from normal (a,b,c), Crohn's disease (d,e,f) and ulcerative colitis (g,h,i) patients. In these and the following plates (Figs. 1, 3, 5, 6 and 10), a, d and g are bright-field photomicrographs of sections stained with hematoxylin and eosin (H&E stain), b, e and h are autoradiograms which show the total binding and c, f and i show the non-specific binding. Control sections (non-specific binding) were treated identically to the sections which show the total binding (b, e and h) except that 1 μM of the appropriate unlabeled peptide (in this case BBS) was added to the incubation medium. In all the darkfield autoradiograms the white silver grains represent concentrations of binding sites. To obtain the specific binding for the normal colon the binding in (c) was subtracted from that in (b), for the Crohn's disease colon (f) was subtracted from (e), and for the ulcerative colitis (U.C.) colon (i) was subtracted from (h). Note that in surgical specimens of the normal, Crohn's disease, and U.C. colon a moderate concentration of specific BBS binding sites are present over the neurons of the myenteric plexus (indicated by arrows) whereas a low concentration is present over the external circular and longitudinal muscle. Also note that there does not appear to be a significant difference in either the location or density of specific BBS binding sites between the tissues from the normal, Crohn's disease and U.C. patients. Line bar = 0.7 mm.

sections were rinsed three times with cold buffer, then rinsed twice with 2 ml of cold 2.5% glutaraldehyde in neat acetone, to minimize dissociation during drying. The total rinse time was approximately 10 sec. (30,31).

Somatostatin

Sections were preincubated in 70 mM Tris-HCl for 5 min and then in an incubation solution of 170 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 1% BSA, 20 mg/l bacitracin, and 100 pM ^{125}I-(Tyr^3)-somatostatin-8 cyclic analog (D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2) (which is similar to Sandostatin^R (97)) for 2 hr. The sections were then washed in 170 mM Tris-HCl buffer (pH 7.4) and 0.01% GSA (4 times, 4 min each) (97).

Neurokinin A (Substance K)

Sections were brought to room temperature and placed in a preincubation medium (19°C for 10 min) consisting of 50 mM Tris-HCl, pH 7.4. They were then incubated at 19°C for 2 hr in a solution of 50 mM Tris-HCl, pH 8.0, containing 3 mM $MnCl_2$, 200 mgl bovine serum albumin, 2 mg/l chymostatin, 4 mg/l leupeptin, 40 mg/l bacitracin, and 100 μM of ^{125}I-Bolton-Hunter Neurokinin A (substance K). Following this incubation, the sections were rinsed with four washes of 50 mM Tris-HCl, pH 7.4 (4°C, 5 min each) and two washes with distilled water (4°C, 5 sec each) (11,64,69,72).

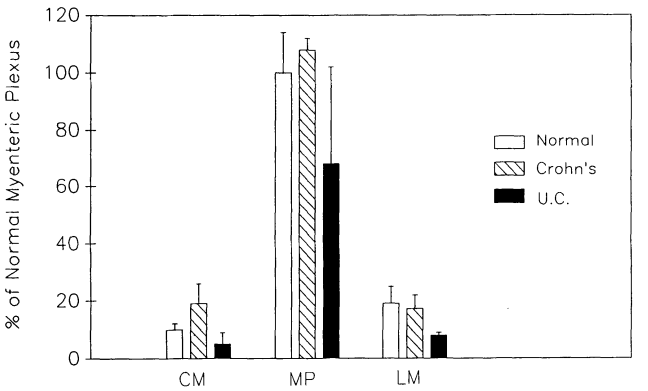


Fig. 2 - Bombesin
Histogram showing relative concentration of bombesin binding sites comparing the normal (open bar), Crohn's disease (hatched bar) and ulcerative colitis (U.C., filled bar) tissue. In this and the following histograms (Figs. 4, 7 and 11) the tissue with the highest density of binding sites for each radioligand in the normal colon is assigned a value of 100% and all other tissues which express similar binding sites are expressed as a percentage of this value. In the case of bombesin the normal tissue with the highest concentration of specific binding sites is the myenteric plexus. Note that there are no significant changes in the density of binding sites for bombesin (when comparing the normal, Crohn's or U.C. tissue) in the external circular muscle (CM), myenteric plexus (MP), or the external longitudinal muscle (LM).

Substance P

Sections were brought to room temperature and placed in a preincubation medium (20°C for 10 min) consisting of 50 mM Tris-HCl, pH 7.4, containing 0.005% (v/v) polyethylenimine. The sections were then incubated at 19°C for 1 hr in a solution of 50 mM Tris-HCl, pH 7.4, containing 3 mM $MnCl_2$, 200 mg/l bovine serum albumin, 2 mg/l chymostatin, 4 mg/l leupeptin, 40 mg/l bacitracin, and 100 pM of ^{125}I-Bolton-Hunter substance P. Following this incubation, the sections were rinsed with four washes of 50 mM Tris-HCl, pH 7.4 (4°C, 2 min each) and two washes of distilled water (4°C, 5 sec each) (11,65,66,68,71).

Vasoactive Intestinal Peptide

Sections were preincubated in 10 mM HEPES buffer (pH 7.4) for 5 minutes at 20°C followed by incubation in a solution of

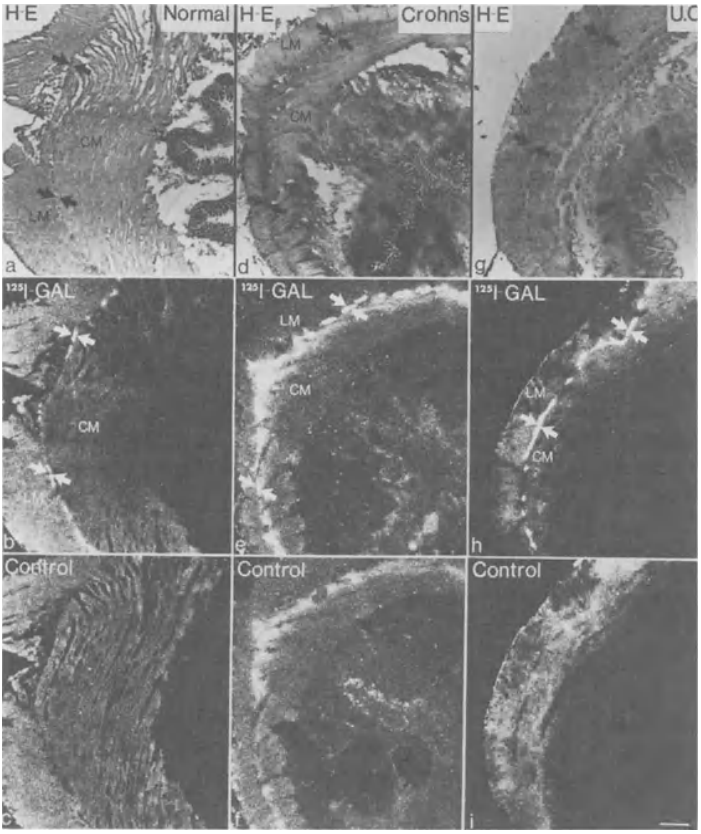


Fig. 3
A series of light and darkfield photomicrographs showing localization of galanin (GAL) receptor binding sites in transverse sections of the human colon from normal (a, b, c), Crohn's disease (d, e, f) and ulcerative colitis (g, h, i) patients. The only specific binding sites are over the myenteric plexus. Note also that the density of binding sites does not appear to differ significantly between the tissue obtained from the normal, Crohn's disease, and ulcerative colitis patients. See Fig. 1 for further explanation. Line bar = 0.7 mm.

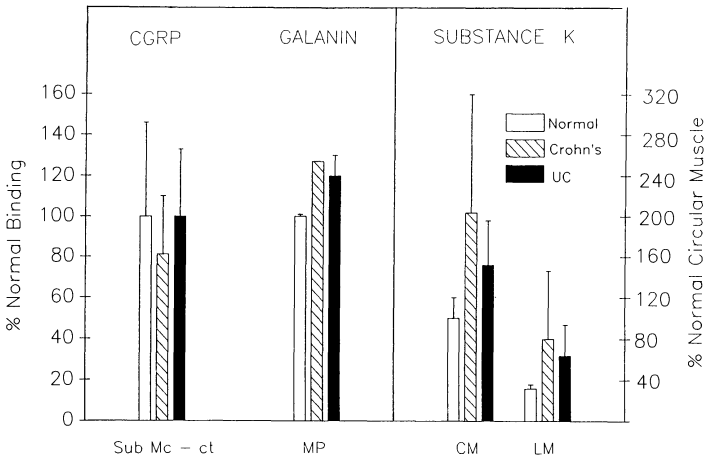


Fig. 4

Histogram showing the changes in the location and concentration of specific binding sites for calcitonin gene-related peptide-α (CGRP), galanin (GAL), and neurokinin A (also known as substance K (SK). Specific CGRP binding sites were located over the connective tissue in the submucosa (Sub Mc - ct), specific binding sites for galanin (GAL) were located over the myenteric plexus (MP), and specific binding sites for neurokinin A (substance K) were located over the external circular (CM) and longitudinal muscle (LM). Note that while each ligand binding site is present over a different set of cell types, there were no significant differences in the densities of CGRP, GAL or SK binding sites between the tissues obtained from the normal, Crohn's disease, or ulcerative colitis patients. See Fig. 2 for further explanation.

10 mM Tris-HCl buffer (pH 7.4), 130 mM NaCl, 4.7 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 1% BSA, 1 mg/ml bacitracin and 100 pM ^{125}I-iodotyrosyl^{10} VIP (Amersham) for 2 hr at 20°C. After this the sections were washed (twice for 15 min, 10°C) in the incubation solution minus the radioligand (103).

Analysis of Autoradiograms

After the final wash all the sections were dipped in distilled water, dried in the cold room (4°C), and stored overnight in desiccant. Quantitative autoradiography analysis was performed by placing the dried, labeled sections in apposition to tritium-sensitive film (Ultrofilm, LKB or Hyperfilm, Amersham) along with iodinated brain mash or commercially available standards (Amersham). After 1-4 weeks, the film was developed in Kodak D-19 developer, fixed, and washed. In sections where a higher degree of histological resolution of the binding sites was sought, the tissue slices were overlaid with emulsion-coated coverslips (119) or processed for standard emulsion-dipped autoradiography (14). After these autoradiograms were developed, the sections were placed in Carnoy's fixative for 3 h, stained with hemotoxylin and eosin (H&E) and mounted with Histoclad. Dark-field or bright-field photomicrographs were then taken of the silver grains and counterstained sections, respectively. Using this approach three complementary images were generated: the au-

toradiograms which were analyzed by quantitative densitometry, the autoradiograms of the emulsion-dipped slides which provided detailed histological resolution of the binding sites, and the counterstained sections which allowed identification of the cell type expressing a specific binding site. Controls for chemographic artifacts were generated by performing the binding exactly as described except that the radioligand was omitted from the incubation medium.

Analysis of Data

To quantitate the density of radiolabeled neurotransmitter binding sites, microdensitometry with tritium-sensitive film was

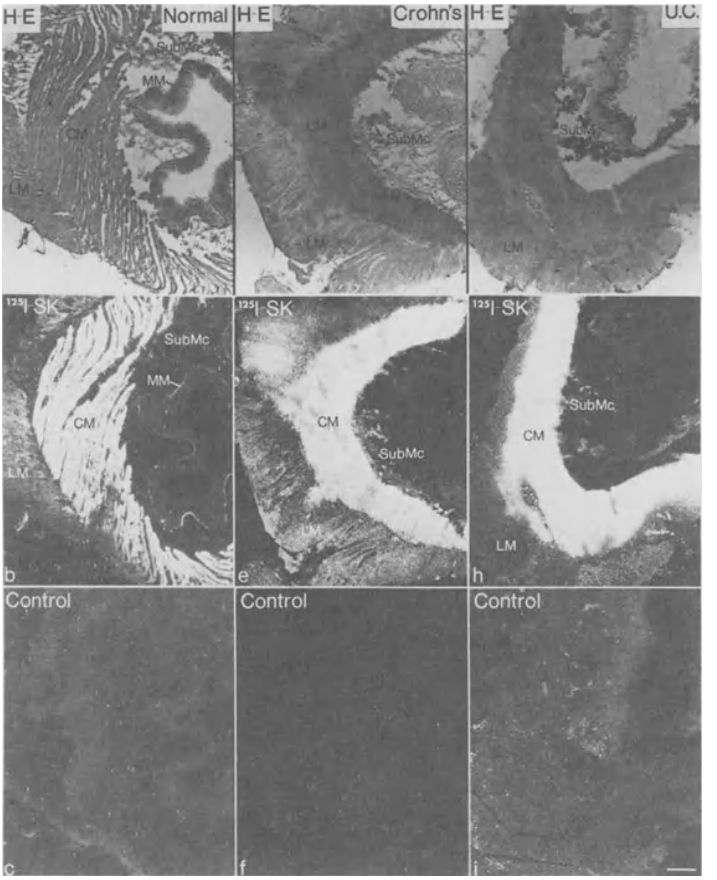


Fig. 5
A series of light and darkfield photomicrographs showing the localization of receptor binding sites for neurokinin A (also known as substance K (SK)) in transverse sections of the human colon from normal (a, b, c), Crohn's disease (d, e, f) and ulcerative colitis (g, h, i) patients. The only specific binding sites are over the external circular and longitudinal muscle. Note also that density of binding sites does not appear to differ significantly between the tissue obtained from the normal, Crohn's disease and ulcerative colitis patients. See Fig. 1 for further explanation. Line bar = 0.7 mm.

performed (96). The exposed film was projected at 20X on a white horizontal surface and the density of the projected image measured with a photocell connected to a digital voltmeter as described (71). At 20X the resolution of the device corresponds to a region 20 μm in diameter on the projected sections. Previous experiments have established that the LKB film does not respond linearly to a linear increase in radioactivity. We therefore constructed a series of standards, exposed these to LKB film, developed and fixed the film, measured this film densitometrically and used these values with a Texas Instruments automatic curve fitting program to obtain a description of film characteristics and correct for the non-linearity. In all cases, specific binding was obtained by subtracting nonspecific binding from the total binding. Nonspecific binding was defined as that binding remaining in the presence of a 1 uM concentration of the unlabeled peptide.

Statistical Analysis

The results were expressed as mean ± one standard error of the mean (SEM) and examined for statistical significance using the Student's t test for independent samples. In all histograms (Figs. 2, 4, 9 and 11) only differences with a significance greater than $p<0.05$ are indicated.

RESULTS

Bombesin

In the normal human colon, receptor binding sites for bombesin (^{125}I-Tyr^{4}-bombesin) are present in high amounts over neurons of the myenteric plexus and in low amounts over both the circular muscle and longitudinal muscle of the muscularis externa (Fig. 1). In comparing histologically normal tissue with tissue obtained from patients with either Crohn's disease or ulcerative colitis, no significant changes were observed in the density of receptor binding sites in the myenteric plexus, circular, or longitudinal muscle (Fig. 2 and Table 2).

Calcitonin Gene Related Peptide-α

In both the histologically normal tissue and the tissues from Crohn's and ulcerative colitis patients, low levels of specific receptor binding sites for CGRP (^{125}I-iodohistidyl^{10}-human CGRP-α) were present over the stroma of the submucosa, which is primarily composed of connective tissue. There was no difference in the density of specific binding sites in comparing the histologically normal tissue and the tissue from Crohn's or ulcerative colitis patients (Fig. 4 and Table 2). It should be noted that while there is a differential expression of CGRP-α and CGRP-β by primary sensory neurons and enteric autonomic neurons in the rat, it has been reported that these two peptides appear to interact with the same receptor binding site in the rat colon (83).

Cholecystokinin

Specific binding sites for cholecystokinin (^{125}I-Bolton-Hunter CCK-8 sulfated) were not observed in any area of the human colon (Table 2) although specific binding sites for the same radioligand were observed in canine fundus and the rat brain under

Table 2

Relative concentration of binding sites for all radioligands tested comparing the normal (N), Crohn's (Cr) and ulcerative colitis (UC) tissue. In the normal colon the tissue with the highest density of binding sites for each radioligand is assigned a value of 1.0 and all other tissue which contains similar binding sites are expressed as a ratio of this value. Tissues which show a significant change in the density of binding sites compared to normal are underlined.

	BBS			CCK	CGRP			GAL			GLU	SK			SOM	SP			VIP		
	N	Cr	UC		N	Cr	UC	N	Cr	UC		N	Cr	UC		N	Cr	UC	N	Cr	UC
Mc																					
lum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	1.0	0.1
bas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.2	0.1
Sub Mc																					
bv	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.7	0.9	-	-	-
ct	-	-	-	-	1.0	0.8	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
lymph	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	1.1	1.1	-	-	-
CM																					
bv	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	0.9	1.2	-	-	-
musc	0.1	0.2	0.1	-	-	-	-	-	-	-	-	1.0	2.0	1.6	-	1.0	0.8	0.6	0.2	0.1	0.2
MP	1.0	1.1	0.7	-	-	-	-	1.0	1.3	1.2	-	-	-	-	-	-	-	-	0.3	0.1	0.3
LM																					
bv	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	1.0	1.2	-	-	-
musc	0.2	0.2	0.1	-	-	-	-	-	-	-	-	0.3	0.8	0.6	-	-	-	-	0.2	0.2	0.2

identical experimental conditions (Mantyh, unpublished observations).

Galanin

Specific binding sites for galanin (^{125}I-porcine galanin) were located over the myenteric plexus in the normal, Crohn's disease, and ulcerative colitis samples (Fig. 3). No significant difference in the density of binding sites was noted among these tissues (Fig. 4 and Table 2).

Glutamate

Specific binding sites for glutamate (^{3}H-glutamate) were not observed in any area of the human colon (Table 2) although specific binding sites using the same radioligand and experimental conditions were observed in the spinal cord and brainstem of the rat, rabbit, cat and monkey (Mantyh, unpublished observations).

Neurokinin A (Substance K)

Specific Neurokinin A (^{125}I-Bolton-Hunter Neurokinin A) receptor binding sites are present in low levels over the external circular and longitudinal muscles from the histologically normal tissue and tissue obtained from Crohn's disease or ulcerative colitis patients (Fig. 5). No change in the density of these receptors was evident in comparing the normal and inflamed colon (Fig. 4 and Table 2).

Somatostatin

Specific somatostatin binding sites (using a cyclic analog of ^{125}I-somatostatin-8) were not observed in any areas of the human colon (Table 2) although specific binding sites for the same radioligand were observed in the brain, spinal cord, and brainstem of the rat, rabbit, cat and monkey using identical experimental conditions (Mantyh, unpublished observations). It should be noted however that this analog, which is not rapidly degraded and is therefore stable enough to be used with receptor autoradiography, is known to bind only to a subpopulation of somatostatin receptors (77,98).

Substance P

In the histologically normal colon, moderate levels of SP (^{125}I-Bolton-Hunder substance P) receptor binding sites are present over the external circular muscle and over smooth muscle cells comprising the tunica media of the large arteries just outside the serosa (Fig. 6). In addition, a low concentration of SP binding sites is found on arterioles and venules in the submucosa.

In tissues obtained from patients with Crohn's disease there was a striking change in the distribution and levels of SP receptor binding sites compared to that seen in the histologically normal surgical specimens obtained at the margins of extensive resections for carcinoma (Fig. 6). The most notable difference is seen in SP receptor binding sites present over arterioles (Figs. 6&7), venules and lymph nodules (Figs. 6&8). In tissues from patients with IBD, arterioles and venules (diameter 0.1-1.0 mm) in all layers of the colon express very high levels of SP

receptor binding sites whereas in normal colon tissue, SP receptor binding sites are undetectable in blood vessels (with the exception of the occasional large arteries just inside the serosa or the arterioles and venules in the submucosa, the latter two of which express low levels of SP binding sites). Since it

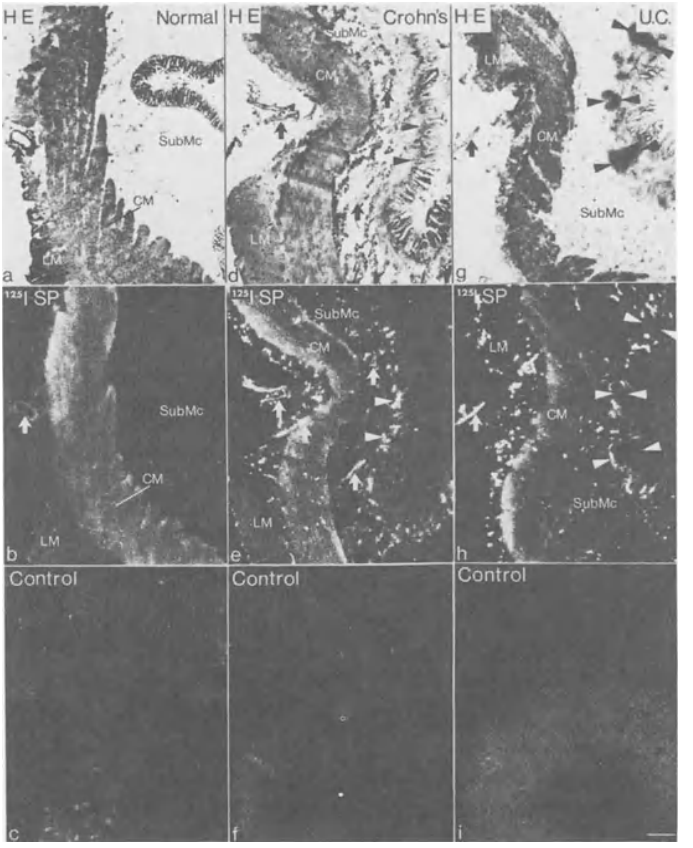


Fig. 6

A series of light and darkfield photomicrographs showing the localization of receptor binding sites for substance P (SP) in transverse sections of the human colon from normal (a, b, c), Crohn's disease (d, e, f) and ulcerative colitis (g, h, i) patients. Note that in the normal tissue a moderate concentration of SP binding sites is present over the circular muscle; this density does not change significantly in tissue obtained from the Crohn's disease and ulcerative colitis patients. In contrast to SP receptor binding sites present over blood vessels (small arterioles and venules, indicated by arrows), the germinal centers of lymph nodules (indicated by arrow heads) are virtually undetectable in the histologically normal tissue but reach the highest density of SP receptor binding sites in the entire human GI tract in the tissues obtained from the Crohn's disease and ulcerative colitis patients. Note also that blood vessels throughout the entire bowel wall display high concentrations of SP receptor binding sites in the Crohn's disease and ulcerative colitis patients. See Fig. 1 for further explanation. Line bar = 0.7 mm.

is difficult to identify the total number of arterioles and venules in H&E stained sections, we have instead compared the total number of blood vessels expressing SP binding sites in the histologically normal vs. disease specimens in a defined comparable area (2.6 mm by 3.5 mm) of the tissue sections. Comparing the average number of arterioles and venules exhibiting SP receptor binding sites, SP receptor binding sites in the samples from 6 normal vs. 4 Crohn's disease patients revealed 6.5 ± 2.4 vs. 14.5 ± 4.9 in the submucosa, 0 vs. 14 ± 1.4 ($p<0.01$) in the external circular muscle and 0 vs. 18.5 ± 3.5 $p<0.01$ in the external longitudinal muscle, respectively.

Coincident with the ectopic expression of SP receptor binding sites by arterioles (Fig. 7) and venules in inflamed tissue is the expression of SP receptor binding sites by the lymph nodules which border the muscularis mucosa. Within each lymph nodule only cells associated with the lightly stained (by H&E) germinal center display detectable SP receptor binding sites (Fig. 8). In 36 lymph nodules found in surgical specimens obtained from patients with Crohn's disease, all 36 display high concentrations of SP binding sites. In contrast, SP binding sites were undetectable in the 24 lymph nodules localized in histologically normal surgical specimens from the 6 patients with carcinoma resection. In IBD tissues, the external circular muscle displays a slightly lower level of SP binding sites than in normal tissues (Figs. 6&9), but this decrease is not statistically significant.

Surgical material from ulcerative colitis patients revealed a pattern and level of SP receptor binding sites similar to that observed in the specimens obtained from Crohn's disease patients (Figs. 6-9). In specimens from ulcerative colitis patients, very high levels of SP receptor binding sites are present over both small arterioles (Fig. 7) and venules located in the muscularis mucosa, submucosa, external circular muscle, external longitudinal muscle, and the serosa. In samples of normal tissue obtained from 6 carcinoma resection patients vs. inflamed tissue from 4 patients with ulcerative colitis, the average number of arterioles and venules in the histologically normal vs. ulcerative colitis tissue in a 2.6 mm by 3.5 mm sample was 6.5 ± 2.3 vs. 13.3 ± 1.5 in the submucosa, 0 vs. 15 ± 6.2 ($p<0.01$) in the external circular muscle and 0 vs. 19 ± 5 ($p<0.01$) in the external longitudinal muscle, respectively.

The germinal centers of lymph nodules in tissue obtained from ulcerative colitis patients also displayed very high levels of SP receptor binding sites compared to those found in normal tissue (Figs. 6-9). Thus, of the 29 lymph nodes in H&E-stained sections of the 4 cases of ulcerative colitis, 28 expressed very high levels of SP binding sites, whereas 24 nodules localized in normal colon failed to express detectable levels of SP binding sites.

Vasoactive Intestinal Polypeptide

In the normal human colon a high level of VIP receptor binding sites is present over the luminal portion of the mucosa whereas a low density of receptor binding sites is present over the basal portion of the mucosa, the myenteric plexus, and the circular and longitudinal muscles (Figs. 10 &11). A comparison of histologically normal tissue with tissue obtained from

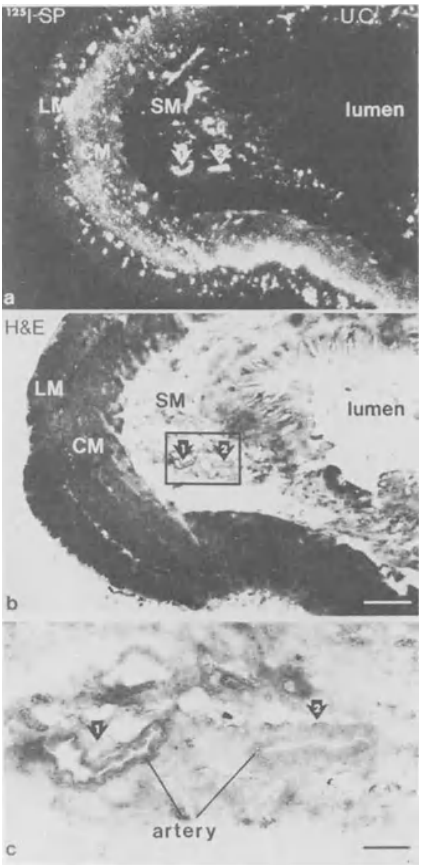


Fig. 7
A series of photomicrographs showing the detailed localization of specific SP binding sites on arteries in an inflamed colon of an ulcerative colitis patient. a. Darkfield autoradiogram showing the localization of SP binding sites on two arteries (arrows). b. Lightfield photomicrograph of the H&E stained section of (a). Note that the two arteries in (b) correspond to the silver grains under the arrows in (a). c. High power enlargement of the inset in (b) showing the two arteries which express the SP binding sites. Line bar = 0.95 mm (b); 0.21 (c).

Crohn's disease patients revealed no significant difference in the density of binding sites in any area of the colon. However, when the histologically normal tissue was compared to tissue obtained from ulcerative colitis patients (Figs. 10&11), there was a marked reduction in the density of VIP binding sites in both the luminal and basal portions of the mucosa. This is in contrast to the binding sites located over the myenteric plexus and circular and longitudinal muscle which showed no significant changes compared to the histologically normal tissue. It should be stressed that the mucosa was present in all the ulcerative colitis tissues (e.g., Fig. 10g), although the density of VIP binding sites was greatly reduced.

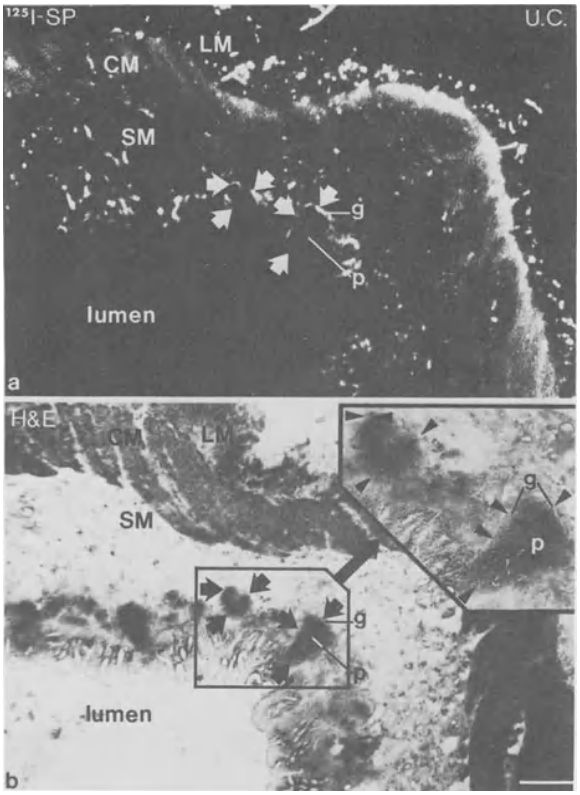


Fig. 8
A series of photomicrographs showing the detailed localization of SP binding sites over the germinal center (g) but not the proliferative zone (p) of lymph nodules (outlined by arrows) in an inflamed colon of an ulcerative colitis patient. a. Darkfield autoradiogram showing the localization of SP binding sites over the lymph nodules. b. Lightfield photomicrograph of the H&E stained section of (a). Inset: High power enlargement of the area outlined in (b). Note that the only part of the lymph nodule that expresses SP binding sites in (a) is the lightly stained germinal center outlined by arrowheads in the inset in (b). Line bar = 0.9 mm (b).

DISCUSSION

Effector Role of Sensory Neuropeptides

The most important observation emerging from the present study is that expression of receptor binding sites for specific sensory neuropeptides is susceptible to functional perturbation in inflamed human tissue. Whereas sensory neuropeptides have been shown to play a role in conveying nociceptive information from peripheral tissues to the spinal cord, recent studies have suggested that sensory neurons also have an efferent function. Initially, it was noted that antidromic stimulation of peripheral sensory nerves caused a marked plasma extravasation dependent on intact sensory nerves (4,48,54,111). With the discovery that several neuropeptides were synthesized by sensory neurons

(36,45), the question arose whether these putative neurotransmitters might be responsible for the neurogenic inflammation. A key observation, with regard to the action of sensory neuropeptides in peripheral tissues, was that sensory neuropeptides are transported not only to the terminals ending in the spinal cord but that the majority of the neuropeptides is transported to the peripheral terminal rather than the central end (8). This data in conjunction with immunochemical data demonstrating that capsaicin (a neurotoxin relatively specific for the small, thinly myelinated or unmyelinated sensory neurons (84)) treatment could eliminate the sensory innervation of vascular beds in parallel with the disappearance of neurogenic inflammation (25,39,40,41, 42), suggested that neuropeptide-containing sensory neurons (and in particular those containing substance P) have the proper anatomical relationship and physiological action on blood vessels to be agents involved in vasodilatation and plasma extravasation in peripheral vascular beds (21,50,57.58).

Relevance to Inflammatory Diseases

The possible relevance of the effector role of sensory neuropeptides to inflammatory diseases of the gastrointestinal (GI) tract (as diagrammed in Fig. 12) was suggested by capsaicin experiments demonstrating that blood vessels throughout the wall of the gastrointestinal tract are densely innervated by neuropeptide-containing sensory neurons (22,23,23, 101,112). These observations and the finding in the canine GI tract that a substantial portion of submucosal arterioles, venules and lymph nodules express receptor binding sites for SP (71,95) suggested

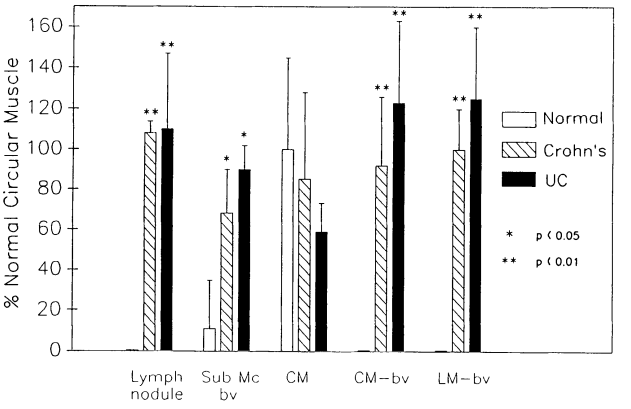


Fig. 9 - SUBSTANCE P
Histogram showing the changes in the location and concentration of SP binding sites in the surgical specimens of normal colon obtained from carcinoma resection (open bar), Crohn's disease (hatched bar), and ulcerative colitis (filled bar). In this histogram 100% specific binding is that concentration of specific SP binding sites observed in the smooth muscle of the normal external circular muscle. Note that whereas in the histolologically normal tissue SP receptor binding sites are undetectable in blood vessels in the circular and longitudinal muscle and in the lymph nodules, in tissue from the Crohn's and ulcerative colitis patients these blood vessels and lymph nodules have the highest density of SP receptor binding sites in the entire human GI tract. See Fig. 2 for further explanation.

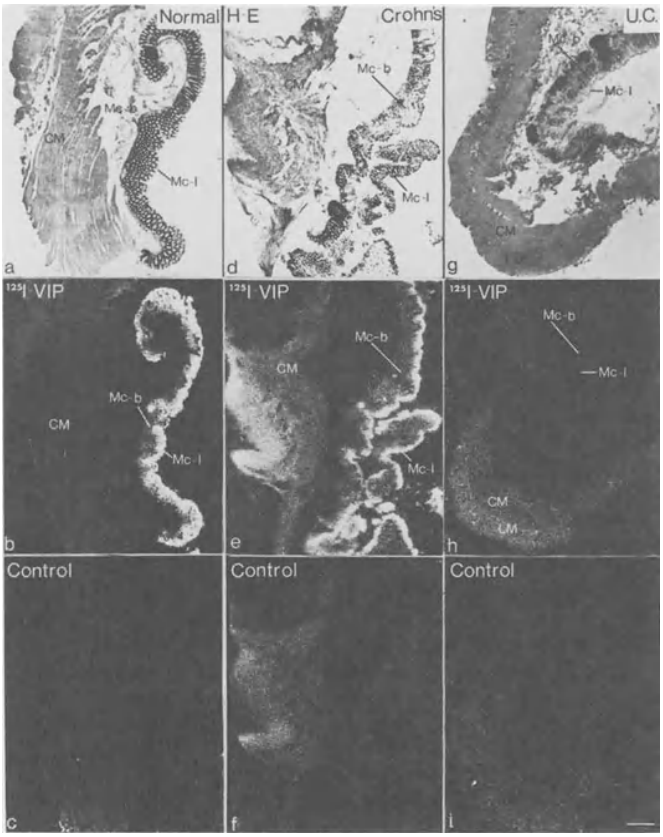


Fig. 10
A series of light and darkfield photomicrographs showing the localization of receptor binding sites for vasoactive intestinal polypeptide (VIP) in the human colon from normal (a, b, c), Crohn's disease (d, e, f), and ulcerative colitis (g, h, i) patients. The highest concentration of specific binding sites is present over the luminal aspect of the mucosa (Mc - l) whereas a low concentration of receptor binding sites is present over the basal aspect of the mucosa (Mc - b) and the external circular (CM) and longitudinal (LM) muscle. Note that the one highly significant difference in the autoradiograms is that the luminal mucosa of the ulcerative colitis tissue contains very low levels of VIP binding sites compared to either the normal or Crohn's disease tissue. See Fig. 1 for explanation. Line bar = 0.7 mm.

that sensory neuropeptides regulate vasodilatation and plasma extravasation in the gastrointestinal tract and that disfunction of this system might lead to an exaggerated inflammatory and immune response.

To test the relevance of this hypothesis to human inflammatory disease, we explored whether receptor binding sites for sensory neuropeptides are altered in two such diseases. While there is considerably more data suggesting that sensory neuropeptides are involved in two other common inflammatory diseases, i.e., arthritis (52,53) and asthma (57,59), we chose to explore

changes in inflammatory bowel disease since this is one of the few human inflammatory diseases where surgical removal of the inflamed tissue is used to ameliorate the disease in severe cases. The use of surgical specimens was critical for the success of the present studies since it allowed us to perform receptor binding experiments on human tissues in which degradation problems associated with long postmortem time delays are not present (64).

Inflammatory bowel disease (IBD) is a generic term that refers to chronic inflammatory dieseases of the intestine of unknown etiology, principally ulcerative colitis and Crohn's disease (100). Both these diseases share a number of clinical, epidemiologic, immunologic, and genetic features including: the most frequent age of onset is 15 to 30 years; there is a higher incidence in females than in males; there is an increasing incidence over the past 20 years; the diseases are more common in whites than non-whites: they are more prevalent among Ashkenazi but not Sephardic Jews (81); and significant emotional events appear to be temporally related to the onset or exacerbation of these diseases (1). Since sensory neurons innervate the GI tract (22,23,112), are involved in regulating the inflammatory response in other disease states (19,52,56,59), and there are other examples where sensory neurons alter their expression in response to stress (e.g. the expression of herpes simplex virus: 13), we investigated whether the location and levels of receptor binding sites for sensory neurotransmitters are significantly altered in IBD.

Of the nine receptor binding sites for sensory neurotransmitters examined, only SP and VIP showed significant changes, and the only cell types which demonstrated a dramatic increase

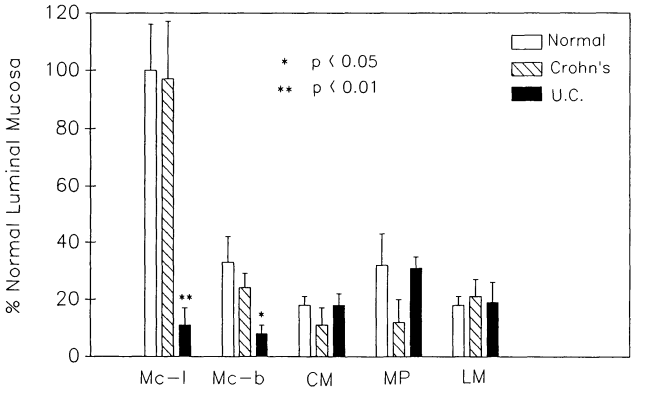


Fig. 11
Histogram showing the changes in the location and density of specific binding sites for vasoactive intestinal polypeptide (VIP). Specific VIP binding sites are over the luminal aspect of the mucosa (Mc - l) whereas a low concentration of receptor binding sites are over the basal aspect of the mucosa (Mc - b) and the external circular muscle (CM), myenteric plexus (MP), and external longitudinal muscle (LM). Note that the mucosa (both the luminal and basal aspect) of the ulcerative colitis tissue contains a very low concentration of VIP binding sites compared to either the normal or Crohn's tissue. See Fig. 1 for further explanation.

in the expression of SP binding sites were those cells involved in regulating the inflammatory and immune responses. These data correlate well with previous findings on the action of SP. for example, SP-containing sensory terminals innervate both arterioles and venules. Upon release SP interacts with the receptor binding sites expressed by the smooth muscle and endothelium of arterioles and venules to produce vasodilatation and plasma extravasation (71,88,111). Thus, SP release would cause vasodilatation and an increase in pressure at the capillary bed resulting in plasma extravasation (92,93). SP release in the vicinity of venules has been shown to cause dilatation (5), to produce changes in the permeability of the venules (50,57,113), and to alter the endothelium of the venules so that the circulating leukocytes attach to these vessels more readily (i.e., pavementing of leukocytes; 33,79,91, 102). These changes alter arteriolar and venular smooth muscle tone and cause changes in the per-

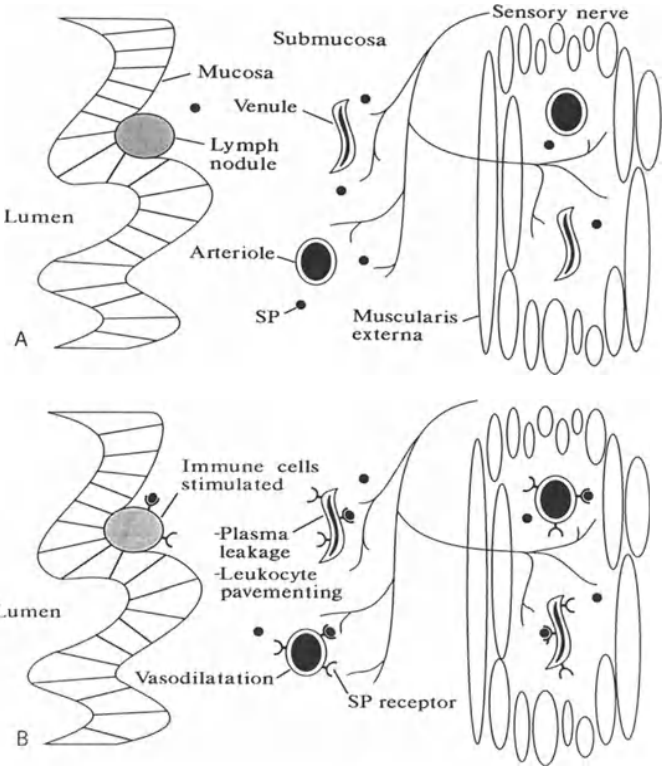


Fig. 12

Schematic diagram of the major differences observed in the present study between (a) the normal human colon and (b) the inflamed colon in inflammatory bowel disease (IBD). The most notable difference is the ectopic expression of high concentrations of SP receptor binding sites by arterioles, venules, and lymph nodules in IBD vs. the normal colon. We hypothesize that this ectopic expression of SP receptor binding sites is involved in regulating the vasodilatation of the arterioles, plasma extravasation and influx of leukocytes through the venules, and a hyperimmune response from direct SP stimulation of leukocytes, all of which are common to these and other human inflammatory diseases.

meability and attachment properties of venules, which contributes to an influx of circulating inflammatory and immune cells into the inflamed area. Among the mobile elements known to invade the inflamed area are macrophages, mast cells, neutrophils, T-lymphocytes, and monocytes; all of these cells have been shown to express binding sites for SP and/or produce a functional response in the presence of SP (2,32,33,75,78,89,90,94,102, 109). These data further suggest that, in addition to promoting edema and the influx of leukocytes, SP may also have a direct action on the function of circulating leukocytes and thus directly regulate the immune response.

Equally suggestive of the contribution of SP to the underlying pathology in the disease is the apparent similarity in the location and concentration of SP binding sites between specimens obtained from patients on corticosteroid treatment vs. those from patients in which the treatment had been stopped for at least 6 months (Table 1). Since corticosteroids are often effective in treating the symptoms of IBD but not the underlying pathophysiology, these data further suggest that the ectopic expression of SP binding sites may be an underlying pathology contributing to the inflammatory state rather than simply being a result of the inflammation response.

Specificity of Receptor - Ligand Expression

Aside from the importance of these neuropeptide binding sites in regulating the inflammatory and immune responses, the present study also suggests that the expression of the appropriate binding site by the target tissue determines, at least in part, whether the released sensory neurotransmitter will produce a functional response. A population of sensory neurons which synthesize SP also synthesize and presumably co-release CGRPα and CGRPβ (28,45,58,83,106,118), SK (16,37,61), CCK (17), and glutamate (18). It is believed that all branches of a neuron contain all the neurotransmitters synthesized by that neuron, regardless of whether or not a postsynaptic receptor is present (15,29). In other words, since many neurons are known to contain multiple neurotransmitters, it appears that the functional action of a given neurotransmitter in a particular branch of the neuron is ultimately dependent upon the presence of the appropriate postsynaptic receptor. Thus, in assessing what neurotransmitters regulate a particular peripheral organ or cell type, it is of paramount importance to first demonstrate that the target tissue expresses a pharmacologically relevant receptor binding site. An example of this is blood vessels in the normal guinea pig gastrointestinal tract, which although extensively innervated by SP-containing sensory nerves (22,23,24, 112), do not contain detectable levels of SP receptors (10,65), and show no increase in vascular permeability to either direct application of SP or to antidromic activation of the sensory nerves innervating these tissues (57). In other words the permeability effects of SP are determined by the presence of SP receptors on postcapillary venules, and this expression of SP receptors (at least on gastrointestinal blood vessels of man) preferentially occurs in an inflammatory state. These data also suggest that receptor binding sites for other sensory neurotransmitters may play a role in regulating the human peripheral tissues in both normal and diseased states, but this role will only be evident when examined in the proper physiological or pathological context.

Receptor - Ligand Mismatch

These results also have clear implications for the receptor-ligand mismatch problem. The presence of a nerve fiber containing a particular transmitter is often not well correlated with the presence of an appropriate postsynaptic receptor (29,34,47, 67,68,74). SP-immunoreactive fibers innervate arterioles and venules throughout the normal human colon, and the distribution of immunoreactive SP fibers and levels of SP change only minimally (2 fold increase; 46) or not at all (7,87,105) in the inflamed colon in patients with ulcerative colitis or Crohn's disease. This is in contrast with dramatically elevated (1000-2000 fold) levels of SP receptor binding sites expressed by arterioles, venules and lymph nodules in inflamed colon. Since it appears that SP binding sites in blood vessels and lymph nodules in the human colon reach detectable levels only in the inflamed state, it is difficult to assess the full repertoire of the target tissues of a particular neurotransmitter unless one knows a priori in what context the expression of binding sites should be examined. It is also conceivable that a receptor is tonically present and only in a disease state is its ligand expressed or released by the presynaptic nerve terminal. Thus, when a mis-match is observed between the presence of a receptor and the presence of its presumed ligand, it should be considered that a proper match may only be apparent in a different physiological or pathological context. The present data also suggest that the coordinated synaptic localization of the native ligand and its receptor must involve factors other than just the presence of the ligand since in the normal colon the innervation of blood vessels by SP apparently does not by itself induce the expression of SP receptors.

Substance P as a Multifunctional Effector Peptide

In the present report we have demonstrated the ectopic expression of high densities of SP binding sites by arterioles, venules, and lymph nodules and suggest that, in a pathological state, SP and its receptors play a role in regulating the inflammatory and immune responses characteristic of inflammatory diseases. A key question that remains to be answered is what is the normal function of the neuropeptide-containing sensory neurons? One observation which suggests a normal function for these neuropeptide-containing sensory neurons is that one of the most pronounced deficits in neonatal capsaicin-treated rats (the neurotoxin destroys primarily the neuropeptide containing C-fibers) is that the skin and fur lose their luster and that these animals lack a normal wound healing response; i.e., these animals have numerous small wounds which do not appear to be healing (9,83). Such a lack of the normal trophic and/or wound healing response after sensory denervation can also be inferred from the ulceration of the cornea which follows trigeminal deafferentation (7). Together the observations that, in a pathological state, SP appears to be involved in regulating a hyperinflammatory and immune response and that, in the normal condition, these sensory neuropeptide-containing neurons may have a trophic action on tissues they innervate and also regulate wound healing after injury.

As reviewed above, SP and other sensory neurotransmitters appear to be released centrally in the spinal cord to signal pain and peripherally to produce vasodilatation, plasma extravasation and homing of leukocytes to the area of injury. Since

sation and homing of leukocytes to the area of injury. Since there cannot be tissue repair until there has been an appropriate inflammatory and immune response (25), the initial action of SP may be to promote and direct the inflammatory and immune responses in the damaged tissue. After the infection and damaged tissue have been cleared, SP and other neuropeptide growth factors continue to be released, but their effects would now be directed towards mitogenesis and tissue remodeling. Two sensory neuropeptides, SP and neurokinin A, have been shown to be potent mitogens for fibroblasts in culture (85). The key to this model is that SP should be both stimulatory and inhibitory towards the same cells, depending on the context of other chemical signals present. This multifunctional role for peptide growth factors has clearly been shown for other peptides including transforming growth factor-β, which stimulates growth of certain fibroblasts in vitro in the presence of platelet derived growth factor, but inhibits the growth of the same cells if epidermal growth factor is present (99,108). Therefore, in human inflammatory diseases it may be that ectopic expression of SP or its receptor is not the primary pathology, but rather that the pathology may involve whatever other factors are needed to switch SP action from the "catabolic" mode, where inflammatory and immune responses are promoted, to the "anabolic" process of tissue growth.

CONCLUSIONS

There is an elegance in the simplicity of assigning the same neuron and perhaps the same neuropeptide for roles in nociceptive information transmission and simultaneous peripheral control of recovery from injury. The normal function of the small thinly myelinated sensory neuron may be to signal tissue damage to the brain and to regulate normal cell turnover and, in the case of injury, the subsequent inflammatory, immune, and wound healing response in the damaged tissue. Such an organization would allow a coordination between the biochemical processes of wound healing and the behavioral response to injury, so that use and further injury of the damaged tissues does not occur during the repair process.

Acknowledgements

Supported by an Alfred P. Sloan Fellowship, and NIH grants NS-239770, NS-22961 and DK-40260.

References

1. Almy, T., 1980, Psychosocial aspects of chronic ulcerative-colitis and Crohn's disease, in: "Inflammatory Bowel Disease", J.B. Kirsner and R.G. Shorter, eds., Lea and Fibiger, Philadelphia.
2. Bar-Shavit, Z., Goldman, R., Stabinsky, Y., Gottlieb, P., Fridkin, M., Teichberg, V.I. and Blumberg, S., 1980, Enhancement of phagocytosis: a newly found activity for substance P residing in its N-terminal tetrapeptide sequence, **Biochem. Biophys. Res. Commun.**, 94:1445.
3. Bartho, L., and Holzer, P., 1985, Search for a physiological role of substance P in gastrointestinal motility, **Neuroscience**, 16:1.
4. Bayliss, V.M., 1901, On the origin from the spinal cord of

the vasodilator fibres of the hind limb, and on the nature of these fibres, **J. Physiol. (Lond)**, 26:173.
5. Benjamin, N., Collery, C. T., Fuller, R. W., Larkin, S., and McEwan, S., 1987, The effects of calcitonin gene-related peptide and substance P on resistance and capacitance vessels, **Br. J. Pharmacol.**, 90:39 (abstract).
6. Beuerman, R.W., and Schimmelpfennig, B., 1980, Sensory denervation of the rabbit cornea affects epithelial properties, **Exp. Neurol.**, 69:196.
7. Bishop, A. E., Polak, J. M., Bryant, M. G., Bloom, S. R., and Hamilton, S., 1980, Abnormalities of vasoactive intestinal polypeptide-containing nerves in Crohn's disease, **Gastroenterology**, 9:853.
8. Brimijoin, S., Lundberg, J. M., Brodin, E., Hokfelt, T., and Nilsson, G., 1980, Axonal transport of substance P in the vagus and sciatic nerves of the guinea pig, **Brain Res.**, 191:443.
9. Buck, S.H., and Burks, T.F., 1983, Capsaicin; hot new pharmacological tool, **Trends Pharm. Sci.**, 4:84.
10. Burcher, E., Buck, S. H., Lovenberg, W., and O'Donohue T. L., 1986, Characterization and autoradiographic localization of multiple tachykinin binding sites in gastrointestinal tract and bladder, **J. Pharm. Exp. Ther.**, 236:819.
11. Burcher, E., Shults, C. W., Buck, S. H., Chase, T. N., and O'Donohue, T.L., 1984, Autoradiographic distribution of substance K binding sites in rat gastrointestinal tract: a comparison with substance P, **Eur. J. Pharm.**, 102:561.
12. Colpaert, F. C., Donnerer, J., and Lembeck, F., 1983, Effects of capsaicin on inflammation and on the substance P content of nervous tissues in rats with adjuvant arthritis, **Life Sci.**, 32:1827.
13. Corey, L., and Spear, P.G., 1986, Infections with herpes simplex viruses, **N. Engl. J. Med.**, 314:686.
14. Cowan, W.M., Gottlieb, D.I., Hendrickson, A.E., Price, J.L., and Woolsey, T.A., 1972, The autoradiographic demonstration of axonal connections in the central nervous system, **Brain Res.**, 37:21.
15. Dale, H.H., 1935, Walter Ernest Dixon Memorial Lecture, **Proc. R. Soc. Med. Therap.**, Sec.28:319.
16. Dalsgaard, C. J., Haegerstrand, A., Theodorsson-Norheim, E., Brodin, E., and Hokfelt, T., 1985, Neurokinin A-like immunoreactivitiy in rat primary sensory neurons: coexistence with substance P, **Histochemistry**, 83:37.
17. Dalsgaard, C. J., Vincent, S. R., Hokfelt, T., Lundberg, J. M., Dahlstrom, A., Schultzberg, M., Dockray, C.J., and Cuello, A.C., 1982, Co-existence of cholecystokinin- and substance P-like peptides in neurons of the dorsal root ganglion of the rat, **Neurosci. Lett.**, 33:159.
18. Debiasi, S., Vaneyck, S.L., Petrusz, P., and Rustioni, A., 1987, Glutamate and substance P co-exist in the same primary afferent terminal in the superficial laminae of the rat spinal dorsal horn, **Neurosci. Abstr.**, 13:434.
19. Devillier, P., Weill, B., Renoux, M., Menkès, C., and Pradelles, P., 1987, Elevated levels of tachykinin-like immunoreactivity in joint fluids from patients with rheumatic inflammatory diseases, **N. Engl. J. Med.**, 314:1323.
20. Eccles, J. C., 1957, "The Physiology of Nerve Cells," Johns Hopkins Press, Baltimore.
21. Foreman, J., and Jordan, C., 1983, Histamine release and vascular changes induced by neuropeptides, **Agents Actions**, 13:105.

22. Franco, R., Costa, M., and Furness, J.B., 1979a, Evidence for the release of endogenous substance P from intestinal nerves, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 301:195.
23. Franco, R., Costa, M., and Furness, J.B., 1979b, Evidence that axons containing substance P in the guinea-pig ileum are of extrinsic origin, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 307:57.
24. Furness, J., Papka, R. E., Della, N. G., Costa, M., and Eskay, R.L., 1982, Substance P-like immunoreactivity in nerves associated with the vascular system of guinea-pigs, **Neuroscience**, 7:447.
25. Gallin, J. I., and Fauci, A. S., 1982, "Advances in Host Defense Mechanisms," Vol. 1 Phagocytic Cells, Raven Press, New York.
26. Gamse, R., Holzer, P., and Lembeck, F., 1980, Decrease of substance P in primary afferent neurons and impairment of neurogenic plasma extravasation by capsaicin, **Br. J. Pharmac.**, 68:207.
27. Gaudreau, P., Quirion, R., St. Pierre, S., and Pert, C.B., 1983, Characterization and visualization of cholecystokinin receptors in rat brain using [^{3}H]pentagastrin, **Peptides**, 4:755.
28. Gibbs, I. L., Furness, J.B., Costa, M., MacIntyre, I., Hillyard, C.J., and Girgis, S., 1985, Colocalization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs, **Neurosci. Lettr.**, 57:125.
29. Goedert, M., Mantyh, P. W., Emson, P. C., and Hunt, S. P., 1984, Inverse relationship between neurotensin receptors and neurotensin-like immunoreactivity in the cat striatum, **Nature**, 307:543.
30. Greenamyre, J.T., Young, A. B., and Penney, J. B., 1983, Quantitative autoradiography of L-[^{3}H]glutamate binding in rat brain, **Neurosci. Lett.**, 37:155.
31. Greenamyre, J.T., Young, A. B., and Penney, J. B., 1983, Quantitative autoradiography distribution of L-[^{3}H]glutamate binding sites in rat central nervous system, **J. Neurosci.**, 8:2133.
32. Hartung, H. P., and Toyka, K.V., 1983, Activation of macrophages by substance P: induction of oxidative burst and thromboxane release, **Eur. J. Pharmacol.**, 89:301.
33. Hartung, H. P., Wolters, K., and Toyka, K.V., 1986, Substance P: binding properties and studies on cellular responses in guinea pig macrophages, **J. Immunol.**, 136:856.
34. Herkenham, M., 1987, Mismatches between neurotransmitter and receptor localization in brain: observations and implications, **Neuroscience**, 23:1.
35. Hokfelt, T., Johansson, O., Ljungdalh, A., Lundberg, J.M., and Schultzberg, M., 1980, Peptidergic neurons, **Nature**, 284:515.
36. Hokfelt, T., Jellerth, J. O., Nilsson, G., and Pernow, B., 1975, Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons, **Brain Res.**, 100:235.
37. Hua, X. Y., Theodorsson-Norheim, E., Brodin, E., Lundberg, J.M., and Hokfelt, T., 1985, Multiple tachykinins, neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory neurons in the guinea pig, **Regulatory Peptides**, 13:1.
38. Hylden, J.L.K., and Wilcox, G. L., 1981, Intrathecal substance P elicits a caudally directed biting and scratching behavior in mice, **Brain Res.**, 217:212.

39. Jansco, N.A., Jansco-Gabor, A., and Szolcsanyi, J., 1967, Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin, **Br. J. Pharmacol.**, 31:138.
40. Jansco, N. A., Jansco-Gabor, A., and Szolcsanyi, J., 1968, The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat, **Br. J. Pharmac.**, 32:32.
41. Jansco, N. A., Kiraly, E., and Jansco-Gabor, A., 1980, Pharmacologically induced elective degeneration of chemosensitive primary sensory neurons, **Nature**, 270:741.
42. Jansco, N.A., Kiraly, E., and Jansco-Gabor, A., 1980, Chemosensitive pain fibers and inflammation, **Int. J. Tiss. Reac.**, 2:57.
43. Jessel, T.M., and Iversen, L.L., 1977, Opiate analgesics inhibit substance P release from rat trigeminal nucleus, **Nature**, 268:549.
44. Johnson, A. R., and Erdos, E.G., 1973, Release of histamine from mast cells by vasoactive peptides, **Proc. Soc. Exp. Biol. Med.**, 142:1252.
45. Ju, G., Hokfelt, T., Brodin, E., Fahrenkrug, J., Fischer, J. A., Frey, P., Elde, R.P., and Brown, J.C., 1987, Primary sensory neurons of the rat showing calcitonin gene-related peptide immunoreactivity and their relation to substance P-, somatostatin-, galanin-, vasoactive intestinal peptide- and cholecystokinin-immunoreactive ganglion cells, **Cell Tissue Res.**, 247:417.
46. Koch, T.R., Carney, J.A., and Go, V.L.W., 1987, Distribution and quantitation of gut neuropeptides in normal intestine and inflammatory bowel diseases, **Dig. Diseases and Sciences**, 32.369.
47. Kuhar, M. J., 1985, The mismatch problem in receptor mapping studies, **Trends Neurosci.**, 8:190.
48. Langley, J. N., 1923, Antidromic action, **J. Physiol.(Lond)**, 57:428.
49. Lembeck, F., Donnerer, J. and Colpaert, F. C., 1981, Increase of substance P in primary afferent nerves during chronic pain, **Neuropeptides**, 1:175.
50. Lembeck, F., and Holzer, P., 1979, Substance P as a neurogenic mediator of antidromic vasodilatation and neurogenic plasma extravasation, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 310:175.
51. Levine, J. D., Clark, R., Devor, M., Helms, C., Moskowitz, M.A., Basbaum, A.I., 1985, Intraneuronal substance P contributes to the severity of experimental arthritis, **Science**, 226:547.
52. Levine, J.D., Collier, D.H., Basbaum, A.I., Moskowitz, M.A., and Helms, C.A., 1985, Hypothesis: the nervous system may contribute to the pathophysiology of rheumatorid arthritis, **J. Rheumatology**, 12:410.
53. Levine, J. D., Dardick, S. J., Roizen, M. F., Helms, C., and Basbaum, A.I., 1986, Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis, **J. Neurosci.**, 6:3423.
54. Lewis, T., and Marvin, H. M., 1927, Observations relating to vasodilatation arising from antidromic impulses, to herpes zoster and trophic effects, **Heart**, 14:27.
55. Llewellyn-Smith, I.J., Furness, J.B., Murphy, R., O'Brien, P.E., and Costa, M., 1984, Substance P-containing nerves in the human small intestine: distribution, ultrastructure, and

characterization of the immunoreactive peptide, **Gastroenterology**, 86:421.
56. Lotz, M., Carson, D.A., Vaughan, J.H.,, 1987, Substance P activation of rheumatoid synoviocytes: Neural pathway in pathogenesis of arthritis, **Science**, 235:893.
57. Lundberg, J.M., Brodin, E., Hua, X., and Saria, A., 1984, Vascular permeability changes and smooth muscle contraction in relation to capsaicin-sensitive substance P afferents in the guinea pig, **Acta Physiol. Scand.**, 120:217.
58. Lundberg, J. M., Franco-Cereceda, A., Hua, X., Hokfelt, T., and Fischer, J.A., 1985, Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin, **Eur. J. Pharmacology**, 108:315.
59. Lundberg, J.M., and Saria, A., 1983, Capsaicin-induced desensitization of the airway mucosa to cigarette smoke, mechanical and chemical irritants, **Nature**, 302:251.
60. Maggio, J.E., 1988, Tachykinins, **Ann. Rev. Neurosci.**, 11:13.
61. Maggio, J. E., and Hunter, J. C., 1984, Regional distribution of kassinin-like immunoreactivity in rat central and peripheral tissues and the effect of capsaicin, **Brain Res.**, 307:370
62. Mantyh, C. R., Gates, T. S., Zimmerman, R. P., Welton, M. L., Passaro, E.P.Jr., Vigna, S.R., Maggio, J.E., Kruger, L., and Mantyh, P.W., 1988, Receptor binding sites for substance P, but not substance K or neuromedin K, are expressed in high concentrations by arterioles, venules and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn Disease, **Proc. Natl. Acad. Sci.**, 85:3235.
63. Mantyh, C. R., and Mantyh, P.W., 1985, Differential localization of cholecystokinin-8 binding sites in the rat vs the guinea pig brain, **Eur. J. Pharmacol.**, 113:37.
64. Mantyh, P. W., 1988, Autoradiographic localization and characterization of receptor binding sites in the brain and peripheral tissues, in: "Receptor Biochemistry and Methodology: Receptor Localization, Ligand Autoradiography," Vol. 13, F.M. Leslie and C.A. Altar, eds., A.R. Liss, New York.
65. Mantyh, P.W., Goedert, M., and Hunt, S. P., 1984a, Autoradiographic visualization of receptor binding sites for substance P in the gastrointestinal tract of the guinea pig, **Eur. J. Pharm.**, 100:133.
66. Mantyh, P.W., and Hunt, S.P., 1985a, Autoradiographic visualization of [^{3}H]-Substance P receptor binding sites in the rat and bovine spinal cord and the rat and cat spinal trigeminal nucleus pars caudalis and the effects of neonatal capsaicin, **Brain Res.**, 332:315.
67. Mantyh, P. W., and Hunt, S. P., 1986, Changes in ^{3}H-substance P receptor binding sites in the rat brain after kianic lesion of the corpus striatum, **J. Neurosci.**, 6:1537.
68. Mantyh, P. W., Hunt, S. P. and Maggio, J. E., 1984b, Substance P receptors: localization by light microscopic autoradiograph in rat brain using [^{3}H]-SP as the radioligand, **Brain Res.**, 307:147.
69. Mantyh, P. W., Maggio, J. E., and Hunt, S. P., 1984c, The autoradiographic distribution of kassinin and substance K binding sites is different from the distribution of substance P binding sites in the rat brain, **Eur. J. Pharmacol.**, 102:361.
70. Mantyh, P. W., Mantyh, C. R., Brecha, N. C., Kruger, L., and

Sternini, C., 1985b), Autoradiographic localization of calcitonin gene-related peptide binding sites in the rat brain, guinea pig periphery and human spinal cord, **Neurosci. Abstr.**, 11:145.
71. Mantyh, P. W., Mantyh, C.R., Gates, T., Vigna, S.R., and Maggio, J.E., 1988, Receptor binding sites for substance P and substance K in the canine gastrointestinal tract and their possible role in inflammatory bowel disease, **Neuroscience**, 25:817.
72. Mantyh, P.W., Mantyh, C. R., and Maggio, J. E., 1987a, Heterogeneity of tachykinin receptors, in: "Substance P and Neurokinins," James L. Henry, ed., Springer-Verlag, New York (in press).
73. Mantyh, P.W., Mantyh, C.R., Popper, P., Vigna, S.R., Kruger, L., Basbaum, A.I., Levine, J.D., and Maggio, J.E., 1987b, Receptors for sensory neuropeptides may be involved in the pathophysiology of rheumatoid arthritis, **Soc. Neurosci. Abstr.**, 13:563.
74. Mantyh, P. W., Pinnock, R. D., Downes, C. P., Goedert, M., and Hunt, S.P., 1984d, Substance P receptors: correlation with substance P induced inositol phospholipid hydrolysis in the rat central nervous system, **Nature**, 309:795.
75. Marasco, W. A., Showell, H.J., and Becker, E.L., 1981, Substance P binds to the formaldehyde chemotaxis receptor on the rabbit neutrophil, **Biochem. Biophys. Res. Comm.**, 99:1065.
76. Massari, V.J., Shults, C.W., Park, C.., Tizabi, Y., Moody, T.W., Chronwall, B.M., Culver, M., and Chase, T.N., 1985, Deafferentation causes a loss of presynaptic bombesin receptors and supersensitivity of substance P receptors in the dorsal horn of the cat spinal cord, **Brain Res.**, 343:268.
77. Maurer, R., and Reubi, J. C., 1985, Brain somatostatin receptor subpopulation visualized by autoradiography, **Brain Res.**, 333:178.
78. Mazurek, N., Pecht, I., Teichberg, V.I., and Blumberg, S., 1981, The role of the N terminal tetrapeptide in the histamine-releasing action of substance P, **Neuropharmacol.**, 20:1025.
79. McGillis, J.P., Organist, M.L., and Payan, D.G., 1987, Substance P and immunoregulation, **Fed. Proc.**, 46:196.
80. Melander, T., Hokfelt, T., Nilsson, S., and Brodin, E., 1986, Visualization of galanin binding sites in the rat central nervous system, **Eur. J. Pharm.**, 124:381.
81. Mendeloff, A. I., 1980, Epidemiology of idiopathic inflammatory bowel disease, in: "Inflammatory Bowel Disease,", J.B. Kirsner and R.G. Shorter, eds., Lea and Fibiger, Philadelphia.
82. Moody, T.W., O'Donohue, T. L., and Jacobowitz, D. M., 1981, Biochemical localization and characterization of bombesin-like peptides in discrete regions of the rat brain, **Peptides**, 2:75.
83. Mulderry, P. K., Ghatei, M. A., Spokes, R. A., Jones, P. M., Pierson, A.M., Hamic, Q.A., Kanse, S., Amara, S.G., Burrin, J.M., Legon, S., Polak, J.M., and Bloom, S.R., 1988, Differential expression of α-CGRP and β-CGRP by primary sensory neurons and enteric autonomic neurons of the rat, **Neuroscience**, 25:195.
84. Nagy, J. I., Hunt, S. P., Iversen, L. L., and Emson, P.C., 1981, Biochemical and anatomical observations on the degeneration of peptide-containing primary afferent neurons after neonatal capsaicin, **Neuroscience**, 6:1923.

85. Nilsson, J., von Euler, A.M., and Dalsgaard, C.J., 1985, Stimulation of connective tissue cell growth by substance P and substance K, **Nature**, 315:61.
86. Oku, R., Saton, M., Takagi, H., 1987, Release of substance P from the spinal dorsal horn is enhanced in polyarthritic rats, **Neurosci. Lett.**, 74:315.
87. O'Morain, C., Bishop, A. E., McGregor, G. P., Levi, A.J., Bloom, S.R., Polak, J.M., and Peters, T.J., 1984, Vasoactive intestinal peptide concentrations and immunocytochemical studies in rectal biopsies from patients with inflammatory bowel disease, **Gut**, 25:56.
88. Orleans-Juste, D'P., Dion, S., Mizrahi, J., and Regoli, D., 1985, Effects of peptides and non-peptides on isolated arterial smooth muscles: role of endothelium, **Eur. J. Pharmacol.**, 114:9.
89. Payan, D. F., Brewster, D. R., and Goetzl, E. J., 1983, Specific stimulation of human T-lymphocytes by substance P, **J. Immunol.**, 131:1613.
90. Payan, D.G., McGillis, J. P., and Goetzl, E. J., 1986, Neuroimmunology, **Adv. Immunol.**, 39:299.
91. Payan, D.G., McGillis, J.P., Organist, M.L., 1986, Binding characteristics and affinity labeling of protein consituents of the human IM-9 lymphoblast receptor for substance P, **J. Biol. Chem.**, 261:14321.
92. Pernow, B., 1983, Substance P, **Pharmacol. Rev.**, 35:85.
93. Pernow, B., 1985, Role of tachykinins in neurogenic inflammation, **J. Immunol.**, 135:812s.
94. Piotrowski, W., Devoy, M.A.B., Jordan, C. C., and Foremen, J.C., 1984, The substance P receptor on rat mast cells and in human skin, **Agents and Actions**, 14:420.
95. Popper, P., Mantyh, C.R., Vigna, S.R., Maggio, J.E., and Mantyh, P.W., 1988, The localization of sensory nerve fibers and receptor binding sites for sensory neuropeptides in canine mesenteric lymph nodes, **Peptides**, 9:257.
96. Rainbow, T. C., Biegon, A., and Berck, D.J., 1984, Quantitative receptor autoradiography with tritium labeled ligands: comparison of biochemical and densitometric measurements, **J. Neurosci. Methods**, 11:231.
97. Reubi, J.C., Corete, R., Maurer, R., Probst, A., Palacios, J.M., 1986, Distribution of somatostatin receptors in the human brain: an autoradiographic study, **Neuroscience**, 18:329.
98. Reubi, J. C., Probst, A., Cortes, R., and Palacios, J. M., 1987, Distinct topographical localization of two somatostatin receptor subpopulations in the human cortex, **Brain Res.**, 406:391.
99. Roberts, A. B., Anzano, M.A., Wakefield, L.M., Roche, N., Stern, D.F., and Sporn, M.B., 1985, Type beta transforming growth factor: a bifunctional regulator of cellular growth, **Proc. Natl. Acad. Sci.**, 82:119.
100. Rosenberg, I. H., 1985, Inflammatory bowel disease, in: "Cecil Textbook of Medicine," J.B. Wyngaarden and L.H. Smith, eds., W.B. Saunders, Philadelphia.
101. Rozsa, Z., Jancso, G., and Varro, V., 1984, Possibile involvement of capsaicin-sensitive sensory nerves in the regulation of intestinal blood flow in the dog, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 326:352.
102. Ruff, M. R., Wahl, S. M., and Pert, C. B., 1985, Substance P receptor-mediated chemotaxis of human monocytes, **Peptides**, 6(suppl.2):107.
103. Schaffer, M.M., and Moody, T. W., 1986, Autoradiographic vi-

sualization of CNS receptors for vasoactive intestinal peptide, **Peptides**, 7:283.
104. Schulzberg, T., Hokfelt, T., Nilsson, G., Terenius, L., Rehfeld, J.F., Brown, M., Elde, R., Goldstein, M., and Said, S., 1980, Distribution of peptide- and catecholamine containing neurons in the gastrointestinal tract of rat and guinea pig: Immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine B-hydroxylase, **Neuroscience**, 5:689.
105. Sjolund, K., Schaffalitzky De Muckadell, O. B., Fahrenkrug, J., Hakanson, R., Peterson, B.G., and Sundler, F., 1983, Peptide-containing nerve fibers in the gut wall in Crohn's disease, **Gut**, 24:724.
106. Skofitsch, G., and Jacobowitz, D. M., 1985a, Autoradiographic distribution of ^{125}I-calcitonin gene-related peptide binding sites in the rat central nervous system, **Peptides**, 4:975.
107. Skofitsch, G., and Jacobowitz, D. M., 1985b, Calcitonin gene-related peptide coexists with substance P in capsaicin-sensitive neurons and sensory ganglia of the rat, **Peptides**, 6:747.
108. Sporn, M. B., and Roberts, A. B., 1988, Peptide growth factors are multifunctional, **Nature**, 332:217.
109. Stanisz, A.M., Befus, D., and Bienenstock, J., 1986, Differential effects of vasoactive intestinal peptide , substance P and somatostatin on immunoglobulin synthesis and proliferation by lymphocytes from Peyer's patches, mesenteric lymph nodes and spleen, **J. Immunol.**, 136:152.
110. Stephenson, J.A., Burcher, E., and Summers, R. J., 1986, Autoradiographic demonstration of endothelium-dependent [^{125}I] Bolton-Hunter substance P binding to dog carotid artery, **Eur. J. Pharmacol.**, 124:377.
111. Stricker, S., 1876, Untersuchungen uber die Gefasswurzeln dis Ischiadicus, **Stitz. Ber. Kaiserl. Akad. Wiss. (Wein)**, 3:173.
112. Su, H. C., Bishop, A. E., Power, R. F., Hamada, Y., and Polak, J.M., 1987, Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas, **J. Neurosci.**, 9:2674.
113. Svensjo, E., Lundberg, J. M., Anggard, A., and Hokfelt, T., 1980, Substance P increase in vascular permeability and presence in the hamster cheek pouch, **J. Microvascular Res.**, 20:38.
114. Tange, A., 1983, Distribution of peptide-containing endocrine cells and neurons in the gastrointestinal tract of the dog: immunohistochemical studies using antisera to somatostatin, substance P, vasoactive intestinal polypeptide, Met-enkephalin and neurotensin, **Biomed. Res.**, 4:9.
115. Tschopp, F.A., Henke, H., Peterman, J.B., Tobler, P.H., Janzer, R., Hokfelt, T., Lundberg, J.M., Cuello, C., and Fischer, J.A., 1985, Calcitonin gene-related peptide and its binding sites in the human central nervous system and pituitary, **Proc. Natl. Acad. Sci.**, 82:248.
116. Van Dijk, A., Richards, J. G., Trzeciak, A., Gillessen, D., and Mohler, H., 1984, Cholecystokinin receptors: Biochemical demonstration and autoradiographical localization in rat brain and pancreas using [^{3}H]cholecystokinin-$_{8}$ as radioligand, **J. Neurosci.**, 4:1021.
116a. Vigna, S. R., Giraud, A. S., Reeve, J. R. Jr., and Walsh, J.H., 1988, Biological activity of oxidized and reduced io-

117. Vigna, S. R., Mantyh, C. R., Giraud, A. S., Soll, A. H., Walsh, J.H., and Mantyh, P.W., 1987, Localization of specific binding sites for bombesin in the canine gastrointestinal tract, **Gastroenterology**, 93:1287.
118. Wiesenfeld-Hallin, Z., Hokfelt, T., Lundberg, J. M., Forssmann, W.G., Reinecke, M., Tschopp, F.A., and Fischer, J.A., 1984, Immunoreactive calcitonin gene-related peptide and substance P coexist in sensory neurons to the spinal cord and interact in spinal behavioral responses of the rat, **Neurosci. Letts.**, 199:2.
119. Young, W.S. III, and Kuhar, M.J., 1979, A new method for receptor autoadiography: [^{3}H]-opioid receptors in rat brain, **Brain Res.**, 179:255.
120. Zarbin, M. A., Innis, R. B., Wamsley, J. K., Snyder, S.H., and Kuhar, M.J., 1983, Autoradiographic localization of cholecystokinin receptors in rodent brain, **J. Neurosci.**, 3:877.

ABBREVIATIONS

A artery
bas basal
BBS bombesin
bv blood vessel (arteriole or venule)
CCK cholecystokinin
CGRP calcitonin gene-related peptide-α
CM circular muscle of the muscularis externa
ct connective tissue
g germinal layer of a lymph nodule
GAL galanin
GLU glutamate
H-E hematoxylin and eosin
LM longitudinal muscle of the muscularis externa
lum luminal
Lym lymph nodule
Mc mucosa
Mc - b mucosa, basal aspect
Mc - l mucosa, luminal aspect
MM muscularis mucosa
MP myenteric plexus
musc muscle
p proliferative zone of a lymph nodule
SK substance K, neurokinin A, α-neurokinin, neuromedin L
SOM somatostatin
SP substance P
SubMc submucosa
U.C. ulcerative colitis
VIP vasoactive intestinal peptide

SENSORY DENERVATION WITH CAPSAICIN REDUCES THE LIVER COLLAGEN DEPOSITION INDUCED BY COMMON BILE DUCT OBSTRUCTION IN RATS

Alessandro Casini[1], Irmgard Th. Lippe[2], Stefano Evangelista[3], Pierangelo Geppetti[4], Paolo Santicioli[3], Carmelo Urso[5], Carlo Alberto Maggi[3], and Calogero Surrenti[1]

Department of Pathophysiology, Gastroenterology Unit (1), Institute of Internal Medicine and Clinical Pharmacology (4) Institute of Pathology (5), University of Florence and Department of Pharmacology, Menarini Pharmaceuticals (3), Florence, Italy. Department of Experimental and Clinical Pharmacology, University of Graz (2), Graz, Austria

INTRODUCTION

Hepatic fibrosis indicates an increased deposition of the normal components of connective tissue in the liver, associated with an altered balance between synthesis and degradation of collagen (Anthony et al., 1978; Hann et al., 1985). This widespread alteration of the liver represents an important stage in the progression of chronic liver disease to cirrhosis (Conn, 1982; Rappaport et al., 1983).

Evidence has been accumulated indicating that capsaicin selectively affects a certain class of primary sensory neurons (Holzer, 1988; Maggi and Meli, 1988). In particular, systemic administration of large doses of capsaicin to intact animals has been repeatedly shown (Jancsó et al., 1985; Jancsó et al., 1987; Ritter and Dinh, 1988) to produce long lasting degeneration and functional blockade of central and peripheral endings of a subset of primary sensory neurons. Systemic capsaicin desensitization produces a long lasting depletion of the sensory neuropeptides of the peripheral nervous system (Holzer, 1988; Maggi and Meli, 1988, Maggi et al., 1987b; Geppetti et al., 1987). An important feature of the capsaicin-sensitive sensory neurons is their ability to release neuropeptides from peripheral terminals, thereby producing an efferent' function (Szolcsányi 1984; Maggi and Meli, 1988).

Recently, the possible influence of some neuropeptides released by peripheral terminals of primary sensory neurons, on the metabolism and proliferation of different connective tissue cells has been emphasized. Substance P (SP) and neurokinin A (NKA) can stimulate cell growth of cultured arterial smooth

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

muscle cells and human skin fibroblasts (Nilsson et al., 1985); CGRP increases survival of musculocutaneous critical flaps in rats in vivo (Kjartansson and Dalsgaard, 1987; Kjartansson et al., 1987); sensory denervation by capsaicin induces a higher incidence of spontaneous skin lesions in rats (Maggi et al., 1987a); a depletion of neuropeptides during wound healing in rat skin has been reported (Senapati et al., 1986). Although these effects may be considered finalistically directed to promote tissue repair at the site of injury, one can also hypothesize their participation in the development and maintenance of certain pathological conditions. Primary afferent fibers containing SP and CGRP have been recently demonstrated in the biliary pathway and liver of guinea-pig and in the liver of the rat (Sasaky et al., 1984; Sasaky et al., 1986; Goheler et al., 1988). All these observations, therefore, suggest that primary afferent neurons might play a role in the development of hepatic fibrosis.

In the present paper, we investigated whether capsaicin-sensitive sensory neurons might participate in the development of liver fibrosis. For this purpose, the expression of hepatic fibrosis induced by common bile duct obstruction (Kountouras et al., 1984) has been evaluated in both vehicle- and capsaicin-pretreated rats.

MATERIALS AND METHODS

Animals

Strain Sprague-Dawley Nossan rats (8 weeks old) were used. The rats were housed under temperature, humidity and illumination controlled conditions. The weight of animals at the time of surgery was 200-300 g. Animals were deprived of food 20 hours before the surgery but allowed free access to tap water.

Sensory Denervation

Four days before surgery, the rats received s.c. capsaicin (125 mg/kg) or an equal volume of the vehicle (10% ethanol, 10% Tween 80, 80% saline, vol/vol/vol). In order to check the effectiveness of the treatment, a drop of 0.33 mM solution of capsaicin was instilled into one eye of the rats and the protective wiping movements were counted (Gamse, 1982). This test was carried out one day before the bile duct obstruction and before the collection of the samples. Vehicle-treated rats responded instantly with wipings while capsaicin-treated rats did not react.

Surgery

Rats were anesthetized with ether. A laparotomy was performed, common bile duct was ligated with a double silk thread and cut by means of an Olympus electrosurgical cutter. Rats which underwent exactly the same surgical protocol except the ligation of common bile duct (sham operation) served as controls. Two days after surgery, we observed a dark-brownish urine, indicating a successful ligation. The mortality rate after this procedure was not more than 8%. Animals were kept in cages of 2 and received standard laboratory rat chow. Food intake, liver weight, serum albumin and liver total protein content were

also evaluated to judge the overall nutritional status of all the studied animals.

Rats were sacrificed 2, 4, 6 and 8 weeks after surgery under CO_2 anesthesia. The abdomen was quickly opened, the liver excised and weighed. Urinary bladder and the common bile duct were also removed and collected for radioimmunoassay (RIA) evaluation of sensory denervation. All these samples were immediately processed for use in the following procedures.

The final groups included 4 vehicle- and 4 capsaicin-treated rats for weeks 2, 4 and 6 after surgery, while 6 capsaicin-treated and 4 control animals were used after 8 weeks. Sham operated rats were killed at weeks 6 and 8. Two vehicle- and 2 capsaicin-treated rats were killed the day of surgery as normal control animals (week 0).

Histological Studies

Specimens were obtained from the right and left lobe of each rat liver. Samples were fixed in buffered formalin and embedded in paraffin. Five micron sections were stained with hematoxylineosin and van Gieson. The amount of collagen was semiquantitatively graded as follows:

0: Normal liver.
1: Mild enlargement of portal tracts. Small stellate or linear collagenous extensions in the lobules.
2: Definite enlargement of portal tracts with thin incomplete septa which do not connect with each other. Widespread intralobular collagen deposition.
3: Formation of complete septa which connect with each other dividing the parenchyma into separate fragments. Diffuse alterations of the parenchyma architecture.

Three sections from the right and left lobe of the liver were evaluated for each animal and the average value was used for the final collagen score of that liver. Samples were blindly examined by three different liver pathologists. When opinions diverged, the score was performed after assessment and discussion of the changes. The average score was then calculated for each group of rats.

Hydroxyproline Determination

Hydroxyproline (Hyp) in the liver samples stored in liquid nitrogen, not submitted to histology, was measured by a modification of a previously described method (Jamall et al., 1981). Briefly, liver specimens were homogenized, extensively dialysed against three changes of PBS for 48 h at 4°C and then precipitated with 40% ammonium sulphate (v/v) for 3 h at 0°C. Precipitates, collected by centrifugation for 1 h at 4°C, were dialysed against three changes of bidistilled water and then lyophilized. Samples were submitted to pepsin digestion (cristalline pepsin, 2798 IU/mg; Worthington Biochemical Co., Freehold NJ, USA) and precipitation of the resistant triple-helical parts of collagen with perchloric acid according to Jalkanen's method (Jalkanen et al., 1980). The resulting precipitates were then hydrolyzed with a 6N HCl solution overnight at 110°C. Hyp content of the samples was assayed by a high-performance liquid chromatographic (HPLC) procedure as described elsewhere (Casini et al., 1982) and expressed as μmols/g of fresh liver tissue.

Substance P-like Immunoreactivity Assay

Substance P-like immunoreactivity (SP-LI) was determined in the rat urinary bladder to check the capsaicin-induced sensory denervation (Maggi et al., 1987b) and in the common bile duct to evaluate the sensory denervation of the liver (Goheler et al., 1988). Urinary bladders and common bile ducts were homogenized in 95°C 2 N acetic acid (1.10; w/v) and kept in a boiling water bath for 10 min. After centrifugation at 20,000 x g for 30 min the supernatant was freeze-dried and stored at -20°C. SP-LI was measured as reported previously (Geppetti et al., 1987). Briefly, SP standard (Peninsula, CA) or samples were incubated overnight at 4°C with anti-SP antiserum (gift of Dr. P. Pradelles, SPILERI, CEN-Saclay, Gyf-sur-Yvette, France) with ^{125}I - Bolton and Hunter conjugated SP (Amersham, UK). Bound from free antigen was separated by the double antibody precipitation. Sensitivity of the assay was 1.1 fmol/tube. Cross-reactivities were 1% with NKA, 0.5 with neurokinin B and less than 0.1% with physalaemin and eledoisin.

Statistical Analysis

Hyp and SP-LI data were analysed using analysis of variance. A linear regression was performed on Hyp values and time after bile duct ligation. The correlation between hyp values and the histology score was evaluated using a Spearman's Rank correlation non-parametric test.

RESULTS

SP-LI levels in vehicle-treated animals were 3.12 ± 0.65 and 3.78 ± 0.7 pmol/g in the urinary bladder and in the biliary tract, respectively. Two weeks after capsaicin pretreatment SP-LI was reduced by 84% in the urinary bladder, while in the biliary tract a 56% reduction was observed. In common bile duct samples obtained at 4, 6 and 8 weeks from the beginning of the experiment SP-LI reduction constanly varied between 50-60%. In the urinary bladder only at the eighth week a partial recovery of peptide level was found, SP-LI being reduced by 53% at this time (tab. 1.).

Table 1. Substance P-like immunoreactivity (SP-LI, μmol/g) in urinary bladder and biliary tract of vehicle- and capsaicin-pretreated rats.

Weeks (a)	Urinary Bladder		Biliary Tract	
	Vehicle	Capsaicin (b)	Vehicle	Capsaicin (b)
2	3.12 ± 0.65	0.51 ± 0.04*	3.78 ± 0.71	1.65 ± 0.40
4	2.96 ± 0.85	0.39 ± 0.05*	3.69 ± 0.77	1.88 ± 0.48*
6	2.58 ± 0.64	0.54 ± 0.16*	4.59 ± 0.81	2.18 ± 0.32*
8	2.91 ± 0.22	1.39 ± 0.23**	4.76 ± 0.92	1.93 ± 0.23*

a: weeks after common bile duct ligation
b: 125 mg/kg of s.c. capsaicin were administered
*: P<0.01
**: P<0.05 Mean values ± SEM

Capsaicin treatment did not significantly affect either the rat food intake and body weight or the serum albumin and liver total protein concentration (tab. 2).

Table 2. Food intake, body weight, serum albumin and liver total protein of vehicle- and capsaicin-treated rats 4 and 8 weeks after surgery.

Ws (a)	Food Intake (g/24 h)	Body Weight (g)	Serum Albumin (g/100 ml)	Liver Prot (μg/mg)
4 Vh	12.9 ± 1.4	357.2 ± 5.8	2.23 ± 0.04	59.9 ± 6.7
4 Cp	16.2 ± 2.3	345.6 ± 12.3	2.48 ± 0.19	55.8 ± 5.5
8 Vh	14.3 ± 2.8	436.8 ± 13.3	2.50 ± 0.21	67.7 ± 7.2
8 Cp	16.7 ± 2.1	425.5 ± 15.6	2.56 ± 0.13	59.9 ± 6.7

a: weeks after common bile duct ligation. Mean values ± SEM.
Vh = vehicle treated
Cp = capsaicin treated

Liver Histopathology

Common bile duct ligation determined a marked liver fibrosis in all the rats. A time-dependent collagen accumulation after the bile duct ligation was observed. The histological changes were more pronounced in vehicle-treated rats in comparison with the group of rats treated with capsaicin. The group of animals sacrificed after 2 weeks exhibited mild or sometimes definite enlargement of portal tracts associated with proliferation of bile duct epithelial cells. The histological alterations were not significantly different, at this time, between the capsaicin- and the vehicle-treated animals. Remarkable differences between the two groups became evident four weeks after the common bile duct ligation. In fact, vehicle-treated rats showed evident portal fibrosis with enlargement of portal tracts associated with widespread intralobular collagen deposition which was even more pronounced after 6 weeks. Diffuse alterations of liver parenchyma structure occurred after 8 weeks: complete septa, connecting with each other, divided the parenchyma into separate fragments and sometimes a nodular regeneration was evident. In capsaicin-treated animals collagen deposition in the liver was less evident than in the vehicle-treated group. However, even in this group a time-dependent development of liver fibrosis was observed. None of the capsaicin-treated rats exhibited formation of complete connective septa, altering the parenchyma architecture, and no nodular regeneration was present after 8 weeks. In sham operated rats, either capsaicin- or vehicle-treated, no histological change was seen in comparison to normal rats.

Tab. 3 shows the histological scores of all the animals studied. Increasing score mean values were evident in the vehicle-treated group in a time dependent manner, while the capsaicin-treated rats had only a slightly increasing value of the histological scores considering the time after bile duct obstruction.

Table 3. Liver histological scores of vehicle- and capsaicin-treated rats

Weeks (a)	Vehicle	Capsaicin
2	1.00 ± 0	1.25 ± 0.25
4	1.75 ± 0.25	1.00 ± 0
6	2.00 ± 0.41	1.25 ± 0.25
8	2.75 ± 0.25	1.33 ± 0.26

a: weeks after common bile duct ligation. Mean values ± SEM

Hydroxyproline Quantitation

The Hyp content of the liver, considered as an index of total collagen deposition, enhanced after common bile duct ligation in a time-dependent manner in both groups of animals. Collagen accumulation was much more evident in vehicle- than in capsaicin-treated rats, and a linear correlation between Hyp values and the time of experiments was recovered (Fig. 1). After two weeks, no significant differences were detected between the two groups. Significantly different values of liver Hyp concentrations were detected after 4 weeks as well as after 6 and 8 weeks. Eight weeks after common bile duct obstruction, vehicle-treated rats showed a 7 fold increase of their liver collagen content in comparison with sham operated animals, while this enhancement was of about 3.5 fold in capsaicin-treated rats. Liver Hyp values of sham operated animals overlapped those of normal rats.

The close association between rat liver Hyp content and the histology score is shown in Fig. 2.

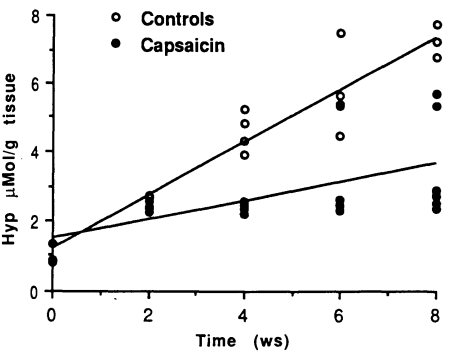


Fig. 1. Relationship between hydroxyproline content of the liver (conc./g of fresh tissue) and time after common bile duct ligation. Values of all the rats studied are reported. White points = Vehicle-treated animals ($r=0.951$; $p<0.001$). Black points = capsaicin-treated animals ($r=0.595$; $p<0.01$). Differences between slope of the curves were statistically significant: $p<0.01$.

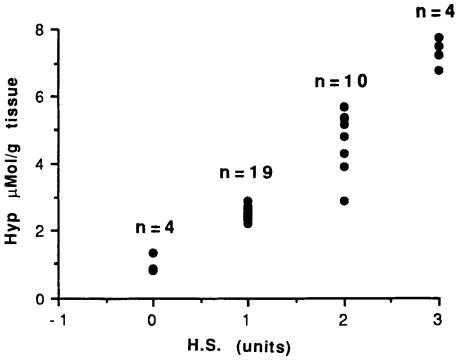


Fig. 2. Correlation between liver hydroxyproline concentration and the histology score. Correlation coefficient = 0.89;$p<0.01$.

DISCUSSION

The influence of capsaicin-sensitive sensory neurons on the development of hepatic fibrosis was evaluated in an experimental model of secondary biliary fibrosis obtained by common bile duct ligation and scission in rats. A progressive deposition of hepatic collagen after bile duct obstruction was evident as demonstrated by the constant increase of hydroxyproline content in the liver of the vehicle-treated rats, which showed values 7 fold higher than sham operated rats 8 weeks after the bile duct obstruction. A liver collagen accumulation was also observed in capsaicin-treated rats, however, in this group of animals the extent of fibrotic changes and the time dependent collagen deposition were significantly reduced when compared with those of vehicle-treated rats. Analogous differences in the extent of hepatic fibrosis between vehicle-treated and capsaicin-treated rats were also observed at the histopathological examination. In fact, the portal and periportal accumulation of extracellular matrix associated with proliferation of ductular structures was clearly more pronounced in the vehicle-treated rats. Capsaicin administration both in newborn and adult animals is known to deplete neuropeptide content of primary sensory neurons (Holzer, 1988; Maggi and Meli, 1988). Reduction of SP-LI by approximately 60%, observed in the biliary tract up to 8 weeks after capsaicin treatment, suggests a long-lasting loss of SP-containing primary afferents at this level. Therefore, these observations raise the possibility that peripheral release of neuropeptides takes place during the development of hepatic fibrosis after common bile duct obstruction and influences, in a positive way, the proliferation of collagen-producing cells.

References

Anthony, P.P., Ishak, K.G., Nayak, N.C., 1978, The morphology of cirrhosis, **J. Clin. Pathol.**, 395:414.

Buck, S.H. Burks, T.F., 1986, The neuropharmacology of capsaicin. Review of some recent observations, **Pharmac. Rev.**, 38:179.

Casini, A., Martini, F., Nieri, S., Ramarli, D., Franconi, F., Surrenti C., 1982, High performance liquid chromatographic

determination of hydroxyprolin after derivatization with 4-dimethyl-aminoazobenzene-4'-sulphonyl chloride, **J. Chromatogr.**, 249:187.
Conn, O.H., 1982, Cirrhosis, in: "Diseases of the Liver", Lippincott, Philadelphia.
Gamse, R., 1982, Capsaicin and nociception in the rat and mouse, **Naunyn Schiedeberg's Arch. Pharmac.**, 320:205.
Geppetti, P., Maggi, C.A., Zecchi-Orlandini, S., Santicoli P., Meli, A., Frilli, S., Spillantini, M.G., and Amenta, F., 1987, Substance P-like immunoreactivity in capsaicin-sensitive structures of the rat thymus, **Regul. Peptides**, 18:321.
Goheler, L.E., Sternini, C., Brecha, N.C., 1988, Calcitonin gene-related peptide immunoreactivity in the biliary pathway and liver of the guinea-pig: distribution and colocalization with substance P, **Cell Tissue Res.**, 253:145.
Hahn, E.G., Schuppan, D., 1985, Ethanol and fibrogenesis in the liver, in: "Alcohol Related Diseases in Gastroenterology", H.K. Seitz and B. Kommerell, eds., Springer-Verlag, Berlin and Heidelberg.
Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve ending: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, **Neuroscience**, 24:739.
Jalkanen, M., Tammi, M., Vihersaari, T., 1980, A rapid assay to measure collagen synthesis in cell cultures, **J. Biochem. Biophys. Methods**, 2:331.
Jamall, I.S., Finelli, V.M., Que Hee, S.S., 1981, A sample method to determine nanogram levels of 4-hydroxyproline in biological tissues, **Anal. Biochem.**, 112:70.
Jancsó, G., Kiraly, E., Such, G., Joo, F., Nagy, A., 1987, Neurotoxic effect of capsaicin in mammals, **Acta Physiol. Hung.**, 69:295.
Jancsó, G., Kiraly, E., Joo., F., Such, G., Nagy, A., 1985, Selective degeneration by capsaicin of a subpopulation of primary sensory neurons in adult rats, **Neurosci. Letters**, 59:209.
Kjartansson, J., Dalsgaard, C.J., 1987, Calcitonin gene-related peptide increases survival of a musculocutaneous critical flap in the rat, **Eur. J. Pharmacol.**, 142:355.
Kjartansson, J., Dalsgaard, C.J., 1987, Decreased survival of experimental critical flaps in rats after sensory denervation with capsaicin, **Plast. Reconstr. Surg.**, 79:218.
Kountouras, J., Billing, B.H., Scheuer, P.J., 1984, Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat, **Br. J. Exp. Path.**, 64:305.
Maggi, C.A., Meli, A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, **Gen. Pharmac.**, 19:1.
Maggi, C.A., Borsini, F., Santicioli, P., Geppetti, P., Abelli, L., Evangelista, S., Manzini, S., Theodorsson-Norheim, E., Somma, V., Amenta, F., Bacciarelli C., and Meli, A., 1987a, Cutaneous lesions in capsaicin-pretreated rats. A trophic role of capsaicin-sensitive afferents? **Naunyn Schmiedeberg's Arch. Pharmacol.**, 336:538.
Maggi, C.A., Santicioli, P., Geppetti, P., Furio, M., Frilli, S., Conte, B., Fanciullacci, M., Giuliani, S., and Meli, A., 1987b, The contribution of capsaicin-sensitive innervation to activation of the spinal vescico-vescical reflex in rats: relationship between substance P levels in the urinary bladder and the sensory-efferent function of capsaicin-sensitive sensory neurons, **Brain Res.**, 415:1.
Nilsson, J., Von Euler, A.M., Dalsgaard, C.J., 1985, Stimulation

of connective tissue cell growth by substance P and substance K, **Nature**, 315:61.
Rappaport, A.M., Mac Phee P.J., Fischer, M.M., Phillips, M.J., 1983, The scarring of the liver acini (cirrhosis), **Virchows Arch.**, 402:107.
Ritter, S., and Dinh, T.T., 1988, Capsaicin-induced neuronal degeneration: silver impregnation of cell bodies, axons and terminals in the central nervous system of the adult rat, **J. Comp. Neurol.**, 271:79.
Sasaki, Y., Kamada, T., Hayashi, N, 1984, Immunohistochemical distribution of glucagon, substance P and vasoactive intestinal polipeptide in hepatic vasculature of the rat, **Hepatology**, 6: 1184.
Sasaki, Y., Hayashi, N., Kasahara, A., 1986, Calcitonin gene-related peptide in the hepatic and splanchnic vascular systems of the rat, **Hepatology**, 6:676.
Senapati, A., Anand, P., McGregor, G..P., Ghatei, M.A., Thompson, R.P.H., Bloom, S.R., 1986, Depletion of neuropeptides during wound healing in rat skin, **Neurosci. Letters**, 71:101.
Szolcsányi J., 1984, Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function, in: "Antidromic Vasodilatation and Neurogenic Inflammation", L.A. Chahl, J. Szolcsányi and F. Lembeck, eds., Akademiai Kiado, Budapest.

AUTONOMIC NEUROPATHY IN LIVER CIRRHOSIS: PREVALENCE AND ASSOCIATION WITH CLINICAL AND LABORATORY FEATURES

S. Giancane, M. Bernardi, F. Trevisani,,
Paola Emanuela D'Intino, Maria Rosa Tame,
A. Gasbarrini, A. Rimondi, and G. Gasbarrini

Istituto di Patologia Medica
University of Bologna, Italy

INTRODUCTION

An impaired autonomic nervous system activity has been described in liver cirrhosis (1,2). Namely, an enhanced sympathoadrenergic activity has been found in patients with ascites, who also showed an impaired cardiovascular responsiveness to such an adrenergic hypertone (2). Moreover, in these patients the cardiovascular response to the administration of exogenous norepinephrine (1), as well as maneuvers stimulating the adrenergic activity (3), was impaired. Recently, abnormalities of vagal activity have also been described and related to the alcoholic etiology of liver disease (4). It has been suggested that vagal neuropathy may result in an inappropriate, non osmotic secretion of antidiuretic hormone, promoting the appearance of hyponatremia (4), an abnormality which is frequently detectable in advanced liver failure.

Taking into account the potential clinical consequences of autonomic neuropathy, we assessed the prevalence of such an abnormality and its relationships with clinical and laboratory features.

PATIENTS AND METHODS

Forty patients (age range 23-80 yr), 29 male and 11 female, with biopsy proved liver cirrhosis, were studied. The cirrhosis was alcoholic in 20 cases and non-alcoholic (postnecrotic or, in 2 cases, cryptogenic) in the remainder. Patients with diabetes, recent gastrointestinal bleeding, cardiac or respiratory diseases and acute infections were excluded. In the 4 days preceding the study, the administration of drugs other than diuretics known to influence the result of the tests was strictly avoided. Diuretic therapy was considered as one of the parameters under study.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

Clinical and Laboratory Parameters

The following parameters were considered: age, sex, Pugh's class (5), presence/absence of ascites, hepatic encephalopathy (II-III degree), hypoalbuminemia (<3.5 g/dl), jaundice (>2 mg/dl), diuretic therapy, reduced glomerular filtration rate (GFR ≤ 50 ml/min), hyponatremia (<132 mmol/dl), muscle wasting, heart rate >80 bpm, mean arterial pressure (diastolic + 1/3 pulse pressure) <90 mmHg and symptoms of peripheral neuropathy.

Evaluation of the Function of Autonomic Nervous System

Autonomic activity was evaluated by tests commonly used in the study of diabetic autonomic neuropathy (6). Vagal function was explored by the "Lying to Standing" and "Deep Breathing" tests. Sympathoadrenergic activity was assessed by the "Sustained Handgrip" test and by the blood pressure response to the assumption of upright posture. The results of each test were classified as normal, borderline and pathological, according to the criteria accepted for evaluation of diabetic neuropathy (6-10). All tests were performed in a quiet and warm room, at the same clock-time. The patients were kept recumbent for 20 minutes before every test. Arterial pressure was determined using Dynamap (EME, Brighton, UK).

LYING TO STANDING TEST (LST) consists of measuring on electrocardiographic tracing the R-R′ interval at the 15th and 30th beat after the assumption of upright posture. A 30th/15th ratio greater than 1.03 was considered normal, borderline from 1.03 to 1.00 and pathological <1.00. The test evaluates vagal activity following the first phase of adrenergic activation after standing (6).

DEEP BREATHING TEST (DBT): the heart rate was measured continuously using R-R′ interval on ECG, whilst the subject breathes deeply (breath frequency of 6-8 times per minute) for one minute. The mean of the differences between the maximum heart rate in inspiration and the minimum in expiration for every breathing cycle was calculated. A difference of 10 or less beats per minute is considered pathological, from 11 to 15 is borderline and >15 normal. Since variations in the heart rate during deep breathing are dependent on an intact vagal function, DBT is one of the most sensitive tests to show a compromised parasympathetic activity (7).

SUSTAINED HANDGRIP TEST (SHT): handgrip normally causes an increase in diastolic arterial pressure. The subject squeezed a dynamometer with the dominant hand for 3 minutes at 1/3 of his maximum strength. An increase in diastolic arterial pressure of 10 mmHg or less is pathological, from 11 to 15 borderline and >15 normal. This test evaluates sympathetic control of the cardiovascular system (8).

BLOOD PRESSURE RESPONSE TO STANDING (BPR): blood pressure was measured during recumbency and every minute, for 3 minutes, after standing. A decrease of systolic arterial pressure of 30 mmHg or more was considered pathological, from 20 to 29 borderline and <20 normal (9). The test checks the integrity of sympathetic pathways and baroceptors.

Vagal and sympathetic activities were considered as: normal in the presence of both tests normal or one test normal and one borderline; borderline in patients with both tests borderline or one test normal and one pathological; pathological in patients with both tests altered or one test pathological and one borderline.

Statistical Analysis

To assess the statistical significance of the relationships between autonomic neuropathy and clinical and laboratory parameters, test and linear correlations were used.

RESULTS

Vagal function was abnormal in 60% of the patients (30% borderline) while sympathoadrenergic function was abnormal in 50% of the cases (45% borderline). The results obtained by performing the single tests are reported in the Table.

Table 1

Prevalence of alterations in the tests exploring the autonomic function

	NORMAL	BORDERLINE	PATHOLOGICAL
LST	22 pt. (55%)	8 pt. (20%)	10 pt. (25%)
DBT	11 pt. (27.5%)	11 pt (27.5%)	18 pt. (45%)
SHT	12 pt. (30%)	9 pt. (22.5%)	19 pt. (47.5%)
BPR	37 pt. (92.5%)	2 pt. (5%)	1 pt. (2.5%)

Pt = patient(s). LST = lying-to-standing test; DBT = deep breathing test; SHT = sustained hand grip test; BPR = blood pressure response to the change of posture.

The altered vagal function was associated with age above 60 ($p<0.05$) and reduced GFR ($p<0.05$); no association was found with the alcoholic etiology. The altered sympathoadrenergic function was associated with reduced GFR ($p<0.05$) and hepatic encephalopathy ($p<0.05$).

Taking into account the single tests, an abnormal LST was associated with age above 60 ($p<0.05$), low GFR ($p<0.05$) and hyponatremia ($p<0.05$). The alteration of DBT was associated with age above 60 ($p<0.05$), reduced GFR ($p<0.005$) and ascites ($p<0.05$).

No associations were found between alterations of clinical and laboratory features and those of the sympathoadrenergic tests. Nevertheless, the extent of blood pressure reduction during the assumption of upright posture was inversely related to GFR ($r=0.32$; $p<0.05$) and directly to serum bilirubin concentration ($r=0.33$; $p<0.05$).

Only one patient, showing a generalized alteration of autonomic function tests, had a clinical sign of autonomic neuropathy (lightheadedness) on the assumption of upright posture.

Only one patient presented clinical evidence of peripheral sensitive neuropathy. In this subject the results of all tests were normal.

DISCUSSION

The prevalence of alterations of tests evaluating autonomic function was surprisingly high in cirrhosis, exceeding that reported in diabetic patients (7). However, it is worth noting that only one patient had a clinical manifestation of autonomic neuropathy, suggesting that these alterations remain subclinical features in a large majority of cirrhotics.

The neurotoxic effect of alcohol abuse has been definitely recognized (1). Nevertheless, our results seem to exclude that the alcoholic etiology of liver disease played a relevant role in determining the observed alterations of autonomic function. Therefore, it is conceivable that the presence of cirrhosis by itself was the main determinant, overcoming a possible alcohol-dependent neurological damage. An alternative explanation may be that the tests commonly utilized to detect autonomic neuropathy are not entirely reliable when they are performed in cirrhotic patients, since they are influenced by factors other than nerve degeneration. Such an assumption can find support in the comparison of our results with those reported from diabetic patients (10). In fact, contrary to what occurs in diabetics, test alterations were never associated with somatic neuropathy. Moreover, the development of diabetic neuropathy depends on the duration of the disease (7), overcoming the age-dependent impairment of baroreceptor sensitivity (12). In contrast, a clear association between test abnormalities and advanced age was disclosed in our patients.

It should be pointed out that the tests exploring the autonomic function are based on hemodynamic adaptations to various stimulations. In cirrhosis, their alteration might represent an inadequate response by the target organs. Such an assumption agrees with the evidence of a reduced cardiovascular adaptation to exercise (13) and postural change (3, 14) in the face of a normal and even enhanced sympathoadrenergic response.

Other results of our study may further corroborate the hypothesis of a target organ failure. The impairment of GFR was the single parameter most frequently associated with the alterations of autonomic function tests. Renal hypoperfusion is a common feature of advanced cirrhosis and is related to abnormal systemic hemodynamics leading to effective hypovolemia (15). In this context, cardiovascular adaptations to physiological stimuli are severely impaired. Therefore, it is conceivable that the association between reduced GFR and presence of abnormal autonomic function tests simply reflected these two running-in-parallel phenomena. According to this interpretation, the association between hyponatremia and abnormal vagal tests may also be due to effective hypovolemia, leading to both GFR reduction and ADH hypersecretion, rather than to the reduced vagal input to hypothalamic nuclei, as suggested by others (4). This formulation can

also explain the observed correlation between the severity of jaundice and the extent of blood pressure reduction upon the assumption of upright posture. In fact, a reduced vascular reactivity to vasoconstrictors has been clearly demonstrated in experimental models of extrahepatic cholestasis (16).

Two further aspects of our study deserve a comment. The association between altered sympathoadrenergic activity and hepatic encephalopathy shown by our patients may be the result of a common pathogenetic background. In fact, weak neurotransmitter hyperproduction and release from nerve endings has been claimed to be involved in the occurrence of both hepatic encephalopathy (17) and systemic hemodynamic disturbances of advanced cirrhosis (3, 17). Finally, it should be noted that the presence of ascites was only associated with alterations in DBT. This finding likely represents the effect of peritoneal effusion on cardiac pre-load and diaphragmatic motility which, in turn, influences the entity of heart rate variations during deep breathing.

In conclusion, the tests commonly used to explore the function of the autonomic nervous system are frequently altered in cirrhosis. Their alterations are likely due to an impaired cardiovascular responsiveness to parasympathetic and sympathoadrenergic afferents rather than to the presence of a true autonomic neuropathy.

References

1. M. R. Lunzer, K.K. Manghani, S.P. Newman, S.P.V. Sherlock, A.G. Bernard, J. Ginsburg, 1975, Impaired cardiovascular responsiveness in liver disease, **Lancet**, 2:382.
2. M. Bernardi, F. Trevisani, R. De Palma, A. Ligabue, F. Capani, M. Baraldini, G. Gasbarrini, 1987, Chronobiological evaluation of sympathoadrenergic function in cirrhosis, **Gastroenterology**, 93:1176.
3. M. Bernardi, F. Trevisani, C. Santini, G. Zoli, M. Baraldini, A. Ligabue, G. Gasbarrini, 1983, Plasma norepinephrine, weak neurotransmitters and renin activity during active tilting in liver cirrhosis: relationship with cardiovascular homeostasis and renal function, **Hepatology**, 3:56.
4. G. Decaux, P. Cauchie, A. Soupart, M. Kruger, F. Delwiche, 1986, Role of vagal neuropathy in the hyponatremia of alcoholic cirrhosis, **Br. Med. J.**, 293:1534.
5. R. N. H. Pugh, I.M. Murray-Lyon, J.L. Dawson, M.C. Pietroni, R. Williams, 1973, Transection of the oesophagus for bleeding oesophageal varices, **Br. J. Surg.**, 60:646.
6. D. J. Ewing, I.W. Campbell, A. Murray, J.M.M. Neilson, B.F. Clarke, 1978, Immediate heart-rate response to standing: simple test for autonomic neuropathy in diabetes, **Br. Med. J.**, 1:145.
7. T. Dyrberg, J. Benn, J. Sandahl Cristiansen, J. Hilsted, J. Nerup, 1981, Prevalence of diabetic autonomic neuropathy measured by simple bedside tests, **Diabetologia**, 20:190.
8. D. J. Ewing, J.B. Irving, F. Kerr, J.A.W. Wildsmith, B.F. Clarke, 1974, Cardiovascular responses in sustained handgrip in normal subjects and in patients with diabetes mellitus: a test of autonomic function, **Clinical Science and Molecular Medicine**, 46:295.

9. D. J. Ewing, 1978, Cardiovascular reflexes and autonomic neuropathy, **Clinical Science and Molecular Medicine**, 55:321.
10. D. M. Fraser, I.W. Campbell, D.J. Ewing, A. Murray, J.M.M. Neilson, B.F. Clarke, 1977, Peripheral and autonomic nerve function in newly diagnosed diabetes mellitus, **Diabetes**, 26:546.
11. G. Duncan, R.H. Johnson, D.G. Lambie, E.A. Whiteside, 1980, Evidence of vagal neuropathy in chronic alcoholics, **Lancet**, 2:1053.
12. M. A. Pfeifer, C.R. Weinberg, D. Cook, J.D. Best, A. Reenan, J.B. Halter, 1983, Differential changes of autonomic nervous system function with age in man, **Am. J. Med.**, 75:249.
13. M. Bernardi, A. Rubboli, C. Cancellieri, R. Montanari, A. Ligabue, F. Trevisani, G. Gasbarrini, 1988, Impact of muscle activity on cardiovascular homeostasis in cirrhosis, **J. Hepatol.**, 7 (suppl.1): S8.
14. M. Bernardi, F. Trevisani, C. Santini, A. Ligabue, M. Capelli, G. Gasbarrini, 1982, Impairment of blood pressure control in patients with liver cirrhosis during tilting. Study of adrenergic and renin-angiotensin systems, **Digestion**, 25:124.
15. O. S. Better, R.W. Schrier, 1983, Disturbed volume homeostasis in patients with liver cirrhosis, **Kidney Int.**, 23:303.
16. O. S. Better, 1983, Bile duct ligation: an experimental model of renal disfunction secondary to liver disease, in: "The Kidney in Liver Disease", M. Epstein, ed., II edition, Elsevier Biomedical, New York.
17. E. J. Fisher, R.J. Baldessarini, 1971, False neurotransmitters and hepatic failure, **Lancet**, 2:75.

2-DEOXY-D-GLUCOSE (2-DG)-INDUCED INCREASE IN GASTRIC ACID SECRETION IS IMPAIRED IN CAPSAICIN-PRETREATED RATS

S. Evangelista, P. Santicioli, and C.A. Maggi

Pharmacology Department, Menarini Pharmaceuticals
Via Sette Santi 3, 50131 Florence, Italy

SUMMARY

This study investigated the increase in gastric acid secretion induced by intravenous administration of 2-deoxy-D-glucose (2-DG; 60 mg kg^{-1}), insulin (5 U kg^{-1}) or by electrical stimulation of the vagus nerve (1 mA, 1 ms, 3 Hz) in urethane-anesthetized rats pretreated when newborn with either capsaicin or the vehicle.

The secretory response to 2-DG was substantially reduced in the capsaicin pretreated rats, while those induced by electrical vagal stimulation or insulin were unaffected.

These findings suggest that capsaicin-sensitive fibers are involved in the afferent branch of the reflex response activated by 2-DG to stimulate gastric acid secretion.

INTRODUCTION

Capsaicin is a known selective neurotoxin which has been used as a tool to study the functional role of primary sensory afferent neurones. These neurones have been reported to innervate the stomach and to play important physiological roles such as in motility and protection from ulcer formation of the stomach (see Maggi and Meli, 1988, for review).

Recently some studies have been carried out on their influence on gastric acid secretion (GAS; Table 1).

Thus, intragastric capsaicin enhances acid gastric elimination by afferent nerve stimulation (Lippe et al., 1989). Sensory denervation produced by high systemic dose of capsaicin had shown to influence histamine (Alfoldi et al., 1986; Raybould and Taché, 1989), but not carbachol (Alfoldi et al., 1986) or bethanechol (Raybould and Taché, 1989) induced increase in gastric acid secretion while controversial results have been reported relative to pentagastrin (Alfoldi et al., 1986; Dugani and Glavin, 1986; Raybould and Taché, 1989) increase in GAS.

Sensory Nerves and Neuropeptides in Gastroenterology
Edited by M. Costa *et al.*, Plenum Press, New York, 1991

Table 1. Effect of capsaicin pretreatment on various stimulants inducing increase in gastric acid secretion in urethane-anesthetized rats.

STIMULATION, DOSE AND ROUTE	CAPSAICIN PRETREATMENT	EFFECT OF CAPSAICIN	REFERENCE
Bethanecol 0.5 mg kg^{-1} s.c.	1 mg perivagal (a)	-	Raybould and Taché, 1989
Carbachol 4-160 μg kg^{-1} s.c.	250 mg kg^{-1} s.c. (a)	-	Alfoldi et al., 1986
Histamine 0.5-5 μg kg^{-1} s.c.	250 mg kg^{-1} s.c. (a)	↓	Alfoldi et al., 1986
Histamine 5 mg kg^{-1} s.c.	1 mg perivagal (a)	↓	Raybould and Taché, 1989
Pentagastrin 25-250μg kg^{-1} s.c.	250 mg kg^{-1} s.c.(a)	-	Alfoldi et al., 1986
Pentagastrin 16μg kg h^{-1} i.v.	1 mg perivagal (a)	-	Raybould and Taché, 1989
Pentagastrin 6 μg kg^{-1} s.c.	65 mg kg^{-1} s.c. (a)	↓	Dugani and Glavin, 1986
2-deoxy-D-glucose 60mg kg^{-1} i.v.	50 mg kg^{-1} s.c.(n)	↓	Evangelista et al., 1989
Insulin 5 U kg^{-1} i.v.	50 mg kg^{-1} s.c. (n)	-	This paper
Stomach distension 5ml $6min^{-1}$	1 mg perivagal (a)	↓	Raybould and Taché, 1989
Elect.vagal stim.(1mA,1ms 3Hz)	50 mg kg^{-1} s.c. (n)	-	Evangelista et al., 1989
Elect.vagal stim.(40V,3ms,6Hz)	1 mg perivagal (a)	-	Thieflin et al., 1989

In parentheses a=adult and n=neonatal pretreatment with capsaicin.

When GAS was stimulated by gastric distension, an indirect vago-vagal reflex was activated and sensory denervation induced by capsaicin inhibited the response (Raybould and Tachè, 1988).

In view of these findings, it appeared worthwhile to determine the influence of systemic capsaicin sensory denervation on the increase in GAS induced by a central stimulant 2-deoxy-D-glucose (2-DG), that is considered to act through vagal stimulation (Colin-Jones and Himsworth, 1970; Hirschowitz and Sachs, 1965).

For comparison, parallel experiments were carried out in rats in which GAS was stimulated by insulin administration, which has been shown to depend upon vagal discharge following the hypothalamic detection of low blood glucose levels (Colin-Jones and Himsworth, 1970).

The stimulation of GAS by electrical stimulation of the vagus nerve was also studied to determine if the vagal efferent branch was sensitive to capsaicin.

METHODS

Male and female albino rats, Sprague-Dawley Nossan strain, were housed at constant room temperature (21±1°C), relative humidity (60%) and with 12 h light-dark cycle (light on at 6.00 a.m.). The animals were deprived of food for 20 h before the experiments but allowed free access to tap water.

Capsaicin was administered subcutaneously (50 mg kg^{-1}), under ether anesthesia, on the second day of life. This dose was reported to cause a permanent degeneration of unmyelinated afferent neurones (Maggi and Meli, 1988) and a marked depletion of stomach's calcitonin gene-related peptide-like immunoreactivity (Sternini et al., 1987). Control animals received equal volumes of vehicle (10% ethanol, 10% Tween 80 and 80% saline, vol/vol/

vol). The animals were used two months thereafter. Acid secretion was determined according to Ghosh and Schild (1958) in urethane (1.5 g kg^{-1} s.c.)-anesthetized rats, maintained at 36-37°C by means of a heating lamp. The animals were tracheotomized and polyethylene tubes inserted into the stomach lumen through the esophagus and the duodenum. The stomachs were flushed with 50 ml of warm saline to remove any solid material and thereafter perfused at a rate of 0.8-0.9 ml min^{-1} by means of a peristaltic pump (De Saga, Heidelberg, F.R.G.). A period of 45 min was allowed for equilibration after all surgical procedures had been completed. After collection of two samples, acid secretion was stimulated by intravenous (via the jugular vein) administration of 2-deoxy-D-glucose (Sigma; 60 mg kg^{-1}) or insulin (5 U kg^{-1}), dissolved in saline in a volume of 1 ml kg^{-1} or by electrical stimulation of the vagus nerve. In this latter case, the animals were artificially ventilated by means of a ventilator (Basile, Varese, Italy) for small rodents (60 strokes min^{-1}, 0.8 ml 100 g^{-1} body wt). The vagi were cut bilaterally at cervical level and the peripheral end of the left vagus was placed on a bipolar hook-shaped platinum electrode and stimulated by means of an electronic stimulator (WPI, New Haven, CT, USA) with square wave pulse of 1 ms duration, at 3 Hz and 1 mA.

Acid output was determined at 15 min intervals by titration of the perfusate with 0.005 N NaOH to pH 7 using a digital pH meter (Radiometer, Copenhagen, Denmark). Statistical analysis was performed by means of Student t test for unpaired data.

RESULTS

Mean basal acid output of controls or that from capsaicin pretreated rats did not differ significantly (Figure 1-3).

Intravenous 2-DG (60 mg kg^{-1}) produced a significant increase in GAS as compared to pre-injection values which reached its maximal values between 75-105 min after administration (Figure 1).

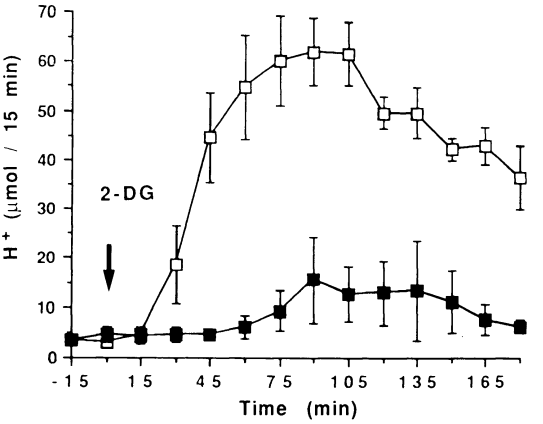


Fig. 1. Effect of 2-deoxy-D-glucose (2-DG, 60 mg kg^{-1} i.v.) on gastric acid secretion (mean ± S.E. of at least 5 animals) in rats pretreated with capsaicin (■) or the vehicle (□). Statistical significance from the control group is shown as * = $P < 0.05$, ** = $P < 0.01$. (Reproduced from Evangelista et al., 1989 by kind permission of Macmillan Press Ltd).

In capsaicin pretreated rats 2-DG elicited only a slight increase in GAS, which was not significantly different as compared to pre-injection values. The increase in GAS induced by 2-DG was significantly different from that in control animals at all time periods (Figure 1).

Increase in GAS induced by insulin (5 U mg kg^{-1} i.v.) reached its maximum at 165 min after the insulin challenge and was unaffected by capsaicin pretreatment (Figure 2).

As shown in Figure 3, the increase in GAS induced by electrical vagal stimulation (1 mA, 1 ms, 3 Hz) reached its maximal values 30 min after the beginning of the stimulation and was not different in capsaicin-pretreated rats as compared to vehicle-treated animals at any time tested (Figure 3).

DISCUSSION

It is known that the central nervous system influences GAS. Electrical or pharmacological stimulation of selective brain areas, in particular the hypothalamus, can induce changes in GAS mediated through the vagus nerve (see Taché, 1987, for review).

Our results show that capsaicin pretreatment abolished 2-DG but not direct vagal stimulation induced increase in GAS. The increase in GAS elicited by 2-DG and insulin has been reported to be due to activation of chemoreceptors in the lateral hypothalamic area (Colin-Jones and Himsworth, 1970) which initiate and sustain a vagally-mediated response (Hirschowitz and Sachs, 1965).

Differences in the mechanisms (peripheral vs central) activating these phenomena can explain the different results obtained between 2-DG and insulin increase in GAS with capsaicin pretreatment.

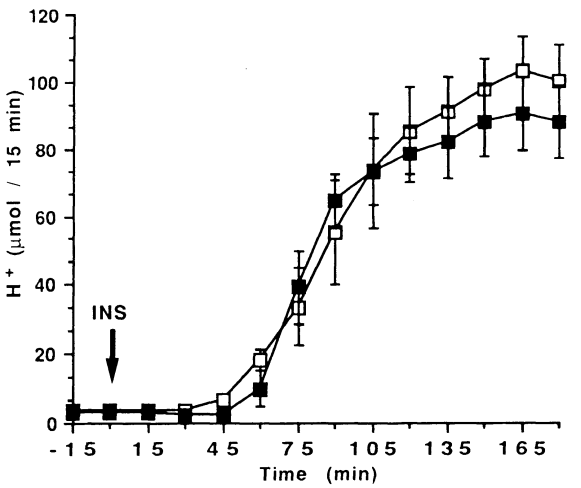


Fig. 2. Effect of insulin (INS: 5 U kg^{-1} i.v.) on gastric acid secretion (mean ± S.E. of at least 5 animals) in rats pretreated with capsaicin (■) or the vehicle (□). Statistical significance from the control group is shown as * = $P < 0.05$, ** = $P < 0.01$.

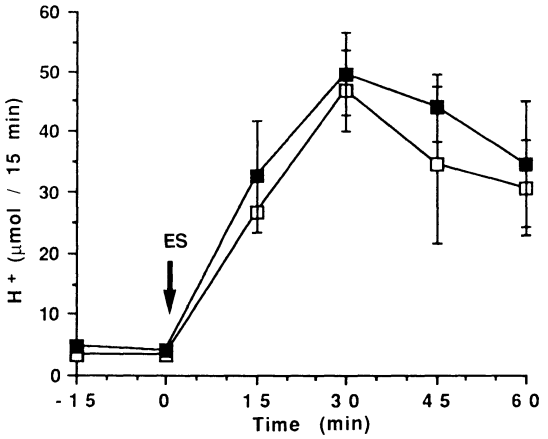


Fig. 3. Effect of electrical stimulation ES) of the vagus nerve on gastric acid secretion (mean ± S.E. of at least 5 animals) in rats pre-treated with capsaicin (■) or the vehicle (□). Statistical significance from the control group is shown as * = $P < 0.05$, ** = $P < 0.01$. (Reproduced from Evangelista et al., 1989 by kind permission of Macmillan Press Ltd).

Hypoglycemia induced by insulin is not only sensed by hypothalamic neurons but also by peripheral glucose receptors through a reflex, involving adrenaline release by the adrenals. These effects are all affected by the ablation of sensory afferents produced by capsaicin (Amann and Lembeck, 1986).

Otherwise systemic hyperglycemia induced by 2-DG, mediated also by adrenalin secretion, is not affected by capsaicin pretreatment (Amann and Lembeck, 1986), indicating that the central components are more important in a 2-DG action.

Neurotoxic damage at hypothalamic level has been described after sensory denervation with capsaicin (Ritter and Dinh, 1988), although a specific effect on the lateral hypothalamic area has not been reported. In conclusion, the present findings indicate that capsaicin-sensitive nerves play a crucial role in the reflex increase in GAS produced by chemically induced glucopenia brought about by 2-DG.

Further studies are needed to assess whether the site of action of capsaicin in preventing the GAS response to 2-DG is central or might also have a peripheral component. In either case, the present data indicate that the efferent vagal control of GAS is unaffected by capsaicin pretreatment.

References

Alfoldi, P., Obal, F., Toth, E., and Hideg, J., 1986, Capsaicin pretreatment reduces the gastric acid secretion elicited by histamine but does not affect the responses to carbachol and pentagastrin, **Eur. J. Pharmacol.**, 123:321.

Amann, R., Lembeck, F., 1986, Capsaicin sensitive afferent neurons from peripheral glucose receptors mediate the insulin-induced increase in adrenaline secretion, **Naunyn-Schmiedeberg's Arch. Pharmacol.**, 334:71.

Colin-Jones, D.G., and Himsworth, R.L., 1970, Location of the chemoceptor controlling gastric acid secretion during hypoglycemia, **J. Physiol.**, 206:397.

Dugani, A.M., and Glavin, G.B., 1986, Capsaicin effects on stress pathology and gastric acid secretion in rats, **Life Sci.**, 39:1531.

Evangelista, S., Santicioli, P., Maggi, C.A., and Meli, A., 1989, Increase in gastric secretion induced by 2-deoxy-D-glucose is impaired in capsaicin pretreated rats, **Br. J. Pharmacol.**, 98:35.

Ghosh, M.N., and Schild, H.O., 1958, Continuous recording of acid secretion in the rat, **Br. J. Pharmacol.**, 13:54.

Hirshowitz, B.I., and Sachs, G., 1965, Vagal gastric secretory stimulation by 2-deoxy-D-glucose, **Am. J. Physiol.**, 209:452.

Lippe, I. Th., Pabst, M.A., and Holzer, P., 1989, Intragastric capsaicin enhances rat gastric acid elimination and mucosal blood flow by afferent nerve stimulation, **Br. J. Pharmacol.**, 96:91.

Maggi, C.A., and Meli, A., 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, **Gen. Pharmacol.**, 19:1.

Raybould, H.E., and Taché, Y., 1989, Capsaicin-sensitive vagal afferent fibers and stimulation of gastric acid secretion in anesthetized rat, **Eur. J. Pharmacol.**, 167:237.

Ritter, S., and Dinh, T.T., 1988, Capsaicin-induced neuronal degeneration: silver impregnation of cell bodies, axons and terminals in the central nervous system of the adult rat, **J. Comp. Neurol.**, 271:79.

Sternini, C., Reeve, J.R., and Brecha, N., 1987, Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, **Gastroenterology**, 93:852.

Taché, Y., 1987, Central nervous system regulation of gastric acid secretion, in: "Physiology of the Gastrointestinal Tract", Vol. 2 (Second edition), L.R. Johnson, ed., Raven Press, New York.

Thieflin, G., Raybould, H.E., Leung, F.W., Taché, Y., and Guth, P.H., 1989, Role of capsaicin-sensitive vagal afferent fibers in increased gastric mucosal blood flow induced by electrical vagal stimulation in the rat, **Gastroenterology**, 96:A509.

INDEX