Reviews of

110 Physiology, Biochemistry and Pharmacology

With 58 Figures and 19 Tables

Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo

ISBN 3-540-18736-7 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-18736-7 Springer-Verlag New York Berlin Heidelberg

Library of Congress-Catalog-Card Number 74-3674

Typesetting: K + V Fotosatz, Beerfelden

Printing and binding: Konrad Triltsch, Würzburg
2127/3130-543210

Contents

Indexed in Current Contents

Rev. Physiol. Biochem. Pharmacol., Vol. 110

The Atrial Natriuretic Factor: Its Physiology and Biochemistry

Edited by
JACQUES GENEST and MARC CANTIN

With the Collaboration of
MADHU B. ANAND-SRIVASTAVA, JEAN R. CUSSON, ANDRÉ DE LÉAN, RAÚL GARCIA, JOLANTA GUTKOWSKA, PAVEL HAMET, OTTO KUCHEL, PIERRE LAROCHELLE, MONA NEMER, ERNESTO L. SCHIFFRIN, PETER W. SCHILLER, GAÉTAN THIBAULT, and JOHANNE TREMBLAY

Contents

From the Clinical Research Institute of Montreal and the Université de Montréal. Clinical Research Institute of Montréal, 110 Pine Avenue West, Montréal, Québec, Canada H2W 1R7

1 Introduction and Historical Data (J. Genest)

In 1981, de Bold et al. (1981) observed a massive and rapid diuresis and natriuresis in recipient rats following the administration of atrial homogenates from other rats. Since this observation, progress has been extraordinary. Few aspects of modern physiology illustrate better the tremendous progress in modern technology for the isolation of proteins, their sequencing and synthesis, the study of structure-activity relationships, the cloning of the cDNA and of the gene, the localization of the latter on the

---

Important note: Measurements of ANF have been expressed in the text as picomoles per liter, which is the same as fentomoles per milliliter. This is obtained by dividing the amount in picograms per milliliter by 3.06. The reverse is 1 pg/ml = 0.324 fmol/ml or 0.324 pmol/l

chromosomal map, the measurement of the mRNA coding for the atrial natriuretic factor (ANF), and the synthesis of analogues (Cantin and Genest 1985; Atlas 1986; Flynn and Davies 1985; Needleman et al. 1985).

This review is the result of the cooperation of the members of the Multidisciplinary Hypertension Research Group (funded by the Medical Research Council of Canada) at the Clinical Research Institute of Montreal, with the help of in-house collaborators: Dr. Mona Nemer from the Laboratory of Molecular Biology of the Eucaryotic Genome; Dr. Ernesto L. Schiffrin, Director of the Laboratory of Experimental Hypertension; Dr. Pierre Larochelle, Director of the Hypertension Clinic and of the Laboratory of Clinical Pharmacology; and Dr. Peter W. Schiller, Director of the Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal.

Kisch (1956) was the first to describe the presence of granules in the atrial cardiocytes of the guinea pig. In their studies of the conductive tissue of rat heart, Bompiani et al. (1959) described the electron-microscopic appearance of very osmophilic, dense bodies close to the Golgi apparatus in those cells. Jamieson and Palade (1964) were the first to demonstrate that these granules are specific and different from other cell organelles such as lysosomes. The granules are adjacent to one and occasionally to both poles of the nucleus of the cardiocytes and are interspersed among the elements of a voluminous Golgi complex. They can number up to 600 per cell in the rat, and their number usually decreases with increasing size of the animal. In the rat, the granules range in diameter from 0.2 to 0.5 μm, while in humans the average diameter is 0.25 μm. Jamieson and Palade (1964) found that these granules were present in all mammalian species studied (bats, hamsters, guinea pigs, mice, rats, rabbits, dogs, pigs, and humans) and observed that they were present exclusively in the atria and not in the ventricles. The finding that the granules are intimately associated with the Golgi complex – which consists mainly of flattened, stacked cisternae which are extensively fenestrated and filled with a fine granular material – suggests that these atrial granules may be formed within the Golgi complex.

Bencosme and Berger (1971) reported that the granules are also present in the ventricular cardiocytes of many nonmammalian vertebrates, such as amphibians, reptiles, and birds. In the 1970s, only about three groups were actively working on these cardiocytes: the first was that of Cantin et al. (1973b) at the Université de Montréal; the second, that of Bencosme and Berger (1971) at Queen's University; and the third, that of Hatt et al. (Marie et al. 1976) in Paris. Cantin's group (Huet and Cantin 1974) was the first one to demonstrate, by ultrastructural chemistry, that the granules can incorporate both [^{3}H]leucine and [^{3}H]fucose, and that protein synthesis takes place in the rough endoplasmic reticulum and in the Golgi complex. This was later confirmed by de Bold and Bencosme (1975). Both Cantin et al. (1973b) and

Table 1. Specific granules in atrial cardiocytes of rats (From Marie et al. 1976)

	Specific granules as a percentage of cell volumes	
	Right atrium	Left atrium
Control group	2.92 ←P<0.01→	1.11
Total water restriction (5 days)	4.14*	1.77
Total Na restriction (3 weeks)	3.63	3.75***
Na loading (1 g% NaCl as drinking fluid – 3 weeks)	1.40*	1.59
Na loading plus DOCA implant (25 mg – 3 weeks)	0.76**	1.19

*p<0.05, **p<0.005, ***p<0.01

de Bold and Bencosme (1975) demonstrated that the granules do not contain any catecholamines. Later, in 1982, Cantin et al. (1982) showed that the atrial granules do not contain any significant amounts of renin and that the reninlike activity is due to cathepsin D.

Hatt's group in Paris (Marie et al. 1976) established that the degree of granulation in atrial cardiocytes varies with the water and sodium intake (Table 1). The group showed the presence of a significantly greater number of granules in the right atrium than in the left atrium in rats. Total water restriction for 5 days significantly increased the number of granules in the right atrium without any significant change in the left atrium. Sodium loading and the administration of deoxycorticosterone acetate (DOCA)+salt significantly decreased the number of granules in the right atrium without any significant change in the left atrium. On the other hand, total sodium restriction for 3 weeks significantly increased the granularity of the left atrium without any significant change in the right atrium. These results of thirst and salt loading were later confirmed by de Bold (1979) and by Thibault et al. (1983a). In 1981, de Bold, with the collaboration of Sonnenberg, Borenstein, and Veress, made the crucial observation that the intravenous administration of rat atrial homogenates to normal rats resulted in rapid, massive, and short-lived diuresis and natriuresis accompanied by a fall in blood pressure (de Bold et al. 1981).

This review will deal exclusively with the natriuretic factor isolated from atria. This does not mean that this is the only natriuretic factor, since there is ample evidence that there are other natriuretic factors involved, such as the cardiac nerves and the sympathetic nervous system, and the inhibitor of Na^+/K^+ adenosine triphosphatase (ATPase) activity or digoxinlike factor.

2 Biochemistry (G. Thibault)

Since the initial discovery by de Bold et al. (1981), the elucidation of the structure of the factor responsible for the natriuretic and diuretic effect of atrial homogenates has been a primordial goal. The initial experiments were, therefore, designed to determine its chemical nature and intracellular localization.

The availability of ANF depends on the tissue source, but also on the method used for its extraction. It rapidly became evident that the natriuretic factor is easily extracted by the homogenization of atrial tissue in an acid solution, such as acetic or hydrochloric acid, since the use of physiological buffers results in a lower yield (de Bold 1982a; de Bold and Flynn 1983; Thibault et al. 1983a). Some researchers also added a boiling step in order to avoid further degradation (Trippodo et al. 1982; Grammer et al. 1983). De Bold and Salerno (1983) showed that acid atrial extract from larger mammalian species, such as the human, contains a relatively lower amount of ANF than that from rodents. The rat, a common laboratory animal, was initially preferred since, in the early days, the only biological assay available was the natriuretic one, this assay required microgramm quantities of the factor for successful detection, and rat atria contain relatively the largest amount of ANF.

The natriuretic factor was rather labile in physiological buffers, suggesting some enzymatic degradation. Moreover, molecular sieving on Sephadex or Bio-Gel columns indicated that it possesses a rather large but heterogeneous molecular weight, ranging from 3 to 25 kDa (de Bold 1982a; Thibault et al. 1983a; Trippodo et al. 1982). On the basis of these data, it was suspected that the natriuretic factor was of a proteinic nature. This was rapidly confirmed by the finding that the incubation of atrial extract in the presence of various proteases such as trypsin, chymotrypsin, or kallikrein destroyed its biological properties (de Bold 1982a; Garcia et al. 1982; Trippodo et al. 1982; Thibault et al. 1983a).

The intracellular localization of the natriuretic factor was also of prime importance since muscular atrial cells contain small, dense bodies which are very similar to specific secretory granules of neuroendocrine tissues (de Bold 1982b). Subcellular fractionation of atrial cardiocytes was undertaken by gentle homogenization of the tissue in isotonic solution (0.25 M sucrose) and subsequently by differential sucrose density gradient centrifugation. Using this technique, it was possible to obtain granule fractions relatively free of lysosomes and mitochondria. The natriuretic activity was closely associated with the presence of these granules (de Bold 1982b; Garcia et al. 1982). It was assumed that they represented a storage site of the natriuretic factor.

2.1 Purification and Amino Acid Sequence

Several groups in Canada, the United States, and Japan attempted to purify and identify the natriuretic factor and contributed to the elucidation of its chemical nature (Tables 2, 3). The major drawback in the isolation of the natriuretic factor was not the purification methodology itself, but rather the biological assay used to detect ANF in the fractions. The diuretic and natriuretic assay performed in anesthetized rats (de Bold et al. 1981) is relatively long, insensitive, tedious, and rather inaccurate, and no more than 30–40 fractions can be measured in the same day, with doubt always remaining about the validity of some results. The discovery by Needleman's group that the natriuretic factor also possesses relaxant activity allowed the introduction of a faster and more reliable assay for the measurement of ANF activity: the chick rectum relaxation assay (Currie et al. 1983). Garcia et al. (1983, 1984a) described similar results using rabbit aorta. Thus, it became possible to detect and quantify ANF activity in more than 100 samples per day.

The purification techniques were essentially the same among the different groups and were based on the fact that ANFs were similar peptides varying in length, with molecular weights ranging from 3 to 15 kDa (Fig. 1). Following the extraction of the atria in acid solution in the presence of protease inhibitors with or without a boiling step, a batchwise purification was performed on octadecyl silica gel (de Bold and Flynn 1983; Thibault et al. 1983b; Napier et al. 1984a) or by ethanol precipitation (Forssmann et al. 1984). This

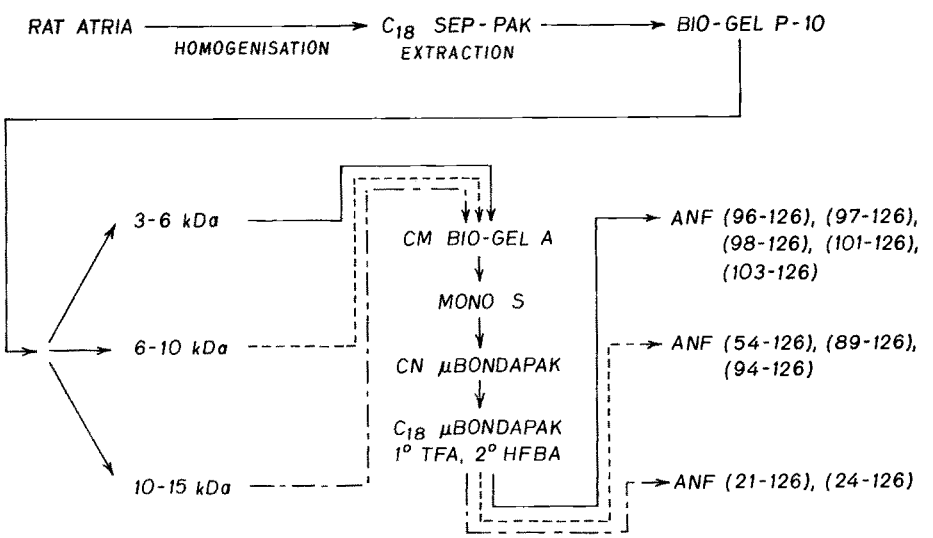


Fig. 1. Purification of rat atrial natriuretic factor. (From Thibault et al. 1983b, 1984b; Lazure et al. 1984; Seidah et al. 1984); TFA: Trifluoroacetic acid; HFBA: Heptafluorobutyric acid

was usually followed by molecular sieving on a Sephadex G-50, or G-75, or a Bio-Gel P-10 column, which allows the separation of low and high molecular weight forms of ANF. Later on, cation exchangers such as SP-Sephadex, CM Bio-Gel A, or Mono S were used, indicating that ANF is probably a basic substance. The last chromatographie steps were performed by reverse-phase chromatography under high pressure, which allows high resolution of microgram quantities of complex mixtures. The overall yield of these purifications was usually nanomoles (micrograms) of pure peptide from hundreds of rat atria. Since we now know that each rat atrium may contain between 5 and 10 μg ANF (Gutkowska et al. 1984b; Nakao et al. 1984), 500 atria would theoretically yield 2.5–5 mg peptide.

The primary structure of short, purified forms of ANF is illustrated in Table 2, together with their original names and the new peptide nomenclature which was adopted in September 1986 at the 11th Scientific Meeting of the International Society of Hypertension. The first peptides sequenced contain fewer than 40 amino acids, and most of them have a tyrosine at the COOH terminal. The diversity in the length of these peptides, ranging from 19 to 35 amino acids, was probably artifactual and may have resulted from nonspecific proteolytic degradation, even though acid solutions, protease inhibitors, and boiling were used. All the peptides were reported to be biologically active.

The presence of basic amino acids in positions 99, 103, and 104, which are known to be potential sites in the maturation of peptide hormones (Lazure et al. 1983), did not seem to be involved in the processing of the various forms isolated from atrial extracts, and the generation of shorter forms appeared to be a rather random process. Probably, even much shorter forms of ANF without any biological activity would have been present in the extract.

One important point is the presence of a disulfide link between Cys-105 and Cys-121. Oxidation of that bridge abolishes almost completely the vasorelaxant (Atlas et al. 1984) and aldosterone inhibitory activity of ANF (Chartier et al. 1984a), indicating that the loop conformation appears essential for the full expression of its biological properties (see Sect. 10).

About 1 year after the elucidation of the rat ANF sequence, the primary structure of human ANF was determined (Kangawa and Matsuo 1984; Thibault et al. 1984a). Human ANF was purified by using the same techniques as those used for rat ANF. A large homology exists between rat and human sequences, the only difference being that an isoleucine in position 110 in the rat sequence is replaced by a methionine in the human one. This high degree of homology, which was later found in rabbit, mouse, dog, porcine, and bovine atria (Table 4), indicates a high evolutionary conservation and emphasizes the importance of the new endocrine system.

The presence of higher molecular weight forms of ANF (longer than 40 amino acids) during gel filtration was originally attributed to the aggregation of ANF molecules, to protein interactions, to a completely different

Table 2. Purification and identification of low-M_r forms of ANF

Sequence	Common names	Peptide nomenclature	References
100 110 120 126			
S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	Cardionatrin I	ANF (99–126)	Flynn et al. 1983
L-A-G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF (1–33)	ANF (94–126)	Thibault et al. 1983b
G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF (3–33)	ANF (96–126)	Seidah et al. 1984
R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF (8–33)	ANF (101–126)	
S-S-C-F... I ...G-C-N-S	Atriopeptin I	ANF (103–123)	
S-S-C-F... I ...G-C-N-S-F-R	Atriopeptin II	ANF (103–125)	
S-S-C-F... I ...G-C-N-S-F-R-Y	Atriopeptin III	ANF (103–126)	Currie et al. 1984b
S-C-F... I ...G-C-N-S	Des-Ser^1-Atriopeptin I	ANF (104–123)	Geller et al. 1984b
C-F... I ...G-C-N-S	Des-Ser^1-Ser^2-Atriopeptin I	ANF (105–123)	
S-S-C-F... I ...G-C-N	Des-Ser^{21}-Atriopeptin I	ANF (103–122)	
R-S-S-C-F... I ...G-C-N-S-F-R	Auriculin A	ANF (102–125)	Atlas et al. 1984
R-S-S-C-F... I ...G-C-N-S-F-R-Y	Auriculin B	ANF (102–126)	
A-L-L-A-G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF-I	ANF (92–126)	
G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF-II	ANF (96–126)	Misono et al. 1984a
G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R	ANF-III	ANF (96–125)	Misono et al. 1984b
R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF-IV	ANF (102–126)	
A-L-L-A-G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	β-rANP (14–48)	ANF (92–126)	
L-A-G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	β-rANP (16–48)	ANF (94–126)	
A-G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	β-rANP (17–48)	ANF (95–126)	
G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	β-rANP (18–48)	ANF (96–126)	Kangawa et al. 1984a
S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	α-rANP	ANF (99–126)	
R-S-S-C-F... I ...G-C-N-S-F-R-Y	α-rANP (4–28)	ANF (102–126)	
S-S-C-F... I ...G-C-N-S-F-R	α-rANP (5–27)	ANF (103–125)	
G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF A	ANF (96–126)	
A-G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF B	ANF (95–126)	Napier et al. 1984a
L-A-G-P-R-S-L-R-R-S-S-C-F... I ...G-C-N-S-F-R-Y	ANF C	ANF (94–126)	
S-L-R-R-S-S-C-F...M...G-C-N-S-F-R-Y	α-hANP	[Met^{110}]ANF (99–126)	Kangawa and Matsuo 1984
S-L-R-R-S-S-C-F...M...G-C-N-S-F-R-Y	human ANF	[Met^{110}]ANF (99–126)	Thibault et al. 1984a
S-L-R-R-S-S-C-F...M...G-C-N-S-F-R-Y	bovine ANF	[Met^{110}]ANF (99–126)	Ong et al. 1986

ANP, atrial natriuretic peptide

Table 3. Purification and identification of high M_r forms of ANF

Sequence	Common names	Peptide nomenclature	References
1 40 80 120			
——————→	ANF-H_1, ANF (1 – 73)	ANF (54 – 126)	Thibault et al. 1984b
——————→	ANF-H_2, pronatriodilatin	ANF (21 – 126)	Lazure et al. 1984
——————→	pro-ANF	ANF (1 – 126)	Thibault et al. 1987
——————→	β-rANP	ANF (64 – 126)	Kangawa et al. 1984b
——————→	γ-rANP	ANF (1 – 126)	Kangawa et al. 1984c
——————→	Atriopeptigen	ANF (16 – 126)	Geller et al. 1984a
——————→	Cardiodilatin-126	[Met^{110}] ANF (1 – 126)	Forssmann et al. 1984
——————→	Cardionatrin IV	ANF (1 – 126)	Flynn et al. 1985
——————→	γ-hANP	[Met^{110}] ANF (1 – 126)	Kangawa et al. 1985
——————→	Rabbit atriopeptigen	ANF (1 – 126)	Wei et al. 1986

ANP, atrial natriuretic peptide

natriuretic substance, or to a longer form of ANF (de Bold 1982a; Trippodo et al. 1982). In fact, further purification and amino acid sequencing of these substances revealed that they represented longer forms of ANF, and that the previously reported peptides were located at its COOH-terminal end (Table 3). These peptides possessed between 63 and 126 amino acids. The first one, as reported by Thibault et al. (1984b), had 73 residues and represented ANF (54–126). Matsuo's group (Kangawa and Matsuo 1984; Kangawa et al. 1984c, 1985) reported the longest isolated forms of rat and human ANF, and Forssmann et al. (1984) that of porcine ANF. Although the sequence homology between species is very well conserved for the last COOH-terminal 45 amino acids (Table 3), this homology decreases considerably on the NH_2-terminal side. Some early studies stated that the NH_2-terminal peptide domonstrated relaxant activity without natriuretic activity (Forssmann et al. 1983); however, this observation could not be reproduced.

A rapid and reliable method of purifying large amounts of ANF(Asn-1 to Tyr-126) has been devised (Thibault et al. 1987a). Secretory granules from rat atrial homogenates were first isolated and thereafter lysed in acetic acid. The soluble proteins were passed onto a $C_{18}\mu$-Bondapak column. The major absorbance peak at 210 nm, which demonstrated immunoreactivity, was identified as ANF(Asn-1 to Tyr-126) by amino acid sequencing and composition. The final yield was about 100–150 μg peptide from the atrial granules of 25 rats. The validity of these sequence structures was subsequently confirmed by the cloning and sequencing of the gene (see Sect. 3).

2.2 Biosynthesis and Molecular Forms of ANF

Although purification studies of ANF have indicated that the atrial tissue contains peptides of various lengths, ranging from 21 to 126 residues, recent evidence suggests that the major intracellular form of ANF is the propeptide of 126 amino acids (Thibault et al. 1987a; Nakao et al. 1984; Lang et al. 1985; Vuolteenaho et al. 1985; Morii et al. 1986; Snajdar and Rapp 1986). Extraction of ANF from small amounts of atrial tissue in the presence of protease inhibitors and with a boiling step demonstrated a 13- to 17-kDa protein. In a more detailed study, Thibault et al. (1987a) isolated atrial granules and purified their ANF content. They clearly demonstrated that the intragranular form was only pro-ANF (Asn-1 to Tyr-126). This peptide was devoid of the signal peptide and, more interestingly, of the two arginines in positions 127 and 128, which are present both on the mRNA and on the gene (Table 4). It is not clear presently whether or not these two codons are translated and the product immediately removed by a carboxypeptidase during the packaging of the granules.

Table 4. Primary structure of pre-pro-ANF of different species (From Vlasuk et al. 1986, Forssmann et al. 1984, Oikawa et al. 1985)

	−25			−20							−10							−1 1							10							20						
human	M	S	S	F	S	T	T	T	V	S	F	L	L	L	A	F	Q	L	L	G	Q	T	R	A	N	P	M	Y	N	A	V	S	N	A	D	L	M	D	F	K	N	L	L	D	H	L	E	E	K
bovine		G		S	A	−	I							F					P			G				V		G	S															R			D		
porcine	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			V		G	S															H			D		
dog		G	−	−		P	I	A	A					F			V						G				V		G	S						L								R			D		
rabbit		G	P				I					F	C				W	H	P	D		I	G				V				M														D	R	
rat		G				−	I		K	G		F		F			W		P		H	I	G				V		S				T															
mouse		G				−	I		L	G		F		V			W		P		H	I	G				V		S				T															

	30										40										50										60										70										
human	M	P	L	E	D	E	V	V	P	P	Q	V	L	S	D	P	N	E	E	A	G	A	A	L	S	P	L	P	E	V	P	P	W	T	G	E	V	S	P	A	Q	R	D	G	G	A	L	G	R	G
bovine							A			S					E	Q							P					S		M			M				N				E			V						
porcine						x	x	x	x	x	x	x	x	x						V			P	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
dog							A	E	S			A			E	Q		A																																	S	
rabbit			F				A					A			E	Q	S	D																										E						S	
rat			V					M				A			E	Q	T	D							S		S									N		S										S	
mouse			V					M				A			E	Q	T	E							S											N		P	L			S		S	R		S	

	80										90										100										110										120										126	
human	P	W	D	S	S	D	R	S	A	L	L	K	S	K	L	R	A	L	L	T	S	P	R	S	L	R	R	S	S	C	F	G	G	R	M	D	R	I	G	A	Q	S	G	L	G	C	N	S	F	R	Y	−	−
bovine			E																																															R	R
porcine	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x																											x	x
dog																				A																															−	−
rabbit	T		E	A		E																									I																			R	R
rat				P																A	G										I																			R	R
mouse				P																A	G										I																			R	R

x indicates that the corresponding amino acids are not known
− no amino-acid in this position
Blank spaces indicate amino acid identity

Although pro-ANF is the only form to have been detected in the granules, the extraction of atrial tissue always reveals the presence of some COOH-terminal 3-kDa peptide of ANF. This peptide may be due to a partial artifactual proteolysis or to a partial intracellular maturation of pro-ANF.

There is at present controversy about the form of ANF secreted by atrial cardiomyocytes. In culture, atrial cardiocytes or even ventricular cardiocytes derived from newborn rats mostly secrete a 15-kDa peptide which has been identified as pro-ANF (Glembotski and Gibson 1985; Bloch et al. 1985, 1986; Cantin et al., unpublished observations). However, the COOH-terminal peptide of 3 kDa is also present and may represent 10% – 20% of the total ANF, suggesting perhaps a partial maturation (Thibault et al., unpublished results). On the other hand, isolated perfused rat or rabbit heart (Langendorf preparation) releases in the effluent, which is free of blood, only the short form of ANF (Lang et al. 1985; Vuolteenaho et al. 1985; Currie et al. 1984a) which was identified as ANF(Ser-99 to Tyr-126) (Thibault et al. 1986).

The cell culture experiments in which pro-ANF was labeled with either [^{35}S]methionine or [^{35}S]cysteine and also the intragranular form of ANF seem to indicate the absence of intracellular maturation. However, experiments using isolated hearts would favor intra-atrial processing. In fact, the former experiments do not exclude such processing, and it can be hypothesized that pro-ANF maturation may take place on the apical side of the plasma membrane through the action of a membrane-bound enzyme or on the extracellular membrane of the endothelium. When in culture, atrial cardiocytes may have lost their capacity to process pro-ANF. Certainly, the maturation of ANF in blood does not appear to be important since even analysis of plasma taken from the coronary sinus demonstrated the absence of pro-ANF (Yandle et al. 1986a).

On the other hand, the generation of COOH-terminal 3-kDa peptide following the incubation of pro-ANF in rat serum has been reported by Bloch et al. (1985), Michener et al. (1986), and Gibson et al. (1987). But no significant conversion of the prohormone has been observed in rat plasma (Michener et al. 1986; Gibson et al. 1987) or even in whole blood (Murthy et al., unpublished observations).

To date, two atrial enzymes have been described as processing pro-ANF. The first one, found in bovine atria, is a thiol-dependent, membrane-bound protease which seems to generate ANF(Ser-99 to Tyr-126) from ANF(Gly-96 to Tyr-126) by removing the first three residues (Baxter et al. 1986). The second one, named IRCM-serine protease 1 (Cromlish et al. 1986a, 1986b), has been found in rat atria and to a lesser extent in ventricles and generates three ANF peptides, (Ser-103 to Tyr-126), (Arg-102 to Tyr-126), and (Ser-99 to Tyr-126), from pro-ANF (Seidah et al. 1986). This same enzyme is found in other endocrine tissues and can specifically process proopiomelanocortin (POMC)-related peptides, β-lipotropin (β-LPH), proinsulin, and proenkepha-

lin. However, more studies are needed, including coimmunolocalization of these enzymes and pro-ANF, before any definite conclusions can be drawn.

Human plasma kallikrein and thrombin have also been reported to generate ANF(99–126) from the precursor (Gibson et al. 1987; Michener et al. 1986), but the physiological meaning of these experiments remains to be elucidated.

The active ANF form found in the rat circulation was recently identified (Thibault et al. 1985; Schwartz et al. 1985). Since the basal level of rat plasma ANF lies at around 16 pmol/l, it is necessary to use very large numbers of rats or to increase its plasma concentration. For the latter objective, Schwartz et al. (1985) used plasma from vasopressin-treated rats, and Thibault et al. (1985) used morphine-treated rats. These drugs were shown to increase the plasma levels of ANF considerably (Horky et al. 1985; Manning et al. 1985). In both cases, ANF was first extracted by means of octadecylsilane columns. Thibault et al. (1985) used an anti-ANF column to further purify the peptide. Subsequently, reverse-phase HPLC was employed. The main circulating form of ANF was identified by amino acid sequencing as the 28-residue peptide ANF(Ser-99 to Tyr-126), which is identical to the form released by isolated heart. Schwartz et al. (1985) also identified the presence of another minor component, ANF(Ser-103 to Tyr-126), which represents about 10% of the total immunoreactive (IR)-ANF. As will be seen below, this peptide probably represents a degradation product of ANF.

In humans, the circulating form has only been identified by its elution profile following reverse-phase HPLC. The retention time is identical to that of the [Met_{110}]ANF(Ser-99 to Tyr-126) (Yandle et al. 1986a; Yamaji et al. 1985b; Anderson et al. 1986d; Burnett et al. 1986; Gutkowska et al. 1986a; Hasegawa et al. 1986). However, the HPLC pattern of extracted human blood is more complex than that of rat blood, indicating that many degradation forms, representing up to 50% of the total IR-ANF, may also be present.

The presence in the circulation of the counterpart of the pro-ANF molecule, namely ANF(1–98), was suggested by recent experiments (Michener et al. 1986). Rabbit antibodies were raised against an ANF fragment, ANF(48–67), and then used to detect and measure the NH_2 terminal of pro-ANF in the rat circulation. Basal levels of immunoreactive NH_2 terminal of 2140 pmol/l were measured (Michener et al. 1986). For our part, we have also developed rabbit antibodies against a different fragment of pro-ANF, ANF(11–37), and basal concentrations of the NH_2 terminal were in the range of 300–600 pmol/l (Thibault et al., unpublished results). Furthermore, preliminary experiments suggest that the NH_2-terminal portion also circulates in the human blood at a concentration about 100-fold higher than that of the COOH terminal, ANF(99–126). If we suppose that both fragments are secreted at an equimolar concentration, the higher NH_2-terminal concentration can only be explained by a much longer half-life and therefore a decreas-

ed elimination of this peptide. Its concentration would represent an index of ANF secretion.

2.3 In Vivo Elimination of ANF

Once in the circulation, peptide hormones are usually eliminated in various ways: uptake by the target tissues and intracellular degradation, inactivation by blood proteases or by the liver, or excretion by the kidneys. The first step in studies of such elimination is to evaluate the distribution of the hormone and its clearance from the circulation. Bianchi et al. (1985) have demonstrated by injecting iodinated ANF(Arg-101 to Tyr-126) into rats that the peptide is widely distributed and that binding sites are found in most organs.

In order to study the inactivation of ANF by blood, 10 µg ANF(Ser-99 to Tyr-126) was incubated at 37 °C in whole rat blood for up to 60 min. After the incubation, plasma ANF was extracted and separated by reverse-phase HPLC, and the immunoreactive peaks were analyzed for their amino acid composition (Murthy et al. 1986a). Results showed that ANF(Ser-99 to Tyr-126) was slowly and gradually transformed into a NH_2-terminal shorter form, ANF(Ser-103 to Tyr-126). No further degradation was observed. The formation of ANF(Ser-103 to Tyr-126) may therefore explain the presence of such peptides in blood, as reported by Schwartz et al. (1985). The blood itself does not appear to be an important pathway in the elimination of ANF.

Bolus injections of ANF(Ser-103 to Tyr-126) into the femoral veins of conscious rats (Luft et al. 1986a) or of ^{125}I-labeled ANF(Ser-99 to Tyr-126) into the jugular vein of anesthetized rats (Murthy et al. 1986b) were used to assess the half-life of the peptide in the circulation. In normal rats, a very fast decay of between 16 and 27 s was calculated (Luft et al. 1986a; Murthy et al. 1986b). This half-life did not seem to be affected by nephrectomy in anesthetized animals. However, Luft et al. (1986a) reported that the half-life doubled in conscious anephric rats. Whether or not the kidneys are important in the elimination of ANF is still uncertain. However, it is more evident that this short half-life is mainly due to the distribution of the ANF and to its uptake by cell membranes. In fact, in humans or in dogs, in which the circulation time is much longer than in rats, a half-life of the order of 1 – 5 min has been found (Jüppner et al. 1986; Verburg et al. 1986; Yandle et al. 1986b).

The degradation and fate of ANF in tissues was investigated in the isolated perfused mesenteric region of the rat (Murthy et al. 1986a). A bolus injection of iodinated ANF was given, and ANF was analyzed in the eluate. After an initial washing period of 2 min, during which about 90% of the radioactivity passed through, an analysis of the material eluting later revealed that the peptide was increasingly degraded. Analysis of the iodinated ANF injected in vivo reveals a similar but somewhat more accentuated pattern of degradation,

indicating that the in vivo elimination of ANF is closely associated with tissue uptake and degradation.

These results are corroborated by incubation of iodinated ANF with cultures of smooth muscle cells. Hirata et al. (1985b) and Napier et al. (1986a) reported in preliminary experiments that ANF binds to high-affinity sites and that this is followed by the internalization of the ANF-receptor complex. In another study (Murthy et al., unpublished results), it was found that ANF degrades intracellularly following internalization, and that degradation is blocked by monensin and chloroquine, two lysosomotropic agents, and by incubation at 4 °C. However, it is not yet clear whether this degradation is due to lysosomal enzymes.

It seems, therefore, that the in vivo removal of ANF is principally mediated through binding, internalization, and intracellular degradation. The blood does not participate to any large extent in such a degradation.

3 Molecular Biology of ANF (M. Nemer)

3.1 Structure of ANF mRNA and Deduced Precursor

Once a partial amino acid sequence of rat ANF was known, it became possible to synthesize several oligonucleotides which were used as probes to screen rat atrial cDNA libraries. Several laboratories were thus able simultaneously to isolate and sequence cDNA clones containing sequences encoding the ANF peptide (Maki et al. 1984; Kangawa et al. 1984c; Seidman et al. 1984b; Yamanaka et al. 1984; Zivin et al. 1984; Flynn et al. 1985). DNA sequence analysis of these clones revealed an open reading frame of 152 amino acids. The first 24 amino acids closely resembled a signal peptide sequence. The ANF sequence was at the carboxyl end of the 126-amino-acid precursor polypeptide (Fig. 2). The DNA sequence analysis also revealed the presence of two arginine residues at the carboxy terminus of the ANF sequence. These residues were never found in any of the purified atrial peptides, and are presumably removed by processing of the precursor polypeptide via a cellular mechanism which remains poorly understood.

Similarly, human ANF cDNA clones were isolated from human atrial cDNA libraries (Oikawa et al. 1984; Nakayama et al. 1984; Zivin et al. 1984). Human ANF is also derived from a larger percursor peptide. Human pre-pro-ANF consists of 151 amino acids, including a 25-amino-acid signal peptide. The DNA sequence encoding human ANF shows a high degree of homology with the rat sequence. The most striking difference is the absence of the two arginine codons found in the rat sequence at the carboxy terminus of the human sequence. However, the isolation of another human cDNA

clone, whose DNA sequence, like that of the rat, contained the two arginine codons, suggested the existence of two alleles which would yield the same mature peptide (Zivin et al. 1984). This observation has recently been confirmed in polymorphism studies of the human ANF gene (see Sect. 3.3).

The human cDNA clone was used as a probe to isolate dog and rabbit ANF cDNA clones (Oikawa et al. 1985). The dog pre-pro-ANF is a 149-amino-acid precursor, containing a 23-amino-acid signal peptide and lacking the two terminal arginine residues. The predicted rabbit precursor would contain 153 amino acids, including a 25-residue signal peptide and the ANF sequence which is followed by the two arginines at the carboxy terminus. A similar structure was also found for mouse (Seidman et al. 1984a) and bovine (Vlasuk et al. 1986) pre-pro-ANF, as deduced from the DNA sequence analysis of genomic clones. Thus, the structure of the ANF precursor appears highly conserved among distantly related mammalian species. However, no biological activity has yet been ascribed to the NH_2-terminal portion of the ANF precursor.

3.2 Structure of ANF Gene

Rat and human ANF cDNA clones have been used as probes to isolate and characterize human (Nemer et al. 1984; Greenberg et al. 1984; Seidman et al. 1984a), mouse (Seidman et al. 1984a), rat (Argentin et al. 1985), and bovine (Vlasuk et al. 1986) ANF genes. The structural organization of the ANF gene is similar in all cases: the gene consists of three exons (Fig. 2). The first exon contains 5′ untranslated sequences, the initiator AUG codon, and sequences encoding the signal peptide and the first 16 amino acids of the ANF precursor; the second exon contains the remaining coding sequences up to the penultimate ANF amino acid; and the third exon contains the last codon for ANF (and in the mouse, rat, and bovine genes, codons for the two arginine residues), the stop codon, and the 3′ untranslated sequences. Altogether, the gene spans about 2000 base pairs (bp), including the two introns.

The ANF gene is present in a single copy per haploid genome, and its overall structural organization is typical of a protein-coding, eukaryotic gene. The sequences at the exon-intron junctions are homologous to the consensus splice site sequence, and a TATA box is found about 30 bp upstream from the site of transcription initiation. This site was precisely determined in rat (Gardner et al. 1986a) and human (Nemer et al. 1984) atria by primer extension, and the initiation was found to be heterogeneous in both species. In the human gene, the second and larger intron contains two repeated sequences of the Alu family. In addition, the second intron contains a potential glucocorticoid receptor binding site which is also present in the rat gene. The presence of this sequence suggests that the ANF gene may be regulated by glucocorti-

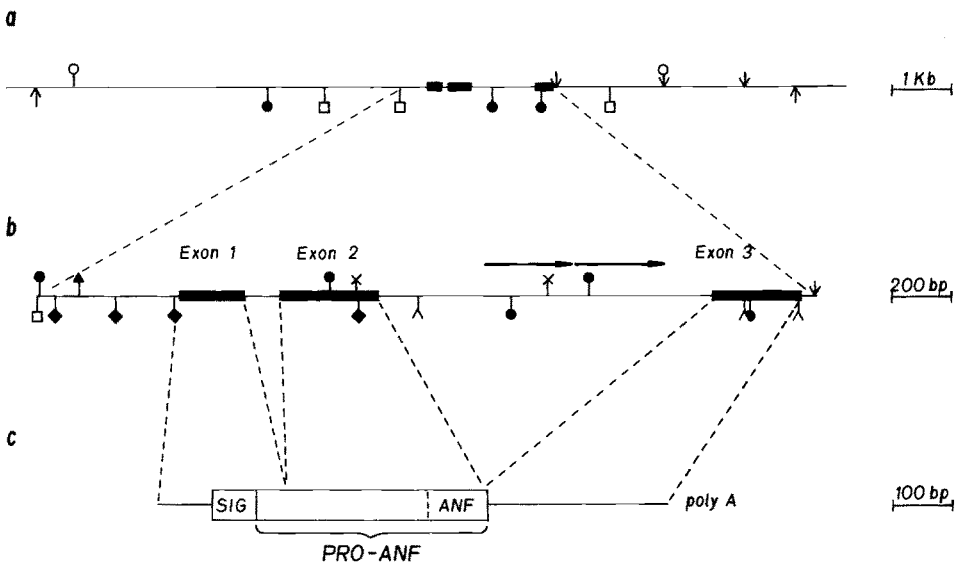


Fig. 2. **a** Structure of the human ANF gene. **b** Detailed restriction map of the 2.6 Kb Bam HI-Eco RI fragment containing the entire gene. The following symbols are used for the restriction enzyme cleavage sites: Eco RI (↓), Hind III (↑), Xba I (⚲), Bam HI (⏃), Pst I (⏃), Ava I (⏃), Kpn I (⏃), Xho II (⏃), Pvu II (⏃), and Nco I (⏃). The two *arrows* in the second intron refer to the two Alu repeated sequences. **c** Schematic diagram of ANF mRNA. The translated portion is represented by the *open box* which includes the signal peptide (*SIG*) and the pro-ANF sequences

coids, a hypothesis which has recently been confirmed (see Sect. 3.4). A comparison of the human and rat genomic sequences shows the highest degree of homology to be in the 5′ flanking sequences ($\simeq 80\%$) and in coding sequences ($\simeq 90\%$). This sequence conservation might reflect a selective pressure to maintain important regulatory sequences.

The cloned ANF cDNAs and genomic sequences provide valuable tools for studying the expression and regulation of the ANF gene in normal and pathological conditions.

3.3 Chromosomal Localization and Polymorphism of ANF Gene

The chromosomal assignment of the human ANF gene was accomplished by in situ hybridization of a ^{3}H-labeled human ANF genomic probe to human chromosomes: specific labeling was observed on the distal short arm of chromosome 1 at band 1p36. This assignment was confirmed by Southern blot analysis of DNA from human×Chinese hamster somatic cell hybrids. The mouse ANF gene was assigned to chromosome 4 by Southern blot hybridization of DNA from mouse×Chinese hamster cell hybrids to a rat

ANF cDNA probe. This assignment adds a seventh locus to the conserved syntenic group of homologous genes located on the distal half of the short arm of human chromosome 1 and on mouse chromosome 4 (Yang-Feng et al. 1985).

Knowledge of the genetic map surrounding the ANF gene may be useful in linkage studies of families with genetically inherited disorders such as essential hypertension. In addition, the allelic polymorphism of the human ANF gene was investigated in the hope of detecting individuals with a genetic predisposition to hypertension secondary to a mutation in the ANF gene.

The first indication of allelic polymorphism in the ANF gene came with the finding of a mutant human cDNA clone which contained at the carboxyl end of the coding region a point mutation which converts the stop codon into an arginine codon (Nemer et al. 1984):

	Arg	Tyr	Stop		
Allele 1:	CGG	TAC	TGA	AGA	TAA
			↓		
	CGC	TAC	CGA	AGA	TAA
Allele 2:	Arg	Tyr	Arg	Arg	Stop

The presence of these two alleles in the human population has just been confirmed, using a pair of oligonucleotides which differ at the single nucleotide residue as hybridization probes. Studies on 60 individuals, including eight families, indicate codominant mendelian segregation with a low frequency for allele 2: about 0.92 for allele 1 and 0.08 for allele 2 (Nemer et al., unpublished data). Restriction fragment length polymorphism (RFLP) was also investigated in the human ANF gene. RFLPs are now widely used to identify genetic loci associated with disease, and the power of this method was first exemplified in Huntington's disease, where identification of a RFLP may allow early diagnosis of afflicted individuals (Gusella et al. 1984). By means of cloned ANF probes, two RFLPs were found at the 5′ end of the human ANF gene locus (Nemer et al. 1986c; Frossard et al. 1986). A RFLP was also found at the 3′ end of the ANF gene, using a cloned genomic DNA fragment (Nemer et al. 1986d). Using one of the RFLPs found at the 5′ end of the ANF gene, Graham et al. (1986) reported that the ANF gene is not linked to Bartter's syndrome, a rare electrolyte disorder.

3.4 Regulation of ANF Gene Expression

Cloned ANF sequences were used to study the regulation of ANF gene expression at the mRNA level. While the measurement of cardiac ANF content represents the difference between synthesis and release, the quantitation of

ANF mRNA levels may be a more direct assessment of ANF synthesis. The effects of hemodynamic and hormonal factors on ANF synthesis were evaluated using this method.

Atrial ANF mRNA levels were found to be severely decreased (by 70%) in rats deprived of water for 4 days (Nemer et al., unpublished data; Takayanagi et al. 1985). When rats were subjected to a low sodium diet for 2 (Takayanagi et al. 1985) or 3 weeks (Nemer et al., unpublished data), atrial ANF mRNA levels were decreased to 30% of control levels. In contrast, rats given a 1% NaCl solution for 21 days (Nemer et al., unpublished data) or a 1.8% NaCl solution for 2, 5, or 10 days (Takayanagi et al. 1985) showed no change in atrial ANF mRNA levels. Volume overload in the rat, whether produced by a mineralocorticoid administration (Nemer et al. 1987, 1986c; Ballermann et al. 1986), bilateral nephrectomy, or bilateral ureteral ligation (Lattion et al. 1986), causes an increase in both atrial (1.5- to 1.75-fold) and ventricular (4- to 11-fold) ANF mRNA levels.

Cardiac ANF synthesis is not only sensitive to volume changes but is also hormonally regulated. Glucocorticoid administration to intact (Gardner et al. 1986b) or adrenalectomized (Nemer et al. 1986a; 1987) rats leads to a two- to threefold increase in atrial, and a four- to fivefold increase in ventricular ANF mRNA levels. The glucocorticoid stimulation of ANF mRNA is due to a direct action of the steroid at the heart level, since ANF mRNA levels in primary cardiocyte cultures are similarly increased by glucocorticoids while being unaffected by mineralocorticoids or other steroid hormones (Nemer et al. 1987). Furthermore, the stimulation of ANF mRNA by glucocorticoids in cardiocyte cultures is dose dependent and is blocked by the glucocorticoid antagonist RU 486.

In addition to glucocorticoids, thyroid hormone also stimulates ANF synthesis, as was reported for rats with hyperthyroidism (Kohno et al. 1986a) and recently confirmed in primary cardiocyte cultures where ANF mRNA levels increased threefold after 48-h treatment with triiodothreonine (T_3) (Nemer et al., unpublished data). Primary cardiocyte cultures are being used to test the effect of other hormones and factors on ANF gene expression.

4 Measurement of IR-ANF in Plasma and Tissues

4.1 Radioimmunoassay (J. Gutkowska)

Various techniques have been developed for the measurement of ANF in plasma and in tissues. The quantitation of the peptide hormone was first attempted via bioassay to assess its diuretic and natriuretic effects (Garcia et al. 1982) or its vasorelaxant action on rabbit aorta (Garcia et al. 1984a) and

Table 5. Human plasma ANF concentrations

References	ANF (pmol/l)	Method	Antibody
Gutkowska et al. 1985	22 ± 1	Direct	Peninsula
Larose et al. 1985	8 ± 2	Sep-Pak	Peninsula
Arendt et al. 1985	8 ± 2	Amberlite X-AD-2	28 AA hANF
Tikkanen et al. 1985a	Undetectable – Low	Direct	Peninsula
Hartter et al. 1985	Range 3 – 23	Sep-Pak	–
Sagnella et al. 1985	2 ± 1	Sep-Pak	Peninsula
Yamamoto et al. 1985	93 ± 4	Direct	28 AA hANF
Hodsman et al. 1985	8 ± 1	Vycor glass	28 AA hANF
Yamaji et al. 1985b	11 ± 3	Affinity chromatography Seph-anti-ANF	28 AA hANF
Shenker et al. 1985	10 ± 1	Sep-Pak	Peninsula
Miyata et al. 1985	77 ± 12	Sep-Pak	28 AA hANF
Espiner et al. 1985	14 ± 1	Sep-Pak	–
Gutkowska et al. 1986a	4 ± 0.4	Sep-Pak	Peninsula

chicken rectum (Currie et al. 1983). However, these bioassays were not sensitive nor reproducible enough to measure ANF accurately in plasma. Several radioimmunoassays (RIAs) (Gutkowska et al. 1987c) subsequently developed to evaluate ANF levels in human and animal plasma generated a wide variability of results. Table 5 enumerates these human plasma ANF values, which range from undetectable or low levels to about 100 pmol/l, obtained by direct RIA, and from 2 to about 67 pmol/l, detected with prior extraction.

The great variability of these data may be due to the direct assay or extraction procedure involved. The values may also be influenced by the ANF recovery method implemented and by the effectiveness of proteolytic enzyme inhibition during the withdrawal of blood and the prolonged storage of the samples. The purity of the standards and the specificity of antibodies are other important factors which may effect the results (Gutkowska et al. 1987c).

There are, however, other conditions which may influence ANF values: the latter may vary depending on water and salt intake (Sagnella et al. 1985; Shenker et al. 1985) and even on the position of the patient when blood is drawn (Larose et al. 1985; Hodsman et al. 1985). Furthermore, plasma ANF values for the same subject in a supine or upright position may vary with changes in sodium consumption (Hollister et al. 1986).

Blood for ANF determination is generally drawn in ice-chilled tubes containing either ethylenediaminetetraacetic acid (EDTA) (as a sodium or potassium salt, 1 – 10 mg/ml) or lithium heparin as an anticoagulant, alone or together with protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF; final concentration 10^{-5} M), aprotinin (varying from 20 to 1500 kallikrein inhibitory units/ml blood), soybean trypsin inhibitor (10 – 500 BAEE units; *N*-benzoyl-*L*-arginine ethyl ester), and pepstatin A (5 μM).

The blood should be centrifuged immediately and the plasma stored at −70 °C. In our laboratory, the blood is collected in tubes containing EDTA (1 mg/ml), PMSF (10^{-5} M), and pepstatin A (5 μM), and the plasma is stored at −70 °C. No degradation of ANF was observed in the pool of aliquoted plasma (2 ml) when it was stored at −70 °C for 10 months. However, if the plasma was stored at −20 °C, the ANF was degraded. It is safer to extract ANF from fresh plasma and then to store the extracts.

The most commonly used method for the preparation of radioactive tracers is the classical one using chloramine-T as an oxidant (Hunter and Greenwood 1962). The iodinated tracer may be purified by HPLC on a C_{18}μ-Bondapak column, with a linear gradient of acetonitrile (10%−60%) in 0.1% trifluoroacetic acid. The purification may be successfully performed by gel filtration on a Bio-Gel P-4 column which has been pretreated with 1% bovine serum albumin or on a Sephadex G-25 superfine or Sephadex G-50 column. A modified chloramine-T method has also been used for the iodination of ANF, in which the standard addition of sodium metabisulfite is omitted in order to avoid the reduction of the disulfide bond of ANF. Iodination by the solid phase Iodo-Gen method has also been applied to ANF. Methods using ANF iodinated by chloramine-T and by lactoperoxidase yield tracers of similar immunoreactivity and affinity for renal glomeruli receptors. The specific activities of iodinated tracers vary from about 300 to 900 μCi/μg. Many laboratories are using commercially available tracers of high specific activities supplied by Amersham International or Peninsula Laboratories.

Alpha-human (h) ANF antibodies in rabbits are usually generated using the 28-amino-acid COOH-terminal ANF peptide conjugated covalently to bovine thyroglobulin, employing as a coupling agent carbodiimide (Skowsky and Fisher 1972) or glutaraldehyde (Reichlin et al. 1968), or, more frequently, the antibodies are obtained commercially from Peninsula Laboratories or Amersham. All the antisera which have been described recognize to a certain extent COOH-terminal peptides of various lengths. No cross-reactivity is noted with unrelated peptides.

In RIA systems, two methods are used to separate free ANF from antibody-bound ANF: Dextran-coated charcoal and the double antibody method. A much higher sensitivity was achieved in our experience with the double antibody method of separation than with the charcoal technique.

The plasma values for ANF obtained by direct RIA are five to ten times higher than with prior extraction. This may be due to the nonspecific interference of plasma proteins. A major part of the "immunoreactive ANF" which is measured directly is not dialyzable, and therefore an extraction step is necessary for an accurate and precise measurement of ANF.

The extraction of ANF from plasma is achieved by C_{18} Sep-Pak cartridges (Waters Associates), by affinity chromatography, or by heat-activated Vycor glass (Gutkowska et al. 1984a, 1987c; Hodsman et al. 1985). Various systems

Table 6. Rat plasma ANF concentrations

References	ANF (pmol/l)	Method	Antibody	Anesthetic
Tanaka et al. 1984	150 ± 14	Sep-Pak	r25AA-thyr	Barbiturate
Gutkowska et al. 1984a	417 ± 70	Vycor glass	r26AA-thyr	Ether
Lang et al. 1985	18 ± 5	ODS	r23AA-thyr	Barbiturate
Horky et al. 1985	20 ± 2	Vycor glass	–	Barbiturate
Schwartz et al. 1985	267	Direct	92AA	Chloral hydrate
Miyata et al. 1985	130 ± 18	Sep-Pak	h28AA-thyr	–
Pettersson et al. 1985	1315 ± 229	Direct	Peninsula	–
Anderson et al. 1986b	24 ± 2	Direct	h28AA-BSA	Diazepam, Fentanyl Fluanisone
John et al. 1986	36 ± 4	Sep-Pak	Monoclonal AG	Decapitation

ODS: octadecasylil

Table 7. Effects of various anesthetics on IR-ANF in rat plasma

Anesthetic	Dose per 100 g body weight	N	IR-ANF pmol/l (Mean ± SE)
Urethane	100 mg	9	14 ± 3
Sodium pentobarbital	6 mg	10	20 ± 2
Ketamine hydrochloride	10 mg	51	65 ± 7
Chloral hydrate	30 mg	20	75 ± 27
Fentanyl	25 g	6	231 ± 38
Diethyl ether		64	269 ± 25
Morphine	10 mg	24	814 ± 94

of solvents may be successfully used for Sep-Pak extraction, such as ethanol (or methanol)-water-acetic acid, acetonitrile-water-trifluoroacetic acid, or acetonitrile-ammonium acetate.

As reported recently by various groups (Anderson et al. 1986b; Solomon et al. 1986; Weidmann et al. 1986a; Cody et al. 1986; Marumo et al. 1986; Hartter et al. 1986; Hollister et al. 1986), the plasma IR-ANF concentration in healthy subjects on a "normal" sodium diet ranges between 2 and 13 pmol/l.

A wide variability of plasma ANF concentrations has been reported in rats, with values ranging from 17 to 1300 pmol/l (Table 6). Furthermore, different anesthetics exert pronounced effects on plasma ANF in this species (Table 7). The mean plasma peptide level is 31 ± 5 pmol/l (n = 30) in rats with catheters implanted in the jugular vein for 24 h, and the level is not significantly different (27 ± 4 pmol/l, n = 29) when the blood is drawn 48 h after inserting the catheter (Horky et al. 1985). Immobilization for 4 h increases plasma ANF

to 84±17 pmol/l (n = 20), which drops to a low of 15±1.4 pmol/l (n = 88) in decapitated rats. Radioimmunological methods used for the measurement of plasma IR-ANF in conscious dogs have revealed a similar basal value of about 17–27 pmol/l (Metzler et al. 1986; Verburg et al. 1986; Wilson et al. 1986). The ANF content of rabbit arterial plasma is reported to be 18±1.3 pmol/l (Wilson et al. 1986).

4.2 Radioreceptor Assay (A. de Léan and H. Ong)

Highly sensitive and specific hormone assays usually depend on the properties of the discriminating protein used, e. g., a specific antibody for RIAs or a specific target tissue for bioassays. The ANF receptor thus displays all the characteristics required for hormone assays. This membrane receptor binds ANF with an extremely high affinity (K_d = 20–50 pM) (de Léan et al. 1984a). It can be easily prepared from bovine adrenal zona glomerulosa at a high density of 0.5–1.0 pmol/mg protein (de Léan et al. 1984a). The physiological receptor, in contrast to most antibodies, is highly specific for biologically active and circulating forms of the hormone. In addition, the ANF receptor from bovine adrenal membrane is especially suitable for assaying human plasma since bovine ANF is identical to the human hormone (Ong et al. 1986). Figure 3 shows a typical dose-response curve for an ANF radioreceptor assay based on bovine adrenal membrane receptor. The assay is characterized by a minimal detectable dose of 0.2 fmol/tube and a standard curve ED_{50} of 12 fmol/tube (de Léan et al. 1985a; Ong et al., unpublished

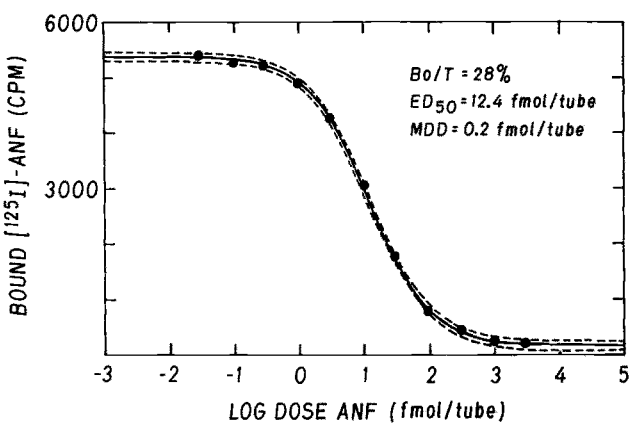


Fig. 3. Dose-response curve for ANF-radioreceptor assay. This representative curve was obtained by using an ANF-receptor preparation from bovine adrenal zona glomerulosa membranes and using ^{125}I-labeled rANF(99–126) and rANF(99–126) as radioligand and standard, respectively (from Ong et al., unpublished observations) B_0/T: percentage of total ligand bound in the absence of unlabeled ligand. MDD: minimal detectable dose

Table 8. Comparative cross-reactivity of ANF fragments in radioreceptor assay (RRA) and radioimmunoassay (from Ong et al., personal communication)

Fragments		Cross-reactivity (%)	
		RRA	RIA
rat ANF	(99–126)	100	100
human ANF	(99–126)	100	100
ANF	(101–126)	78	100
ANF	(103–126)	2.4	100
ANF	(103–125)	1.4	5
ANF	(103–123)	0.003	0
ANF	(111–126)	0	1
ANF	(116–126)	0	60

observations). Table 8 compares the cross-reactivity of ANF fragments assayed in the radioreceptor assay and in the commonly used commercial RIA. In contrast with the RIA, only the biologically active forms of ANF significantly cross-react in the radioreceptor assay; even the short inactive fragments ANF(111–126) and ANF(116–126) still cross-react in the RIA. The radioreceptor assay is thus highly specific for ANF(99–126), the biologically active and major circulating form of the hormone, while the shorter form ANF(103–126) in which the two important arginine residues 101 and 102 are lacking only cross-reacts at 2.4% in this assay (Table 8 and Ong et al., unpublished observations).

A further demonstration of the specificity of the assay is provided in Fig. 4, showing a reverse-phase HPLC profile of human plasma. The figure shows that virtually all the ANF activity detected in the radioreceptor assay is in a single peak coincident with ANF(99–126), while the commercial RIA also detects shorter and less active fragments eluting earlier in the chromatogram.

As with other assays, ANF radioreceptor assay requires the proper extraction of plasma in order to concentrate the low amounts of hormone circulating and to remove proteins and other interfering molecules. Bürgisser et al. (1985) reported on the application of this assay using an extraction procedure based on C_{18} Sep-Pak cartridges (Waters) washed and eluted with methanol/trifluoroacetic acid. Their results indicated plasma ANF levels in normal volunteers of 70 fmol/ml, a value clearly above that measured by RIAs (Bürgisser et al. 1985). Ong et al. (unpublished observations) described the application of an extraction procedure based on cyano Sep-Pak cartridges (Waters) eluted with methanol/acetic acid, and they reported mean normal plasma ANF levels of 3 fmol/ml and an excellent correlation with the results obtained by RIA.

ANF radioreceptor assay is applicable not only to human plasma but also to the detection of the hormone in tissues and cell cultures. It was successfully

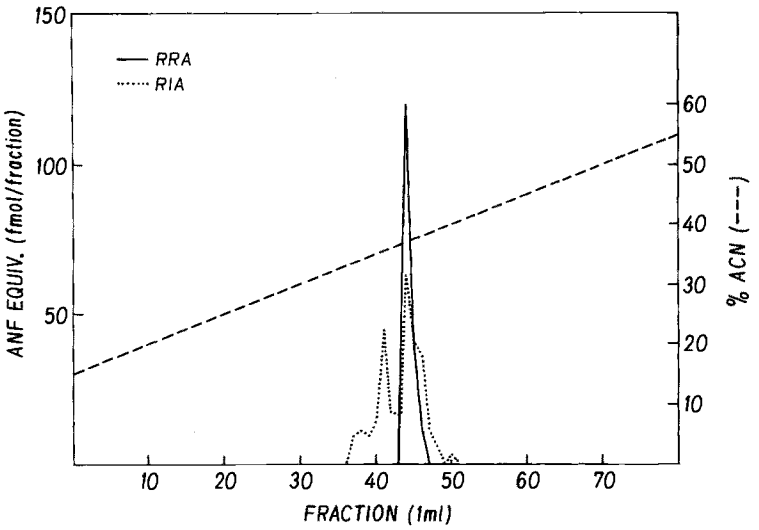


Fig. 4. Elution profile of human plasma on reverse-phase HPLC. Plasma (100 ml) was injected on a Bondapak C_{18} column and eluted with a gradient of 15%–55% acetonitrile (*ACN*) in 0.1% trifluoroacetic acid. ANF was assayed by radioreceptor assay (*RRA*) using bovine adrenal zona glomerulosa membranes or by radioimmunoassay (*RIA*) using a commercial antibody (Peninsula)

applied to the detection of an ANF-like polypeptide factor in adrenal medulla (de Léan et al. 1985a) and in cultured chromaffin cells (Nguyen et al., unpublished observations), and it was used during the purification of bovine ANF (Ong et al. 1986). The radioreceptor assay is thus an attractive alternative to the RIA, and because of its specificity and sensitivity it offers advantages for clinical and experimental studies.

5 ANF in Extra-atrial Tissues (J. Gutkowska and U. Nemer)

Following the discovery of ANF bioactivity (de Bold et al. 1981), it was generally thought that ANF synthesis was exclusive to atria. This belief was reinforced by previous findings of secretorylike granules present in atria but not in ventricles (Jamieson and Palade 1964), and in early bioassays ventricular extracts were used as negative controls (de Bold et al. 1981).

With the development of specific immunocytochemical assays and RIAs and the availability of cDNA probes, it became possible to assess the distribution of ANF mRNA and peptides in extra-atrial tissues. The ANF gene now appears to be expressed in a number of tissues including heart ventricles, anterior pituitary, lung, aortic arch, and various brain regions. In addition,

ANF immunoreactivity has been detected in a number of other tissues in man and in various animal species.

5.1 ANF Gene Expression in Heart Ventricles

When rat or human cDNA clones are used as hybridization probes in Northern blot analysis, a single band of about 950 nucleotides is detected in RNA prepared from the atrial tissues of various mammalian species. In rat atria, the abundance of the mRNA species hybridizing to ANF probes is estimated to be 1%–3% of the total mRNA, indicating that the ANF gene is very efficiently transcribed in atrial tissues. Furthermore, Northern blot analysis of RNA prepared separately from right and left atria reveals that ANF mRNA concentrations are about twofold higher in the right than in the left atria of rats and hamsters (Nemer et al., unpublished observations); this ratio is similar to that of IR-ANF in the atria.

As well as RNA from atria, RNA prepared from rat ventricles also contains a weak hybridizing band which comigrates with the atrial transcripts, suggesting that the ANF gene is also expressed in ventricles, albeit to a much lesser extent (Zivin et al. 1984). The expression of the ANF gene in ventricular tissues was further analyzed using in situ hybridization to rule out any contamination from atrial tissues. With the aid of a ^{32}P-labeled ANF cDNA probe in in situ hybridization experiments, specific labeling was observed in both atrial and ventricular cardiocytes, confirming the presence of ANF transcripts in heart ventricles (Nemer et al. 1986b). ANF transcripts are identical in size in atria and ventricles, as was revealed by Northern blot analysis (Zivin et al. 1984; Nemer et al. 1986b; Gardner et al. 1986a; Lattion et al. 1986). The initiation of transcription is similar in both heart compartments, as was revealed by nuclease mapping and primer extension (Nemer et al., unpublished data; Gardner et al. 1986a). The abundance of ANF transcripts in ventricles was found to be 100–150 times lower than in atria (Nemer et al. 1986d). The ANF gene is expressed in all three ventricular compartments, as was demonstrated by the Northern blot analysis of total RNA prepared separately from right and left ventricles and the interventricular septum. ANF mRNA levels are highest in the left ventricle and lowest in the right. The relative abundance of ANF mRNA in left ventricle vs intraventricular septum vs right ventricle is 8:3:1 (Nemer et al., unpublished data). Given the much larger mass of the ventricles, the ratio of total ventricular to atrial ANF transcripts is about 1:5.

The molecular weight distribution of ventricular ANF peptides is identical to that of atrial peptides, as was revealed by HPLC analysis. However, ventricular ANF content is 1000-fold lower than atrial ANF content (Nemer et al. 1986d). Taken together with the absence of granules and the lower accu-

mulation of ANF in the ventricles, the tenfold discrepancy between the abundance of ventricular ANF transcripts and that of peptides suggests that ANF secretion from ventricular cardiocytes is of the constitutive type (Bloch et al. 1986). Thus, it appears that the ANF gene is expressed in all heart compartments and that ANF is a cardiac and not an exclusively atrial hormone, as was originally thought.

5.2 ANF in Brain and Central Nervous System

The finding of both high and low molecular weight forms of IR-ANF in the rat hypothalamus was the first demonstration of the extracardiac presence of ANF peptides (Tanaka et al. 1984). An elevated intake of sodium was shown to decrease the ANF content of the hypothalamus, in contrast to the effect of such an increased intake on the plasma content.

Jacobowitz and his coworkers (1985) studied IR-ANF-containing neurons in the rat brain, using rabbit antiserum generated against synthetic rat 25-amino-acid COOH-terminal peptide coupled to thyroglobulin. These investigators discovered ANF-containing nerve fibers and cell bodies in the preoptic-hypothalamic area, amygdala, mesencephalon, and pons. This same group undertook a detailed immunohistochemical mapping of ANF in the rat brain and spinal cord (Skofitsch et al. 1985). ANF-like immunoreactivity was evident throughout the rat nervous system. Relatively high densities of IR-ANF were observed in the anteroventral third ventricular area. A similar localization of ANF-like immunoreactivity in the rat brain was reported by other groups (Saper et al. 1985; Kawata et al. 1985a, 1985b; Standaert et al. 1986a) which employed immunocytochemical methods or RIAs. The largest collection of IR-ANF-containing neurons was found in the hypothalamus, with stained bipolar ANF neurons being demonstrated in the anteroventral periventricular preoptic nucleus adjacent to the anteroventral extreme of the third ventricle. Prominently stained neurons were also observed in the pons (Saper et al. 1985). Furthermore, ANF was colocalized in the neurons of the pontine tegmentum and in lateral dorsal tegmental nuclei with choline acetyltransferase- and substance P-like immunoreactivity (Standaert et al. 1986b).

Specific RIAs revealed the highest levels of IR-ANF in the hypothalamus (23.2 ± 3.6 ng/g wet tissue), septum (20.7 ± 1.27 ng/g wet tissue), and midbrain (8.2 ± 0.64 ng/g wet tissue), with small quantities in the cerebral cortex, olfactory bulb, thalamus, pons-medulla, and hippocampus and with trace amounts in the striatum, cerebellum, and pituitary (Kawata et al. 1985b; Morii et al. 1985).

The presence of IR-ANF in the brain suggests that the ANF gene may be expressed therein. However, few data are available on the presence of ANF

mRNA in the brain, probably as a result of technical difficulties in detecting low levels of transcripts. By means of sensitive cRNA probes, ANF transcripts similar in size to their atrial counterparts were recently detected in the rat hypothalamus, amygdala, and brain stem (Hang et al. 1986). The finding that these transcripts originate in the brain was also confirmed by the in situ hybridization of tissue sections with ^{35}S-labeled ANF cRNA probes (Nemer et al., unpublished observations).

The molecular weight forms of brain atrial natriuretic peptides are still unknown. A recent study (Shiono et al. 1986) using reverse-phase (RP) HPLC coupled with RIA revealed two major components of IR-ANF in extracts of whole rat brain. The HPLC elution patterns of both IR-ANF forms were similar to COOH-terminal peptides composed of 24 and 25 amino acids, respectively (Shiono et al. 1986). The 24-amino-acid COOH terminal was also found in extracts of rat preoptic-hypothalamic tissues (Zamir et al. 1986). In contrast to these results, Glembotski et al. (1985) reported the presence of a 2.8-kDa ANF-like peptide in the hypothalamus. Since ANF synthesis takes place in at least some brain regions, it would not be surprising if high molecular weight precursor forms of ANF were found there. However, only amino acid analysis of IR-ANF in various parts of the brain could resolve this controversy. ANF peptides are present not only in the rat brain. IR-ANF-containing neurons have been detected in lateral parts of the nucleus periventricularis and amygdaloid complex of *Tupaia belangeri* (Forssmann and Mutt 1985).

The possible role of ANF as a neurotransmitter and neuroregulator was suggested by finding IR-ANF in the peripheral autonomic nervous system. A biologically active ANF-like peptide was demonstrated in rat ganglia nodosa (Debinski et al. 1986) and in superior cervical ganglia (Debinski et al., unpublished data; Gutkowska et al. 1987b). The discovery of ANF-like material in the neurocardiac system of the snail has added an interesting dimension to the phylogenesis of ANF. A high molecular weight peptide (presumably the prohormone) has been noted in the subesophageal ganglion, and a low molecular weight form has been demonstrated in both this same tissue and the atria.

The function of ANF in the brain is not known, but the largest concentration of positively stained ANF-containing cells is located in the anteroventral third ventricle (AV3V), a region associated with blood pressure regulation and volume homeostasis (Hartle and Brody 1984). These observations suggest that ANF may be involved in the central regulation of fluid and salt balance. Moreover, its presence in the nucleus periventricularis suggests that ANF could modulate cardiovascular centers.

5.3 ANF in the Eye

The eye appears to be a target organ for ANF, as both ANF receptors and peptides have been found therein. IR-ANF has been demonstrated in the rat anterior uvea and retina with an abundance of 31 and 8 ng/g wet tissue, respectively (Stone and Glembotski 1986). This is comparable with the concentration of IR-ANF in the hypothalamus (approximately 50 ng IR-ANF/g wet tissue). The RP-HPLC elution pattern of extracts of both tissues indicates that the main component is the 28-amino-acid COOH-terminal peptide. The presence of an ANF-like peptide in the anterior uvea, and of ANF receptors in this tissue, implies that this peptide may participate in the regulation of intraocular fluid pressure.

5.4 ANF in the Kidney

The kidney is a major target organ for ANF (Sakamoto et al. 1985; McKenzie et al. 1985), and not surprisingly IR-ANF has been detected in various parts of the kidney. Most of the IR-ANF determined by RIA has been found in the renal cortex. Analysis of rat kidney extracts by high-performance gel permeation chromatography (HP-GPC) coupled with RIA has revealed that only low molecular weight ANF fragments are present in the rat kidney. Water deprivation significantly decreases renal IR-ANF levels and a substantial diminution is observed after perfusion with saline. The concentration of IR-ANF in the kidney is only about 5 ng/g tissue. It is therefore difficult to assess whether ANF is derived from circulating blood or from ANF receptors in this organ. Nevertheless, the detailed immunocytochemical studies made by McKenzie et al. (1985) have revealed intense intracellular staining in the cortical and medullary collecting tubules and ducts, not only in rat but also in horse, pig, monkey, and human kidneys. The localization of ANF in renal collecting tubules and ducts may have important implications for its proposed action sites. Recent investigations strongly suggest that the peptide may act distally in renal collecting ducts (Briggs et al. 1982; Sonnenberg et al. 1981).

5.5 ANF in Other Tissues

ANF transcripts and ANF immunoreactivity have also been detected in the lung, anterior pituitary, and aortic arch. In all these tissues, the ANF transcripts are similar to cardiac transcripts, though present in a relative abundance of about 0.01% (Gardner et al. 1986a, 1987). Just as in the atria, water deprivation decreases ANF peptide levels in the lung (Sakamoto et al. 1986), where both high (Gutkowska et al. 1987a) and low (Sakamoto et al.

1986) molecular weight forms have been detected. No information is available on the molecular weight forms of anterior pituitary IR-ANF, which is found in pituitary gonadotrophs (McKenzie et al. 1985; Gardner et al. 1986a). ANF immunoreactivity has also been found in the posterior lobe of the pituitary (Samson 1985a; Gutkowska et al. 1987b). In the aortic arch, IR-ANF has been localized to the adventitia of the arch in regions thought to contain the aortic baroreceptors (Gardner et al. 1987). This suggests a role for ANF in blood pressure regulation through the baroreceptor reflex.

In addition, ANF immunoreactivity has been detected in adrenal medullary chromaffin cells (McKenzie et al. 1985) at a 20000-fold lower concentration than in the right atrium (Matsuo et al. 1985). Small amounts of IR-ANF have also been found in the submaxillary gland (Cantin et al. 1984c; Matsuo et al. 1985) and in the thyroid gland (Matsuo et al. 1985).

The finding of ANF peptides in different tissues suggests that this cardiac hormone ends up in a number of target organs, possibly to regulate blood pressure and fluid balance. Furthermore, the presence of low amounts of ANF mRNA and peptides in extracardiac tissues suggests local functions which remain to be elucidated.

6 Mechanisms of Release (R. Garcia)

The diuresis and natriuresis resulting from left atrial distension are attributed to stretch receptors in the left atrium which would be responsible for a homeostatic mechanism linking changes in circulatory volume with renal responses (Henry et al. 1956a). However, the distension of the right atrium (Kappagoda et al. 1973) and of both atrial appendages (Kappagoda et al. 1972) has similar effects, and these are enhanced in blood volume-expanded animals (Gupta et al. 1982).

The diuresis elicited by left atrial distension has been ascribed partly to an inhibition of arginine vasopressin release (Gupta et al. 1982; Baisset and Montastruc 1957; De Torrente et al. 1975; Kaczmarczyk et al. 1983; Ledsome and Wilson 1984), but the natriuretic response is more difficult to explain. It has also been suggested that the diuresis induced by the distension of the left atrium is probably due to a diuretic factor of unknown origin (Linden 1979). The discovery of ANF in secretorylike atrial specific granules (de Bold 1982a; Garcia et al. 1982) has provided new perspectives in our understanding of the mechanisms controlling diuresis and natriuresis induced by increased intra-atrial pressure. The first indirect evidence that ANF can be released by the atria came from Dietz (1984). Using an isolated rat heart-lung preparation, Dietz demonstrated the presence of a natriuretic substance in the perfusate fluid, arising in response to increases in right atrial pressure. This finding was

confirmed by a specific ANF RIA. It was further demonstrated that the peptide can be released not only by an isolated preparation but also into the circulation in vivo following saline volume expansion (Cantin et al. 1984a; Lang et al. 1985). In anesthetized dogs, raising left atrial pressure by means of a distensible balloon releases ANF into the circulation. This is not modified by vagotomy, β-blocking, or direct sympathetic nerve stimulation (Ledsome et al. 1985, 1986). Thus, the ANF release appears to be stimulated by mechanisms producing atrial distension and to be related more to a direct stretch than to a reflex triggered by vagal or sympathetic efferents. However, the fact that ANF released in the dog is stimulated by atrial stretch does not necessarily mean that the peptide is involved in the natriuresis observed during atrial distension. Experiments in conscious dogs designed to raise right or left atrial pressure by atrial stretch have failed to demonstrate a relationship between plasma ANF and the renal response. Left atrial distension increased sodium excretion and ANF release, whereas right atrial distension produced an increase in ANF release of the same magnitude but no change in either diuresis or natriuresis (Goetz et al. 1986). Moreover, cardiac denervation abolishes the natriuresis induced by left atrial distension, but not the release of ANF (Goetz et al. 1986). This also suggests that ANF could not be the mediator of the natriuresis induced by dilating the left atrium with a balloon. However, cardiac denervation does not affect the natriuresis induced by an acute volume expansion in the conscious dog (Kaczmarczyk et al. 1981; Fater et al. 1982), which is accompanied by an increase in atrial pressure (Kaczmarczyk et al. 1981) and ANF release (Salazar et al. 1986).

Since the atrial pressure created by distending the atria with a balloon is higher than that generated during moderate changes in blood volume (Henry et al. 1956b), it is quite possible that the natriuresis induced in conscious dogs by such mechanical atrial distension is mediated by the triggering of a neural mechanism, whereas the natriuresis induced by moderate volume changes (volume expansion) could be mediated by a humoral factor (ANF).

In intact anesthetized rats, acute volume expansion with either isotonic saline (Lang et al. 1985), a colloidal solution, or whole blood (Anderson et al. 1986a) produces a marked and rapid release of ANF into the circulation. Right (Veress and Sonnenberg 1984) or bilateral (Kobrin et al. 1985) atrial appendectomy and the administration of ANF antibodies (Cantin et al. 1984c) blunt both the natriuretic response to volume expansion and, in bilateral operations, ANF release (Villarreal et al. 1986a). It has recently been demonstrated (Garcia et al. 1987c) that graded blood volume expansion (10% – 30% of total blood volume) in conscious rats induces gradual increases in natriuresis and in ANF release which are completely abolished by right, but not left, atrial appendectomy (Fig. 5). These findings suggest that in the rat it is the right atrium which has an important role in regulating sodium and water balance during volume expansion. This hypothesis is supported by the

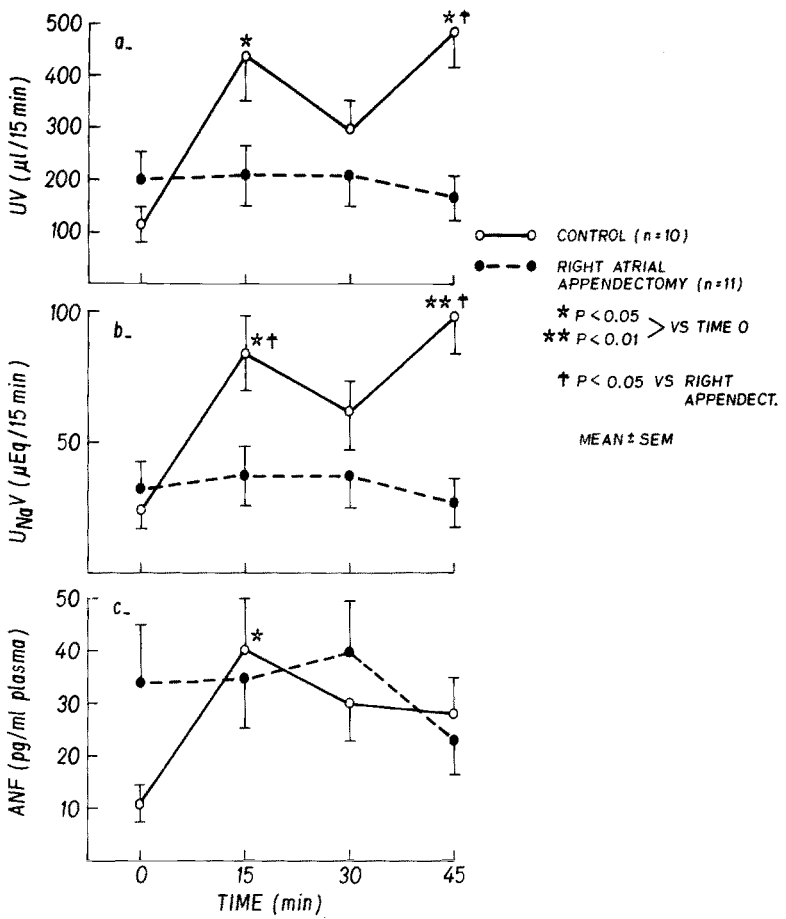


Fig. 5a–c. Effect of right atrial appendectomy; **a** on diuresis, **b** on natriuresis, and **c** on plasma ANF during graded blood volume expansion. *UV*, urine volume; $U_{Na}V$, urinary sodium volume

fact that when right atrial pressure is prevented from rising during volume expansion, natriuresis and diuresis are abolished (Ackermann and Rudolph 1983). Moreover, the renal response can be abolished during volume expansion in rats by the administration of ANF antiserum (Cantin et al. 1984c; Cantin and Genest 1985; Hirth et al. 1986). This supports the hypothesis that ANF may be the humoral mediator of the atrial regulation of body fluid homeostasis in rats. The published data on dogs are more conflicting; this may reflect species differences and the relative importance of neural as against humoral systems in sodium excretion.

The involvement of ANF during chronic changes in water and sodium homeostasis in the rat has been more difficult to evaluate. Water deprivation decreases plasma levels of ANF (Takayanagi et al. 1985; Januszewicz et al.

1986c; Schwartz et al. 1986), which are rapidly restored by rehydration (Januszewicz et al. 1986c). Such deprivation increases the atrial ANF content (Schwartz et al. 1986; Takayanagi et al. 1985), while reducing ANF mRNA levels (Takayanagi et al. 1985). A high-salt diet lasting several weeks increases plasma ANF levels and decreases the atrial ANF content (Takayanagi et al. 1985; Schwartz et al. 1986), while the atrial ANF mRNA content is unchanged. These results suggest that chronic changes in blood volume induced by diet manipulation may modify not only ANF release but also its synthesis in atrial tissue. However, contradictory results have also been reported. Luft et al. (1986b) demonstrated that rats kept for 3 weeks on either a high, low, or normal sodium diet modified their urinary sodium excretion, plasma renin activity (PRA), and urinary aldosterone secretion accordingly, but failed to show any change in plasma ANF levels. This suggests that the sodium metabolism may well be regulated by humoral mechanisms other than ANF during chronic changes in sodium intake. A similar lack of modifications in plasma ANF during chronic changes in sodium ingestion was also described in the dog (Salazar et al. 1986b). Whether or not these apparently contradictory data are the result of different experimental protocols remains to be elucidated.

The effects that changes in blood volume induced by mineralocorticoid administration may have on plasma ANF levels have been equally inconsistent. Both normal (Luft et al. 1986b; Gardner et al. 1986b) and high (Schiffrin and St-Louis 1987; Ballermann et al. 1986) plasma ANF levels have been reported in the rat. The results obtained with the administration of glucocorticoids have been less conflicting: these have been shown to stimulate not only the release of ANF into the circulation but also its synthesis by atrial tissue (Gardner et al. 1986b; Garcia et al. 1985a; Lachance et al. 1986; Nemer et al. 1986a, 1987).

Hypophysectomy blocks the release of ANF induced by volume expansion, but the response can be restored if an anterior pituitary is reimplanted. Since bilateral adrenalectomy does not modify ANF release induced by the same stimulus, it has been suggested that hormones (yet to be determined) from the anterior pituitary may be required for the secretion of ANF during acute volume loading (Zamir et al. 1987).

Assays to study the possible effects of the in vivo administration of various agonists on ANF release in the rat have also been controversial. The bolus administration of pressor doses of arginine vasopressin, phenylephrine, and angiotensin II produces a significant increase in plasma ANF levels (Manning et al. 1985), which has been attributed to changes in right atrial pressure (Katsube et al. 1985a). Experiments in our own laboratory have demonstrated that chronic infusions of subpressor doses of phenylephrine into rats do not modify plasma ANF levels (Garcia et al. 1986b). This suggests that α-adrenergic receptors are not involved in ANF release. Phenylephrine infusions

into six normal subjects at low pressor doses (BP from basal levels of $113 \pm 8/66 \pm 6$ mmHg to $128 \pm 7/78 \pm 3$ mmHg) and high pressor doses (BP to $144 \pm 3/90 \pm 7$ mmHg) resulted in significant increases in plasma IR-ANF (from 4.8 ± 1.7 to 9.8 ± 3.4 and 13.6 ± 8.4 pmol/l, respectively) (Closas et al., unpublished observations). Clonidine, an α_2-adrenergic receptor agonist, has been shown to induce a marked dose-related increase in plasma ANF levels which correlates well with the increased urine output in normal rats (Baranowska et al. 1987a). This effect is partially inhibited both by the selective α_2-adrenergic antagonist, yohimbine, and by naloxone. Moreover, yohimbine decreases basal plasma ANF levels, while verapamil has an effect on basal or clonidine-stimulated ANF (Baranowska et al. 1987b). However, since the clonidine dose administered could be sufficient to induce significant hemodynamic changes, the mechanism involved in ANF release is difficult to interpret.

The infusion of angiotensin II into the rat stimulates ANF release only when there is a rise in left ventricular end-diastolic pressure and even in the absence of any change in right atrial pressure (Lachance et al., unpublished observations). This observation, the findings in volume-expanded animals with a right atrial appendectomy (Veress and Sonnenberg 1984; Garcia et al. 1987b), and the finding that in hypertensive rats only the left atrial tissue has a lower ANF content (Imada et al. 1985; Gutkowska et al. 1986b; Garcia et al. 1987d) suggest that preload (increased venous return) may stimulate ANF release mainly from the right atrium, whereas afterload (increased left ventricular end-diastolic, hence, left atrial pressure) may stimulate ANF release from the left atrium.

Several anesthetics and central nervous system depressants have been reported to enhance ANF release (Horky et al. 1985). For example, pharmacological doses of morphine induce a substantial rise in plasma ANF levels, which is blocked by specific antagonists (Gutkowska et al. 1986d). Other opioid receptor agonists such as fentanyl, pentazocine, and ethylketocyclazocine induce similar increases in plasma ANF concentration, which are significantly correlated with an enhanced urinary output (Gutkowska et al. 1987a). However, both the mechanisms of morphine-stimulated ANF secretion and the question of whether or not endogenous opioids play any physiological role in the regulatory mechanisms of ANF release need further investigation.

In order to avoid the hemodynamic effects that agonist administration may have in the intact animal, in vitro ANF release studies have also been carried out on atrial minces. It was reported (Sonnenberg and Veress 1984; Sonnenberg et al. 1984) that α-adrenergic, cholinergic, and vasopressin stimulation release ANF from atrial minces. This suggests a direct, receptor-mediated release. Other investigators (Lachance et al. 1986; Arjamaa and Vuolteenaho 1985) failed to reproduce these results. One explanation could be that dif-

ferent methods were used to assess ANF in the media. Sonnenberg and Veress (1984; Sonnenberg et al. 1984) used a bioassay, hence an unspecific effect may have been induced by the injected media, whereas the other investigators used a specific RIA.

Since the isolated perfused rat heart has been shown to release ANF in response to changes in atrial pressure (Lang et al. 1985), and since it is independent of any influence from the nervous system, it has been widely used in attempts to clarify the release mechanisms of the cardiac peptide. The α_1-adrenergic receptor agonist phenylephrine has been reported to stimulate ANF release (Currie and Newman 1986); however, this finding has not been confirmed by in vivo experiments involving either agonist infusion (Garcia et al. 1986b) or direct sympathetic nerve stimulation (Ledsome et al. 1986). The same preparation has been used in the attempt to establish which intracellular pathways are involved in the secretion of ANF by atrial cardiocytes. It now seems clear enough that any maneuver conducive to a direct stretch of the atrial wall stimulates ANF release; however, it is not yet known which internal signal responds to this stretch. Recently (Ruskoaho et al. 1985), it has been reported that phorbol ester and a calcium ionophore stimulate the secretion of ANF from isolated perfused hearts. This suggests that calcium mobilization and a calcium-activated protein kinase C may have a role in the secretion of ANF by atrial cardiocytes. It has also been reported (Ruskoaho et al. 1986; Saito et al.1986) that Bay K 8644, a voltage-sensitive calcium channel agonist, and forskolin (Ruskoaho et al. 1986), which is known to stimulate adenylate cyclase, increase the release of ANF by the perfused rat heart. This suggests that the intracellular pathway stimulating the secretion of ANF by atrial cardiocytes could be mediated by the phosphoinositide and the cyclic adenosine monophosphate (cAMP) systems.

It was first reported over 30 years ago that polyuria may follow prolonged episodes of paroxysmal supraventricular tachycardia, which are often accompanied by hypotension, normal cardiac output, and increases in right and left atrial pressure (Borst 1954; Wood 1963). More recently, levels of plasma ANF were found to increase during such episodes and to return rapidly to normal once the tachycardia is subdued (Schiffrin et al. 1985b; Yamaji et al. 1985a; Tikkanen et al. 1985b). The question raised by these findings is whether ANF release is stimulated by the increase in heart rate per se or by the changes in intracavitary pressure that tachycardia may induce. The in vitro electrical pacing of isolated rat atria demonstrates a positive relationship between the frequency of stimulation and the release of ANF into the media. This suggests that the frequency of atrial contraction in the absence of changes in intracavitary pressure may be an important factor in the release of ANF during tachycardia (Schiebinger and Linden 1986b). These results are not affected by the administration of α- and β-adrenergic or cholinergic antagonists, so the effect of pacing may not be due to the release of agonists by the atrial nerve

terminals. These results contrast with other in vitro and in vivo investigations. When isolated perfused rat heart is paced to induce heart rates within physiological limits, 300–500 beats per minute, no change in ANF release is observed (Lachance et al., unpublished observations). Cardiac pacing in the intact rat (Rankin et al. 1986) results in an increase in plasma ANF levels, which is always accompanied by a rise in right atrial pressure. Tachycardia in the dog leads to a rise in plasma ANF levels when, and only when, there is a concomitant increase in atrial pressure (Ledsome et al. 1986). Moreover, during volume depletion in the rat, an increase in heart rate and a depression of ANF release have been observed (Garcia et al. 1987b). These results suggest that the stimulation of ANF release observed during tachycardia is dependent not on the heart rate itself, but on the changes in atrial pressure that changes of heart rate may induce.

In humans, procedures that increase venous return have been demonstrated to elicit ANF release. Thus, acute volume expansion by means of a saline infusion (preload) induces a rapid and marked release of ANF in normal subjects (Anderson et al. 1986b; Weil et al. 1985; Sagnella et al. 1985). The changes in the levels of plasma ANF are significantly correlated with those in right atrial pressure (Anderson et al. 1986b). This is consistent with the hypothesis that in both humans and experimental animals, atrial distension is the main stimulus for ANF release. Moderate oral sodium loading for 3 days increases plasma ANF levels in normal subjects, and this increase is positively correlated with urinary sodium excretion and a weight gain (Hollister et al. 1986; Gutkowska et al. 1986d). Because sodium loading increases plasma volume, we may suppose that venous return and right atrial pressure are also elevated. Since atrial pressure and sodium excretion may also be modified by changes in body posture, several groups have investigated whether the same is true for plasma ANF. Leg raising, body tilting, or upright – supine movements (Larose et al. 1985; Hollister et al. 1986; Ogihara et al. 1986a; Solomon et al. 1986) are all maneuvers conducive to modifications of plasma ANF levels. This suggests that ANF plays a moment-to-moment volume regulatory role.

Thermoneutral, head-out water immersion causes an increase in central blood volume, raising central venous pressure (this being due to a shift of blood from the periphery) and inducing diuresis and natriuresis (Epstein 1978). The natriuresis observed during head-out water immersion was thought to be due to a decrease in plasma aldosterone levels. But recently, with the discovery of ANF, the natriuresis induced by this maneuver has received renewed attention. Several studies (Anderson et al. 1986c; Ogihara et al. 1986b; Gerber et al. 1986) on normal volunteers have demonstrated that head-out water immersion elicits a significant increase in plasma ANF levels, parallel to the observed natriuresis. These findings suggest once again that ANF is released into the plasma in response to an increased venous return and atrial distension.

7 ANF Receptors

7.1 Localization of Binding Sites (M. Cantin)

Binding sites for ANF have been localized by in vivo and in vitro radioautography in a wide variety of tissue and cell types.

7.1.1 Periphery

Kidney. The injection of ^{125}I-labeled ANF (Arg-101 to Tyr-126) into the rat aorta (through the carotid artery) revealed the presence of numerous binding sites in the whole renal cortical vasculature, but particularly in the glomeruli, where capillary loops were heavily labeled (Bianchi et al. 1985), in the outer medullary descending vasa recta, and in inner medullary collecting duct cells (Bianchi et al. 1987).

Comparative ultrastructural study of ^{125}I-labeled ANF and ^{125}I-labeled angiotensin II binding in rat glomeruli after their intra-aortic injection revealed that most of the former peptide (63%) binds to visceral epithelial cell podocytes, while most of the latter peptide (60%) binds to mesangial cells (Bianchi et al. 1986c) (Fig. 6).

Some of these results have been confirmed by radioautography in vitro using various iodinated ANF fragments. Thus ^{125}I-labeled ANF (atriopeptin III – Ser-103 to Tyr-126) has been localized in the glomeruli and papillae of rat kidney (Murphy et al. 1985; von Schroeder et al. 1985). Using ^{125}I-labeled ANF (Ser-99 to Tyr-126), binding to rat glomeruli and inner medullary collecting ducts has also been shown (Koseki et al. 1986b, 1986c; Lynch et al. 1986; Healey and Fanestil 1986). The ultrastructural localization (Fig. 7) of ^{125}I-labeled ANF binding sites in glomerular epithelial podocytes, the vasa recta, and medullary duct cells has also been confirmed (Koseki et al. 1986b, 1986c).

In vitro radioautographic studies in guinea pig and human kidneys revealed a pattern of binding sites localized in glomeruli, renal arteries, and the outer medulla (cortical collecting tubules) (Mantyh et al. 1986).

Adrenal Cortex and Medulla. Light-microscope radioautographic studies involving ^{125}I-labeled ANF (Arg 101 to Tyr-126) injected in vivo have confirmed that receptors are present in the rat zona glomerulosa (Bianchi et al. 1985). They are also present in the endothelial and smooth muscle cells of adrenal cortical arterioles, veins, and venules, and on the endothelium of the zona glomerulosa capillaries (Bianchi et al. 1985). A small number of binding sites have been localized on norepinephrine- and epinephrine-storing cells of the adrenal medulla (Bianchi et al. 1985). Here again, these results have been con-

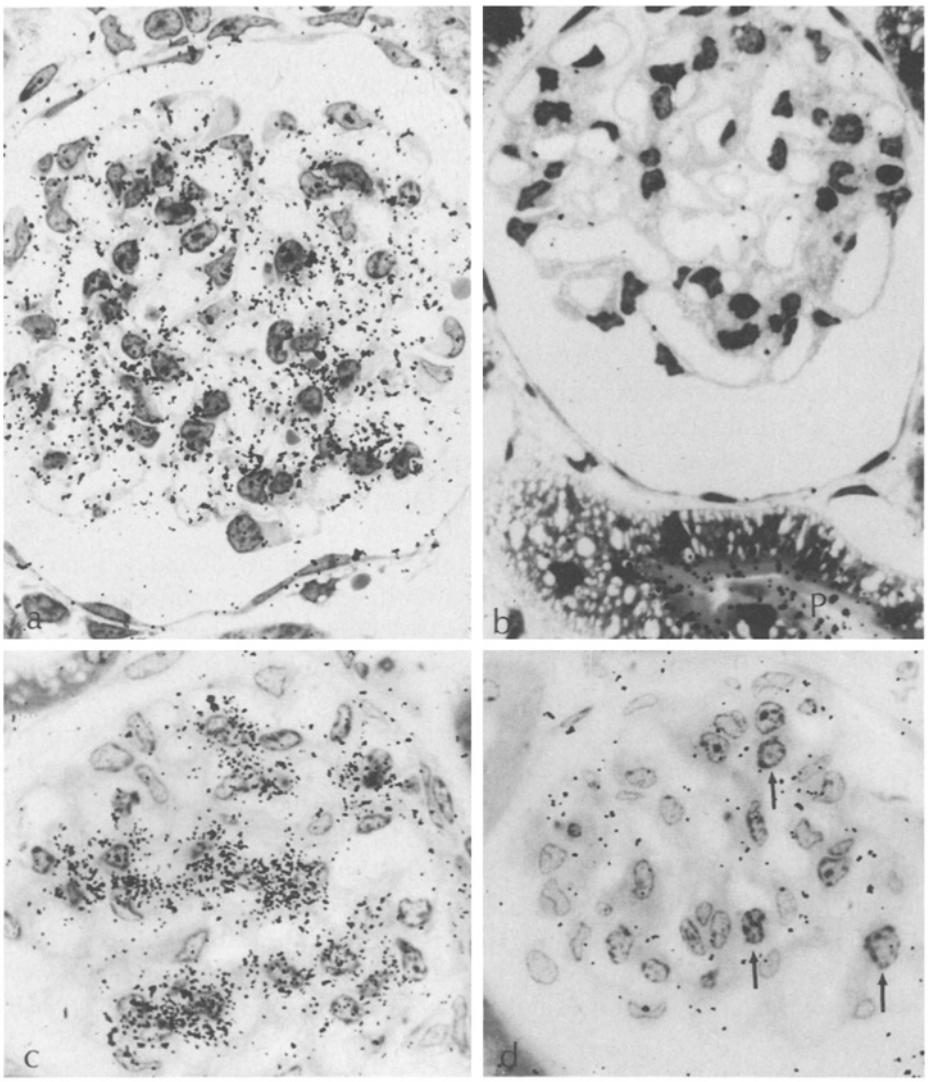


Fig. 6. **a** Glomerulus after injection of ^{125}I-labeled ANF. The silver grains follow the contour of glomerular capillaries ×1000. **b** Glomerulus after injection of ^{125}I-labeled ANF with an excess of ANF. While silver grains are almost totally absent over glomerular structures, they are still present over the lumen and brush border of proximal tubules (*P*)×1000. **c** Glomerulus after injection of ^{125}I-labeled angiotensin II (AII). Silver grains are localized in clusters over the axial region of glomerulus ×1000. **d** Glomerulus after injection of ^{125}I-labeled AII following 30-min infusion with saralasin (Sar^1-Ala^8-AII). Note paucity of silver grains located over mesangial cells (*arrows*) ×1000. (Reproduced with the permission of the authors and the editors of Am J Physiol 1986, 251:F594–F602)

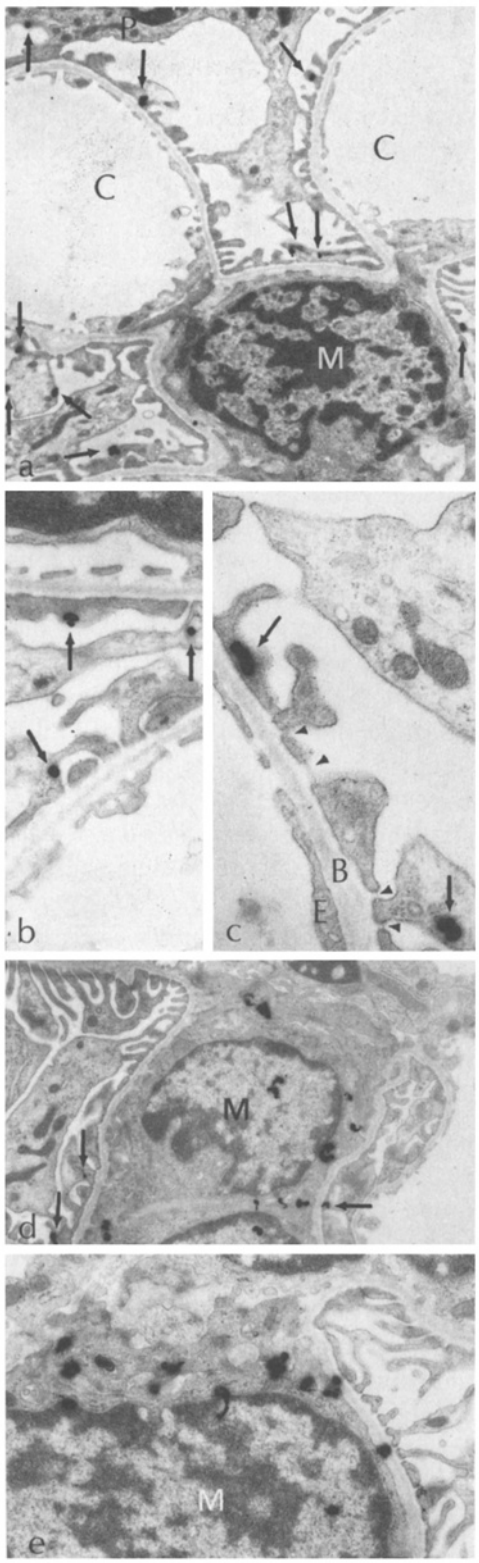


Fig. 7. **a** Part of glomerulus after injection of ^{125}I-labeled ANF. Note silver grains over the podocytes (*arrows*) of epithelial visceral cells (*P*). *M*, mesangial cell; *C*, capillary lumen ×7800. **b** Detail of glomerulus after injection of ^{125}I-labeled ANF. Silver grains are located over podocytes of epithelial cells (*arrows*) ×18390. **c** Detail of glomerulus after injection of ^{125}I-labeled ANF. Silver grains are located over podocytes of epithelial cell (*arrows*). Slit diaphragm (*arrow heads*); *E*, endothelium; *B*, basement membrane ×25430. **d** Part of glomerulus after injection of ^{125}I-labeled AII. Silver grains are mostly localized over mesangial cells (*M*). Three grains (*arrows*) are located over podocytes of visceral epithelial cells ×7880. **e** Detail of glomerulus after injection of ^{125}I-labeled AII. Most of the silver grains are located at the periphery of a mesangial cell (*M*) ×13030. (Reproduced with the permission of the authors and the editors of Am J Physiol 1986, 251:F594–F602)

firmed in the rat by in vitro radioautography (Murphy et al. 1985; von Schroeder et al. 1985; Lynch et al. 1986; Mantyh et al. 1986). In the guinea pig, binding sites are exclusively localized in the zona glomerulosa (Mantyh et al. 1986).

Other Tissues. Binding sites have been found in the rat in the endothelial lining of the four heart chambers (Bianchi et al. 1985). In the liver, they are localized on the smooth muscle and endothelial cells of all vascular beds, at a density proportional to the size of the vessels: the density of labeling is inersely proportional to vessel size. The endothelial cells of the sinusoids are less densely labeled, as are those of capillaries in portal spaces. Silver grains are also present on all parenchymal cells (Bianchi et al. 1985). The smooth muscle layer of guinea pig gallbladder contains abundant binding sites (Mantyh et al. 1986). The endothelial and smooth muscle cells of arteries, arterioles, veins, and venules of the lungs are consistently labeled. Label is also associated with the endothelial cells of all alveoli. Bronchi and bronchioles are not labeled (Bianchi et al. 1985). ANF binding sites have also been described in the lung parenchyma of the guinea pig (Mantyh et al. 1986). In the digestive tract of the rat, binding sites have been described close to the base of the mature columnar epithelium of villi in the duodenum, jejunum, and ileum (Bianchi et al. 1985). In the colon, label was exclusively associated with the smooth muscle cells of the muscular layer (Bianchi et al. 1985). In the guinea pig, label has also been found associated with the muscular layer of the colon and with the smooth muscle cells of the aorta (Mantyh et al. 1986). The posterior pituitary of the guinea pig revealed a substantial concentration of ANF binding sites (Mantyh et al. 1986).

7.1.2 Brain and Related Structures

Using in vitro radioautography in rats and guinea pigs, the distribution of binding sites was found to be unique and quite distinct from any other peptide receptor distribution previously visualized. A high density of binding sites was found in the subfornical organ, median eminence, area postrema, and nucleus tractus solitarii. A low density of binding sites was found in the lateral olfactory tract, subthalamic nucleus and locus ceruleus. Other areas rich in ^{125}I-labeled ANF binding sites are the olfactory bulb, choroid plexus, corpus callosum and ependyma (Quirion et al. 1984, 1986). With an in vivo radioautographic technique in the rat (Fig. 8), it was confirmed that circumventricular organs (the pineal gland, area postrema, subfornical organ, and organum vasculosum of the lamina terminalis, but not the subcommissural organ) were heavily labeled. The median eminence was also labeled, as were all cerebral vessels, the choroid plexus, and arachnoid (Bianchi et al. 1986b).

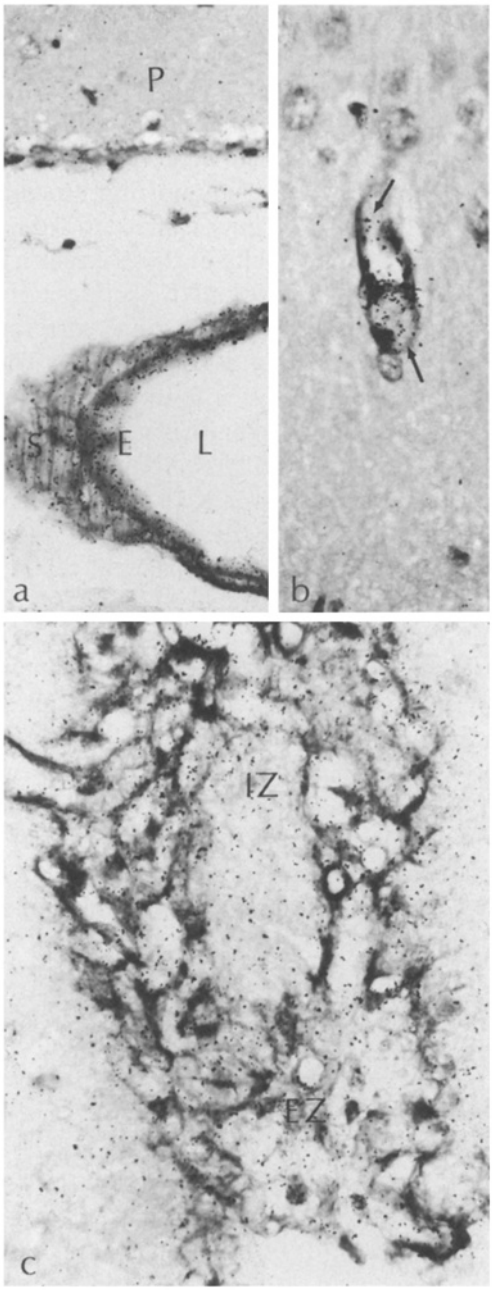


Fig. 8. a Coronal section of the brain of a rat 2 min after injection of ^{125}I-labeled ANF. Silver grains are localized on smooth muscle cells (*S*) and on endothelial cells (*E*) of a subarachnoid artery at the level of the amygdala cortex. *L*, lumen; *P*, cerebral parenchyma ×400. **b** Coronal section of the cortex of a rat injected with ^{125}I-labelled ANF alone. Silver grains (*arrows*) are localized on the endothelium of a capillary ×630. **c** Coronal section at the level of the organum vasculosum of the lamina terminalis. Silver grains are present on the parenchymal cells of the internal zone (*IZ*) and on the capillaries of the external zone (*EZ*) of a rat injected with ^{125}I-labeled ANF alone ×360. (Reproduced with the permission of the authors and publisher from Neuroendocrinology 1986, 44:365–372)

7.2 Vascular Receptors (E. L. Schiffrin)

Receptors for ANF in blood vessels have been described by Napier et al. (1984b) in rabbit aorta and by Schiffrin et al. (1985a) in rat mesenteric and renal arteries. These receptors have a high affinity (K_d of 50–500 pM) and low capacity (100–300 fmol/mg protein). The steady state binding data are in line with the affinity calculated from kinetic experiments (Schiffrin et al. 1985a). The specificity of these sites has been investigated. It was demonstrated that the potency of ANF peptides to relax phenylephrine-contracted aortic strips or mesenteric artery rings corresponds with the potency of these peptides in the binding assay (Table 9). The potency of ANF to relax agonist-precontracted vessels is lower than the affinity of ANF in the binding assay if a large dose of agonist is used (EC_{90}). However, at low agonist concentrations (EC_{50}), the EC_{50} of ANF in relaxing precontracted aorta is 30–50 pM. This contrasts with a K_d of 50–500 pM in the radioligand binding assay, possibly suggesting the presence of "spare receptors" for ANF. The density of ANF binding sites in different rabbit arteries and veins corresponds with the potency with which ANF relaxes these blood vessels (Winquist et al. 1985). Thus, a large number of sites were detected in tissues which respond well to low levels of ANF, such as the renal artery and facial vein; an intermediate number were detected in the pulmonary artery and vein; and few receptors were found in the ear and in femoral arteries.

After the initial studies on homogenates of blood vessels (Napier et al. 1984b; Schiffrin et al. 1985a), ANF receptors were demonstrated in cultured rat vascular smooth muscle cells (Hirata et al. 1984b; Schiffrin et al. 1986b) or in bovine aortic endothelial and smooth muscle cells (Schenk et al. 1985a). While in some studies the affinity of ANF smooth muscle receptors was 1–2 nM (Hirata et al. 1984b; Schenk et al. 1985a), in other studies it was

Table 9. Comparison of potency of atrial natriuretic peptides (ANP) on binding and relaxation of rat mesenteric artery and aorta

Peptide	Mesenteric artery		Aorta	
	Binding K_I (nmol/l)	Relaxation IC_{50} (nmol/l)	Binding K_I (nmol/l)	Relaxation IC_{50} (nmol/l)
ANP (99–126)	0.31	0.33	0.40	0.19
ANP (101–126)	0.24	0.46	0.29	1.06
ANP (103–126)	1.50	0.62	1.02	1.06
ANP (103–125)	1.70	0.62	1.22	1.34
ANP (103–123)	5.27	3.51	3.62	3.28

Means of 3–5 binding experiments and 8–12 relaxation experiments are represented. Vascular tissue was precontracted with an EC_{90} of phenylephrine (1 μM for mesenteric artery rings and 30 nM for aortic strips).

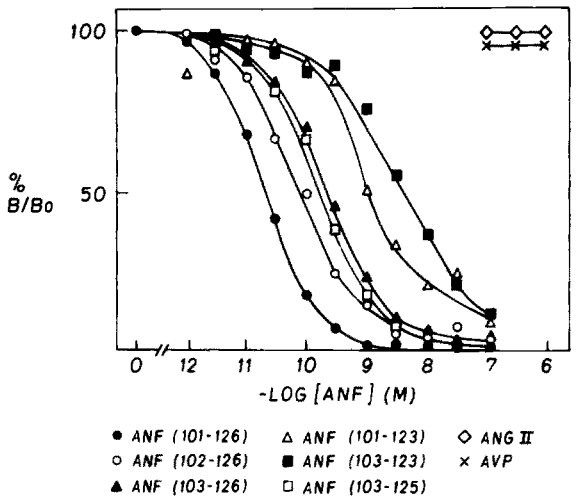


Fig. 9. Representative competition binding curve for ^{125}I-labeled ANF (101–126) vs. different ANF peptides on membranes prepared from cultured mesenteric artery-derived smooth muscle cells. (From Schiffrin et al. 1986d) %B/B_0: Labeled ANF bound at each concentration of unlabeled peptide as percent of labeled ANF bound in absence of unlabeled peptide

much higher (10–40 pM) (Schiffrin et al. 1986b). The latter study (Schiffrin et al. 1986b) was performed at 4 °C, in contrast to the former, carried out at 22 °C (Hirata et al. 1984b; Schenk et al. 1985a), and the absence of degradation during the incubation period was verified. Furthermore, the affinity found in saturation studies depends crucially on an accurate estimate of the specific activity of the radioligand, which was reported to be 100 μCi/μg in one study (Hirata et al. 1984b), in contrast to most other investigations, in which a specific activity of 400–600 μCi/μg is calculated for ^{125}I-labeled ANF (Napier et al. 1984b; Schiffrin et al. 1985a, 1986b; Winquist et al. 1985). This may have led to underestimation of the K_d, which is probably close to 50–100 pM. The potency of different ANF peptides to displace labeled ANF from these receptor sites (Fig. 9) is comparable with their potency to relax vascular smooth muscle (Table 9) and to induce cyclic guanosine monophosphate (GMP) formation (Hirata et al. 1985b).

However, a recent study by Scarborough et al. (1986) has shown that the deletion of amino acids at the carboxyl terminus of ANF significantly decreases the potency of these peptides to stimulate cyclic GMP production, but does not affect their affinity for the receptor in radioligand binding assays. These findings suggest the existence of multiple subpopulations of ANF receptors, some coupled to guanylate cyclase, others not. The exposure of cultured vascular smooth muscle cells to ANF over an 18-h period results in the down-regulation of ANF receptors (Hirata et al. 1985b; Schiffrin et al. 1986b) (Fig. 10) and of cyclic GMP responses (Hamet et al. 1986). Thus, the density of vascular ANF sites may be regulated by the ambient concentration of ANF; this has also been shown in vivo, as is further discussed below. ANF is rapidly internalized after binding, through receptor-mediated endocytosis, and is delivered to lysosomal structures, where it is degraded (Hirata et al. 1985a).

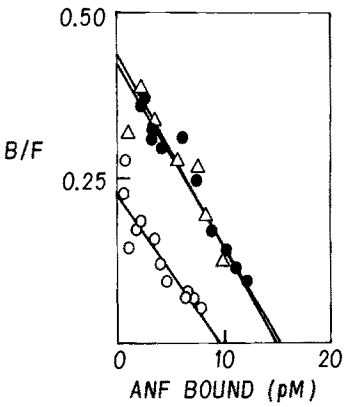


Fig. 10. Scatchard plots of saturation binding curves for ^{125}I-labeled ANF to membranes from cultured vascular smooth muscle cells. Control (●); cells exposed for 24 h to 10^{-8} M ANF (○); or cells exposed for 24 h, washed, and allowed to recover for 24 h (△) are depicted. Affinity was unchanged (40 pM), but B_{max} decreased from 775 fmol/mg protein to 465 fmol/mg protein after exposure to ANF for 24 h and after recovery reached a density of 800 fmol/mg protein. *B/F*, bound/free. (From Schiffrin et al. 1986d)

Progress has been made towards the purification of the vascular ANF receptor. Vandlen et al. (1985) used a photoreactive analogue of ANF or attached ^{125}I-labelled ANF covalently to the ANF receptor from rabbit aorta membranes, using a bifunctional cross-linking agent. They demonstrated the presence of three peptides, of 60, 70, and 120 kDa, which bind ANF with a K_d of 200 pM. Vandlen et al. (1986) found that, when solubilized with the nonionic detergent dodecyl-β-maltoside, the ANF receptor from rabbit aorta has a conserved affinity (K_d of 220 pM) and a molecular mass of 300–350 kDa. Using covalent cross-linking with disuccinimidyl suberate, Schenk et al. (1985a) demonstrated two proteins in cultured bovine vascular smooth muscle, one of 66 kDa and a minor species of 180 kDa. In cultured A10 smooth muscle cells, Napier et al. (1986a) reported the binding of ANF to a 60 kDa polypeptide with a K_d of 80 pM, using both a photoaffinity analogue and cross-linking. Interestingly, this receptor has a high affinity for shorter peptides, e. g., ANF (103–123), and for linear analogues of ANF, in contrast to the rabbit aorta receptor, which has a high affinity for ANF (101–126) and a lower affinity for the shorter ANF (103–123) and for the linear ANF peptides (Napier et al. 1986b). This suggests that there are two receptor subtypes in smooth muscle, present in varying proportions in different tissues.

Distinct high-affinity ANF receptors functionally coupled to particulate guanylate cyclase have been demonstrated in cultured bovine aortic endothelial cells (Leitman and Murad 1986; Schenk et al. 1985a). In these cells, atriopeptin I, ANF (103–123), has a very low potency as an agonist to increase cyclic GMP production (EC_{50} of 0.5 μM vs 0.3 nM for ANF (101–126), atriopeptins II and III), while it competes in the radioligand binding assay with a K_d of 0.45 nM vs 0.1 nM for ANF (101–126). This suggests the presence of multiple and functionally distinct ANF receptors (Leitman and Murad 1986).

In another study, Leitman et al. (1986) used a cross-linking reagent to occupy ANF sites in bovine endothelial cells irreversibly with tyrosine-atriopeptin I, and they found that the dose-response curve of ANF(101–126) for cyclic GMP accumulation was unchanged. If ANF is cross-linked to the sites on the endothelium, the responses of cyclic GMP to ANF are significantly reduced. A 66-kDa site (94% of sites) and a 130-kDa site (6%) can be labeled with ^{125}I-labeled ANF. Tyrosine-atriopeptin I competes with high affinity (K_d of 0.9 nM) for the 66-kDa site and with low affinity (>100 nM) for the 130-kDa site. Since the affinity of tyrosine-atriopeptin I coincides with the potency of this peptide to stimulate cyclic GMP accumulation, the authors concluded that the less abundant 130-kDa site is coupled to guanylate cyclase.

The regulation of vascular ANF receptors has also been investigated. Sodium loading decreases the density of ANF vascular sites in the mesenteric arteries of uninephrectomized rats (Fig. 11) (Schiffrin et al. 1986c). After mineralocorticoid treatment (subcutaneous infusion of deoxycorticosterone or DOCA for 2 weeks), vascular ANF sites are also down-regulated, and the response of norepinephrine-precontracted aorta to ANF decreases in sensitivity (Schiffrin and St-Louis 1985). This is in line with a concomitant increase in the plasma concentration of ANF; together with the reversible down-regulation of ANF sites in cultured vascular smooth muscle (Schiffrin et al. 1986b), it suggests that increased plasma ANF concentrations directly down-regulate ANF vascular sites and result in the decreased responsiveness of blood vessels to ANF. In two models of volume-expanded experimental hypertension – the one-kidney, one-clip (1K–1C) Goldblatt and the DOCA-

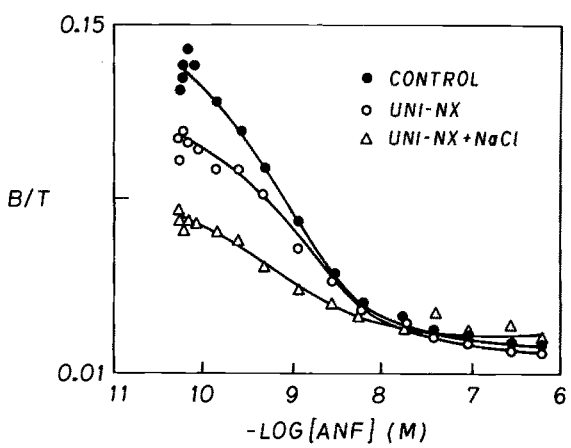


Fig. 11. Representative experiment of a competition binding curve for ^{125}I-labeled ANF and unlabeled ANF(101–126) to membranes from mesenteric vessels of rats after uninephrectomy and sodium loading. Results are represented as bound/total (*B/T*) vs total (labeled + unlabeled). The density of binding sites was: control, 198 fmol/mg protein (K_d of 0.26 nM); uninephrectomized (*Uni-NX*), 100 fmol/mg protein (K_d of 0.45 nM); *Uni*-NX + NaCl, 90 fmol/mg protein (K_d of 0.31 nM). (From Schiffrin et al. 1986c)

salt hypertensive rat – it was found that plasma ANF is increased, vascular ANF receptors in the mesenteric arteries and aorta are decreased, and the relaxation response of precontracted aorta to ANF becomes less sensitive (Schiffrin et al. 1986d; Schiffrin and St-Louis 1987). The reduced density of ANF receptors and the decreased vascular sensitivity are in line with a decreased depressor response to the intravenous infusion of ANF into 1K–1C hypertensive rats (Garcia et al. 1985b) and DOCA-salt hypertensive rats (Schiffrin and St-Louis 1987). In two-kidney, one-clip (2K–1C) Goldblatt hypertensive rats, a lesser elevation of plasma ANF was found (Schiffrin and St-Louis 1987) and there was a tendency to a reduction in density of ANF vascular sites which was not significant; however, the relaxation responses of the aorta to ANF are also reduced (Schiffrin et al. 1986d). This indicates that the mechanism of diminished sensitivity to ANF may be more complex than it seemed at first, and that the reduced relaxation of large arteries does not necessarily predict decreased hypotensive effects of ANF, since these rats are highly responsive to intravenous ANF (Garcia et al. 1985d).

7.2.1 Platelet ANF Receptors in Man

Platelets are a readily accessible tissue in man and are known to bear receptors for vasoactive peptides such as angiotensin II and vasopressin. Schiffrin et al. (1986a) characterized ANF sites on platelets and showed that the potency of different ANF analogues to bind to platelets is similar to their potency to bind to vascular membranes from rats (Table 10). Although these sites do not have a known biological action, they are regulated similarly to rat vascular sites; after an increased dietary sodium intake, ANF binding sites on platelets are decreased concomitantly with an increase in the plasma concen-

Table 10. Potency of ANF peptides competing with labeled human or rat ANF for binding to human platelets or to rat mesenteric artery (from Schiffrin et al. 1986a)

Peptide	Potency K_I (pM)		
	Human platelets		Rat mesenteric artery
	^{125}I-labeled human ANF	^{125}I-labeled rat ANF	^{125}I-labeled rat ANF
hANF (99–126)	36	73	137
rANF (101–126)	58	66	85
rANF (103–126)	170	170	680
rANF (103–125)	190	370	800
rANF (103–123)	530	–	2900

Results are means of 2–4 experiments

tration of ANF (Schiffrin et al. 1986a). Furthermore, in patients with severe congestive heart failure who have very high plasma ANF concentrations, there is a significant decrease in the density of platelet ANF sites (Schiffrin 1986).

7.3 Molecular Properties (A. de Léan and H. Ong)

The effects of ANF in its various target tissues are mediated by specific receptors in the cell membranes (de Léan et al. 1984b). Such receptors have been documented in vascular tissue, e. g., in rat aorta (Napier et al. 1984b), in mesenteric arteries (Schiffrin et al. 1985a), in dog and rat kidney glomeruli (de Léan et al. 1985c), in bovine adrenal zona glomerulosa (de Léan et al. 1984b), in rat capsular tissue (Schiffrin et al. 1985a), in human placenta (Sen 1986), and in cultured arterial smooth muscle and endothelial cells (Schenk et al. 1985a; Schiffrin et al. 1986b). All these receptor binding sites display a high degree of specificity and a high affinity (K_d of 20–500 pM) typical of hormone receptors. In bovine adrenal zona glomerulosa, detailed studies of structure-activity relationships showed that the structure of specific receptor binding sites correlated with biological activity, i. e. the inhibition of aldosterone secretion (de Léan et al. 1985b). In other tissues, only the correlation with guanylate cyclase or cyclic GMP production was investigated (Scarborough et al. 1986).

In all the systems studied, ANF binding is highly promoted by divalent cations, e. g., Mg^{2+} or Mn^{2+}, but only slightly enhanced by monovalent cations (de Léan et al. 1984b). Guanosine triphosphate (GTP) or its nonhydrolyzing analogues, which typically modulate hormone binding to receptors coupled to adenylate cyclase, are inactive in ANF binding (de Léan et al. 1984b). Computerized data analysis of detailed competitive ANF binding assays revealed that the results are compatible with the existence of two apparent classes of binding sites with typically higher (K_d of 50 pM) and lower (K_d of 5 nM) affinity for the hormone (de Léan et al. 1984b). Interestingly, the potency of ANF in inhibiting aldosterone production correlates with the higher-affinity binding component, while the potency of the hormone in affecting cyclic GMP production correlates better with the lower-affinity binding component, suggesting that more than one specific receptor might be involved in mediating the effects of ANF. Another piece of evidence in favor of the potential heterogeneity of ANF receptors is the range of molecular weights of the receptor protein demonstrated by affinity cross-linking and by photoaffinity labeling. Table 11 shows that protein bands both in the range of M_r 60000–70000 and of M_r 110000–140000 were reported by various authors.

The heterogeneity of ANF binding sites was also demonstrated in cultured arterial smooth muscle and endothelial cells (Schenk et al. 1985b). In these

Table 11. Molecular weight (MW) of the atrial natriuretic factor receptor

Tissue	MW	Techniques	References
Kidney	140000	SDS gel	Yip et al. (1985)
Adrenal	130000	SDS gel	Hirose et al. (1985)
	70000		
Adrenal	124000	SDS gel	Misono et al. (1985)
Aorta	120000	SDS gel	Vandlen et al. (1985)
	70000		
	60000		
VSMC	180000	SDS gel	Schenk et al. (1985b)
	66000		
Aorta	140000	SDS gel	Koseki et al. (1986a)
Adrenal	140000	SDS gel	
Kidney	65000		
Placenta	160000	SDS gel	Sen et al. (1986)
Lung	120000	SDS gel	Kuno et al. (1986)
Adrenal	130000 (68000)	SDS gel	Meloche et al. (1986)
Adrenal	109000	Hydrodynamic	Meloche et al. (1986)

VSMC, vascular smooth muscle cells

Table 12. Hydrodynamic properties of the solubilized ^{125}I-labeled ANF receptor complex (from Meloche et al. 1986)

Physical parameter	Value
Stokes radius, a (Å)	50.8 ± 0.9 (7)
Partial specific volume, $\bar{v}$ (ml/g)	0.770 ± 0.009 (16)
Sedimentation coefficient, $S_{20,w}$ (S)	6.34 ± 0.17 (4)
Relative molecular mass M_r	158000
Frictional ratio, f/f_0	1.25
Detergent bound (g/g protein)	0.45 (0.66 – 0.22)
Molecular weight of protein	109000 (95000 – 130000)

systems, analysis of the structure-activity relationships for ANF stimulation of cyclic GMP production showed that smaller fragments, e. g., ANF(103 – 123), display very low potency in stimulating cyclase activity, while they are equipotent with ANF(99 – 126) in radioligand binding assays. ANF binding sites in these systems have been characterized by Schenk et al. (1985 b) and are apparently a dimer of binding proteins of M_r 60000. In contrast, the particulate form of guanylate cyclase characterized by Kuno et al. (1986) has a M_r of 130000 and copurifies with ANF binding activity. Although the identity of one of the forms of ANF receptor protein with guanylate cyclase enzyme is still debated, the heterogeneity of molecular sizes and of structural specificity still suggests that two distinct proteins are involved, at least in some target tissues.

The ANF receptor has been successfully extracted from cell membranes with various detergents. This allows the hydrodynamic properties of the receptor to be determined. Table 12 shows that the bovine adrenal ANF receptor solubilized with octyl-glucoside is characterized by a Stokes radius of 50.8 Å, a sedimentation coefficient of 6.3 S, and a calculated M_r of 109000, in line with the calculated size of the protein in sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after affinity cross-linking (Meloche et al. 1986). Interestingly, the bovine adrenal zona glomerulosa appears to contain only this larger-sized receptor protein, which displays a high structural specificity towards ANF fragments (Meloche et al., unpublished observations). In contrast with its apparent homogeneity as documented by affinity cross-linking, the bovine adrenal ANF receptor displays some heterogeneity in gel filtration after solubilization with detergent (Meloche et al., unpublished observations). Two forms are partially resolved by HPLC on a Superose 6 column. Interestingly, the larger form, which elutes earlier on gel filtration, is enhanced in the presence of amiloride. This diuretic also enhances ANF binding in intact membrane receptor preparations (de Léan 1986) by promoting the conversion of the binding sites to a higher-affinity form (Fig. 12). This suggests that the ANF receptor protein might be tightly

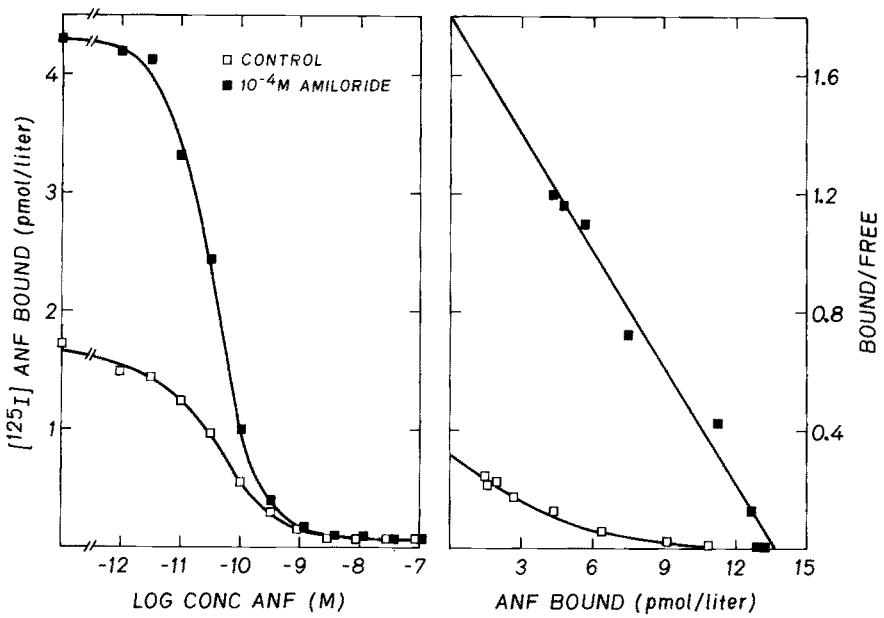


Fig. 12. Effect of amiloride on ANF binding to adrenal membranes. Bovine adrenal zona glomerulosa membranes were incubated with ^{125}I-labeled ANF at various concentrations (*CONC*) of ANF (99–126) in the presence or absence of 100 μM amiloride. The results are portrayed both as competition curves (*left panel*) and Scatchard plots (*right panel*). (From de Léan 1986)

associated with an amiloride-sensitive protein which may be involved in mediating its cellular action. Since amiloride potentiates the cellular effects of ANF and even mimics its effect on aldosterone secretion, it is tempting to postulate that an amiloride-sensitive sodium transporter is associated with at least some of its cellular effects. In fact, several authors have shown that ANF modulates sodium influx or transport in kidney cells (Cantiello and Ausiello 1986), flounder intestine (O'Grady et al. 1985), and kidney proximal tubules (Hammond et al. 1985).

8 Second Messengers

8.1 Particulate Guanylate Cyclase/cGMP (P. Hamet and J. Tremblay)

8.1.1 Intracellular cGMP

Atrial (but not ventricular) extracts and synthetic ANF(Arg-101 to Tyr-126) increase cGMP levels in kidney slices and in primary cultures of renal cortical cells (Hamet et al. 1983, 1984). In the course of the purification of ANF, the rise in specific natriuretic activity is correlated with enhanced cGMP formation in the primary renal cortical cell cultures. Furthermore, ANF antibodies are capable of inhibiting both the natriuretic and diuretic effects of ANF, as well as its ability to elevate cGMP levels. These results have rapidly been confirmed by many groups in vascular smooth muscles (Hirata et al. 1984b; Hamet et al. 1986; Fiscus et al. 1985; Cohen and Schenk 1985; Hirata et al. 1985c; Sato et al. 1986), lungs (Waldman et al. 1984), testes (Pandey et al. 1986), kidneys (Inui et al. 1985; Tremblay et al. 1985), adrenals (Matsuoka et al. 1985; Jaiswal et al. 1986; Waldman et al. 1985), cultured neural and astroglia cells (Friedl et al. 1985, 1986), cardiomyocytes (Aiton and Cramb 1985), and other cell types (Waldman et al. 1984; Leitman and Murad 1986). In most of these tissues, a functional correlation has been established between the increases in cGMP and vasodilation (Winquist et al. 1984b; Rapoport et al. 1985; Masters et al. 1985; Ohlstein and Berkowitz 1985), steroidogenesis (Matsuoka et al. 1985), glomerular filtration (Ballermann et al. 1985; Tremblay et al. 1985), and renin secretion (Obana et al. 1985b).

It has also been observed that the efficacy of ANF in augmenting cGMP levels differs from tissue to tissue and does not correspond to the potency of other cGMP agonists and general vasodilators such as sodium nitroprusside (SNP) (Hamet et al. 1984). SNP, which is neither diuretic nor natriuretic, does not interfere with the functions of ANF (Hamet et al. 1984). Using isolated segments of dog and rat kidneys, the glomerulus was shown to be the major action site for ANF. In this fraction, cGMP increased 50-fold with only a two-

to threefold elevation in distal tubules and collecting ducts, and no effect in proximal tubules (Tremblay et al. 1985). The studies by Ardaillou et al. (1986) recently confirmed this finding. They determined that the epithelial cell is the target for ANF in glomerulus (Bianchi et al. 1985, 1986c). Inui et al. (1985) also examined the efficiency of various ANF fragments in the LLC-PK_1 cell line.

The different types of responses to ANF and SNP led to the study of the effects of ANF on particulate and soluble guanylate cyclases. It is well known that SNP is an agonist of soluble guanylate cyclase (Bohme et al. 1977), which is a heme protein distributed throughout most of the body (Gerzer et al. 1981). On the other hand, the particulate enzyme is a glycoprotein which does not have any known endogenous agonist (Garbers and Radany 1981). The only stimulators of this enzyme in mammalian systems are *E. coli* enterotoxin in the intestine (Field et al. 1978) and mitogenic agents in lymphocytes (Coffey 1986). ANF-induced increases in cGMP levels were shown to be correlated with the distribution of particulate guanylate cyclase (Tremblay et al. 1985). One of the tissues richest in this enzyme appears to be the glomerulus, which possesses 95% of guanylate cyclase in particulate form. SNP does not modify cGMP levels in this tissue (Tremblay et al. 1985). At the other extreme, ANF does not increase cGMP in blood platelets, which are rich in soluble guanylate cyclase, whereas SNP is a potent inhibitor of platelet aggregation (Hamet et al. 1986).

8.1.2 Extracellular cGMP

Atrial extracts and ANF(Arg-101 to Tyr-126) increase cGMP levels in the plasma and urine of normal Wistar rats (Hamet et al. 1984). Similar results have been found in monkeys and humans (Hamet et al. 1986). A dose-response relationship can be demonstrated between ANF and cGMP levels in human plasma and urine (Gerzer et al. 1985; Cusson et al. 1987) following a bolus injection of the peptide hormone. The enhancement of cGMP precedes the diuretic and natriuretic responses. Although the ANF concentration required to increase cGMP in vitro appears to be high, it has to be emphasized that this apparent lack of a dose-response correlation is certainly not the case in vivo.

The rise in extracellular cGMP results from the egression of this nucleotide from the cell. The egression of cGMP appears to be analogous to that of cAMP, yet its mechanism has not been well studied hitherto (Tjornhammar et al. 1983). It has been observed that the transitory intracellular increase in cGMP is accompanied by its prolonged egression, which in turn contributes to a persistent stimulation in the extracellular medium (Hamet et al. 1986; Pang et al., unpublished observations). The primary effect of ANF on the vas-

cular wall occurs at the level of the endothelium. Cyclic GMP can be released from endothelial cells, which are rich in particulate guanylate cyclase. Whether or not the cGMP that reaches the extracellular space can have a paracrine effect remains to be explored. Studies of extracellular cGMP have identified it as a biological marker of ANF in both normal and hypertensive animals and humans (Hamet et al. 1986). Furthermore, it can be a reflection of the physiological stimulation of endogenous ANF release that can be induced by, for example, changes in posture (Hamet et al. 1986).

It is now well established that in experimental or human congestive heart failure, ANF levels are elevated in parallel with the severity of the failure (Arendt et al. 1986; Burnett et al. 1986; Cody et al. 1986; Kouz et al. 1986b; Naruse et al. 1986; Osborn et al. 1986; Pasternac et al. 1986; Raine et al. 1986; Riegger et al. 1986; Shenker et al. 1985; Tikkanen et al. 1985a). On the other hand, ANF infusions yield variable results in these conditions, and the relative lack of potency of the peptide must be interpreted judiciously. One view is that ANF receptors could be desensitized. However, recent investigations have revealed highly significant increases in cGMP both in cardiomyopathic hamsters (Fig. 13) and in patients with cardiac insufficiency (Hamet, Tremblay, Cantin, unpublished observations). High doses of ANF may thus be unable to induce diuresis or natriuresis in these edematous states, which are beyond the scope of interaction between the peptide, membrane receptors, and cGMP production. Such considerations may have direct therapeutic implications, and hence defects of the cGMP system must be explored.

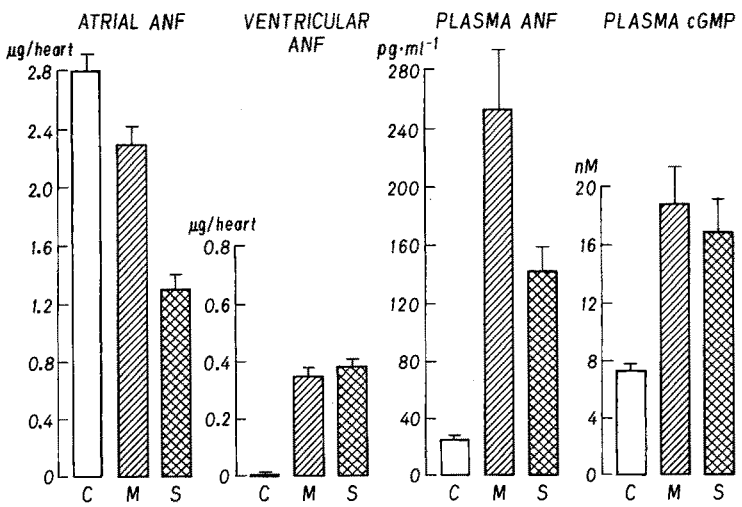


Fig. 13. ANF heart content and plasma concentration, and cGMP plasma levels in cardiomyopathic hamster. ANF heart content is expressed as total content per atrium or ventricle, respectively. *C*, control hamster; *M*, moderate heart failure; *S*, severe heart failure

The abnormal response to ANF in hypertension could be explained by modifications of peripheral ANF receptors in vessels (Schiffrin et al. 1986d) or the brain (Saavedra et al. 1986a, 1986b). In spontaneously hypertensive rats (SHR), a decreased density of ANF receptors and an increased stimulation of particulate guanylate cyclase have been noted in vascular smooth muscles (Appel and Dunn 1986; Hamet et al. 1986; Khalil et al. 1986; Takayanagi et al. 1986a, 1986b). Since the enhanced reaction of cGMP to ANF is significant in SHR as well as in humans and persists in vitro in cultured cells, the abnormality of particulate guanylate cyclase may be genetically determined in this model. However, the defect is probably not generalized in hypertension, as opposite results have been obtained in Dahl rats (Appel and Dunn 1986).

8.1.3 Particulate Guanylate Cyclase

As mentioned earlier (Kimura and Murad 1975), particulate guanylate cyclase is a 130-kDa glycoprotein distributed unequally throughout the body. This enzyme has been purified from the mammalian lung (Kuno et al. 1986) and sea urchin sperm (Garbers and Radany 1981). Antibodies produced against it do not crossreact with soluble guanylate cyclase. In preliminary experiments, the cross-linking of ^{125}I-labeled ANF and photoaffinity labeling with azido [^{32}P] GTP indicated a similar electrophoretic mobility for ANF receptors and particulate guanylate cyclase (Carrier et al. 1987).

As soon as it was established that ANF stimulates particulate guanylate cyclase activity (Waldman et al. 1984; Tremblay et al. 1985, 1986), several studies were designed to determine the relationship between ANF receptors and the enzyme. The copurification of rat lung ANF receptors and particulate guanylate cyclase has been achieved (Kuno et al. 1986). The protein has an apparent molecular weight of 120 kDa. However, at least in some cells, such as endothelial cells, the existence of other ANF binding sites (60–70 kDa) appears not to be associated with particulate guanylate cyclase stimulation. Furthermore, the copurification of ANF receptors with particulate guanylate cyclase does not preserve the peptide's ability to stimulate the enzyme. Nevertheless, recent results suggest that the two activities are closely linked, at least after the solubilization of membrane proteins from the adrenal cortex with Triton X-100, in which guanylate cyclase retains its affinity for ANF (Tremblay et al. 1986; Ishido et al. 1986). Current investigations have demonstrated that particulate guanylate cyclase can still be stimulated by ANF even after further purification by GTP-agarose affinity chromatography (Pulido, Tremblay, Thibault, Hamet, unpublished results).

8.1.4 Functional Implications of Particulate Guanylate Cyclase

Two types of particulate guanylate cyclase modulation have been reported which may have physiological consequences. In the first type, cultured endothelial cells exposed to ANF persistently produce cGMP even after extensive washing. In vitro, the enzyme remains active despite washing and solubilization with Triton X-100. The fact that particulate guanylate cyclase stimulation is relatively irreversible could explain the huge increments in plasma and urinary cGMP levels, which stay elevated even after the values for circulating ANF have returned to baseline. Following an acute bolus or prolonged infusion of ANF, diuresis and vasodilation continue beyond the increases in ANF. It is conceivable that the prolonged effectiveness of ANF is linked to the relatively irreversible stimulation of particulate guanylate cyclase (Tremblay et al. 1987).

In the second type of modulation, the density of ANF receptors is decreased after the prolonged exposure of endothelial and vascular smooth muscle cells to ANF (Schiffrin et al. 1986d). In this situation, the basal cGMP levels remain elevated, but concomitantly the responsiveness of particulate guanylate cyclase is diminished although its basal activity is enhanced. A 24-h recovery period restores ANF receptor levels and the receptor stimulability of particulate guanylate cyclase (Schiffrin et al. 1986d; Tremblay et al. 1987). This observation appears to be related to physiological and pathophysiological conditions in which a prolonged elevation of ANF levels increases cGMP and leads to the apparent inefficiency of both endogenous and exogenous ANF, as occurs in congestive heart failure.

8.1.5 Role of ANF and cGMP in the Regulation of Cytosolic Ca^{2+} Levels and Sodium Transport

Although the role of cGMP in the mode of action of ANF appears to be well established, the mechanism by which this nucleotide functions remains unknown. Fiscus et al. (1985) recently demonstrated that the activity of cGMP-dependent protein kinase is stimulated by ANF. Several cGMP functions could be expressed via the phosphorylation of specific protein substrates which in turn could modifiy the activity of contractile proteins similarly to the effect of cAMP, leading to vasodilation (Rapoport et al. 1983). On the other hand, since the increases in cGMP do not have the same functional consequences as increases in cAMP, different mechanisms could be implicated. The different consequences could be due to the phosphorylation of different substrates and also to an entirely dissimilar mode of action. In the retina, it has now been clearly demonstrated that cGMP can work directly, without any involvement of phosphorylation, via its physical interaction with sodium

channels (Fesenko et al. 1985). In this tissue, the occupancy of sodium channels by cGMP decreases sodium influx through the cooperative binding of two cGMP molecules to the regulatory site of the transport protein (Koch and Kaupp 1985). The hydrolysis of cGMP leads to the opening of the channels (Fesenko et al. 1985) with a consequent influx of calcium and sodium. Calcium movements which occur when the channels are closed by cGMP are related to preexistent cytosolic calcium levels (George and Hagins 1983).

ANF has no influence on any known sodium transport system described up to the present time (Beliveau et al. 1985). It is conceivable that ANF plays its natriuretic and diuretic roles mainly via hemodynamic effects on vasodilation (Camargo et al. 1984), yet the tubular aspect must also be considered since ANF appears to impact on medullary collecting tubules (Chai et al. 1986; Healy and Fanestil 1986; Koseki et al. 1986a). Most recently, a direct action of ANF on sodium transport has been demonstrated in LLC-PK_1 cells (Inui et al. 1985), which possess an amiloride-sensitive sodium transport system, and in which sodium is exchanged for hydrogen ions. In the growing phase of these cells, ANF, like amiloride, can inhibit sodium movement, while in the resting phase, ANF can specifically suppress conductive transport, but not sodium-proton exchange. Using lithium as a marker of proximal tubule absorption, Biollaz et al. (1986a) recently showed that ANF increases diuresis partly by inhibiting sodium reabsorption at the level of the collecting duct. Finally, although ANF has no effect on sodium-potassium ATPase in suspensions of collecting tubule cells, it is able to reduce the entry of sodium into these cells isolated from the internal part of rabbit kidney medulla (Zeidel et al. 1986a). The in vivo excretion of sodium may thus be explained by the diminution of its luminal entry at the level of the collecting duct.

The effectiveness of ANF in modifying free cytosolic calcium levels remains controversial. Some studies have suggested that it diminishes the increases in calcium induced by angiotensin concomitantly with a rise in cGMP (Knorr et al. 1986). On the other hand, the striking increases in cGMP elicited by ANF in juxtaglomerular cells, correlating with the inhibition of renin secretion, are not accompanied by changes in cytosolic calcium levels (Yamamoto et al. 1986). One investigation has revealed a suppressive effect of ANF on the efflux of calcium stimulated by vasopressin (Meyer-Lehnert et al. 1986).

Generally, it has not been possible to demonstrate any influence of ANF on phosphoinositide turnover or protein C kinase activation. As already mentioned, several studies have failed to show an effect of ANF on sodium-potassium ATPase. However, recent experiments indicate that there is an indirect relationship since the actions of ANF can be inhibited by ouabain (Sybertz and Desiderio 1985). This inhibition is reminiscent of the blocking effect of ouabain on SNP-induced increases in cGMP described several years ago.

Therefore, cGMP is an excellent marker of the action of ANF in tissues, and its levels in extracellular fluids in general reflect accurately the biological

activity of ANF in vivo. Cylic GMP also appears to be significantly involved as a second messenger in the mediation of the action of ANF. The search for mechanisms by which cGMP induces vasorelaxation and sodium transport may provide a better understanding of the physiological and pathophysiological implications of ANF.

8.2 Adenylate Cyclase/cAMP System (M. B. Anand-Srivastava)

8.2.1 Effect of ANF on Vascular Smooth Muscle

ANF inhibits adenylate cyclase activity in a concentration-dependent manner in vascular smooth muscles from the aorta, renal artery, and mesenteric artery, with an apparent K_i between 10^{-10} and 10^{-9} M (Anand-Srivastava et al. 1984). Adenylate cyclase from the mesenteric and renal arteries is inhibited to a greater extent ($\simeq 50\% - 60\%$) than that from the aorta ($\simeq 40\%$). ANF can also inhibit the stimulatory effects of hormones on adenylate cyclase activity and those of agents such as F^- and forskolin which activate adenylate cyclase by a receptor-independent mechanism. In addition, ANF shows an additive effect with the inhibition of adenylate cyclase from rat aorta of angiotensin II (AII).

8.2.2 Effect of ANF on Adrenal Cortical Membranes

ANF also inhibits adenylate cyclase activity in adrenal cortical membranes in a concentration-dependent manner, with an apparent K_i between 5×10^{-11} and 10^{-10} M. The maximal effect observed was between 25% and 35% (Anand-Srivastava et al. 1985b). Various agents such as isoproterenol, dopamine, prostaglandins, and adrenocorticotropic hormone (ACTH) stimulate adenylate cyclase activity to various degrees, and ANF inhibits but never completely abolishes the stimulatory effects of all these agents. In addition, ANF also inhibits the stimulatory effect of forskolin on adenylate cyclase. A similar inhibitory effect of ANF on adenylate cyclase in adrenal cortical membranes has also been reported by Waldman et al. (1985). Furthermore, ANF has been reported to decrease cAMP levels and aldosterone production in rat adrenal capsular cells (Matsuoka et al. 1985) and in the human adrenal gland (Naruse et al. 1987). These data suggest that the inhibition of steroidogenesis by ANF may partly be mediated by the cAMP/adenylate cyclase system.

8.2.3 Anterior and Posterior Pituitary

ANF inhibits the activity of adenylate cyclase from anterior and posterior pituitary homogenates in a concentration-dependent manner (Anand-Srivastava et al. 1985 a). The maximal inhibitions observed were 42% with an apparent K_i of 10^{-10} M in anterior pituitary and 25% with an apparent K_i of 10^{-11} M in posterior pituitary. ANF can also inhibit to various degrees the stimulatory effects of corticotropin-releasing factor (CRF), vascoactive intestinal peptide (VIP), prostaglandins (PGE_1), NaF, and forskolin on adenylate cyclase from anterior pituitary homogenates. However, the inhibition is more pronounced in cultured anterior pituitary cells, where VIP- and CRF-stimulated adenylate cyclase activity is completely abolished by ANF. Similarly, ANF can inhibit the stimulatory responses to *N*-ethylcarboxamide adenosine (NECA), NaF, and forskolin of adenylate cyclase from posterior pituitary homogenates. ANF has also been shown by Obana et al. (1985 a) to inhibit vasopressin release and cAMP levels in posterior pituitary.

8.2.4 Kidney

ANF inhibits adenylate cyclase activity in various nephron segments such as glomeruli, loops of Henle, and collecting ducts, but not in proximal tubules and whole kidney membranes from dog kidney (Anand-Srivastava et al. 1986; Waldman et al. 1984). The maximal inhibitions observed were about 45% in glomeruli and collecting ducts, with an apparent K_i between 10^{-11} and 5×10^{-10} M, and about 30% in loops of Henle, with an apparent K_i between 10^{-11} and 5×10^{-10} M. ANF also inhibits to various extents the stimulatory responses to various hormones such as vasopressin, parathyroid hormone (PTH), and AII, which have been shown to play an important role in the regulation of renal function. However the stimulation is never completely abolished. On the other hand, ANF interacts differently with different hormones; for example, the inhibitory effect of ANF on PGE_1-stimulated adenylate cyclase activity appears to be associated with an increase in K_a (control 0.7 μM, with ANF 2.0 μM), but not with a decrease in V_{max}, whereas the inhibition of PTH-stimulated adenylate cyclase activity is associated with a decrease in V_{max}.

8.2.5 Cultured Cardiocytes

ANF also inhibits adenylate cyclase activity in cultured cardiocytes from rat atria (left and right) and ventricles from neonatal rats in a concentration-dependent manner (Anand-Srivastava and Cantin 1986). However, the inhibi-

tion is greater in atria (55% in right and 65% in left) than in ventricles (35%), with an apparent K_i between 10^{-10} and 10^{-9} M. ANF is also able to inhibit the stimulatory effects of NECA, isoproterenol, prostaglandins, forskolin, and NaF on adenylate cyclase in cultured atrial and ventricular cardiocytes; however, the degree of inhibition varies for each agent. For example, dopamine- and PGE_1-stimulated enzyme activities are completely abolished by ANF in ventricular cardiocytes, whereas isoproterenol-, NECA-, and forskolin-sensitive adenylate cyclase activities are slightly inhibited. Cyclic AMP has recently been shown to be one of the mediators of ANF release from isolated perfused hearts (Ruskoaho et al. 1986). This suggests that ANF may act as a feedback regulator of its own release through its interaction with the adenylate cyclase/cAMP system.

8.2.6 Ciliary Processes of the Eye

ANF inhibits adenylate cyclase activity in a concentration-dependent manner, with an apparent K_i between 10^{-9} and 10^{-8} M (Bianchi et al. 1986a). The maximal inhibition observed is about 20%. In addition, ANF also inhibits the stimulatory effects of epinephrine, norepinephrine, dopamine, isoproterenol, and forskolin on adenylate cyclase; however, the stimulations are never completely abolished.

8.2.7 Platelets and Lungs

ANF is also able to inhibit adenylate cyclase activity in rat platelets and lung membranes (Table 13). The maximal inhibitions observed are about 40% and 30%, respectively. ANF also inhibits the stimulatory responses to some hormones such as NECA, catecholamines and prostaglandins in these membranes (Anand-Srivastava et al., unpublished observations).

8.2.8 Lack of Effect of ANF on Adenylate Cyclase in Some Tissues

ANF does not show any inhibitory effect on adenylate cyclase activity in some tissues such as spleen, skeletal muscle, adrenal medulla, testis from the rat (Anand-Srivastava et al. 1984) and proximal tubules of dog kidney (Anand-Srivastava et al. 1986). This suggests that ANF receptors may not be present in these tissues, or if they are present, they may not be coupled to the adenylate cyclase system.

Table 13. Effect of ANF on adenylate cyclase activity in various tissues

Tissue	Adenylate cyclase activity pmol cAMP (mg protein)$^{-1}$ (10 min)$^{-1}$		
	Basal	ANF (10^{-8} M)	Inhibition (%)
Vascular smooth muscle			
Aorta	59±3	37±1	37
Renal artery	57±2	30±3	48
Mesenteric artery	36±1	15±2	59
Pituitary			
Anterior pituitary	82±4	50±3	39
Posterior pituitary	120±2	98±4	19
Adrenal cortical membranes	40±5	25±2	38
Kidney			
Glomeruli	78±3	43±6	45
Collecting ducts	100±4	54±7	46
Loops of Henle	41±3	29±4	30
Cardiocytes			
Left atrial cardiocytes	145±5	105±2	28
Right atrial cardiocytes	220±10	120±10	46
Ventricular cardiocytes	210±2	160±2	34
Platelets	239±18	137±13	43
Lungs	490±15	325±17	34

Adenylate cyclase activity was determined as previously described (Anand-Srivastava et al. 1984). Values are mean ±SEM of triplicate determinations from one of two to three experiments

8.2.9 Effect of ANF on cAMP Levels

ANF also decreases cAMP levels in cultured cardiocytes from left and right atria and from ventricles by about 50%, 60%, and 40%, respectively (Anand-Srivastava and Cantin 1986). ANF-induced decreases in cAMP levels have also been reported in various other tissues by other investigators (Matsuoka et al. 1985; Obana et al. 1985a; Pandey et al. 1985). Such decreases may be the result of the inhibition of adenylate cyclase activity in these different tissues.

8.2.10 Dependence on Guanine Nucleotides of Inhibition of Adenylate Cyclase by ANF

The inhibitory effect of ANF on adenylate cyclase is absolutely dependent on the presence of guanine nucleotides (Anand-Srivastava et al. 1986; Anand-

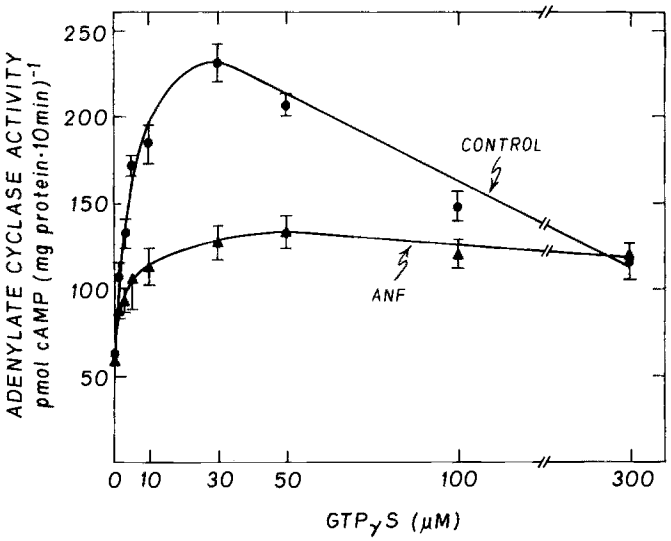


Fig. 14. Dependence on guanine nucleotides of the inhibition of adenylate cyclase by ANF in anterior pituitary homogenates. Adenylate cyclase activity was determined at various concentrations of Guanosine 5-O-(Thiotriphosphate) (GTP y S) in the absence (control, ●) or presence (▲) of 10^{-8} M ANF. Values are means ±SEM of three separate experiments

Srivastava and Cantin 1986). Maximal inhibition is observed in the presence of 10 μM concentrations of guanine nucleotides in glomeruli and cultured cardiocytes (Anand-Srivastava et al. 1986; Anand-Srivastava and Cantin 1986). However, in anterior pituitary, the maximal effect is observed between 10 and 30 μM, and at higher concentrations (300 μM) the inhibition is completely abolished, as shown in Fig. 14. A lack of inhibitory effect of ANF on adenylate cyclase in anterior pituitary has recently been reported by Heisler et al. (1986). This may be due to the fact that these investigators used a very high concentration of GTP (300 μM) which completely abolished the expression of the inhibitory effect of ANF on adenylate cyclase in these membranes. The requirement for guanine nucleotides in eliciting the inhibitory effect of ANF on adenylate cyclase suggests that such inhibition is a receptor-coupled phenomenon, and that ANF receptors are coupled to adenylate cyclase through the guanine nucleotide regulatory protein.

8.2.11 Involvement of Guanine Nucleotide Regulatory Protein in Coupling of ANF Receptors to Adenylate Cyclase

Ninhibin, a sperm factor isolated from bovine sperm (Johnson et al. 1985), has been reported to attenuate the α-adrenergic inhibition of platelet adenylate cyclase by inactivating the guanine nucleotide regulatory protein

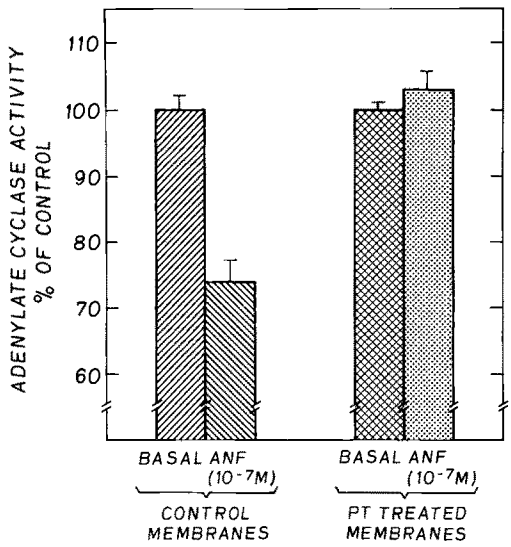


Fig. 15. Effect of pertussis toxin (*PT*) on ANF-mediated inhibition of adenylate cyclase in washed rat aorta particles. Washed particles (20–30 µg protein) were incubated in 25 mM glycylglycine buffer, pH 7.5, containing 1 mM nicotinamide adenine dinucleotide (NAD), 0.4 mM ATP, 0.4 mM GTP, 15 mM thymidine, 10 mM dithiothreitol, and ovalbumin (0.1 mg/ml), with 5 µg/ml PT (PT-treated membranes) or without PT (control membranes), for 30 min at 30 °C in a total volume of 100 µl. The particles were washed two to three times with 10 mM Tris and 1 mM EDTA buffer, pH 7.5, and finally suspended in the same buffer and used for adenylate cyclase activity determination. Values are means ±SEM of three separate experiments. The basal enzyme activities in control and PT-treated washed aorta particles were 23±4 and 30±7 pmol cAMP (mg protein)$^{-1}$ (10 min)$^{-1}$, respectively

(Gi) (Jakobs et al. 1983). Ninhibin also attenuates the ANF-mediated inhibition of adenylate cyclase in aorta membranes (Anand-Srivastava et al. 1985 c). In addition, the inhibition by ANF of isoproterenol-stimulated adenylate cyclase activity is also abolished by ninhibin, suggesting that ANF receptors may be coupled to adenylate cyclase through Gi. Furthermore, pertussis toxin, which attenuates the GTP-dependent and receptor-mediated inhibition of adenylate cyclase by adenosine diphosphate (ADP) ribosylation of the 41-kDa membrane protein Gi (Katada and Ui 1982), also attenuates the ANF-mediated inhibition of adenylate cyclase in aorta membranes (Fig. 15). Moreover, pertussis toxin treatment attenuates the ANF-mediated inhibition of isoproterenol- and forskolin-stimulated adenylate cyclase activity (Anand-Srivastava et al. 1987). These data strongly suggest that ANF receptors are coupled to adenylate cyclase through Gi.

These studies demonstrate, as shown in Fig. 16, that ANF receptors are negatively coupled to adenylate cyclase through inhibitory Gi. The inhibition of the adenylate cyclase/cAMP system in various tissues may be one of the important mechanisms whereby ANF exerts its physiological effects, such as

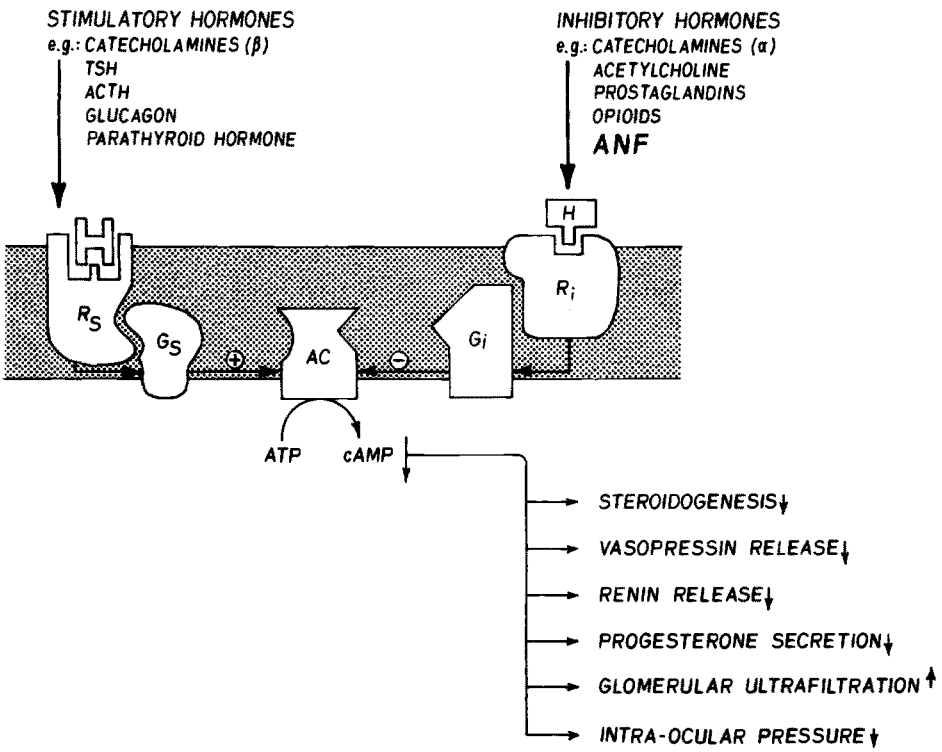


Fig. 16. The coupling of ANF receptor (*Ri*) to adenylate cyclase through inhibitory guanine nucleotide regulatory protein (*Gi*)

the inhibition of vasopressin release from posterior pituitary, of steroidogenesis in adrenals, of progesterone secretion from Leydig tumor cells, and of renin release from kidney, the lowering of intraocular pressure, and the increase in the glomerular filtration rate.

8.3 Calcium (E. L. Schiffrin)

Calcium is a major intracellular mediator in excitation-response coupling in many tissues, for example, in smooth muscle (Deth and Van Breemen 1977; Van Breemen et al. 1982) and in the adrenal zona glomerulosa (Fakunding and Catt 1980; Fakunding et al. 1979; Kojima et al. 1985; Schiffrin et al. 1981), two tissues in which ANF inhibits the response to stimulating agents. For this reason, it is not surprising that different investigators have attempted to relate the vasorelaxant effects of ANF and its inhibition of aldosterone secretion to effects on cytosolic calcium concentration or on calcium fluxes. Indeed, in the adrenal gland, the pattern of inhibition by ANF of aldosterone secretion stimulated by angiotensin II, ACTH, or potassium suggests the pos-

sibility of an effect on calcium fluxes (Chartier et al. 1984b; Schiffrin et al. 1985a). It was shown that ANF does not interact with receptors for dihydropyridines (calcium channel blockers such as nifedipine) in the adrenal (Schiffrin et al. 1985a).

When the effects of ANF and nifedipine on aldosterone secretion by the adrenal are compared (Chartier and Schiffrin 1987b), both ANF and nifedipine decrease the maximal response to angiotensin II and potassium and displace the dose-response curve for ACTH to the right, as had previously been shown after exposure to organic or inorganic calcium channel blockers, such as verapamil and lanthanum (Fakunding and Catt 1980), or after lowering extracellular calcium (Fakunding et al. 1979). ANF also inhibits noncompetitively the aldosterone output induced by the calcium channel activator Bay K 8644 (Chartier and Schiffrin 1987b). When calcium influx into adrenal zona glomerulosa cells is examined, it is shown that ANF does not affect basal, but inhibits angiotensin II-, ACTH- and potassium-induced calcium influx (Fig. 17), as well as Bay K 8644-induced calcium influx, similarly to the effects of nifedipine (Chartier and Schiffrin 1987b). In contrast to these findings, Capponi et al. (1986) found that ANF does not affect

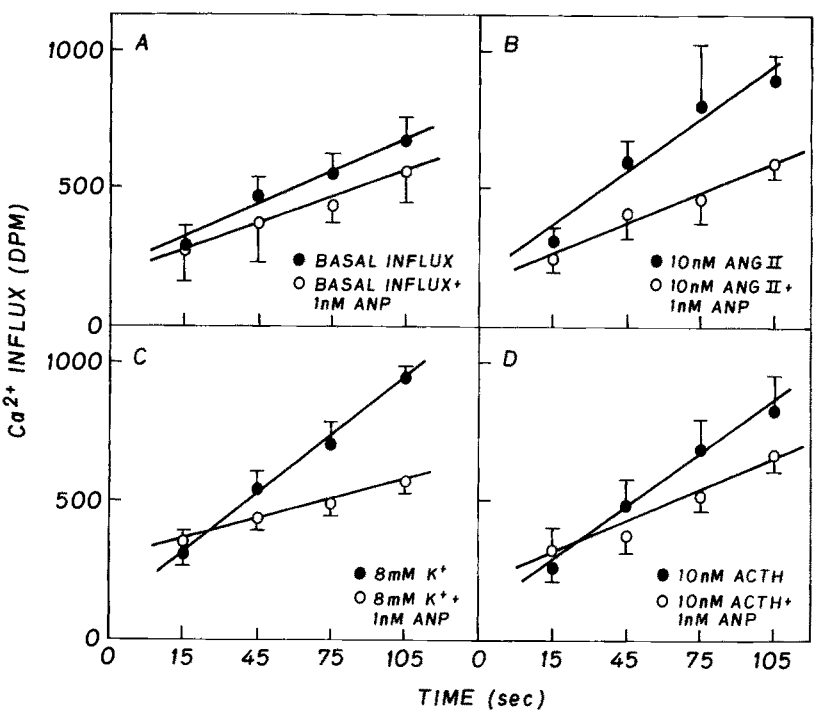


Fig. 17. Calcium (Ca^{2+}) influx into isolated rat adrenal glomerulosa cells in the presence of angiotensin (*ANG*) II, ACTH, or potassium (K^+) with or without ANF. (Modified from Chartier and Schiffrin 1987b)

angiotensin II- or potassium-induced changes in cytosolic calcium in the adrenal. These authors conclude that ANF does not affect calcium fluxes in the adrenal, similarly to the study reported by Goodfriend et al. (1984), who did not find that ANF induces changes in ^{45}Ca uptake. However, these investigators used a low-calcium medium for incubation and found that angiotensin II lowers calcium uptake, in contrast to the findings of other laboratories (Kojima et al. 1985). Calcium uptake, as measured by this technique, may be more a measure of total cell calcium than of calcium influx. The discrepancies between studies measuring ^{45}Ca influx and showing effects of ANF (Chartier and Schiffrin 1987b) and studies using the Quin-2 method (Capponi et al. 1986) may be due to the fact that both methods measure different events. Indeed, these authors have shown that nifedipine does not block changes in cytosolic calcium induced by angiotensin II (Capponi et al. 1984), while it does block calcium influx as measured by the ^{45}Ca technique (Chartier and Schiffrin 1987b; Kojima et al. 1985).

Several studies have implicated calcium in the effects of ANF in vascular smooth muscle. Garay et al. (1985) showed that ANF inhibits ouabain- and bumetanide-insensitive K^+ efflux stimulated by the calcium ionophore A 23187. This suggests that ANF counteracts the rise in cytosolic calcium induced by A 23187. Bergey and Kotler (1985) found that atriopeptins II and III have a vasorelaxant effect on rabbit aortic strips contracted by norepinephrine, a high potassium concentration of A 23187. This suggests that ANF acts by modulating intracellular events involving calcium, either through the inhibition of calcium-calmodulin-myosin light-chain kinase interactions or through the increased extrusion of calcium by the enhancement of a cGMP-dependent protein kinase. In contrast to the previous study, Chiu et al. (1986) reported that atriopeptin II is preferentially antagonistic to the norepinephrine and angiotensin II responses of rabbit aorta strips in comparison with the high-potassum response and significantly decreases the ^{45}Ca influx and efflux from aorta (in a calcium-free medium) induced by norepinephrine and angiotensin II. ^{45}Ca efflux after stimulation by high potassium is not increased by atriopeptin, thus suggesting that ANF blocks the increase in cytosolic calcium induced by angiotensin II and norepinephrine by inhibiting calcium influx and release from intracellular storage sites, but has no effect on calcium extrusion. Similar results were reported by Meisheri et al. (1986), who found that atriopeptin II inhibits norepinephrine-, histamine-, or caffeine-induced ^{45}Ca efflux from rabbit aorta. The same authors reported that in rabbit aorta, atriopeptin II blocks ^{45}Ca influx stimulated by norepinephrine but has less effect on K^+-induced ^{45}Ca influx (Taylor and Meisheri 1986). This indicates that atriopeptin II is a powerful antagonist of agonist-induced calcium influx through "receptor-operated" calcium channels. Using the calcium indicator Fura-2, Hassid (1986) demonstrated that atriopeptin II decreases both basal cytosolic-free calcium concentrations in cultured vascu-

lar smooth muscle cells and the sustained response, but not the transient initial rise, induced by angiotensin II.

Atriopeptin II also decreases the cytosolic calcium levels elevated by 50 mM KCl, suggesting that ANF relaxes vascular smooth muscle by decreasing basal and vasoconstrictor-induced increases in cytosolic-free calcium and may act as an antagonist of calcium-mediated processes (Hassid 1986). In contrast to these data, Capponi et al. (1986), using the Quin-2 technique, did not find any effects of ANF on angiotensin II-induced changes in cytosolic calcium concentration in cultured vascular smooth muscle cells. The reasons for these discrepancies remain to the established. In rabbit and guinea pig renal arteries, human ANF inhibits norepinephrine- and caffeine-induced contractions in a calcium-free medium and norepinephrine-induced hydrolysis of phosphatidylinositol 4,5-biphosphate. This led Fujii et al. (1986) to conclude that ANF inhibits calcium release from intracellular storage sites by inhibiting the synthesis of inositol 1,4,5-triphosphate and accelerates the extrusion of calcium by stimulating the production of cGMP.

Data on the involvement of calcium as a mediator of ANF are contradictory, but it would appear that calcium influx and, probably, calcium-dependent phosphorylation in rat adrenal zona glomerulosa, as well as calcium influx and release from intracellular storage sites in rabbit aorta and renal arteries, may be inhibited by ANF. The mechanism whereby ANF, by stimulating cGMP production, interacts with calcium influx or intracellular release remains to be established. The effects of ANF on calcium extrusion also require further investigation.

9 Biological Effects of ANF

9.1 Kidney (M. Cantin)

Atrial extracts of various mammals such as cattle, pig, dog, hamster, mouse, rat, monkey and man possess diuretic and natriuretic activity (de Bold et al. 1981; Trippodo et al. 1983; Forssmann et al. 1983; de Bold and Salerno 1983; Nemeh and Gilmore 1983; Chimoskey et al. 1984). The activity is also present in the ventricle of a nonmammalian vertebrate, the frog (de Bold and Salerno 1983).

The observation of the rapid onset (within 1–2 min) and short duration (less than 20 min) of the renal effects has been confirmed by all subsequent studies. The intravenous (i. v.) injection of an atrial extract to rats not only produces diuresis and natriuresis but also increases renal excretion of potassium, calcium, magnesium, and phosphate (Keeler and Azzarolo 1983). These observations have been confirmed by direct injection into the dog renal

artery of synthetic ANF (Arg-101 to Tyr-126) (Seymour et al. 1985a). Sustained (5 days) infusion of ANF in the dog, however, produces only a transient effect on sodium excretion and has no significant long-term effect on glomerular filtration rate (GFR) (Granger et al. 1986).

Radioautographic studies have now shown that most of the ANF binding sites in the kidney are localized in glomeruli (Bianchi et al. 1986c; Fig. 6). They are also present, to a lesser degree, in the outer medullary descending vasa recta and inner medullary collecting duct cells (Bianchi et al. 1987). They are present on the endothelium and smooth muscle cells of all renal vessels (Bianchi et al. 1985). At the electron microscope level, the binding sites in glomeruli are selectively localized on the podocytes of visceral epithelial cells (Bianchi et al. 1986c; Fig. 7). This also seems to be the case in humans, where the exposure of cultured glomerular visceral epithelial cells to ANF leads to more cGMP formation than in the case of mesangial cells (Ardaillou et al. 1986). ANF may also modulate the tonus of medullary arteries and/or the capillary hydraulic permeability of vasa recta in the outer medulla. In fact, in the dog, doses of ANF which do not alter GFR induce a redistribution of renal blood flow from the superficial to the juxtamedullary cortex (Salazar et al. 1986a). A whole series of investigations has been conducted on purified dog kidney fractions: glomeruli, proximal tubules, thick ascending limbs of Henle loops, and collecting ducts (Vinay et al. 1981, 1987). Typical competition curves display an IC_{50} for ANF in the glomerulus of 250–300 pmol/l. A class of receptors with high affinity displays a pK of 9.9 ± 0.3 and a binding capacity of 205 ± 102 fmol/mg protein, while a lower-affinity component is characterized by a pK of 8.0 ± 0.3 and a larger capacity of 4800 ± 3200 fmol/mg protein. No acceptor could be identified in proximal tubules (de Léan et al. 1985c). In the thick ascending limbs of Henle loops, binding sites with a pK of 9.4 ± 0.1 and a total binding capacity of 36 ± 8 fmol/mg protein can be found. In the collecting ducts, the pK is 9.4 ± 0.2 and the total binding capacity slightly higher: 148 ± 43 fmol/mg protein. Only high-affinity binding sites could be documented in these two preparations (de Léan et al. 1985b). The stimulation of particulate guanylate cyclase with the concomitant formation of cGMP and the inhibition of adenylate cyclase activity closely parallel the above results.

The effect of ANF on the metabolism of dog and rat kidney tubules has been studied in vitro and compared to that of furosemide (0.1–1 mM), hydrochlorothiazide (0.5 mM) or amiloride (0.1 mM). The substrate uptake (O_2, lactate, glutamine, glucose) and production of metabolites (glutamate, ammonium, alanine, glucose) by these nephron segments were measured in the absence or presence of the diuretic agent or the vehicle of ANF (acetate, 1 mM). The total ATP turnover and the contribution of identified metabolic pathways for this turnover were calculated. It was expected that a molecule with diuretic properties reducing the permeability of cell membranes to NaCl

would secondarily reduce the Na^+/K^+-ATPase activity and therefore the oxygen and substrate utilization by affected cells. It was shown (a) that each nephron segment presents the expected metabolic characteristics, (b) that furosemide markedly inhibits the oxidative metabolism of thick ascending limbs, (c) that acetate displaces the oxidation of glutamine and lactate in nephron segments with aerobic metabolism, and (d) that ANF has no effect on the metabolism of the nephron segments despite the presence of cGMP-generating receptors in the distal nephron. Thus, ANF must exert its natriuretic effect by a mechanism which is different from that of classical diuretics (Vinay et al. 1987).

Physiologically, ANF acts directly on the kidney to increase the glomerular filtration rate (GFR) (Atlas et al. 1984; Burnett et al. 1984; Camargo et al. 1984, 1986; Cogan 1986; Huang et al. 1985; Kleinert et al. 1984; Sosa et al. 1986) and to reduce inner medullary hypertonicity (Borenstein et al. 1983; Hirata et al. 1985a; Needleman et al. 1985; Zeidel et al. 1986b). In regard to the renal vasculature, ANF does not behave as a classical vasodilator, but rather as a powerful antagonist of vasoconstriction with a small direct or indirect agonist (vasoconstrictive) action of its own (Camargo et al. 1984; Maack and Kleinert 1986). While ANF dilates arcuate and interlobular arteries and afferent arterioles, it constricts efferent arterioles. This leads to an increase in glomerular capillary hydrostatic pressure, which in turn explains, at least in part, the increase in GFR and filtration fraction (Camargo et al. 1984; Fried et al. 1986; Maack and Kleinert 1986; Marin-Grez et al. 1986).

As we have seen above, ANF has a direct effect on glomerular cells: binding sites are localized on the podocytes of visceral epithelial cells, which probably indicates that the stimulation of cGMP by ANF is due to the activation of particulate guanylate cyclase which is located on the surface of these cells. This is what has been shown on isolated human visceral epithelial cells, although a small increase in mesangial cells has also been noted. Binding sites for ANF are also present on mesangial cells, and the inhibition of AII-induced contractions of rat glomeruli by ANF probably depends on interaction between the two peptides at this level (Bianchi et al. 1986c). These results strongly suggest that one of the main effects of ANF is to increase the ultrafiltration coefficient (Fried et al. 1986) by dilating the afferent arterioles, constricting the efferent arterioles, and modulating the size of "pores" and the shape of foot processes.

In addition to its vascular and hemodynamic effects, ANF acts directly or indirectly to inhibit the load-reabsorption balance in inner medullary collecting ducts. This phenomenon may be due to an increase in the passive permeability of capillary collecting ducts to sodium and/or to an alteration in inner medullary hemodynamics (Sonnenberg 1986; Sonnenberg et al. 1986).

A proximal tubular action of ANF has also been postulated using lithium (Burnett et al. 1984), but direct micropunctures and isolated perfused tubule

experiments failed to unveil a sodium transport inhibitory action of the peptide in nephron segments from proximal convoluted tubules to cortical collecting ducts (Baum and Toto 1986; Briggs et al. 1982, Cogan 1986; Huang et al. 1985; Kondo et al. 1986). It cannot be ruled out, however, that ANF may indirectly inhibit sodium reabsorption in proximal tubules of premedullary nephrons. In addition, ANF inhibits sodium reabsorption in epithelial cells, which exhibit a cGMP-inhibitable sodium transport process (Cantiello and Ausiello 1986; Inui et al. 1985; O'Grady et al. 1985; Zeidel et al. 1986a, 1986b). The questions whether the intact mammalian nephron has such a sodium transport process and, if so, to what extent this action of ANF contributes to its natriuretic effect remain to be elucidated. The prevention of ANF-mediated renal hemodynamic effects by partial clamping of the renal arteries results in the loss of the natriuretic action of ANF. This suggests that ANF-induced natriuresis is mainly due to its hemodynamic effect, rather than to a putative direct tubular action (Sosa et al. 1985a, 1985b).

Studies in various tissues, including the kidney, have revealed major discrepancies between the kinetics of specific binding and the dose-response curve for ANF-induced increases in cGMP. Thus the half-maximal increase in cGMP occurs at significantly higher doses than the half-maximal binding of ANF, and cGMP continues to increase at concentrations of ANF which are well beyond the saturation of binding (Ballerman et al. 1985; Hirata et al. 1984b; Schenk et al. 1985a; Stokes et al. 1986).

Finally, ANF consistently decreases inner medullary hypertonicity (Borenstein et al. 1983; Hirata et al. 1985a; Sosa et al. 1986). Although the exact mechanism of this action remains to be elucidated, there is solid evidence to indicate that a decrease in inner medullary hypertonicity magnifies the natriuretic effect of the ANF-induced increase in sodium load to the distal nephron. Due to the countercurrent exchange mechanism, it is possible that the concentration of ANF in the inner medulla in vivo is greater than in the cortex, a phenomenon which could partly compensate for the lower affinity to ANF of papillary limbs.

Recent studies indicate that the hemodynamic effects of ANF are manifest not only at the glomerular level but also at the level of the papilla. Measurements of hydraulic pressure in the Munich-Wistar rat renal papilla with the servo-null technique before and after i. v. injection of rat (r) ANF(Arg-102 to Tyr-126) revealed, in the latter case, a marked increase in pressure in both descending and ascending vasa recta with lesser increments in the loops of Henle and collecting ducts at the base or mid portion of the exposed papilla (Dunn et al. 1986).

In highly purified preparations of rabbit inner medullary collecting duct (IMCD), outer medullary collecting duct (OMCD), and thick ascending limb (TAL) cells, human (h) ANF(Arg-102 to Tyr-126) inhibits the ouabain-sensitive oxygen consumption (QO_2) by $27.4 \pm 1.6\%$ in IMCD but gives no sig-

nificant inhibition in OMCD or TAL cells. Concentration-response curves for QO_2 give the following apparent K_i values: hANF(Arg-102 to Tyr-126) and rANF(Ser-103 to Tyr-126), 6.6 ± 10^{-10} M; rANF(Ser-103 to Gly-125), 8.0 ± 10^{-9} M; rANF(Asp-111 to Tyr-126), no effect. The inhibition of QO_2 by hANF is prevented by pretreatment with ouabain, while hANF has no effect on QO_2 in the presence of the metabolic inhibitor amphotericin. These results indicate that hANF does not directly inhibit Na^+/K^+-ATPase or cellular metabolism but reduces sodium entry into IMCD cells (Zeidel et al. 1986b).

These and previous radioautographic results tend to indicate that the effects of ANF on the kidney are mediated by both hemodynamic and tubular effects. The hemodynamic effects may or may not induce (depending on the circulating levels of ANF) an increase in GFR by acting on pre-and postglomerular arterioles and epithelial visceral cell podocytes; but they do produce an increase in the medullary blood flow by acting on descending vasa recta and a tubular effect at the level of IMCD cells. While the increased blood flow, by increasing inner medullary hydraulic pressure, may induce sodium entry into the lumen of IMCDs, a direct effect of ANF on their luminal permeability may inhibit luminal sodium entry into IMCD cells. This general hypothesis would explain the diuresis and natriuresis which have been reported in the absence of changes in GFR, and it would also explain the fact that ANF, unlike other loops and osmotic diuretics, produces a urinary sodium concentration in excess of plasma sodium values (Mendez et al. 1986).

9.2 Cardiovascular System (R. Garcia)

Crude atrial extracts (Deth et al. 1982; Currie et al. 1983) and partially purified ANF (Garcia et al. 1983; Grammer et al. 1983; Garcia et al. 1984a) have been shown to possess a vasorelaxant effect on precontracted vascular smooth muscle. The relaxant effect is observed regardless of the agent used to constrict the vessels, and it is specially marked with norepinephrine and angiotensin II (Garcia et al. 1984a; Kleinert et al. 1984). That the relaxant effect is indeed due to ANF, and not to contaminants present in the atrial extracts, was confirmed once a synthetic peptide (26 AA, Arg-101 to Tyr-126) was made available by Nutt et al. at Merck Co., Inc., in September 1983 (Garcia et al. 1984b).

Heterogeneity in the responses of different vascular preparations to ANF suggests (Garcia et al. 1984a) that they could be due to differences in the quantity or sensitivity of specific receptor sites for the peptide. The heterogeneity in the responses of different vascular segments has been confirmed by several investigators (Winquist et al. 1984a; Faison et al. 1985; Cohen and Schenk 1985), and the presence of high-affinity vascular receptors for ANF

has been firmly established (Napier et al. 1984b; Schenk et al. 1985b; Schiffrin et al. 1985a, 1986b). The vasorelaxant effect of ANF on vascular smooth muscle does not depend on the integrity of the endothelium, a characteristic which differentiates this peptide from other vasodilators such as nitroprusside (Winquist et al. 1984b; Scivaletto and Carvalho 1984). Resistance vessels from the brain or mesenteric territory appear in vitro to be more resistant than the renal vascular bed to the relaxant effect of ANF (Garcia et al. 1984a; Aalkjaer et al. 1985; Osol et al. 1986). In vivo, however, ANF infusion decreases vascular peripheral resistance in dogs when compensatory autonomic reflexes are attenuated (Holtz et al. 1986), indicating an effect of ANF on resistance vessels.

The fact that ANF induced an increase in tissular levels of cGMP (Hamet et al. 1983) and its vasorelaxant effect was mimicked by sodium nitroprusside (Garcia et al. 1983, 1984a), a known stimulant of cGMP (Schultz et al. 1977), suggested a role for the latter in mediating the effects of ANF. The finding that methylene blue, an inhibitor of soluble guanylate cyclase (Gruetter et al. 1979, 1981), did not inhibit the relaxation induced by ANF (Garcia et al. 1984a) suggests another pathway of cGMP intracellular increase. It was later demonstrated that ANF increases tissular cGMP through the stimulation of particulate guanylate cyclase (Winquist et al. 1984b; Waldman et al. 1984) which is not inhibited by methylene blue. So it seems likely that ANF vasorelaxation may be mediated by the intracellular accumulation of cGMP, though direct evidence has yet to be provided.

There is no clear evidence, either, that ANF modifies intracellular calcium concentration or intra- or extracellular calcium fluxes. Recently, it has been reported that calcium extrusion from the cell seems not to be a primary effect of ANF (Chiu et al. 1986), however, ANF inhibits the agonist-stimulated release of intracellular calcium (Meisheri et al. 1986) decreasing basal and stimulated cytosolic free calcium levels (Hassid 1986), which could be an important mechanism in the vasorelaxant effect of ANF.

9.2.1 Blood Pressure and Systemic Hemodynamics

Because of the vasorelaxant and natriuretic activities of ANF, its effects on a variety of models of experimental hypertension have received ample attention. Acute administration of synthetic ANF has been shown to have a profound and prolonged hypotensive effect in anesthetized (Garcia et al.1985f) or conscious (Seymour et al. 1985b, 1987; Pegram et al. 1986) 2K-1C and 1K-1C hypertensive rats. Normotensive animals were less sensitive to ANF and 2K-1C animals presented a greater diuretic and natriuretic response to the administration of the peptide (Garcia et al. 1985f; Seymour et al. 1985b). An increase in the urinary excretion of cGMP, such as had been previously re-

ported in intact animals (Hamet et al. 1984), was observed in 2K-1C rats (Garcia et al. 1985 f). DOCA-salt hypertensive animals were less responsive than renovascular hypertensive rats (Seymour et al. 1985 b; Schiffrin and St-Louis 1987). Volpe et al. (1985) reported that the hypotensive response to ANF of 2K-1C animals was greater when the animals were saralasin responsive. They also found that 1K-1C animals were responsive to ANF infusion only when they were sodium depleted and hence renin dependent. The differences in the responsiveness to ANF in renovascular hypertensive animals (Garcia et al. 1985 f; Seymour et al. 1985 b; Volpe et al. 1985) may well be due to the diversity of the injected doses of the peptide. Surprisingly, the hypotensive response to ANF in renin-dependent 2K-1C animals described by Volpe et al. (1985) was accompanied by a further rise in an already high PRA. In spite of this increase, plasma aldosterone was depressed, a finding which was interpreted as a direct effect of ANF on adrenal cells.

The studies of the effects of ANF on spontaneously hypertensive rats (SHR) have yielded contradictory results. Acute bolus or short-infusion administration of ANF has been described as inducing a higher (Pang et al. 1985; Kondo et al. 1985; Kihara et al. 1985) or similar (Marsh et al. 1985) natriuretic response. In the latter experiments, a bellshaped response was observed in SHR, higher doses of ANF inducing a lower sodium excretion. The fall in blood pressure in SHR has been reported to be greater than (Pang et al. 1985; Kondo et al. 1985; Marsh et al. 1985) or equal to (Kihara et al. 1985) that in normotensive Wistar-Kyoto (WKY) rats. Whether these discrepancies are the result of the different doses and ways of ANF administration, bolus (Pang et al. 1985; Kihara et al. 1985) versus short infusion (Kondo et al. 1985; Marsh et al. 1985), or the effect of anesthesia (Pang et al. 1985; Kondo et al. 1985; Kihara et al. 1985) remains to be elucidated. However, the evidence seems to point to a vascular hyperresponsiveness of SHR to ANF. The chronic infusion of low doses of ANF normalizes blood pressure in SHR without having any effect on that of WKY animals or on sodium excretion in either strain (Garcia et al. 1985 e). Cyclic GMP urinary excretion after acute ANF administration has been reported to be minimally (Kihara et al. 1985) or equally changed (Pang et al. 1985) in SHR when compared with that in normotensive animals. Whether this cyclic nucleotide is involved in the in vivo natriuretic or hypotensive effect of ANF remains, however, to be further investigated.

The hemodynamic mechanism(s) by which ANF reduces blood pressure in normotensive and hypertensive animals has not been less debated. Ackermann et al. (1984) reported that the injection of atrial extracts into normotensive anesthetized intact rats induced a fall in blood pressure which was mainly due to a depressed cardiac output. When the animals were vagotomized the hypotensive effect was less marked, but was entirely due to a reduction in total peripheral resistance. This reduction was greater when the carotid sinus was denervated. This suggests that the hypotensive effect of ANF in the rat is the

result of a complex direct peripheral vasodilatory effect which is not compensated by an increase in cardiac performance, and in which reflex mechanisms play an important role.

These results were later confirmed in the dog using a synthetic peptide (Koyama et al. 1986). Using conscious rats chronically instrumented with electromagnetic flow probes, a decrease in cardiac output and increase in vascular peripheral resistance have been reported (Lappe et al. 1985) in normotensive animals, the increase in peripheral resistance being ascribed to reflex compensatory mechanisms. Similar results have been reported in conscious sheep, where a decreased cardiac output accompanied by a rise in total peripheral resistance have been described following a short-period infusion of atriopeptin II (Breuhaus et al. 1985). Total peripheral resistance decreases slightly in sympathectomized rats after ANF administration but is unchanged in intact animals (Sasaki et al. 1986). These effects on blood pressure and cardiac output seem not to be mediated by a direct negative inotropic effect of ANF on cardiac muscle (Natsume et al. 1986; Criscione et al. 1987). On the other hand, ANF has been found to reduce both cardiac output and vascular peripheral resistance in normotensive rats (Hirata et al. 1985a) and cardiac output alone in the dog (Kleinert et al. 1986). On the other hand, Shapiro et al. (1986) have described a significant increase in cardiac output and heart rate following the administration of ANF in conscious dogs. These hemodynamic changes, which were interpreted as due to basoreflex withdrawal of vagal tone and activation of the efferent adrenergic nervous system, were accompanied by a slight but significant decline in blood pressure and an increased flow to different organs.

At this time, it is necessary to emphasize the fact that all these experiments have been performed under acute administration of ANF. Almost nothing is known about the effects of chronic infusions of ANF on either cardiac output or total peripheral resistance.

Using a different technology, that of microspheres, several groups of investigators have reported completely different results from those described above. ANF infusion induced a fall in blood pressure in normotensive rats without changing cardiac output and there was a significant increase not only in renal blood flow, but in several other organs as well, accompanied by a decrease in total peripheral resistance (Wakitani et al. 1985b; Garcia et al. 1985c; Fujioka et al. 1985; Caramelo et al. 1986). Either a decrease in cardiac output, a decrease in vascular peripheral resistance, or a combination of both may well explain the hypotensive effect of ANF. It is not clear whether the differences in the hemodynamic effects observed during the acute administration of ANF can be explained solely by the use of different methods in conscious or anesthetized animals. One finding is uniform: ANF administration, even when reducing blood pressure, does not modify heart rate.

The acute hemodynamic effect of ANF in the hypertensive rat may depend on the model of experimental hypertension used. Volpe et al. (1986b) demonstrated that short infusions of ANF reduces vascular peripheral resistance in renin-dependent 2K-1C rats without modifying cardiac output. Equivalent doses of ANF do not reduce blood pressure in DOCA-salt hypertensives in spite of a marked drop in cardiac output. Higher doses, however, decrease cardiac output in 2K-1C rats, stressing the influence that the dose of ANF administered may have on the hemodynamic effects of the peptide. In the SHR animals, the ANF-induced hemodynamic effects reported are quite variable. Whereas some investigators have described no modifications of cardiac output with a decrease in peripheral resistance (Fujioka et al. 1985; Koike et al. 1984), others (Lappe et al. 1985; Sasaki et al. 1985) have reported significant decreases in cardiac output. More work is obviously needed to clarify this important aspect of ANF. Interestingly, it has recently been reported (Saavedra et al. 1986b) that SHR have a decreased number and affinity of specific binding sites for ANF in the subfornical organ, which, because of the latter's relationship of the anteroventral third ventricle region (AV3V), could suggest a connection to the pathogenesis of hypertension in SHR.

Another factor which could partially explain the drop in cardiac output is the decrease in plasma volume described following the acute administration of ANF to nephrectomized rats (Almeida et al. 1986; Fluckiger et al. 1986). This decrease was explained as a shift of fluids from the intravascular to the extravascular space. This effect of ANF has not been observed during its chronic administration to SHR, even in the presence of a marked hypotensive response (Garcia et al. 1987a).

The chronic administration of ANF lasting for several days may have completely different effects on blood pressure and natriuresis than those seen after acute administration. It has recently been demonstrated (Garcia et al. 1985b, 1986c, 1987d) that the chronic infusion of ANF for up to 12 days reduces blood pressure in models of experimental hypertension with different causal mechanisms, without having any blood pressure-lowering effect in the normotensive controls. The hypotensive effect is however, much more marked in 2K-1C and in SHR than in 1K-1C animals. The lesser effect in the latter model could be secondary to a decreased vascular sensitivity to the peptide. This hypothesis is supported by the findings of specific ANF vascular receptors (Schiffrin et al. 1985a) which are down-regulated in 1K-1C rats (Schiffrin et al. 1986c). The larger reduction of blood pressure in SHR and in 2K-1C animals during chronic, rather than acute, experiments strongly suggests that the mechanisms involved are different. As previously discussed, a reduction in cardiac output and/or vascular peripheral resistance may be the acute effect of ANF, but the mechanisms involved in its chronic hypotensive effect are not clear.

Different patterns of sodium excretion were observed during the chronic infusion of ANF. Whereas 2K-1C animals (Garcia et al. 1985d, 1986c) reacted

with the normalization of an initially higher pressure, diuresis, and natriuresis, no change in urinary volume and sodium excretion was observed in SHR (Garcia et al. 1985e) and an increased natriuresis was observed in the volume-dependent 1K-1C animals (Garcia et al. 1985b). A reduction in circulatory volume may play a role in the hypotensive effect of ANF in the latter animals, but this is not true for SHR, where no changes in body fluids are observed (Garcia et al. 1985d). The chronic infusion of ANF decreases PRA (Garcia et al. 1985d, 1986c) only in 2K-1C hypertensive animals, suggesting the possibility that the hypotensive effect of ANF in this model of hypertension could be related to an inhibition of renin release. However, since ANF inhibits the in vitro contraction induced in vascular smooth muscle by various agonists including angiotensin II (Garcia et al. 1984a), the possibility of a direct inhibitory effect in the effector site – vascular smooth muscle – cannot be excluded. However, that ANF infusion decreased but did not normalize blood pressure in angiotensin II-induced hypertension in the rat (Yasujima et al. 1986), although it reduced blood pressure to normal levels in norepineph-

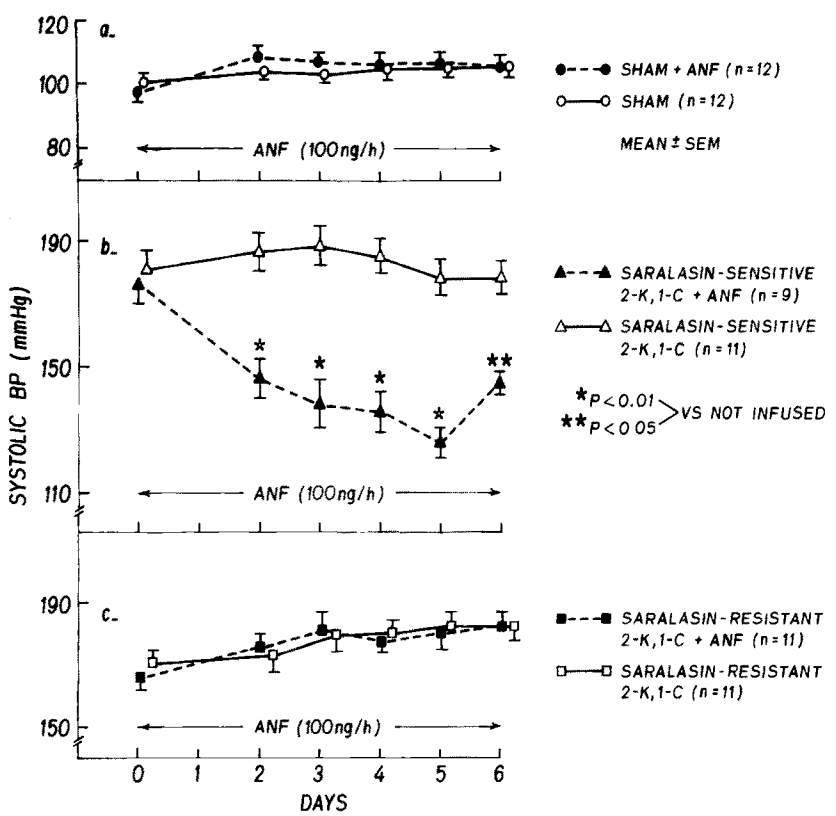


Fig. 18a–c. Effect of chronic administration of ANF on blood pressure **a** in sham-operated normotensive, **b** in saralasin-sensitive, and **c** in saralasin-resistant 1K-1C hypertensive rats

rine-induced hypertension (Yasujima et al. 1985), suggests that the depression of PRA release could be the major factor in the chronic hypotensive effect of ANF. The finding that chronic infusion of ANF decreases blood pressure, PRA, and aldosterone only in the renin-dependent 2K-1C rats (Garcia et al. 1986c) adds support to this hypothesis (Fig. 18).

The action of ANF on PRA has been controversial. Acute experiments in animals with stimulated renin release (Burnett et al. 1984; Maack et al. 1984) have demonstrated that ANF administration depresses PRA and renin release. These findings have been interpreted as the result of either an increased sodium load to the macula densa or a direct inhibitory effect on juxtaglomerular cells (Maack et al. 1984; Obana et al. 1985b). The finding that renin release from kidney slices can also be suppressed by ANF (Obana et al. 1985b) gives support to the latter explanation, as do our results (Garcia et al. 1987b) of decreased renin in the absence of the increased urinary excretion of sodium. No change in PRA was observed in animals with normal renin levels when they were chronically infused with ANF (Garcia et al. 1985d, 1986c, 1987d). This suggests that in order to be inhibited, renin has to be previously stimulated. On the other hand, acute administration of ANF to renin-dependent 2K-1C rats has been reported to produce further elevation of an already high PRA (Volpe et al. 1985). This serves well to emphasize the completely different hemodynamic or hormonal-inhibitory effects that ANF may have when administered either acutely or chronically in normotensive and hypertensive animals. Hemodynamic effects can even be observed during chronic ANF infusion in SHR without discernable changes in plasma ANF levels (Garcia et al. 1987a).

9.3 Adrenal Cortex (E. L. Schiffrin)

Since it had been shown that ANF antagonizes the action of angiotensin II on the vasculature (Garcia et al. 1984a), the effect of synthetic ANF(101–126) on another target tissue for angiotensin II, the adrenal cortex, was studied (Chartier et al. 1984b, 1984c). The aldosterone output stimulated by angiotensin II, ACTH, and potassium in isolated rat adrenal zona glomerulosa cells is nonselectively inhibited by nanomolar concentrations of ANF (Fig. 19). The inhibitory effect of ANF on aldosterone secretion depends on the intramolecular disulfide bond remaining intact (Chartier et al. 1984a). ANF also inhibits the response of aldosterone to the intravenous infusion of angiotensin II in vivo in the conscious rat, as confirmed by Atarashi et al. (1985). Simultaneously and independently, Atarashi et al. (1984) showed that atrial extracts inhibit the aldosterone response to angiotensin II, ACTH, and potassium in isolated rat adrenal zona glomerulosa cells, and Goodfriend et al. (1984) showed similar results with bovine adrenal cells stimulated by angiotensin II.

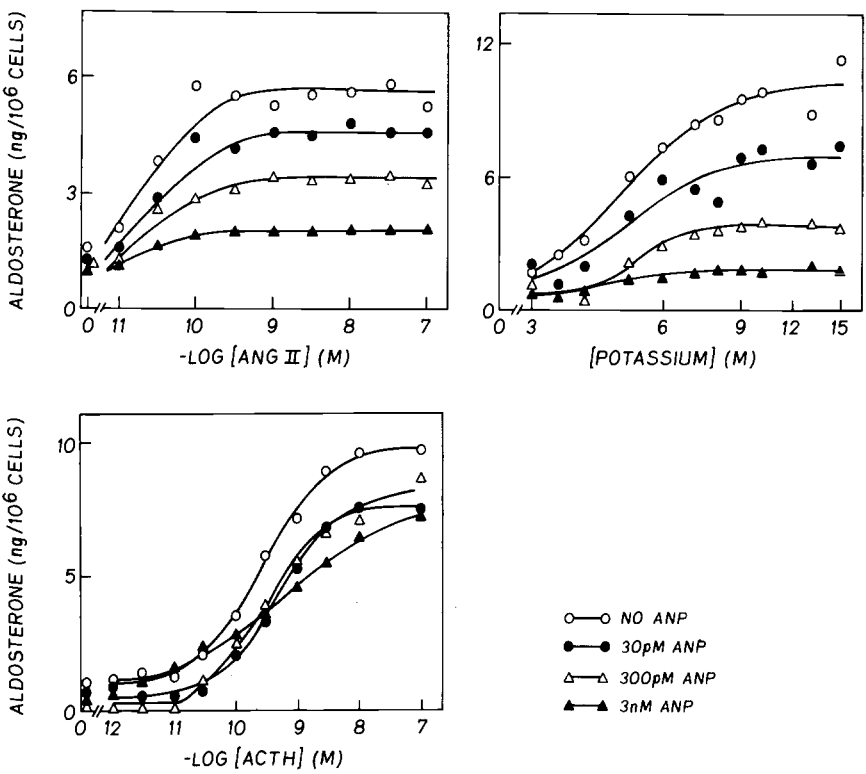


Fig. 19. Inhibition of aldosterone secretion by isolated rat adrenal zona glomerulosa cells stimulated by angiotensin (*ANG*) II, ACTH, or potassium

These studies were extended by de Léan et al. (1984b), who found that ANF inhibited angiotensin II-, ACTH-, potassium-, and prostaglandin-stimulated aldosterone and ACTH-stimulated cortisol secretion in bovine adrenal cells in primary culture. They also demonstrated the presence of specific receptors for ANF in bovine adrenal cells with an affinity (K_d of 0.1 nM) which is in line with the inhibitory potency of ANF on aldosterone secretion (de Léan et al. 1984a). The inhibition of aldosterone secretion was confirmed by other studies in vitro (Campbell et al. 1985; Kudo and Baird 1984). Atriopeptin II, ANF(103–124), (Atarashi et al. 1985), or ANF(101–126) (Chartier and Schiffrin 1986b, 1987b) decreases the maximum response to angiotensin II and potassium and displaces the dose-response curve for ACTH to the right, suggesting that ANF may act in the adrenal via the blockade of calcium influx (Fig. 19) (Chartier et al. 1984b; Chartier and Schiffrin 1986a; see Sect. 8.3).

The effect ANF has of displacing the dose-response curve for ACTH to the right on isolated adrenal capsular cells in vitro (Chartier and Schiffrin 1986a, b) can be reproduced in vivo in conscious rats. The results on the effect of

ANF on basal aldosterone secretion are divergent, with some studies showing an inhibition (Atarashi et al. 1984, 1985; Goodfriend et al. 1984) and others showing little or no effect (Chartier et al. 1984a, b, c), which may depend on the magnitude of the basal output. In some preparations, basal output appears to be stimulated, perhaps by the ambient concentration of potassium (5.6 mmol/l in some media), and thus the effect of ANF on basal secretion may actually represent the effect on potassium-stimulated aldosteronogenesis. ANF does not decrease the basal aldosterone plasma concentration in vivo in the conscious rat (Atarashi et al. 1985; Chartier et al. 1984b; Chartier and Schiffrin 1986a), but ANF infused in vivo not only decreases the response to angiotensin II (Atarashi et al. 1985; Chartier et al. 1984b) and ACTH (Chartier and Schiffrin 1986a), as described above, but also blocks the aldosterone response to the infusion of potassium (Takagi et al. 1986). Furthermore, the hypersecretion of aldosterone characteristic of the sodium-depleted state is inhibited by the infusion of ANF into conscious rats for 30–120 min, producing concentrations of ANF in plasma of 65.3 pmol/l, within the high physiological range (Fig. 20) (Chartier and Schiffrin 1987a). Thus, ANF preferentially inhibits endogenous angiotensin II-stimulated aldosterone secretion, as was previously shown for exogenously administered angiotensin II.

Similar findings have been reported in another form of endogenous angiotensin II-induced aldosterone hypersecretion, namely, that found in the 2K-1C Goldblatt hypertensive rat (Volpe et al. 1984) and also in conscious

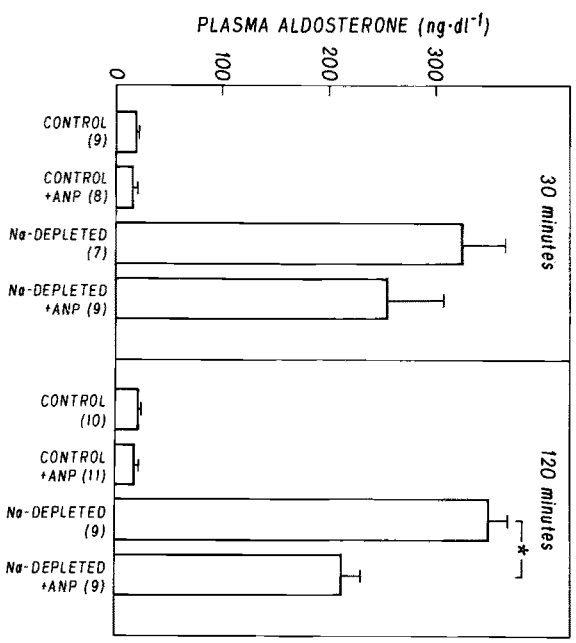


Fig. 20. Effect of intravenous ANF infusion at 50 ng/min for 30 or 120 min on plasma aldosterone concentration in control and sodium-depleted rats. (From Chartier and Schiffrin 1987a)

dogs with low-output cardiac failure due to thoracic inferior vena cava constriction (Freeman et al. 1985). In anesthetized sodium depleted rats, intravenous infusions of ANF (350 ng kg^{-1} min^{-1}) reduce the aldosterone secretion rate and plasma renin activity (Vari et al. 1986). In anesthetized nephrectomized rats, these authors found that the dose required to inhibit the aldosterone secretory rate is fivefold higher.

In in vivo studies on the conscious rat, plasma renin activity is unaffected by ANF infusion (Atarashi et al. 1985; Chartier et al. 1984b; Chartier and Schiffrin 1986a, 1987a; Takagi et al. 1986), while in the anesthetized rat, Vari et al. (1986) found that renin release is inhibited by ANF. This had previously been reported by Burnett et al. (1984) in the anesthetized dog and by Obana et al. (1985b) in the anesthetized rat. However, taking together the evidence from in vitro and in vivo studies and the demonstration of specific adrenal receptors for ANF in rat (Schiffrin et al. 1985a) and bovine adrenal zona glomerulosa (de Léan et al. 1984a, b), it appears evident that ANF exerts an independent nonselective inhibitory effect on stimulated aldosterone secretion. The inhibition of aldosterone secretion has been reproduced in human adrenal cells (Higuchi et al. 1986b) and in one study on cells from an aldosterone-producing adenoma (Hirata et al. 1985d), but ANF failed to inhibit aldosteronogenesis in cells from another aldosterone-producing adenoma (Higuchi et al. 1986c). In one report, in vivo aldosterone secretion in man is unaffected in the sodium-replete state by the intravenous injection of 100 µg ANF (Richards et al. 1985c), but it is decreased in another report at a dose of 100 ng kg^{-1} min^{-1} for 45 min (Weidmann et al. 1986b). Plasma aldosterone is also decreased in hypertensive patients receiving 100 µg ANF intravenously (Richards et al. 1985b). Aldosterone levels are reduced by ANF infusion in the sodium-depleted state (Cuneo et al. 1986; Weidmann et al. 1986b). The response of aldosterone, but not of blood pressure, to angiotensin II in normal men is abolished by ANF in one study (Vierhapper et al. 1986), while increases in both blood pressure and plasma aldosterone induced by angiotensin II (10 ng kg^{-} min^{-1}) infused for 30 min are blunted by an infusion of α-human ANF (15 pmol kg^{-1} min^{-1}) for 45 min (to achieve a plasma concentration of 300 pmol/l (Anderson et al. 1986e).

As mentioned above, specific receptors for ANF have been demonstrated in the rat (Fig. 21) (Schiffrin et al. 1985a) and bovine adrenal gland (de Léan et al. 1984a, b). The structure-activity relationship for aldosterone production and the potency of ANF peptides in the radioligand binding assay from bovine adrenal cells investigated by de Léan et al. (1985a), as well as studies on the molecular characterization of the peptides, are described elsewhere in this review. Receptor occupation in the adrenal zona glomerulosa is associated with cGMP production (Matsuoka et al. 1985; Tremblay et al. 1986; Waldman et al. 1985) and may affect calcium fluxes (Chartier and Schiffrin 1987b). The relationship between these events and the mechanism of action

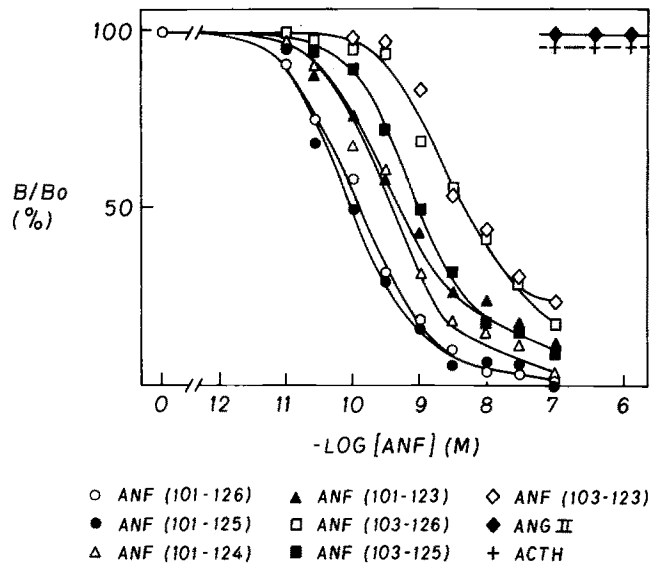


Fig. 21. Competition binding experiment of ^{125}I-labeled ANF competing with different ANF peptides (From Schiffrin et al. 1985a), % B/B_0, see Fig. 9

of ANF are discussed below. The precise site of aldosterone biosynthesis affected by ANF is controversial. Goodfriend et al. (1984) found evidence that ANF inhibits the early pathway of steroidogenesis (the conversion of cholesterol to pregnenolone in the presence of trilostane to inhibit the latter) in basal conditions and after stimulation by angiotensin II in bovine adrenal cells. The late pathway (the conversion of exogenous progesterone to aldosterone) is not stimulated by angiotensin II or altered by ANF. Racz et al. (1985) reported that ANF is equipotent in reducing aldosterone, deoxycorticosterone, and corticosterone production by bovine adrenal cells stimulated by angiotensin II, ACTH, prostaglandin E_1, forskolin, phorbol ester, or potassium, indicating that ANF inhibits the early pathway of aldosterone biosynthesis. In contrast, Campbell et al. (1985) reported that in rat adrenal cells both the early and the late pathway stimulated by angiotensin II are inhibited by ANF.

De Léan et al. (1984b) reported that cortisol secretion in cultured bovine adrenal fasciculata cells stimulated by angiotensin II or ACTH is inhibited by ANF. Similar effects on basal and ACTH-stimulated cortisol production by cultured bovine zona fasciculata cells were found by Higuchi et al. (1986a). Binding sites in the bovine adrenal cortex appear, however, very sparse in the fasciculata and are concentrated mainly in the zona glomerulosa, as was demonstrated by radioautography. Racz et al. (1985) showed that these effects in bovine adrenal fasciculata cells are due to an action of ANF on the early pathway of aldosterone biosynthesis. In contrast, no ANF receptors are de-

tected in rat adrenal fasciculata cells (Schiffrin et al. 1985a). Corticosterone production is unaffected by ANF (Campbell et al. 1985) in vitro, and the corticosterone response to ACTH in vivo in conscious rats is not altered by ANF (Chartier and Schiffrin 1986a). Thus, as for the distribution of angiotensin II receptors and responses, there are differences in the localization of ANF receptors and responses between the bovine and rat adrenal. An intriguing observation was reported by Jaiswal et al. (1986), who found that ANF increases cGMP levels in isolated rat adrenal fasciculata cells and also increases the corticosterone output. Higuchi et al. (1986a) also localized binding sites for ANF in the zona reticularis, using in vitro radioautography, and they reported that ANF inhibits the basal and ACTH-stimulated secretion of dehydroepiandrosterone. Finally, ANF has been reported to stimulate [^{3}H]thymidine incorporation into DNA of bovine adrenal glomerulosa cells in primary culture, which may indicate a growth-stimulating activity of ANF in the adrenal (Horiba et al. 1985).

No changes in the density or affinity of adrenal ANF receptors are detected after changes in sodium balance in rats by examining binding to homogenates from adrenal capsules (Schiffrin et al. 1986c) or by in vitro autoradiography (Lynch et al. 1986). In the latter study, water deprivation, which may lower ANF levels in plasma, results in an increase in the density of ANF sites. In renal and DOCA-salt hypertensive rats, the adrenals exhibit little change in ANF receptor density (Schiffrin et al. 1986c; Schiffrin and St-Louis 1985) in spite of the increases in plasma ANF concentration found in these rats (Schiffrin and St-Louis 1987). In SHR, Lynch et al. (1986) did not find differences in the density of ANF sites as compared with WKY at 7–10 weeks of age, using in vitro autoradiography. In line with this, Racz et al. (1986a) found that the potency of ANF to inhibit aldosterone output in zona glomerulosa cells from SHR, WKY, and DAHL salt-sensitive and salt-resistant rats is similar.

9.4 Pituitary Gland and Eye (M. Cantin and J. Gutkowska)

9.4.1 Anterior Lobe of the Pituitary

ANF(Arg-101 to Tyr-126) has been shown by in vitro radioautography to bind to specific receptors in the anterior pituitary (Quirion et al. 1984a) and to inhibit adenylate cyclase activity in anterior pituitary homogenates and cultured cells (Anand-Srivastava et al. 1985a). In monolayer cultures of anterior and intermediate lobe cells of the pituitary, rat ANF(Ser-103 to Tyr-126) suppresses the release of ACTH, β-endorphin, and γ-melanocyte-stimulating hormone (γ-MSH) in a dose-dependent manner, as well as the release of these peptides under the influence of CRF (Shibasaki et al. 1986). In rat anterior

pituitary cells in culture, the effects of rat ANF (Ser-103 to Tyr-126; Ser-103 to Ser-123; Ser-102 to Arg-125) and of human ANF (Ser-99 to Tyr 126) were examined on the secretion of ACTH, growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), and luteinizing hormone (LH) and on the cellular production of cAMP and cGMP in basal conditions and during stimulation with 10 nM CRF, gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), and growth hormone-releasing factor (GRF) (Abou-Samra et al. 1987). The only significant effect of ANF is the stimulation of cellular cGMP. The stimulation of LH release was also found, but it was attributed to the contamination of Bachem rat ANF (Ser-103 to Tyr-126; Ser-103 to Ser-125) by a potent GnRH agonist (Abou-Samra et al. 1987). The stimulation of cGMP production by ANF in culture is not due to contamination with fibroblasts or endothelial cells since it occurs in an ACTH-secreting mouse tumor cell line (AT-t20) which is entirely made up of secretory cells (Heisler et al. 1986).

Although some investigators have reported that ANF inhibits CRF-stimulated ACTH release (Shibasaki et al. 1986) in rat anterior pituitary, this has not been confirmed (Abou-Samra et al. 1987; Heisler et al. 1986). It has also been reported that human ANF(Ser-99 to Tyr-126) (Bachem) stimulates LH and follicle-stimulating hormone (FSH) release from superfused rat anterior pituitary cells (Horvath et al. 1986). Whether this is the result of contamination requires further study. At the moment, the available results indicate that ANF markedly stimulates cGMP production in rat anterior pituitary cell cultures without modifying the secretion of anterior pituitary hormones. ANF does not decrease the activity of adenylate cyclase when studies are carried out with high concentrations (300 μM) of GTP which completely abolish the expression of the inhibitory response (Anand-Srivastava et al. 1985a; Heisler et al. 1986).

9.4.2 Vasopressin Release

Because of the effects of vasopressin on the kidney and blood vessels and its role in the control of volumes, several studies have been done on the possible interrelationships of ANF with the regulation of the release of this peptide from the posterior pituitary. In vitro binding studies with ^{125}I-labeled ANF revealed the presence of high-affinity receptor sites displaying a pK of 9.9, a K_d of 0.14 mM, a B_{max} of 20 fmol/posterior lobe, and an IC_{50} of 200 pM (Januszewicz et al. 1985). ANF also inhibits adenylate cyclase in posterior pituitary homogenates (Januszewicz et al. 1985). In addition, ANF inhibits the stimulatory effect of N-ethylcarboxamide adenosine (NECA), NaF, and forskolin on adenylate cyclase from rat pituitary homogenates (Januszewicz

et al. 1985). In the frog, IR-ANF has been localized by the immunogold technique in dense-core vesicles of nerve endings in the posterior pituitary (Netchilailo et al. 1986).

The first studies using quartered posterior lobes of the rat pituitary indicated that ANF(Arg-101 to Tyr-126) produces the stimulation of arginine vasopressin (AVP) release (Januszewicz et al. 1985). In the isolated posterior pituitary (with the neurointermediate lobe), ANF(Arg-103 to Tyr-126) was shown to have no effect during a 15-min test period, but at concentrations of 10^{-10} or 10^{-9} M it slightly but significantly reduced AVP release during the recovery period (Crandall and Gregg 1986). When the superfused rat posterior pituitary gland is used (Obana et al. 1985a), ANF(Arg-102 to Tyr-126) ($10^{-6}-10^{-10}$ M) significantly inhibits basal and also KCl- (50 nM) or angiotensin II-stimulated AVP release. In the same conditions, AVP also decreases cAMP and increases cGMP secretion from the posterior pituitary.

The effect of ANF(Arg-101 to Tyr-126) has also been evaluated in an in vitro model of rat hypothalamoneurohypophysial complex in organ culture, in which part of the hypothalamus is separated from the posterior pituitary by a fluidtight barrier, with an intact stalk connecting both structures. ANF does not change basal AVP release from the posterior pituitary, but when the osmolality of the hypothalamic side is increased (324 ± 2 mosmol/kg H_2O), ANF(Arg-101 to Tyr-126) significantly reduces the ensuing release of AVP on the pituitary side (Januszewicz et al. 1986b). Using an identical preparation, it was found that ANF(Arg-101 to Tyr-126) significantly inhibits basal AVP release after a delay of at least 15 min (Crandall and Gregg 1986). When the exposure period is increased to 30 min, ANF (10^{-10} M) significantly decreases angiotensin II-stimulated AVP release in a dose-dependent manner. The same concentrations of ANF do not significantly depress acetylcholine-stimulated AVP release (Crandall and Gregg 1986).

The first report on ANF-AVP interactions in vivo in the rat revealed that ANF(Ser-103 to Tyr-126) injected i. v. at doses ranging from 0.02 to 2.0 nmol significantly inhibits the increase in plasma AVP induced by 3 days of water deprivation (Samson 1985b). The increase in plasma AVP produced by hemorrhage is also inhibited by the i. v. injection of 2.0 nmol ANF ($\simeq 5$ µg) (Samson 1985b). In conscious euvolemic rats injected with 10 µg ANF(Arg-101 to Tyr-126), there is a significant decrease in the resting levels of AVP (Januszewicz et al. 1986a). Lower doses ($1-5$ µg) have no significant effect. In the same manner, the increases in plasma AVP produced by osmotic stimuli such as NaCl (600 mM) or mannitol (900 mM) are only significantly reduced by 10 µg ANF and not by smaller doses (Januszewicz et al. 1986a). The increase in AVP produced by the injection of sucrose (900 mM) is not inhibited by 10 µg ANF (Januszewicz et al. 1986a). This dose of ANF produces high plasma levels of IR-ANF (39.3 ng/ml, 1.01 ng/ml, and 0.32 ng/ml at 1, 5, and 10 min, respectively), which is in line with the well-known fast disap-

pearance of ANF (Murthy et al. 1986b). These concentrations are far above the normal values reported for circulating IR-ANF in the rat.

It has recently been shown that in conscious rats, intracerebroventricularly administered ANF can inhibit AVP release without any change in mean arterial pressure, heart rate, plasma osmolality, and sodium levels (Share et al. 1986). Other investigators have also shown that the lateral ventricular infusion of ANF stimulates urine flow in both normally hydrated and sodium-depleted conscious rats, while severalfold higher doses of ANF injected i. v. fail to exert similar effects (Fitts et al. 1985).

All these observations suggest that ANF in the central nervous system may exert physiological effects on AVP release. Pharmacological levels of ANF would be needed to exert, from the periphery, similar effects on brain structures involved in the regulation of AVP release and devoid of blood brain barrier.

9.4.3 Eye

The presence of binding sites for ANF(Arg-101 to Tyr-126) has been ascertained by in vitro (Quirion et al. 1984a) and in vivo (Bianchi et al. 1985) photonic microscopy (Fig. 22a, b) in the ciliary body ephithelium of the eye. It was shown by electron microscope radioautography that these binding sites are exclusively localized (at 2 min after the injection of ANF) on the plasmalemma of the basilar infoldings of the "pigmented" ciliary body epithelium (Fig. 22c) (Bianchi et al. 1986a). The receptor has a pK (-log K_d) of 10.4 (39 pM) and a B_{max} of 28 fmol/mg protein. It is negatively coupled to adenylate cyclase (Bianchi et al. 1986a). The intraocular (intracameral or intravitreous) injection of ANF(Arg-101 to Tyr-126) produces a significant and long-lived (more than 5-h) decrease in intraocular pressure, while the topical application of ANF on the eye surface produces smaller and more inconsistent effects (Sugrue and Viader 1986). As regards the intravitreous injection of ANF in the rabbit, these results have now been confirmed (Steardo and Nathanson 1986) and extended to the brain. It was shown that ANF stimulated particulate guanylate cyclase activity in purified rabbit and pig cerebral microvessels and in membrane fractions purified from whole rabbit choroid plexus. In arachnoid villi and in pia-arachnoid, only high concentrations of ANF produces a small stimulation of guanylate cyclase. In membrane preparations from epithelial cells isolated from the choroid plexus, the stimulation of guanylate cyclase by ANF is much greater than in membranes prepared from vascular cell-enriched choroid tissue. The study of the production of cerebrospinal fluid by ventricular cisternal perfusion shows that ANF produces a 35% decrease in the rate of production of this fluid in the rabbit (Steardo and Nathanson 1986).

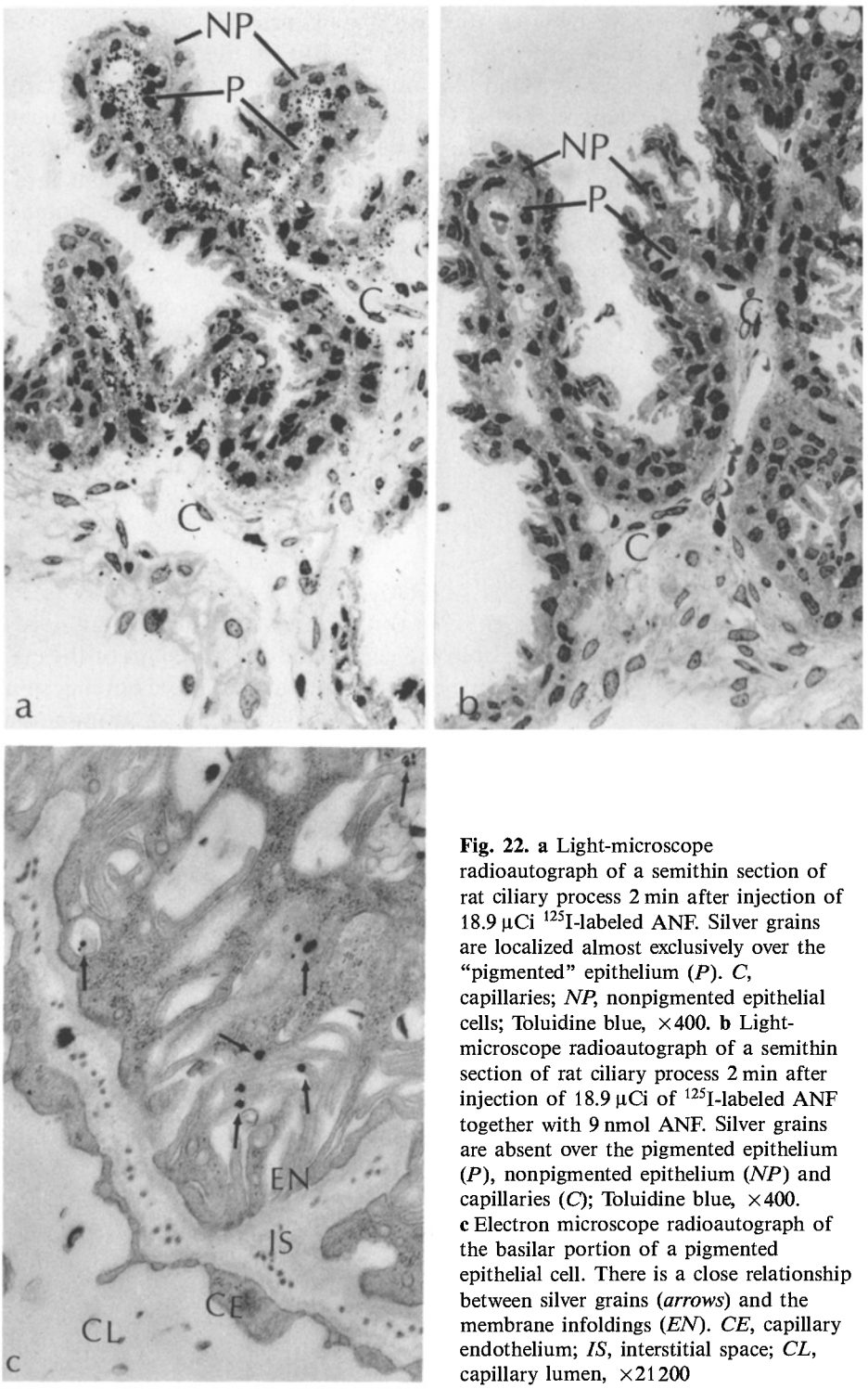


Fig. 22. a Light-microscope radioautograph of a semithin section of rat ciliary process 2 min after injection of 18.9 μCi ^{125}I-labeled ANF. Silver grains are localized almost exclusively over the "pigmented" epithelium (*P*). *C*, capillaries; *NP*, nonpigmented epithelial cells; Toluidine blue, ×400. **b** Light-microscope radioautograph of a semithin section of rat ciliary process 2 min after injection of 18.9 μCi of ^{125}I-labeled ANF together with 9 nmol ANF. Silver grains are absent over the pigmented epithelium (*P*), nonpigmented epithelium (*NP*) and capillaries (*C*); Toluidine blue, ×400. **c** Electron microscope radioautograph of the basilar portion of a pigmented epithelial cell. There is a close relationship between silver grains (*arrows*) and the membrane infoldings (*EN*). *CE*, capillary endothelium; *IS*, interstitial space; *CL*, capillary lumen, ×21200

10 Structure-Activity Relationships (G. Thibault)

Many atrial peptides, varying in length from 21 to 33 amino acids, were sequenced and synthesized in 1983 and 1984. The first synthetic one was the 26-residue peptide (Arg-101 to Tyr-126) produced in September 1983 (Nutt et al. 1986; Seidah et al. 1984) after its isolation by the group at the Clinical Research Institute of Montreal in June 1983 (Cantin and Genest 1985). Physiological studies of some of these peptides were undertaken without a precise knowledge of their biological potency. Extensive studies of the influence of the length of various ANF peptides on different physiological parameters were conducted. Peptides truncated either at the COOH terminal or at the NH_2 terminal were systematically assayed in a variety of biological and binding assays. The results are presented in Tables 14, 15.

All the NH_2-terminal extended or truncated peptides demonstrate a comparable degree of natriuretic activity. The variability of the natriuretic assay is such that a twofold difference is not significant. Only deletions of the residues 122–126 at the COOH terminal, as in ANF(Arg-101 to Cys-121), result in a marked reduction in natriuretic activity. In the dog, as reported by Needleman et al. (1985), natriuresis is not affected by the deletion of residues 99–102. The further removal of the NH_2-terminal residues or amino acids 124–126 at the COOH terminal reduces sodium excretion by a factor of 10. In fact, in vivo assays, such as natriuresis, are not good systems for assessing peptide potency since either proteolysis in the circulation or the physiological state of each animal may affect the response.

ANF is a potent relaxant of precontracted vascular and nonvascular smooth muscle. The relaxation of precontracted chick rectum proves to be a useful biological assay for screening ANF activity (Wakitani et al. 1985a). The sensitivity of this assay is at least ten times greater than that of the natriuretic one since levels as low as 10 pmol ANF can be detected. The potency of the peptides evaluated by this assay is shown in Table 14. ANF(Ser-99 to Tyr-126), ANF(Arg-101 to Tyr-126), and ANF(Cys-105 to Tyr 126) demonstrate the highest activity. Interestingly, the deletion of Arg 101 and 102 decreases the activity, but the further removal of Ser 103 and 104 seems to restore it completely. Here again, the deletion of Phe-Arg-Tyr at the COOH terminal is critical.

The relaxation of precontracted vascular tissue, such as the rabbit aorta, gives essentially the same results as the relaxation of intestinal smooth muscle (Garcia et al. 1985g; Fok et al. 1985; Olins et al. 1986). These results are further supported by studying the vasodilative effects of ANF. In isolated rat or dog kidney, the longer peptides, such as ANF(Ser-99 to Tyr-126) are the most effective in increasing renal blood flow; this increase is secondary to a decrease in renal vascular resistance (Wakitani et al. 1985a; Katsube et al. 1985b). However, most of the peptides produce an equivalent fall in blood

Table 14. Relative biological potency of ANF-related peptides

ANF peptides	Na Excretion[a]	Relaxation				Aldosterone inhibition		Cyclic GMP production	
		Chick rectum[a]	Rabbit aorta[b]	Rabbit aorta[c]	Rabbit aorta[d]	Bovine adrenal cells[e]	Rat adrenal cells[f]	Rat glomeruli[g]	Bovine endothetial cells[h]
(54–126)	44	–	–	0.2	–	63	–	–	–
(96–126)	57	–	–	32	–	253	–	–	–
(99–126)	51	122	100	127	129	100	100	–	–
(101–126)	100	100	100	100	100	100	100	100	100
(102–126)	133	36	50	100	114	100	–	15	–
(103–126)	95	15	20	10	48	100	20	–	6
(104–126)	86	76	–	8	–	32	–	–	–
(105–126)	143	162	15	32	–	32	–	126	–
(101–125)	116	74	–	4	–	1000	150	–	–
(101–124)	75	–	–	0.4	–	–	–	–	–
(101–123)	21	43	–	0.2	–	3	5	76	–
(101–121)	3	21	–	0.1	–	1	–	22	–
(103–125)	42	9	40	0.6	71	63	20	–	7
(103–123)	22	5	0.3	0.3	0.5	0.3	5	–	0.1
(106–125) Met^{110}	–	0.5	–	0.004	–	0.1	–	–	–
(99–126)	–	–	–	–	–	–	30	–	86

All potencies were calculated by comparison to ANF (Arg-101 to Tyr-126)
[a]Thibault et al. 1984c. [b]Fok et al. 1985. [c]Garcia et al. 1985g. [d]Olins et al. 1986. [e]De Lean et al. 1985b. [f]Schiffrin et al. 1985a. [g]Hamet et al. 1986. [h]Leitman and Murad 1986

pressure in intact animals (Wakitani et al. 1985b), suggesting that, as for the natriuretic assay, in vivo experiments may not be adequate to evaluate accurately the potency of such peptides.

When cells of adrenal zona glomerulosa are incubated in the presence of ANF, an inhibition of aldosterone secretion is observed. This inhibition is seen on both the basal and the stimulated secretion. Fifty percent inhibition is observed with ANF concentrations of 50–200 pM (de Léan et al. 1985a; Schiffrin et al. 1985a). The deletion of the last three COOH-terminal residues drastically reduces the inhibitory effects of ANF on aldosterone secretion. However, the removal of the last two NH_2-terminal residues preceding the Cys 105 only slightly affects this activity (Table 14).

The biological response to ANF appears to be in part mediated by an activation of particulate guanylate cyclase followed by an enhanced production of cGMP (Waldman et al. 1984; Hamet et al. 1984). Whether cGMP is the prime and exclusive messenger of the biological action remains, however, to be determined. Partial data (Table 14) indicate that a lower biological response to ANF peptides is also accompanied by a lower efficiency of these peptides in stimulating the production of cGMP (Hamet et al. 1986; Leitman and Murad 1986).

Observations of the various physiological effects of ANF were later followed by the finding that binding sites for ANF are located in the same target tissues. Binding sites for ANF have been reported in many tissues. In Table 15, the relative potency of the dissociation constant of related ANFs in some tissue preparations is presented. In general, the truncated ANFs demonstrate the same degree of potency in all these tissue preparations. The potency is only slightly affected by the removal of Arg-101, but further deletion of residues at the NH_2 terminal decreases it. The deletion of Phe-Arg at the COOH-terminal side decreases binding.

Analysis of the data presented in Tables 14 and 15 indicates a fairly good correlation between the binding affinities to the truncated ANFs and the levels of biological responses, which in turn indicates that the binding sites for ANF correspond to true receptors. These receptors are associated in all cases with a biological response, which may be the production of cGMP, sodium excretion, smooth muscle relaxation, or the inhibition of steroidogenesis.

The following general comments can thus be made on the basis of these results. The maximal potency is obtained with ANF(Arg-101 to Tyr-126) and ANF(Ser-99 to Tyr-126) (Garcia et al. 1986a). The addition of amino acids at the NH_2 terminal, as in ANF(Glu-54 to Tyr-126), only slightly affects the biological effect and the binding potency. The ANF propeptide is about five to ten times less potent (Kangawa et al. 1985; Hayashi et al. 1986). These data seem also to indicate that, with the assays used, there is no tissue or species specificity. Truncated peptides demonstrate comparable potency on rat, human, rabbit, bovine, or chicken tissues. This is further exemplified with

Table 15. Relative binding potency of ANF-related peptides

ANF peptides	Binding to membranes of						
	Rat adrenal cells[a]	Rat glomeruli[b]	Rat mesenteric arteries[a]	Bovine aorta endothetial cells[c]	Bovine adrenal cells[d]	Rabbit lung[e]	Human platelets[f]
(54–126)	107	–	148	–	32	–	–
(96–126)	122	–	150	–	100	–	–
(99–126)	100	–	200	–	158	91	–
(101–126)	100	100	100	100	100	100	100
(102–126)	24	–	20	–	100	61	–
(103–126)	20	2	20	42	10	30	41
(104–126)	13	–	18	–	13	–	–
(105–126)	11	6	29	–	8	–	–
(101–125)	190	–	150	–	250	–	–
(101–124)	37	–	39	–	6	–	–
(101–123)	35	5	40	–	0.3	–	–
(101–121)	31	19	26	–	0.08	–	–
(103–125)	6	–	15	54	8	48	18
(103–123)	11	–	5	17	0.02	2	7
(106–125) Met^{110}	–	–	–	–	0.004	–	–
(99–126)	81	–	77	96	63	–	90

All values were calculated by comparison to ANF (Arg-101 to Tyr-126)
[a]Schiffrin et al. 1985a. [b]Carrier et al. 1985. [c]Leitman and Murad 1986. [d]De Lean et al. 1985b. [e]Olins et al. 1986. [f]Schiffrin et al. 1986

human ANF, which gives an identical response on rat, bovine, and human tissue.

Human and rat ANF show a similar potency, suggesting that the methionine residue in position 110 may not be involved in the binding of ANF to its receptor (Hayashi et al. 1986; Chino et al. 1985). This was further confirmed by Seymour et al. (1986), who observed comparable degrees of vasodilation, sodium excretion, and renin inhibition in the conscious dog.

Studies done with ANF(Phe-106 to Try-126) suggest the importance of the disulfide bridge in the conformational structure of ANF and therefore for its binding (Schiller et al. 1985; see Sect. 11). The linear peptide has a very low biological activity, being about 1000 times less potent than ANF(Arg-101 to Tyr-126). However, it is still active at concentrations in the micromolar range, indicating that the loop conformation is not an absolute requirement for biological activity.

The removal of NH_2-terminal residues from Arg-101 to Cys-105 progressively reduces the potency by a factor of 10. These amino acids, in particular Arg-102 and Ser-103, are probably important in the stabilization of

ANF binding to its receptors. On the other side, the deletion of COOH-terminal residues, with the exception of Tyr-126, decreases by a factor of 100 the binding and consequently the biological responses.

Interestingly, the simultaneous deletion of amino acids at both ends, as in atriopeptin I, ANF(Ser-103 to Ser-123) causes additive decreased effects. ANF(Ser-103 to Arg-125) presents similar responses to those of ANF(Ser-103 to Tyr-126) since the Tyr-126 does not seem to be necessary for the activity.

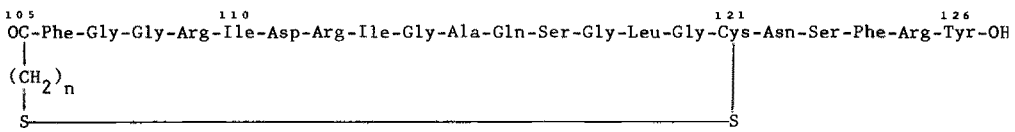
Therefore, the amino acids flanking the disulfide bridge, in particular those at the COOH terminal, as well as the disulfide bridge itself, are necessary to express the full biological activity. The circulating forms, ANF(Ser-99 to Tyr-126) and ANF(Arg-101 to Tyr-126) are equipotent and induce maximal physiological responses.

11 **Analogues** (P. W. Schiller)

Analogues of ANF are designed and synthesized for two purposes: (a) they will serve as indispensable tools for the further clarification of the physiological role(s) of ANF and (b) they may serve as new drugs in such disorders as hypertension, congestive heart failure, and renal failure. As with any newly discovered peptide, important initial goals are the preparation of analogues with enhanced stability against enzymatic degradation, the development of antagonists, and the synthesis of receptor-specific analogues.

The question of the importance of the ring structure for biological activity has been addressed by several investigators. The observation that the linear fragment ANF(106–125) is a full agonist with about 100- to 1000-fold lower potency than ANF(103–125) in various in vitro assay systems indicates that the disulfide linkage stabilizes the bioactive conformation of ANF but is not an absolute requirement for biological activity (Schiller et al. 1986). The latter finding was confirmed by the observation that the linear fragment ANF(106–120) shows low but significant diuretic/natriuretic activity (Kiso et al. 1985). It is also of interest to note that the side-chain-linked parallel and antiparallel dimers of human ANF(99–126) have about half the potency in the rabbit aorta assay and a slightly reduced diuretic/natriuretic activity in comparison with monomeric ANF(99–126) (Kambayashi et al. 1986). In addition, a slower onset of the biological effect was observed with the dimers. The different conformational constraints present in the monomer and in the dimers may be responsible for the observed differences in the activity profile.

Structure-activity studies with various ANFs truncated at both the NH_2-terminal and the COOH-terminal end had shown that the NH_2-terminal exocyclic peptide segment is relatively less important for activity than the COOH-terminal exocyclic segment (Thibault et al. 1987b). Thus, the



n = 1, [Mac^{105}]ANF(105-126) (I)

n = 2, [Mpr^{105}]ANF(105-126) (II)

n = 3, [Mbu^{105}]ANF(105-126) (III)

Fig. 23. Structural formulas of desamino ANF(105–126) analogues

NH_2-terminally truncated peptide ANF(105–126) was found to be still quite potent in all assay systems. This observation led to the design of an analogue of ANF(105–126) which lacks the NH_2-terminal amino group. Such a desamino analogue was obtained through the substitution of 3-mercaptopropionic acid (Mpr) for the cysteine residue in position 105 of the peptide sequence (Fig. 23, analogue II) (Schiller et al. 1987).

To assess the effect of subtle ring contraction or expansion on the activity profile, analogues of Mpr^{105}ANF(105–126) in which the side-chain in position 105 is shortened or lengthened by one methylene group were prepared by the substitution of 2-mercaptoacetic acid (Mac, analogue I) or 4-mercaptobutyric acid (Mbu, analogue III), respectively, for Cys^{105}. All three analogues show very potent diuretic/natriuretic effects (Table 16). The highest diuretic activity is observed with analogue II, which is about five times more active than ANF(101–126). Similarly, the desamino analogues show two to four times higher activity than ANF(101–126) and 1.5–2.5 times higher activity than ANF(105–126) in the natriuresis assay. These results indicate that analogues I–III display higher diuretic and natriuretic activity than any other ANF peptide or analogue reported to date. The hypotensive effect of these analogues is found to be comparable to that of ANF(101–126) (Table 16).

Table 16. Natriuretic/diuretic and blood pressure-lowering effects of ANP analogues

No.	Analogue	Urine volume (µl/20 min)	Sodium excretion (µEq/20 min)	Blood pressure decrease (mmHg)
I	Mac^{105} ANP (105–126)	1079 ± 283	151 ± 55	22.5 ± 3.2
II	Mpr^{105} ANP (105–126)	1587 ± 566	123 ± 75	22.5 ± 5.2
III	Mbu^{105} ANP (105–126)	1321 ± 131	232 ± 75	13.7 ± 3.1
IV	ANP (101–126)	334 ± 269	63 ± 17	18.3 ± 6.0

Bolus injections of 1 nmol of each compound; mean of 3 determinations ± SEM

Recently, the structurally related analogue Mpr^{105}, *D*-Ala^{107}ANF(105–126) has also been reported to have a potent blood pressure-lowering effect (Mogannam et al. 1986). In an in vitro cGMP assay, the desamino analogues are also about as potent as ANF(101–126) (S. C. Pang, J. Tremblay, P. Hamet, unpublished results). On the other hand, relatively lower potencies are observed with these compounds in the rabbit aorta assay and in the bovine zona glomerulosa aldosterone suppression and receptor binding assays (Schiller et al. 1987). In the latter assays, analogues II and III are about equipotent and three to six times more potent than analogue I. The extraordinarily high activity of the desamino analogues in vivo may be due to their stability against degradation by aminopeptidases. Furthermore, these peptides have a lower overall positive charge than natural ANF, due to the omission of the α-amino group, and therefore nonspecific ionic adsorption to negatively charged sites in the vascular bed may be reduced. Such nonspecific binding may be a major factor responsible for the rapid disappearance of natural ANF from circulation (Murthy et al. 1986b).

The replacement of the two half-cystine residues in ANF(105–126) with α-aminosuberic acid results in a dicarba analogue which is quite potent in the chick rectum assay but has relatively low activity in the diuresis/natriuresis assay and in the rabbit aorta assay (Chino et al. 1985). Low diuretic/natriuretic activity was also observed with a dicarba analogue of the bare ring structure in which the two cysteine residues had been replaced by 8-aminocaprylic acid (Kiso et al. 1985).

Several analogues characterized by amino acid deletions within the ring structure of ANF have been prepared. The successive omission of Gly-120, Leu-119 and Gly-118 in ANF(103–126) results in a progressive loss of potency in various in vitro assay systems (Schiller et al. 1986). The deletion of Gly-107, the tripeptide segment encompassing residues 106–108, or the hexapeptide segment comprising residues 115–120 also drastically reduces potency (Chino et al. 1985), as does the removal of Phe-106 or the dual omission of Gly-107 and Gly-118 (Adams 1986). Even more drastic reductions in ring size are achieved by changing the position of the half-cystine residues in the peptide sequence (Albus et al. 1986). Thus, Cys-105 to Cys-114 and Cys-108 to Cys-114 analogues of ANF were prepared; however, these compounds turned out to be inactive. These results indicate that ring contractions are not well tolerated and that the ring size present in native ANF is optimal for high agonist activity.

A number of ANF analogues were prepared through amino acid substitutions in various positions of the peptide sequence. In one study (Nutt et al. 1986), *D*-amino acid residues were systematically substituted in positions 104 through 124 and the vasorelaxant potencies of the resulting analogues were determined. High potency was retained by the configurational inversion of Gln-116 and Ser-123 and by the substitution of *D*-alanine for glycine in posi-

tions 106, 114, 118, and 120. On the other hand, the configurational inversion of Phe-106, Ile-110, Asp-111, Arg-112, Ile-113, Ser-117, and Leu-119 produced a major drop in potency. This study thus provides valuable information regarding *D*-amino acid substitutions in efforts aimed at improving the metabolic stability of ANF. High potency is retained if one or both arginine residues in positions 101 and 102 are moved closer to the ring; thus, Arg^{104}ANF(103–125) and $Arg^{103,104}$ANF(103–125) are found to be very potent in the rabbit aorta assay (Adams 1986). The substitution of tyrosine for Phe-106 results in a fourfold loss of potency (Misono et al. 1985).

Several substitutions were made in position 110, where isoleucine and hionine are located in rat and human ANF, respectively. Nearly equal potency is observed with analogues containing methionine, isoleucine, or norleucine in that position, whereas the Met(0)-110 analogue (methioninesulfoxide) is considerably less potent (Chino et al. 1985). In the COOH-terminal exocyclic peptide segment, the importance of the Phe-124 for vascular smooth muscle relaxation activity is documented by the observation that the substitution of N-methylphenylalanine in position 124 of ANF(103–126) results in an analogue at least two orders of magnitude less potent than the parent peptide in the rabbit aorta assay (P. W. Schiller, L. A. Maziak, unpublished results). Arg-125 represents another crucial residue for natriuretic/diuretic and vascular smooth muscle relaxation activity since analogues containing *D*-arginine, lysine, or glycine in position 125 are found to be only weakly active (Fok et al. 1985; Adams 1986). However, Phe-124 and Arg-125 are relatively less important for intestinal smooth muscle relaxation activity (Currie et al. 1984a). Tyr-126 can be omitted without loss of activity (Thibault et al. 1987b). Finally, ANF analogues with a COOH-terminal carboxamide function are shown to be more potent than corresponding peptides with a free COOH-terminal carboxyl group (Adams 1986), presumably due to enhanced resistance against attack by carboxypeptidases.

Little is known about the conformation(s) of ANF. The results of a ^1H nuclear magnetic resonance (NMR) study are consistent with a β-structure or an averaging of conformations (Fesik et al. 1985). There is little doubt that these large cyclic peptides exist in a conformational equilibrium in solution, and this idea has recently been confirmed by a second NMR study (D. Veber, personal communication). Thus, at this stage, conformational considerations are of little help for analogue design.

12 Relationship with the Autonomic Nervous System (O. Kuchel)

Volume overload and increased atrial pressures, two stimuli which induce ANF release, result equally in adaptive responses of the autonomic nervous

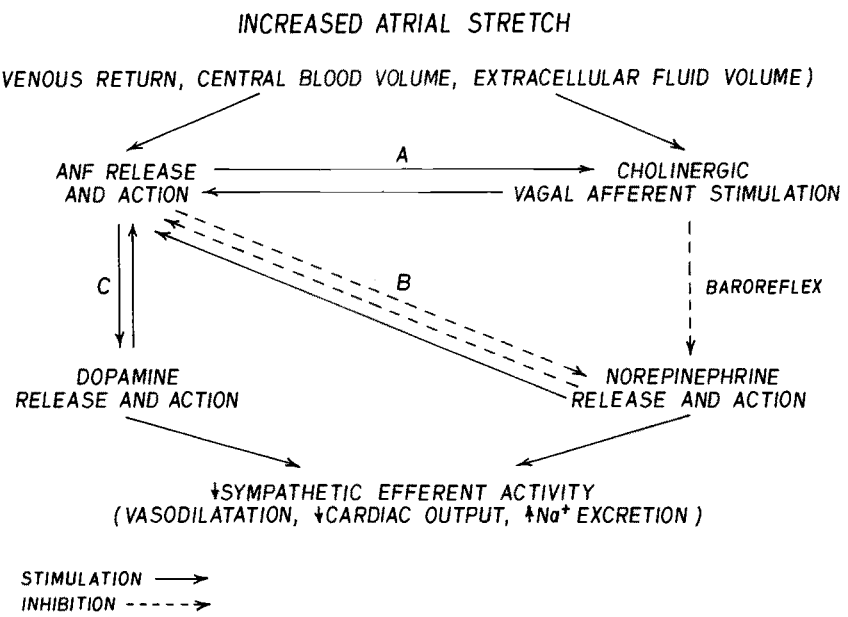


Fig. 24. Schematic view of ANF-catecholamine interactions in the control of body fluid balance. *A*, *B*, and *C* correspond to Sections 12.1 – 12.3, respectively. Note the triangular relationship between atrial stretch, ANF release, and afferent autonomic responses. Norepinephrine (*NE*) may also stimulate ANF release but inhibits its target action. The indirect, catecholamine-mediated efferent mechanisms by which ANF may cause vasodilation, decreased cardiac output, and increased Na^+ excretion are of a stimulatory and inhibitory nature

system (ANS). Afferent signals, sensing the pressure changes and "fullness" within the circulation, originate in arterial and cardiopulmonary baroreceptors, travel through glossopharyngeal and vagal fibers to central nuclei, and culminate in decreased peripheral sympathetic outflow (Abboud 1982). Volume expansion by saline evokes a reduction in norepinephrine (NE) excretion and plasma dopamine-β-hydroxylase (DβH) activity, accompanied by enhanced urinary dopamine (DA) excretion (Alexander et al. 1974). The possible interaction of ANF with these concomitant events within the ANS is summarized in Fig. 24.

12.1 ANF Release and Volume- and Baroreceptor-Mediated Modulation of Peripheral Sympathetic Nervous System Activity

The reflex response to volume and pressure overload is rapid and lasts only a few seconds. The time course of ANF release indicates, however, that it peaks only between 2 min (Ledsome et al. 1986) and 5 – 10 min (Agnoletti et al. 1986) of atrial stretching and then declines to preceding levels within

30 min despite the continuation of the load. The baroreceptor-mediated inhibition of sympathetic outflow which can be induced by exogenous ANF (Thoren et al. 1986) does not necessarily imply a physiological role for this mechanism; however, it can be conceived as a humoral extension of the initially purely neurogenic reflex response. The ANF gene found to be expressed in the aortic arch (Gardner et al. 1987) points to a previously unsuspected and possible role for ANF in blood pressure regulation through the baroreceptor reflex. On the other hand, the evidence for neurogenic signals being directly implicated in ANF release is controversial. There are some indications that cholinergic (Garcia et al. 1986b) and α_1-receptor-mediated (Schiebinger and Linden 1986a) and β-adrenergic receptor-mediated (Gibbs 1987) stimuli may be involved. Denervation experiments and in vitro or perfusion studies suggest, however, that ANF release is due to local stretching of the atrial wall, and not to the stimulation of atrial adrenergic receptors, reflex vagal activation, or the suppression of sympathetic efferents (Ledsome et al. 1986). However, the role of sympathetic stimuli which could additionally participate in the initial release of ANF cannot be entirely eliminated.

12.2 ANF and Efferent Sympathetic Response

Several experimental and clinical investigations (see review by Kuchel et al. 1987a) support the notion that ANF may act as an inhibitory modulator of neurotransmission, including catecholamine (CA) synthesis, release, and receptor action (Table 17). The inhibition of stimulated NE release by ANF in rat mesenteric arteries is reportedly due to a prejunctional mechanism (Nakamura and Inagami 1986). However, ANF has little influence on vascular responses to sympathetic nerve stimulation and exogenous NE in vivo (Herzer et al. 1987). This contradicts human infusion studies suggesting that the blood pressure (BP)-decreasing effect of ANF is predominantly due to an interaction with noradrenergic BP control mechanisms (Uehlinger et al. 1986b). ANF interferes with the expression of postsynaptic α_1-, but not α_2-receptor-mediated functions (Kyncl et al. 1987). Consequently, its inhibitory effect on NE-induced vasoconstriction may be more pronounced in arteries with α_1-receptor-mediated functions than in veins, where NE can produce contraction via both receptor subtypes. ANF applied locally to individual neurons inhibits their firing rate (Wong et al. 1986). The association between a reflex sympathetic discharge induced by carotid baroreceptor deactivation (neck compression) and reduction in ANF release independent of changes in atrial pressure (Volpe et al. 1986a) also indicates that the activation of efferent sympathetic stimuli may play an inhibitory role in the secretion of ANF.

Table 17. Some observations suggesting an inhibitory action of ANF on the sympathetic nervous activity (SNA)

In the whole organism
Suppressed SNA due to vagal stimulation in response to ANF bolus injection.
Absence of tachycardia following ANF infusion despite a profound fall in blood pressure.
Neck compression-induced carotid baroreceptor deactivation and sympathetic discharge inhibits ANF release independently of changes in atrial pressure.
ANF infusion suppresses the increased urinary NE excretion following adrenalectomy or in one kidney-one clip (1 K-1 C) renovascular hypertension.
ANF antagonizes the pressure action of NE.
Despite comparable BP decrease, less splanchnic nervous activity and NE release following ANF than after administration of systemic vasodilators.
In organ perfusion or local application studies
ANF causes a prejunctional inhibition of arterial NE release and interferes with the expression of postsynaptic α_1-receptor-mediated functions.
ANF locally applied to an individual neuron inhibits the neural firing rate.
ANF inhibits NE-induced vasoconstriction in vascular strips.
In vitro:
ANF inhibits CA synthesis in human pheochromocytoma cell cultures, in rat ganglia, and in bovine adrenomedullary cells.

The suppressive action of ANF on plasma NE levels may be particularly evident when plasma NE is elevated, as it is in the case of severe congestive heart failure (Viquerat et al. 1985). Hourly stepwise increases in ANF infusion (with corresponding increases in plasma IR-ANF in two patients with severe congestive heart failure) result in a transitory decrease in plasma NE (Kuchel et al., unpublished observations). Other workers did not find any change in plasma NE in response to ANF in similar but possible less severe degrees of congestive heart failure (Riegger et al. 1986). In such instances, ANF infusion does not increase urinary Na^+ excretion, suggesting that high sympathetic tone may entirely override the natriuretic action of ANF or that the kidneys have become less responsive to ANF. An overriding renal sympathetic tone may also explain the lack of natriuretic action of ANF infused into SHR presenting a sympathetic discharge induced by decreased BP (Kuchel et al. 1986). The findings that ANF does not dilate small arteries in vitro (Osol et al. 1986) and that lowered cardiac output may be a factor in the hypotensive action of ANF (Ackermann et al. 1984) are both compatible with the possibility that its hypotensive action in vivo is not only direct but also attributable to a withdrawal of sympathetic outflow to the heart and small arteries. The theory of bidirectional negative interactions between ANF and NE is supported by observations that a NE infusion completely suppresses ANF-induced vasodilation (Bolli et al. 1986) and vice versa, and that

renal sympathetic nerves attenuate the natriuretic effect of ANF (Morgan et al. 1986).

12.3 Dopamine-ANF Interactions

The interactions between DA and ANF are mutual, and in contrast to NE, on appears to potentiate the other (Kuchel et al. 1987b). Supported by some preliminary evidence (Racz et al. 1986b), DA may be a stimulant for ANF release. The heart has the capacity of releasing DA and harbors high-affinity DA receptor binding sites (Drake et al. 1982). As regards the target action of ANF, pretreatment of rats with dopaminergic receptor inhibitors abolishes the natriuretic action of ANF (Marin-Grez et al. 1985); this suggests an involvement of dopaminergic mechanisms, at least in this effect of the peptide. However, more recent studies using specific dopaminergic inhibitors indicate that this action may result from renal hemodynamic changes provoked by dopaminergic suppression, rather than from dopaminergic inhibition itself (Webb et al. 1986).

The mutual positive interaction between DA and ANF deserves further attention because of the striking similarities between their homeostatic purpose, their effects, and their target tissues. DA and ANF are probably involved synergistically in the defense against volume expansion and hypertension, DA release being complementary to, or even synonymous with, the withdrawal of sympathetic tone. The latter elicits a suppression of DβH activity, with DA synthesis increasing and DA not only exerting effects opposite to those of NE but itself inhibiting NE release (Whitfield et al. 1980). Idiopathic edema is a hypodopaminergic state (Kuchel et al. 1977) with decreased plasma IR-ANF concentrations (Naruse et al. 1986). We have observed that some patients with refractory idiopathic edema and chronic abuse of diuretics have an inability to stimulate both DA and ANF by saline expansion (Kuchel et al. 1987c). Idiopathic edema may be as yet the only clinically documented state of a combined DA and ANF deficiency. Interestingly, this is also the only salt-retaining condition which appears to qualify as a genuine ANF deficiency (Naruse et al. 1986).

13 Physiopathology of ANF

13.1 Physiology and Pharmacokinetics in Normal Subjects
(P. Larochelle and J.R. Cusson)

With the exception of one study, in which the intranasal administration of ANF was followed by increased diuresis and natriuresis (Shionoiri and Kaneko 1986), ANF has always been given intravenously.

13.1.1 Hemodynamic Effects

Richards et al. (1985a) reported that in normal volunteers, ANF (100 µg i. v. bolus) produces a decrease in blood pressure, an increase in heart rate, and enhanced diuresis and natriuresis. The blood pressure-lowering effect of ANF was confirmed with continuous infusions at rates of 70–200 ng kg^{-1} min^{-1} (equivalent to 23–65 pmol kg^{-1} min^{-1}) (Weidmann et al. 1986a; Biollaz et al. 1986b; Fujio et al. 1986; Bussien et al. 1986) and with the i. v. bolus administration of 50 and 100 µg (Larochelle et al. 1987d). At lower infusion rates, blood pressure remained unchanged (Waldhausl et al. 1986; Anderson et al. 1986f, 1987; Biollaz et al. 1986b; Cuneo et al. 1986; Cody et al. 1986; Cusson et al. 1987). In cases where blood pressure decreases, heart rate increases, with the exception of some control subjects in whom ANF produces symptomatic hypotension, often accompanied by bradycardia (Bussien et al. 1986; Waldhausl et al. 1986; Weidmann et al. 1986a; Biollaz et al. 1986b; Cuneo et al. 1986; Cusson et al. 1987). The mechanism of this phenomenon (Fig. 25) is unclear at the present time.

There have been some studies aimed at elucidating the mechanisms whereby ANF reduces blood pressure. Bussien et al. (1986) reported that ANF increases skin blood flow in a dose-dependent fashion, suggesting that the vasorelaxant properties of ANF might be physiologically or pharmacologically relevant. Bolli et al. (1987) reported that ANF given intra-arterially increases forearm blood flow. Central hemodynamic effects of ANF in normal man were recently studied by Cody et al. (1986), using the i. v. infusion of ANF at 100 ng kg^{-1} min^{-1} (33 pmol kg^{-1} min^{-1}) for 60 min. Blood pressure, heart rate, cardiac index, and systemic vascular resistance are unchanged despite an increase in plasma ANF levels up to 710±118 pmol/l. However, the pulmonary capillary wedge pressure is significantly decreased from 11±1 to 7±1 (SEM) mmHg, whereas right atrial pressure tended to decrease from 4±2 to 3±1 mmHg.

13.1.2 Renal Effects

Diuresis and natriuresis are consistently produced following the administration of ANF either as a single bolus (Richards et al. 1985a; Kuribayashi et al. 1985), if the doses given are greater than 25 µg (Larochelle et al. 1987d) or as continuous infusions (Struthers et al. 1985; Tikkanen et al. 1985a; Waldhausl et al. 1986; Weidmann et al. 1986a; Anderson et al. 1986f; Biollaz et al. 1986b; Cuneo et al. 1986; Cody et al. 1986; Gnädinger et al. 1986; Fujio et al. 1986; Cusson et al. 1987). The extent of these effects is dependent on other experimental conditions, such as sodium intake (Uehlinger et al. 1985) and the extent of the reduction in blood pressure (Weidmann et al. 1986a),

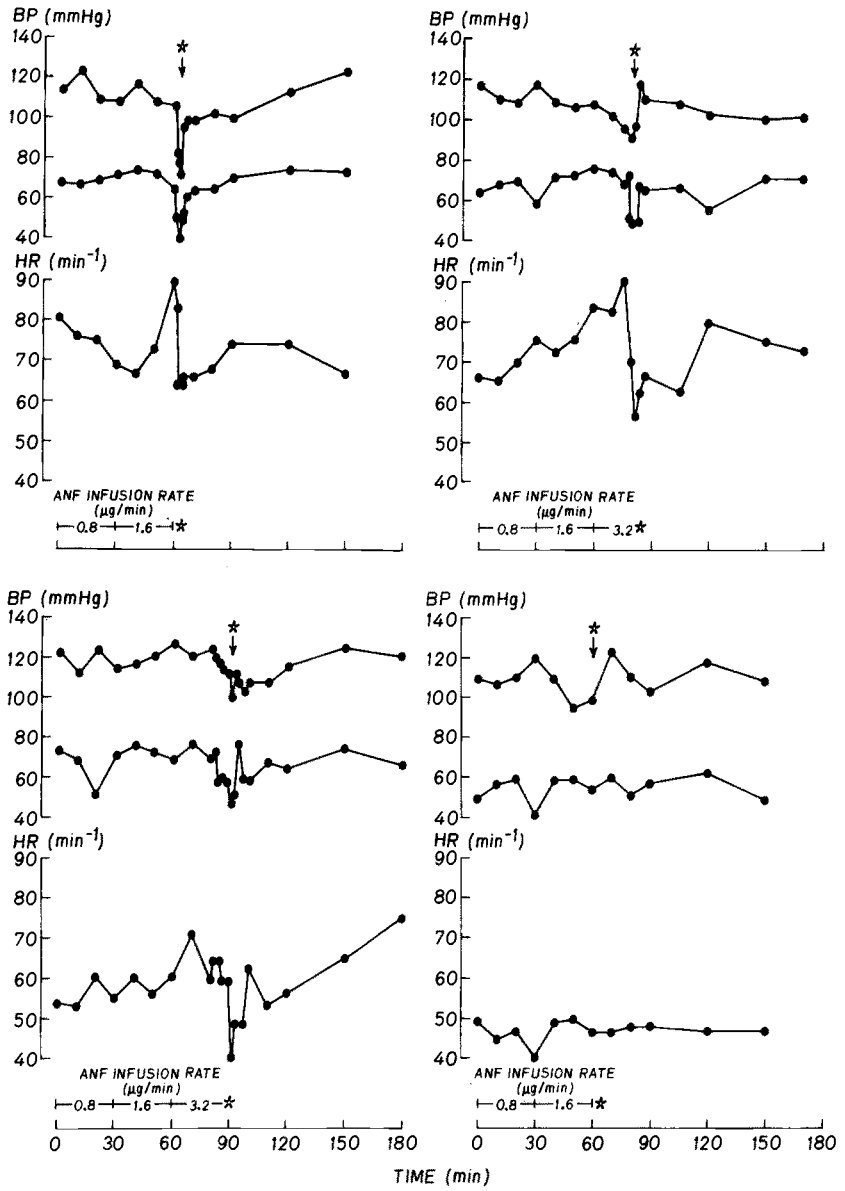


Fig. 25. Blood pressure (*BP*) and heart rate (*HR*) in four seated, healthy volunteers given ANF in stepped infusions. The *asterisk* indicates that sudden lightheadedness, with nausea, occurred. The infusion of ANF was then immediately discontinued. (From Cusson et al. 1987)

since hypotension can activate antidiuretic and antinatriuretic responses. ANF also increases the urinary excretion of chloride, calcium, magnesium, and phosphorus (Richards et al. 1985a; Weidmann et al. 1986a; Biollaz et al. 1986b; Cuneo et al. 1986), but it does not affect (Tikkanen et al. 1985a; Weidmann et al. 1986a; Cody et al. 1986) or even reduces (Biollaz et al. 1986b;

Cusson et al. 1987) potassium excretion. GFR, as measured by inulin clearance (Biollaz et al. 1986b; Cody et al. 1986) or by ^{51}Cr-labeled ethylenediaminetetraacetate (EDTA) clearance (Weidmann et al. 1986a) remains unchanged, whereas the filtration fraction increases and effective renal plasma flow decreases. ANF also increases osmolar and free water clearance (Weidmann et al. 1986a; Biollaz et al. 1986b; Cody et al. 1987).

ANF has no apparent effect on plasma electrolyte levels, but it increases plasma albumin and hematocrit levels due to hemoconcentration (Weidmann et al. 1986a; Cody et al. 1986; Gnädinger et al. 1986), thus confirming findings in animals (Almeida et al. 1986).

13.1.3 Hormonal Effects

The hormonal effects of ANF have been repeatedly studied. A reduction in plasma aldosterone concentration (when baseline levels are not suppressed) and no change or a slight decrease in PRA have been reported (Espiner et al. 1985; Weidmann et al. 1986a; Cuneo et al. 1986; Cody et al. 1986). The aldosterone response to the infusion of angiotensin II is attenuated (Vierhapper et al. 1986) or abolished (Anderson et al. 1986f) by ANF. Thus, in humans, ANF antagonizes the secretion of aldosterone, but further studies are needed to clarify its possible effects on PRA. ANF does not change plasma cortisol levels (Richards et al. 1985a; Shionoiri and Kaneko 1986; Cuneo et al. 1986) or ACTH levels (Cuneo et al. 1986).

Preliminary evidence suggests that ANF increases insulin secretion in man (Uehlinger et al. 1986a), and that the increase in plasma norepinephrine levels seen following ANF administration is most likely due to baroreflex sympathetic stimulation by its blood pressure-lowering action (Weidmann et al. 1986a; Uehlinger et al. 1986a; Larochelle et al. 1987d). Finally, ANF given as a continuous infusion over 20 min at the rate of 100 ng kg^{-1} min^{-1} (33 pmol kg^{-1} min^{-1}) clearly decreases plasma AVP levels (Fujio et al. 1986). This supports the experimental evidence of the inhibitory effect of ANF on AVP production (see Sect. 9.4).

13.1.4 Effect on Plasma and Urinary cGMP

Bolus (Gerzer et al. 1985) and continuous infusions (Ohashi et al. 1986a, b) of ANF increase plasma and urinary levels of cGMP in a dose-dependent fashion (Larochelle et al. 1987d; Cusson et al. 1987). Measurements of cGMP in plasma and urine are seen as useful indices of ANF secretion and action (see Sect. 8.1). However, their specificity and their correlation with ANF plasma levels under physiological circumstances remain to be established.

Table 18. Pharmacokinetic parameters of ANF (adapted from Larochelle et al. 1987d)

	Dose (μg)	
	50	100
Distribution half-life (min)	1.2 ± 0.2	1.8 ± 0.2
Elimination half-life (min)	4.7 ± 0.7	10.7 ± 3.8
Pseudo-steady-state volume of distribution (l)	33.2 ± 6.4	27.5 ± 12.7
Volume of distribution of the central compartment (l)	7.5 ± 2.1	5.5 ± 1.4
Volume of distribution of the peripheral compartment (l)	25.7 ± 6.6	22.1 ± 11.6
Plasma clearance (ml/min)	5948 ± 1211	3389 ± 948

13.1.5 Pharmacokinetics of ANF

Only a few studies have attempted specifically to characterize ANF pharmacokinetics (Yandle et al. 1986b; Nakao et al. 1985; Larochelle et al. 1987d; Cusson et al. 1987), whereas the effect of ANF administration on its plasma concentration has been studied by many groups.

Differences in RIA methods and study design probably account for the wide range of plasma ANF levels obtained after continuous infusions of ANF. For instance, infusions of ANF at a rate of 3.3 μg/min (Yandle et al. 1986b) and of 12.5 μg/min (Weidmann et al. 1986a) result in plasma ANF levels close to 400 pmol/l. More recently, low rates of infusion such as 1.2 pmol kg^{-1} min^{-1} (Anderson et al. 1987) and 0.8 μg/min (Cusson et al. 1987) produced plasma ANF levels close to high physiological levels.

Following the cessation of a continuous infusion of ANF or after a bolus of ANF, plasma ANF levels quickly return to baseline values. One or two phases of decline in plasma ANF levels are found, with half-lives of less than 11 min (Nakao et al. 1985; Yandle et al. 1986b; Larochelle et al. 1987d; Cusson et al. 1987). ANF appears to be widely distributed, perhaps due to the many recognition sites for ANF in blood vessels and other tissues. These receptors are perhaps also responsible for the high metabolic clearance rate of ANF because of their binding and internalization of ANF (see Sect. 2). The pharmacokinetic parameters found after the bolus administration of 50 and 100 μg ANF are given in Table 18 (according to Larochelle et al. 1987d). The longer elimination half-life of ANF after the highest dose suggests that ANF might not follow linear kinetics.

13.1.6 Side Effects

A sensation of flushing is felt by many subjects, and there is also a feeling of lightheadedness upon standing in a few of them. A sudden fall in blood

pressure, often with a bradycardic response and nausea, has been described in several studies (Bussien et al. 1986; Waldhausl et al. 1986; Weidmann et al. 1986a; Biollaz et al. 1986b; Cuneo et al. 1986; Cusson et al. 1987). An example of such a reaction is shown in Fig. 25 (Cusson et al. 1987). Whether ANF interferes with the normal baroreflex mechanisms remains to be clarified, but this is suggested by the occurrence of hypotension with bradycardia.

13.2 Physiopathology of ANF in Animals (M. Cantin and R. Garcia)

13.2.1 ANF and Experimental Hypertension

The hypotensive effect of ANF in both normotensive and several spontaneous or non spontaneous models of experimental hypertension in the rat is a well-established fact. However, whether or not the cardiac peptide plays a causal or regulatory role in the rise of blood pressure in these models has yet to be determined.

Experimental hypertension in the rat has been described as producing varying concentrations of ANF in the atria, in plasma, or in both. Sonnenberg et al. (1983) were the first to report a decrease in bioassayable ANF in the atrial tissue of SHR. This finding was later confirmed by the RIA of atrial ANF (Garcia et al. 1985e). Higher levels of plasma ANF have been demonstrated in SHR, coinciding with the onset of hypertension, the difference being greater with advanced age and higher levels of blood pressure (Imada et al. 1985; Gutkowska et al. 1986b; Morii et al. 1986). In these studies (Imada et al. 1985; Gutkowska et al. 1986b; Morii et al. 1986), the atrial tissue content of ANF is found to be lower in the left atria of SHR. This suggests a depletion of ANF secondary to an increase in left atrial pressure, as reported by Noresson et al. (1979), resulting in the stretch of the atrial walls and the consequent release of the peptide. The lesser distensibility of the left atrium in SHR (Ricksten et al. 1980) may contribute to an exaggerated response in this release.

Atrial and plasma ANF levels in stroke-prone SHR are not significantly different from those of non stroke-prone SHR (Morii et al. 1986) in spite of their higher blood pressure. Whether this increased release of ANF is intended as a compensatory mechanism requires further investigation. However, not all the investigators agree on the reported levels of plasma and atrial tissue ANF in SHR and WKY rats. Some (Pettersson et al. 1985; Haass et al. 1986) have reported equal levels of plasma ANF in SHR and WKY rats. The origin of this discrepancy is not apparent since at least one group (Haass et al. 1986) used animals of the same age and origin as those used by another (Gutkowska et al. 1986b). The effect of acute plasma expansion in SHR is not yet conclusive either, since equal (Haass et al. 1986) or attenuated (Pettersson et al. 1985) ANF release has been reported.

Another model of experimental hypertension which has received ample attention is the DAHL salt-sensitive (S) hypertensive rat. Early publications (Hirata et al. 1984a; Snajdar and Rapp 1985) reported a high atrial ANF content in DAHL "S" rats, with a renal hyporesponsiveness to the peptide (Hirata et al. 1984a). This suggests that the decreased renal response could be a factor in the sensitivity to sodium load. Once specific RIAs for ANF became available, the increased content of atrial ANF could not be confirmed (Gutkowska et al. 1986c; Tanaka and Inagami 1986). Whether this difference can be attributed to the method employed, to the duration of the sodium load, or to the degree of hypertension needs to be established. However, there is general agreement that plasma levels of ANF in DAHL S rats are elevated (Gutkowska et al. 1986c; Snajdar and Rapp 1986; Tanaka and Inagami 1986).

Since DAHL S rats have an expanded blood volume which may precede the establishment of high blood pressure (Snajdar and Rapp 1985), the release of ANF could be stimulated by two mechanisms. One would be the increase in blood pressure, with the consequent rise in left ventricular end-diastolic pressure, and the other the expanded extracellular volume, a well-known stimulus for ANF release. In contrast to plasma IR-ANF, which appears to be elevated in both hypertensive SHR and DAHL S rats, the celiac ganglionic content of ANF exhibits distinct patterns in the two hypertensive models. In the normotensive stage, Kuchel et al. (1987d) reported no difference in celiac IR-ANF content; however, when comparably hypertensive, SHR have higher and DAHL S rats lower celiac ganglionic IR-ANF content than their respective controls. This dissociation of plasma and ganglionic IR-ANF content in genetic hypertension suggests that ANF in neural tissues may have a neuromodulatory role distinct from the "hormonal" role of ANF circulating in the blood.

A nonspontaneous, volume-dependent model of hypertension such as is produced by DOCA-salt administration has recently been reported (Sugimoto et al. 1986; Schiffrin and St-Louis 1987) to have higher plasma ANF levels and a lower total atrial ANF content. Plasma ANF is significantly but poorly correlated with the level of systolic blood pressure.

Among the nonspontaneous models of experimental hypertension, 1K-1C rats have been shown to possess low left atrial IR-ANF levels (Garcia et al. 1985b). We have recently reported that plasma ANF is higher in this model of hypertension than in a control group; the increase appears during the early stage of hypertension (1 week), and levels remain elevated until the end of the period of observation (8 weeks). The increased plasma levels of ANF at weeks 1 and 2 after the clipping of the renal artery are accompanied by a lower total atrial ANF content and concentration in the left but not in the right atrium. In the more advanced stages, the differences in atrial ANF disappear, but they reappear at week 8, not only in the left but in the right atrium as well. After unclipping, the blood pressure in 1K-1C rats rapidly normalizes but plasma

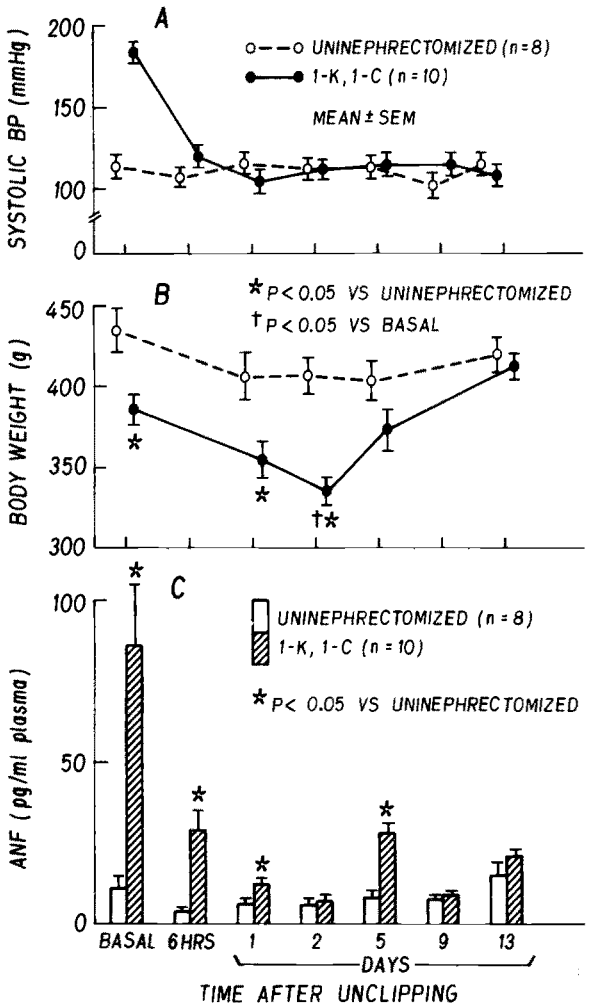


Fig. 26A–C. Effect of unclipping on **A** blood pressure (*BP*), **B** body weight, and **C** plasma ANF in 1K-1C hypertensive rats

ANF levels, although lower than before unclipping, remain higher than in the controls (Fig. 26). This suggests that ANF may play a role in the natriuretic response to unclipping known to occur in this model of hypertension (Liard and Petters 1973).

In 2K-1C hypertensive rats, a model which may be renin and not volume dependent, atrial ANF content was not found to be significantly different from that of controls after 4 weeks of hypertension (Garcia et al. 1987d); however, the plasma ANF levels were higher than in normotensive controls (Garcia et al. 1987e).

It seems, then, that independently of the pathogenic mechanism, all models of spontaneous or nonspontaneous experimental hypertension heretofore investigated show increased levels of plasma ANF and normal or reduced atrial

ANF content, especially in the left atrium (Garcia et al. 1987e). The atrial ANF content seems to depend on the stage of development of high blood pressure, but it appears that high plasma levels are present in the early stages.

The high levels of plasma ANF could be the result of an increase in left atrial pressure leading to atrial distension and ANF release. The increase in left atrial pressure is correlated with that in mean arterial pressure, which increases left atrial pressure by the rise in left ventricular end-diastolic pressure. Models such as 1K-1C and DOCA-salt may have an increased plasma volume as well, which by itself may also stimulate ANF release. It appears reasonable to think that, because of its natriuretic and hemodynamic effects, the increased stimulation of ANF release in hypertension could be a regulatory mechanism to prevent further deleterious effects of high blood pressure.

13.2.2 ANF and Experimental Congestive Heart Failure

The cardiomyopathic hamster has been found to be a useful model of congestive heart failure for at least two decades. The implications of ANF in the development of this disease are beginning to emerge. All investigators now agree that during congestive heart failure in these animals, the amount of IR-ANF decreases significantly in the atrial tissue (Chimoskey et al. 1984; Edwards et al. 1986; Franch et al. 1986; Ding et al. 1987). The plasma levels of IR-ANF are also markedly elevated in animals with moderate or severe heart failure (Edwards et al. 1986; Franch et al. 1986; Ding et al. 1987). Congestive heart failure in the hamster also produces a significant increase in ventricular IR-ANF (Ding et al. 1987). While the HPLC pattern of IR-ANF is of the high molecular weight type in atria and ventricles of both control and cardiomyopathic hamsters, high molecular weight forms of ANF appear in the plasma of animals with severe congestive heart failure, but not in controls (Ding et al. 1987).

Since the central venous pressure of these animals is markedly increased during congestive heart failure (Cantin et al. 1973a), the decrease in atrial IR-ANF is likely to be related to the increased pressure in the chambers, which leads to stretch and enhanced secretion by atrial cardiocytes. The same general mechanisms are probably also operative at the ventricular level. In congestive heart failure, cardiomyopathic hamsters show a significant elevation of end-diastolic pressure in both left and right ventricles (Jeffrey et al. 1970). In moderate and severe heart failure, these hamsters show a normal increase in ANF mRNA in the ventricular cardiocytes (Lavigne et al., unpublished observations).

At the ultrastructural level, atrial cardiocytes of cardiomyopathic hamsters show a picture of intense secretory stimulation in severe heart failure. The increase in the amount of rough endoplasmic reticulum, the tremendous in-

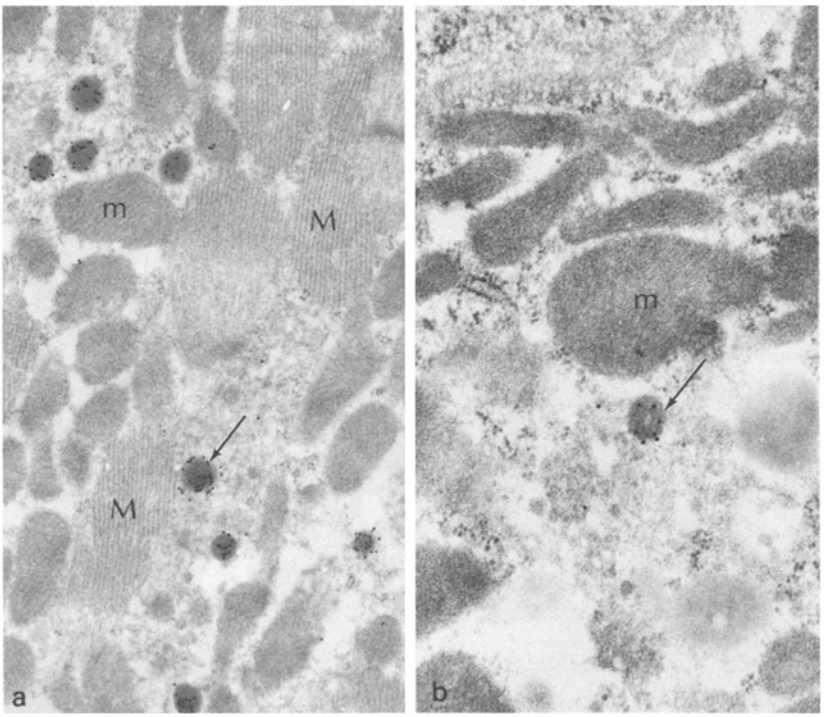


Fig. 27. **a** Ventricular cardiocyte from cardiomyopathic hamster in severe congestive heart failure, embedded in Lowicryl K_4M and reacted according to the immunogold technique. *m*, mitochondria; *M*, myofilaments. Secretory granules (*arrow*) are decorated with gold particles ×31430. **b** Ventricular cardiocyte from cardiomyopathic hamster in severe congestive heart failure embedded and reacted as in **a**. *m*, mitochondria. Secretory granules (*arrow*) are decorated with gold particles ×54280

crease in the size and complexity of the Golgi complex, and the decrease in the number and size of secretory granules are indicative of such a process. This has been well documented in the chronically stimulated exocrine pancreas (Bieger et al. 1976; Kern 1980; Webster et al. 1977), in the prolactin cells of the anterior pituitary (Antakly et al. 1980; Hausler et al. 1978), and in juxtaglomerular cells during acute malignant experimental hypertension (Cantin et al. 1984b). The increased size of the Golgi complex and the increased number of secretory granules in ventricular cardiocytes may be signs of the same phenomenon in these cells. While no granules containing IR-ANF can be found in ventricular cardiocytes of control hamsters, the immunogold technique reveals the presence of IR-ANF in typical secretory granules (Ding et al. 1987) (Fig. 27). Why, in the case of atrial cardiocytes, these stimuli lead to an actual decrease in IR-ANF content, while the reverse is true in ventricular cardiocytes, remains to be determined. It may be due to the fact that the secretory process is different in atrial and ventricles.

While the constant presence of secretory granules in atrial cardiocytes seems to indicate that the secretion of ANF by these cells is regulated (Tartakoff et al. 1978; Gumbiner and Kelly 1982; Willingham and Pastan 1984; Moore et al. 1983), this does not seem to be the case in ventricular cardiocytes. The absence of secretory granules in control rat ventricular cardiocytes – which, at least in culture, secrete ANF (Cantin et al. 1987) – tends to indicate that the basal secretion of ANF in these cells is of the constitutive type (Tartakoff et al. 1978; Gumbiner and Kelly 1982; Willingham and Pastan 1984; Moore et al. 1983). Stimulation may thus lead to be formation of secretory granules and to hypersecretion by an alternative pathway. Whether atrial or ventricular cardiocytes or both release higher molecular weight peptide(s) observed in the hamster circulation also remains to be determined. A similar finding has been described in severely hyperglycemic, hypoinsulinemic patients, in whom nearly 40% of the serum immunoreactive insulin was proinsulinlike material. This has been attributed to the liberation of immature β-cell granules and to "pancreatic exhaustion" (Ding et al. 1987).

13.2.3 ANF and Renin-Angiotensin System

Recent investigations have indicated that ANF is implicated in the regulation of the renin-angiotensin-aldosterone system. There is overwhelming evidence that ANF directly inhibits aldosterone secretion and most of the effects of angiotensin II on its target cells, but its effects on renin release are still controversial. Maack et al. (1984) have shown that synthetic auriculin (ANF 24 AA, Ser-103 to Tyr-126) infusion reversibly decreases the renin secretory rate, with a more gradual fall in peripheral plasma renin levels.

Similarly, a significant suppression of renin release has been reported during the intrarenal infusion of ANF in dogs with acute heart failure (Burnett et al. 1984; Scriven and Burnett 1985). ANF infusion in dogs with a single, denervated, nonfiltering kidney also produces a marked inhibition of renin secretion (Villarreal et al. 1986b). Contrary to these data, Opgenorth et al. (1986) have demonstrated that in the nonfiltering kidney without a functioning macula densa, ANF infusion does not suppress renin release. All these studies have, however, been performed on anesthetized animals in which renin activity is probably stimulated.

In conscious rats with 2K-1C hypertension, chronic infusions (with minipumps) of synthetic ANF(Arg-101 to Tyr-126) produce a decrease not only in blood pressure but also in PRA (Garcia et al. 1985d). In fact, in this model of hypertension, ANF lowers PRA only in renin-dependent hypertensive animals (Garcia et al. 1987d). ANF is also able to suppress renin levels in sodium-depleted 1K-1C hypertensive rats, possibly reflecting an increased sensitivity of the macula densa following sodium depletion (Volpe et al. 1985). It has been shown, however, that acute ANF treatment of renin-depen-

dent 2K-1C hypertensive rats produces a further elevation of the already high PRA (Volpe et al. 1984). In this condition, ANF is probably unable to alter the intrarenal hemodynamics of the clipped, renin-secreting kidney, and consequently the renin-stimulating effect of a fall in systemic pressure predominates. The finding has been confirmed in dogs with acute unilateral renal constriction, where ANF not only fails to decrease renin but actually increases it (Sosa et al. 1985a).

In vitro studies on the influence of ANF on renin release have produced conflicting data. Working with kidney slices, Obana et al. (1985b) reported that the inhibitory effect of ANF may be partly mediated by an increase in cGMP and a decrease in cAMP. Hiruma et al. (1986), also working with kidney slices, observed a stimulation of renin release following exposure to ANF without any change in cAMP levels. These authors also reported an inhibition by ANF of isoproterenol-stimulated renin release (Hiruma et al. 1986). Other workers have reported no effects of ANF on basal renin secretion in kidney slices but a potentiation of the inhibitory effect of angiotensin II (AII) (Antonipillai et al. 1986).

In highly pure (80%–90%) cultures of rat juxtaglomerular cells, ANF (10^{-13} to 10^{-9} M) inhibits renin release in a dose-dependent manner. In parallel with the inhibition of renin release, ANF leads to an increase in intracellular cGMP levels. ANF (0.1 nM) does not stimulate calcium influx into these cells, whereas in the same conditions, AII does (Kurtz et al. 1986).

13.3 Physiopathology of ANF in Humans
(J. Genest, P. Larochelle, and J.R. Cusson)

There is unanimous agreement that plasma IR-ANF is significantly higher (three to ten times) in blood from the coronary sinus than in blood from any other locations, indicating that its major source is the atria. There is no tendency for plasma IR-ANF to increase with age in normal subjects (Larochelle et al. 1987c; Weil et al. 1986), although Ohashi et al. (1987) reported a significant increase in the older age group (64–91 years) when compared with a younger group (24–29 years). This contrasts with the findings of Richards et al. (1986a, b), who found a significant correlation of plasma IR-ANF and age in normotensive, but not in hypertensive subjects. Opposite results were reported by Larochelle et al. (1987c). The administration of hANF by infusion (0.1 $\mu g\,kg^{-1}\,min^{-1}$ for 20 min) was reported to decrease plasma AVP to undetectable levels in six normal subjects (Fujio et al. 1986), as was also shown in rats by Januszewicz et al. (1986c; Samson 1985a). Studies done by Yamaji et al. (1986a) on plasma IR-ANF in the umbilical cord blood of human fetus showed significantly higher levels in arterial than in cord venous or maternal venous blood.

13.3.1 Hypertension

Several groups have measured plasma IR-ANF concentrations in hypertensive patients (Larochelle et al. 1987b, c; Genest et al. 1987; Zachariah et al. 1987; Burnett et al. 1986b; Sugawara et al. 1985, 1987; Arendt et al. 1985, Sagnella et al. 1986; Kohno et al. 1986b; Naruse et al. 1986; Nozuki et al. 1986; MacGregor et al. 1986; Nishiuchi et al. 1986; Richards et al. 1986; Yamaji et al. 1986b; Andersson et al. 1986; Hedner et al. 1986). Two groups, those of Larochelle et al. (1987c), and of Zachariah et al. (1987), have studied strictly defined groups of patients with mild essential hypertension – defined as patients without organ damage, without left ventricular hypertrophy, and with diastolic pressures below 105 mmHg. Both groups reported no differences in plasma IR-ANF levels (after Sep-Pak extraction) in patients with mild essential hypertension as compared with groups of normal subjects (Fig. 28). Six other groups have reported no significant differences in plasma IR-ANF (after Sep-Pak extraction) in patients with hypertension, undefined as to type and severity, as compared with control subjects (Naruze et al. 1986; Nozuki et al. 1986; Andersson et al. 1986; Hedner et al. 1986; Nishiuchi et al. 1986). Using affinity chromatography for plasma ANF extraction, Yamaji et al. (1986b) could not find any difference. On the other hand, in similar undefined groups of hypertensive patients, Sugawara et al. (1985), Sagnella et al. (1986), Kohno et al. (1986b), and Richards et al. (1986) found plasma IR-ANF levels (after

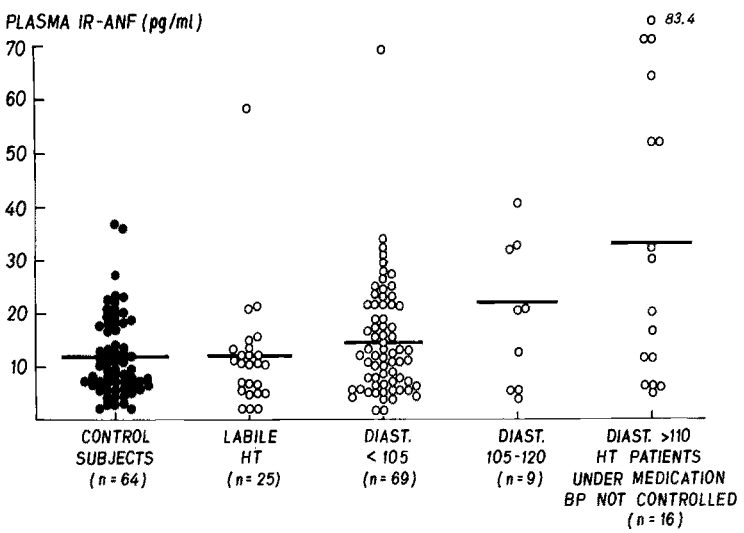


Fig. 28. Plasma IR-ANF concentration following Sep-Pak purification in 64 control subjects, 25 patients with labile borderline hypertension (*HT*), 69 patients with mild essential hypertension and a diastolic pressure (*DIAST*) below 105 mmHg, 9 patients with essential hypertension and a diastolic pressure between 105 and 120 mmHg, and 16 patients with severe hypertension under medication and with a diastolic pressure above 110 mmHg

Table 19. Plasma IR-ANF (after Sep-Pak extraction) in human hypertension

References	Plasma IR-ANF (pmol/l) (mean ± SEM)		
	Control subjects		Mild essential HT
Patients with mild essential hypertension (HT)			
Larochelle et al. 1987c	3.6 ± 0.3 (n = 64)	N.S.	4.2 ± 0.5 (n = 92)
Zachariah et al. 1987	11.5 ± 0.7	N.S.	10.1 ± 1.0 (n = 29)
Patients with undefined hypertension (as to type and/or severity)			
No significant difference	Control subjects		Undefined HT
Naruse et al. 1986	58.8 ± 5.5 (n = 34)		65.7 ± 11.1 (n = 31)
Andersson et al. 1986	11.4 ± 3.3 (n = 6)		9.5 ± 3.9 (n = 12)
Hedner et al. 1986	9.9 ± 2.9 (n = 29)		10.8 ± 2.9 (n = 19)
Nishiuchi et al. 1986	6.3 ± 0.3 (n = 54)		13.7 ± 2.3 (n = 23)
Nozuki et al. 1986	No difference		
Yamaji et al. 1986b[a]	12.4 ± 0.5 (n = 108)		12.6 ± 0.9 (n = 41)
Significant difference	Control subjects		Undefined HT
Sugawara et al. 1985	12.3 ± 1.9 (n = 14)	$p < 0.01$	25.4 ± 3.1 (n = 14)
Sugawara et al. 1987	11.8 ± 1.6 (n = 20)	$p < 0.05$	22.9 ± 4.8 (n = 50)
Sagnella et al. 1986	2.7 ± 1.2 (n = 24)	$p < 0.005$	5.6 ± 4.5 (n = 28)
MacGregor et al. 1986	2.7 ± 0.2 (n = 25)	$p < 0.001$	5.2 ± 0.5 (n = 48)
Kohno et al. 1986b	– (n = 17)	$p < 0.05$	– (n = 43)
Arendt et al. 1985[b]	8.5 ± 2.3 (n = 13)	$p < 0.02$	58.8 ± 18.3 (n = 17)
Richards et al. 1986a	7.5 ± 4.6 (n = 55)		17.3 ± 16.3 (n = 33)

[a] Affinity chromatography on antiANF-coupled agarose, Class I & II pts, WHO
[b] IR-ANF after extraction with Amberlite XAD-2 resin

Sep-Pak extraction) to be slightly but significantly higher in hypertensive patients than in control subjects. Using amberlite XAD-2 resin for the extraction of plasma IR-ANF, Arendt et al. (1985) also found significantly higher plasma IR-ANF levels in hypertensive patients than in control subjects (Table 19). In some of these latter groups, patients with moderate and severe hypertension, possibly of renal or renovascular origin, were included, hence the higher plasma levels of IR-ANF. Plasma IR-ANF was within the normal range in all patients with labile, borderline hypertension and in patients with mild essential hypertension, that is with diastolic pressure below 105 mmHg (Larochelle et al. 1987b, c; Fig. 28). This would suggest that the increased plasma IR-ANF levels reported by some came from patients with severe hypertension, possibly with some degree of heart failure.

In almost all cases, the plasma levels of IR-ANF after Sep-Pak purification were below 65.3 pmol/l. It is important, here, to relate this latter finding to the observations of Larochelle et al. (1987 d), Cusson et al. (1987), and Genest et al. (1987): there was no effect on blood pressure following bolus injections of ANF (25 µg) in control subjects despite peak plasma levels of up to 359.9 pmol/l, nor was there any effect with infusion of ANF at 3.2 µg/min for 30 min in control subjects and in patients with mild hypertension and constant plasma levels of 81.7 pmol/l. Only with bolus injections of 50 and 100 µg human ANF (Larochelle et al. 1987 d;, Sugawara et al. 1986) and infusion rates of 6.5 µg/min for 45 min, as used by Weidmann et al. (1986 a), was there a significant fall in blood pressure in the group of patients with esential hypertension from an average of 181/127 mmHg to 165/109 mmHg.

One interpretation of the above results has been proposed by Genest (1986; Genest et al. 1987), namely, that patients with mild essential hypertension show hyporesponsiveness of the atria in releasing ANF, since several workers have demonstrated that patients with essential hypertension, even in the mild phase, have higher left and right atrial pressures than normal subjects (Guazzi et al. 1979; Ferlinz 1980; Olivari et al. 1978; Atkins et al. 1977; Safar et al. 1974; Tarazi et al. 1966; Frohlich et al. 1971; London et al. 1985). It must be repeated here that increases in atrial pressure are the strongest stimuli for the release of ANF, and it has been demonstrated that increases in the pressure of the right or left atria as small as 1 mmHg, according to Goetz (1987), are accompanied or followed by an increase of about 10.5 pmol/l in plasma IR-ANF both in humans and in experimental animals.

The absence of any increase in plasma IR-ANF concentrations in patients with *mild* essential hypertension (Larochelle et al. 1987 b, c; Zachariah et al. 1987; and also Naruze et al. 1986; Nozuki et al. 1986; Anderson et al. 1986 c; Hedner et al. 1986) is consistent with the findings of Ogawa et al. (1981) and of Tsuchiga et al. (1980). These groups demonstrated that the plasma levels of cGMP, which is the marker of ANF activity, were not increased in 68 patients with essential hypertension, as compared with normal subjects. On the other hand, groups of patients with severe essential hypertension, with diastolic pressures above 110 mmHg, and uncontrolled with antihypertensive medication have significantly elevated mean plasma IR-ANF levels (Larochelle et al. 1987 c) (Fig. 28).

According to the hypothesis put forward by Genest (1986; Genest et al. 1987), such hyporesponsiveness of the atria in releasing ANF in patients with mild essential hypertension would be compatible with (a) a decreased ability of the hypertensive kidney to excrete sodium loads, as postulated by Guyton et al. (1980), except by increasing the blood pressure ("pressure natriuresis"), as was demonstrated in rats by Tobian et al. (1974, 1975); (b) a decreased inhibition of aldosterone secretion and the inappropriately high levels of secretion, plasma levels, and excretion of aldosterone in patients with essential

hypertension during high salt intake, as was demonstrated by Collins et al. (1970), Luetscher et al. (1972), and Genest et al. (1960, 1976; Genest 1983, 1984); (c) an increased peripheral resistance characteristic of essential hypertension because the concentration of circulating ANF is insufficient to prevent or decrease the vasoconstrictor activity associated with either norepinephrine or AII; (d) an absence of any increase in plasma cGMP levels in patients with essential hypertension; (e) the blunting of the ANF response to saline load (1 l isotonic saline in 30 min) in seven patients with essential hypertension, as compared with control subjects (Pecker et al. 1987).

Plasma IR-ANF, which was slightly but significantly elevated in aortic blood, was not increased in the peripheral blood of 24 patients with renovascular hypertension; nor was there any difference in the plasma IR-ANF measured directly in the renal venous blood of both kidneys in these patients, despite a significant increase in PRA in the renal venous blood of the stenotic kidney (Larochelle et al. 1987c). Patients with primary aldosteronism show a significant increase in plasma levels of IR-ANF (Tunny et al. 1986; Yamaji et al. 1986b).

13.3.2 Congestive Heart Failure

Many workers (Nakaoka et al. 1985; Shenker et al. 1985; Hartter et al. 1985; Tikkanen et al. 1985a; Burnett et al. 1986; Ogawa et al. 1986; Singer et al. 1986; Cody et al. 1986; Raine et al. 1986; Katoh et al. 1986; Nicklas et al. 1986; Schiffrin and Taillefer 1986) have demonstrated significant increases in plasma IR-ANF concentrations in patients with congestive heart failure. The increase in plasma IR-ANF levels is parallel to the severity of the congestive failure. Patients in Class I of the New York Heart Association classification (that is, patients with cardiac disease in whom ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain) had plasma IR-ANF levels within the normal range. Plasma IR-ANF levels fell in all patients as treatment improved their symptoms (Katoh et al. 1986).

A close correlation has been found between the right atrial and pulmonary wedge pressure on the one hand and plasma IR-ANF concentrations on the other (Raine et al. 1986; Kouz et al. 1986a, b; Rodeheffer et al. 1986; Nicklas et al. 1986; Ogawa et al. 1986). Such results are consistent with the observation that the plasma levels of cGMP, a marker of ANF activity, are significantly increased in patients with congestive heart failure (Ogawa et al. 1986). A negative correlation has been demonstrated between the left ventricular ejection fraction and the plasma IR-ANF concentration (Schiffrin and Taillefer 1986).

ANF administration to patients with congestive heart failure has been studied (Cody et al. 1986; Raine et al. 1986; Crozier et al. 1986; Xu et al. 1986;

Riegger et al. 1986). Generally, a good correlation is found between plasma IR-ANF levels and mean right atrial and pulmonary wedge pressure. An increase in cardiac output is observed, with a decrease in mean systemic arterial, pulmonary, and right atrial pressure. Variable results are reported for PRA and aldosterone and for urinary volume and sodium excretion.

13.3.3 Other Conditions

The administration of ANF at 50 ng kg^{-1} min^{-1} for 60 min to a patient with severe treatment-resistant ascites due to terminal alcoholic cirrhosis resulted in a significant increase in urinary volume and sodium excretion, a marked decrease in plasma aldosterone concentration, and a fall in blood pressure to 85–90/60 mmHg (Fyhrquist et al. 1985).

High plasma ANF levels have been reported in patients with inappropriate secretion of antidiuretic hormone (Sakamoto et al. 1986; Cogan et al. 1986). Paroxysmal atrial tachycardia is accompanied, as was first demonstrated by Schiffrin et al. (1985b) and confirmed by others (Tikkanen et al. 1985b; Yamaji et al. 1985a; Nicklas et al. 1986), by an increase in plasma IR-ANF concentration. This increase is possibly responsible for the polyuria noted during the episodes of tachycardia (Borst 1952).

Patients with severe chronic renal failure undergoing dialysis show significantly increased plasma IR-ANF levels, which decrease significantly following hemodialysis and loss of weight (Larochelle et al. 1987a; Rascher et al. 1985; Yamamoto et al. 1985; Anderson et al. 1986d; Hasegawa et al. 1986). Insulin-dependent diabetic patients have normal plasma IR-ANF levels, but 4 out of 12 with "cardiac autonomic neuropathy" had elevated levels (Kahn et al. 1986). Plasma IR-ANF levels are lower ($p < 0.005$) in hypothyroid patients (Zimmerman et al. 1987).

Plasma IR-ANF concentrations are significantly increased in patients during head-out water immersion (Anderson et al. 1986c; Ogihara et al. 1986b) and during exercise (Tanaka et al. 1986; Somers et al. 1986). High levels of plasma IR-ANF have also been reported in patients with Bartter's syndrome (Tunny et al. 1986; Yamada et al. 1986) and low levels in patients with Gordon's syndrome (Tunny et al. 1986). Increases in plasma IR-ANF have been found in mineralocorticoid "escape" during the administration of 9-α-fluorohydrocortisone in normal man (Cappuccio et al. 1986). Patients with idiopathic edema had low plasma IR-ANF levels (Naruse et al. 1986).

14 Summary and Conclusion (J. Genest)

In the history of medical research, few advances have been more rapid – in all aspects – than those in the research on the atrial natriuretic factor since

the original observation by de Bold et al. in 1981 of the marked natriuresis, diuresis, and vasorelaxation following the i. v. administration of crude atrial extracts. The atrial factor responsible for these findings has been isolated and sequenced, the cDNA coding for ANF has been cloned, and the gene has been localized on the chromosomal map. Most of its biological activities have been determined, and these clearly provide a balance to the activities of the renin-angiotensin system. Many points remain to be elucidated, such as the role of ANF in patients with essential hypertension with congestive heart failure; the participation of ventricular ANF in pathological states such as hypertension and congestive heart failure; the interplay of ANF and angiotensin II in brain regions involved in the regulation of sodium, water, and blood pressure such as the AV3V region and the subfornical organ; the role of ANF in the ciliary bodies of the eyes; the relationship of particulate guanylate cyclase stimulation and adenylate cyclase inhibition with vasorelaxation; the neuromodulatory role of ANF in neural transmission; and many others. Fundamental questions remain to be answered and offer a field for innovative researches.

Acknowledgements. The work reported from our laboratories was supported by several grants from the Medical Research Council of Canada, the National Research Council of Canada, the Ministère de la Science et de la Technologie du Gouvernement du Québec, the Fonds FCAR du Gouvernement du Québec, the Canadian Kidney Foundation, the Canadian Heart Foundation, the Quebec Heart Foundation, Bio-Mega Inc., Quebec, Pfizer Company, England, and La Fondation de Sève de Montréal. The authors wish to gratefully acknowledge the invaluable help of Miss Lise Lanthier in the preparation of the manuscript.

References

Aalkjaer C, Mulvany MJ, Nyborg NC (1985) Atrial natriuretic factor causes specific relaxation of rat renal arcuate arteries. Br J Pharmacol 86:447–453

Abboud F (1982) The sympathetic system in hypertension. State-of-the-art Review. Hypertension 4(suppl II):II-208–II-225

Ackermann U, Rudolph JR (1983) Control of right atrial pressure at constant cardiac output suppresses volume natriuresis in anesthetized rats. Can J Physiol Pharmacol 62:798–801

Ackermann U, Irizama TG, Milojevic S, Sonnenberg H (1984) Cardiovascular effects of atrial extracts in anaesthetized rats. Can J Physiol Pharmacol 62:819–826

Adams SP (1986) Atrial natriuretic factor: structural activity studies. Work presented at the 191st Annual Meeting of the American Chemical Society, New York, NY, April 13–18

Agnoletti G, Ferrari R, Rodella A, Quinzanini M, Albertini A, Harris P (1986) Time-course of release of atrial natriuretic factor from stretched rat atrium. In: X World Congress of Cardiology, American Heart Association, September 14–19, Abstract 74

Aiton JF, Cramb G (1985) Atrial natriuretic factor stimulates cGMP production in rabbit ventricular myocytes. J Physiol 367:101P

Albus U, Breipohl G, Geiger R, Hropot M, Knolle J, König W, Schölkens B, Usinger P (1986) Atrial natriuretic factors. Work presented at the 9th International Symposium on Medicinal Chemistry, Berlin (West), September 14–18

Alexander RW, Gill JR Jr, Yamabe H, Lovenberg W, Keiser HR (1974) Effects of dietary sodium and acute saline infusion on the interrelationship between dopamine excretion and adrenergic activity in man. J Clin Invest 54:194–200

Almeida FA, Suzuki M, Maack T (1986) Atrial natriuretic factor increases hematocrit and decreases plasma volume in nephrectomized rats. Life Sci 39:1193–1199

Anand-Srivastava MB, Cantin M (1986) Atrial natriuretic factor receptors are negatively coupled to adenylate cyclase in cultured atrial and ventricular cardiocytes. Biochem Biophys Res Commun 138:427–436

Anand-Srivastava MB, Franks DJ, Cantin M, Genest J (1984) Atrial natriuretic factor inhibits adenylate cyclase activity. Biochem Biophys Res Commun 121:855–862

Anand-Srivastava MB, Cantin M, Genest J (1985a) Inhibition of pituitary adenylate cyclase by atrial natriuretic factor. Life Sci 36:1873–1879

Anand-Srivastava MB, Genest J, Cantin M (1985b) Inhibitory effect of atrial natriuretic factor on adenylate cyclase activity in adrenal cortical membranes. FEBS Lett 181:199–202

Anand-Srivastava MB, Johnson RA, Picard S, Cantin M (1985c) Ninhibin: a sperm factor attenuates the atrial natriuretic factor mediated inhibition of adenylate cyclase: possible involvement of inhibitory guanine nucleotide regulatory protein. Biochem Biophys Res Commun 129:171–178

Anand-Srivastava MB, Vinay P, Genest J, Cantin M (1986) Effect of atrial natriuretic factor on adenylate cyclase in various nephron segments. Am J Physiol 251:F417–F423

Anand-Srivastava MB, Srivastava AK, Cantin M (1987) Pertusis toxin attenuates atrial natriuretic factor-mediated inhibition of adenylate cyclase: involvement of inhibitory-guanine nucleotide regulatory protein. J Biol Chem 262:4931–4934

Anderson JV, Christofides ND, Bloom SR (1986a) Plasma release of atrial natriuretic peptide in response to blood volume expansion. J Endocrinol 109:9–13

Anderson JV, Donckier J, Mckenna WJ, Bloom SR (1986b) The plasma release of atrial natriuretic peptide in man. Clin Sci 71:151–155

Anderson JV, Millar ND, O'Hare JP, MacKenzie JC, Corrall RJM, Bloom SR (1986c) Atrial natriuretic peptide: Physiological release associated with natriuresis during water immersion in man. Clin Sci 71:319–322

Anderson JV, Raine AE, Proudler A, Bloom SR (1986d) Effect of haemodialysis on plasma concentrations of atrial natriuretic peptide in adult patients with chronic renal failure. J Endocrinol 110:193–196

Anderson JV, Struthers A, Christofides N, Bloom SR (1986e) Atrial natriuretic peptide: an endogenous factor enhancing sodium excretion in man. Clin Sci 70:327–331

Anderson JV, Struthers AD, Payne NN, Slater JDH, Bloom SR (1986f) Atrial natriuretic peptide inhibits the aldosterone response to angiotensin II in man. Clin Sci 70:507–512

Anderson JV, Donckier J, Payne NN, Beacham J, Slater JDH, Bloom SR (1987) Atrial natriuretic peptide: Evidence of action as a natriuretic hormone at physiological plasma concentrations in man. Clin Sci 72:305–312

Andersson OK, Persson B, Aurell M, Granerus G, Wysocky M, Hedner J, Hedner T (1986) Basal and stimulated levels of immunoreactive atrial natriuretic peptide (α-hANP) levels in relation to central venous pressure, renal and central hemodynamics and sodium excretion in normotensive and hypertensive man. J Cardiovasc Pharmacol 8:1300

Antakly T, Pelletier G, Zeytinoglu T, Labrie F (1980) Changes in cell morphology and prolactin secretion induced by 2 Br-α-ergocryptine, estradiol and thyrotropin-releasing hormone in rat anterior pituitary cells in culture. J Cell Biol 86:377–387

Antonipillai I, Vogelsang J, Horton R (1986) Role of atrial natriuretic factor in renin release. Endocrinology 119:318–322

Appel RG, Dunn MJ (1986) Renal papillary collecting tubule (RPCT) cell sensitivity to atrial natriuretic factor (ANF) in Dahl-sensitive (S) and salt-resistant (R) rats. Clin Res 34, 2:588A

Ardaillou N, Nivez MP, Ardaillou R (1986) Stimulation of cyclic GMP synthesis in human cultured glomerular cells by atrial natriuretic peptide. FEBS Lett 204:177–182

Arendt RM, Stangl E, Zahringer J, Liebisch DC, Herz A (1985) Demonstration and characterization of alpha-human atrial natriuretic factor in human plasma. FEBS Lett 189: 57–61

Arendt RM, Gerbes AL, Ritter D, Stangl E, Bach P, Zahringer J (1986) Alpha-atrial natriuretic factor in cardiovascular disease. J Am Coll Cardiol 7:75A

Argentin S, Nemer M, Drouin J, Scott GK, Kennedy BP, Davies PL (1985) The gene for rat atrial natriuretic factor. J Biol Chem 260:4568–4571

Arjamaa O, Vuolteenaho O (1985) Sodium ion stimulates the release of atrial natriuretic polypeptides (ANP) from rat atria. Biochem Biophys Res Commun 132:375–381

Atarashi K, Mulrow PJ, Franco-Saenz R, Snajdar R, Rapp J (1984) Inhibition of aldosterone production by an atrial extract. Science 224:992–994

Atarashi K, Mulrow PJ, Franco-Saenz R (1985) Effect of atrial peptides on aldosterone production. J Clin Invest 76:1807–1811

Atkins JM, Mitchell HC, Pettinger WA (1977) Increased pulmonary vascular resistance with systemic hypertension. Am J Cardiol 29:802–807

Atlas SA (1986) Atrial natriuretic factor: a new hormone of cardiac origin. Recent Prog Horm Res 42:207–249

Atlas SA, Kleinert HD, Camargo MJ, Januszewicz A, Sealey JE, Laragh JH, Schilling JW, Lewicki JA, Johnson LK, Maack T (1984) Purification, sequencing and synthesis of natriuretic and vasoactive rat atrial peptide. Nature 309:717–719

Baisset A, Montastruc P (1957) Polyurie par distension auriculaire chez le chien: rôle de l'hormone antidiurétique. J Physiol (Paris) 49:33–36

Ballermann BJ, Hoover RL, Karnovsky MJ, Brenner BM (1985) Physiologic regulation of atrial natriuretic peptide receptors in rat renal glomeruli. J Clin Invest 76:2049–2056

Ballermann BJ, Bloch KD, Leidman JG, Brunner BM (1986) Atrial natriuretic peptide transcription, secretion and glomerular receptor activity during mineralocorticoid escape in the rat. J Clin Invest 78:840–843

Baranowska B, Gutkowska J, Cantin M, Genest J (1987a) Plasma immunoreactive atrial natriuretic factor (IR-ANF) increases markedly after α_2-adrenergic stimulation with clonidine in normally-hydrated rats. Biochem Biophys Res Commun 143:159–163

Baranowska B, Gutkowska J, Talbot B, Genest J, Cantin M (1987b) Plasma immunoreactive atrial natriuretic factor is inhibited by selective blockade of α_2-adrenergic receptors in conscious Sprague-Dawley rats. Neurosci Lett 76:119–123

Baum M, Toto RD (1986) Lack of direct effect of atrial natriuretic factor in the rabbit proximal tubule. Am J Physiol 250:F66–F69

Baxter JH, Wilson IB, Harris RB (1986) Identification of an endogenous protease that processes atrial natriuretic factor at its amino terminus. Peptides 7:407–411

Beliveau R, Bernier M, Vinay P, Tremblay J, Pang SC, Hamet P (1985) Atrial natriuretic factor does not alter sodium transport in purified luminal membrane from rat kidney cortex. IRCS Med Sci 13:402–403

Bencosme SA, Berger JM (1971) Specific granules in mammalian and non-mammalian vertebrate cardiocytes. Methods Achiev Exp Pathol 5:173–213

Bergey JL, Kotler D (1985) Effects of atriopeptins I, II and III on arial contractility, sinus nodal rate (guinea pig) and agonist-induced tension in rabbit aortic strips. Eur J Pharmacol 110:227–281

Bianchi C, Gutkowska J, Garcia R, Thibault G, Genest J, Cantin M (1985) Radioautographic localization of ^{125}I-atrial natriuretic factor (ANF) in rat tissues. Histochemistry 82:441–452

Bianchi C, Anand-Srivastava MB, de Léan A, Gutkowska J, Forthomme D, Genest J, Cantin M (1986a) Localization and characterization of specific receptors for atrial natriuretic factor in the ciliary processes of the eye. Curr Eye Res 5:283–293

Bianchi C, Gutkowska J, Ballak M, Thibault G, Garcia R, Genest J, Cantin M (1986b) Radioautographic localization of ^{125}I-atrial natriuretic factor binding sites in the brain. Neuroendocrinology 44:365–372

Bianchi C, Gutkowska J, Thibault G, Garcia R, Genest J, Cantin M (1986c) Distinct localization of atrial natriuretic factor and angiotensin II binding sites in the glomerulus. Am J Physiol 251:F594–F602

Bianchi C, Gutkowska J, Garcia R, Thibault G, Genest J, Cantin M (1987) Localization of ^{125}I-atrial natriuretic factor (ANF)-binding sites in rat renal medulla. A light and electron microscope autoradiographic study. J Histochem Cytochem 35:149–153

Bieger W, Seybold J, Kern HF (1976) Studies on intracellular transport of secretory proteins in the rat exocrine pancreas. Cell Tissue Res 170:203–219

Biollaz J, Bidiville J, Diezi J, Waeber B, Nussberger J, Brunner HR, Brunner-Ferber F (1986a) Synthetic human atrial natriuretic peptide: renal site of the natriuretic effect in normal volunteers. Clin Res 34, 2:590A

Biollaz J, Nussberger J, Porchet M, Brunner-Ferber F, Otterbein ES, Gomez H, Waeber B, Brunner HR (1986b) Four-hour infusions of synthetic atrial natriuretic peptide in normal volunteers. Hypertension 8(suppl. II):II-96–II-105

Bloch KD, Scott JA, Zisfein JB, Fallon JT, Margolies MN, Seidman CE, Matsueda GR, Homey CJ, Graham RM, Seidman JG (1985) Biosynthesis and secretion of proatrial natriuretic factor by cultured rat cardiocytes. Science 230:1168–1171

Bloch KD, Seidman JG, Naftilan JD, Fallan JT, Seidman CE (1986) Neonatal atria and ventricles secrete atrial natriuretic factor via tissue specific secretory pathways. Cell 47:695–702

Bohme E, Graf H, Hill HU, Arsenow W (1977) Stimulation of guanylate cyclase by sodium nitroprusside. Naunyn Schmiedebergs Arch Pharmacol 297:R12

Bolli P, Müller FB, Resink TJ, Linder L, Raine AEG, Erne P, Kiowski W, Bühler FR (1986) Cardiopulmonary volume changes and atrial natriuretic peptide release in normal subjects and patients with essential hypertension. Proceedings, 11th Scientific Meeting of the International Society of Hypertension, Heidelberg, abstract 649

Bolli P, Müller FB, Linder L, Raine AEG, Resink TJ, Erne P, Kiowski W, Ritz R, Bühler FR (1987) The vasodilator potency of atrial natriuretic peptide in man. Circulation 75:221–228

Bompiani GD, Ramilles CH, Hatt PY (1959) Le tronc commun du faisceau de His et les cellules claires de l'oreillette droite. Arch Mal Coeur 52:1257–1274

Borenstein HB, Cupples WA, Sonnenberg H, Veress AT (1983) The effect of a natriuretic atrial extract on renal haemodynamics and urinary excretion in anaesthetized rats. J Physiol 334:133–140

Borst JGG (1954) The characteristic renal excretion patterns associated with excessive or inadequate circulation. In: Ciba Symposium on the Kidney. Editors: Lewis AAG, Wolstenholme GEW. J. A. Churchill, London, pp 255–284

Breuhaus BA, Saneii HH, Brandt MA, Chimoskey JE (1985) Atriopeptin II lowers cardiac output in conscious sheep. Am J Physiol 249:R776–R780

Briggs JP, Steipe B, Schubert G, Schnermann J (1982) Micropuncture studies of the renal effects of atrial natriuretic substance. Pflügers Arch 395:271–276

Bürgisser E, Raine AEG, Erne P, Kamber B, Bühler FR (1985) Human cardiac plasma concentrations of atrial natriuretic peptide quantified by radioreceptor assay. Biochem Biophys Res Commun 133:1201–1209

Burnett JC Jr, Granger JP, Opgenorth TJ (1984) Effects of synthetic atrial natriuretic factor on renal function and renin release. Am J Physiol 247:F863–F866

Burnett JC Jr, Kao PC, Hu DC, Heser DW, Heublein D, Granger JP, Opgenorth TJ, Reeder GS (1986) Atrial natriuretic peptide elevation in congestive heart failure in the human. Science 231:1145–1147

Bussien JP, Biollaz J, Waeber B, Nussberger J, Turini GA, Brunner HR, Brunner-Ferber F, Gomez HJ, Otterbein ES (1986) Dose-dependent effect of atrial natriuretic peptide on blood pressure, heart rate, and skin blood flow of normal volunteers. J Cardiovasc Pharmacol 8:216–220

Camargo MJ, Kleinert HD, Atlas SA, Sealey JE, Laragh JH, Maack T (1984) Ca-dependent hemodynamic and natriuretic effects of atrial extract in isolated rat kidney. Am J Physiol 246:F447–F456

Camargo MJ, Atlas SA, Maack T (1986) Role of increased glomerular filtration rate in atrial natriuretic factor-induced natriuresis in the rat. Life Sci 38:2397–2404

Campbell WB, Currie MG, Needleman P (1985) Inhibition of aldosterone biosynthesis by atriopeptins in rat adrenal cells. Circ Res 57:113–118

Cantiello HF, Ausiello DA (1986) Atrial natriuretic factor and cGMP inhibit amiloride-sensitive Na^+ transport in the cultured renal epithelial cell line, LLC-PKl. Biochem Biophys Res Commun 134:852–860

Cantin M, Genest J (1985) The heart and the atrial natriuretic factor. Endocr Rev 6:107–126

Cantin M, Forthomme D, Bajusz E (1973a) Renal glomerulus in experimental congestive heart failure: ultrastructural and functional studies. In: Cardiomyopathies, vol 2. Edited by Bajuz E., University Park Press, Baltimore, pp 467–492

Cantin M, Veilleux R, Huet M (1973b) Electron and fluorescence microscopy of the hamster atria after administration of 6-hydroxydopamine. Experientia 29:582–584

Cantin M, Michelakis AM, Ong H, Ballak M, Beuzeron J, Benchimol S (1982) Relationship of specific granules to renin activity in the myocardium. In: Chagor E, Smirnov V, Dhalla NS (eds) Advances in Myocardiology, vol 3. Plenum, New York, pp 519–529

Cantin M, Gutkowska J, Januszewicz P, Thibault G, Garcia R, Genest J (1984a) Effect of blood volume expansion on the plasmatic concentration of immunoreactive atrial natriuretic factor in the rat. Proceedings 10th meeting, International Society of Hypertension, Interlaken, June 1984, Abstract

Cantin M, Gutkowska J, Lacasse J, Ballak M, Ledoux S, Inagami T, Beuzeron J, Genest J (1984b) Ultrastructural immunocytochemical localization of renin and angiotensin II in the juxtaglomerular cells of the ischemic kidney in experimental renal hypertension. Am J Pathol 115:212–224

Cantin M, Gutkowska J, Thibault G, Milne RW, Ledoux S, MinLi S, Chapeau C, Garcia R, Hamet P, Genest J (1984c) Immunocytochemical localization of atrial natriuretic factor in the heart and salivary glands. Histochemistry 80:113–127

Cantin M, Ding J, Thibault G, Gutkowska J, Salmi L, Ballak M, Garcia R, Genest J (1987) Immunoreactive ANF is present in both atria and ventricles. Mol Cell Endocrinol 52:105–113

Capponi AM, Lew PD, Jornot L, Vallotton MB (1984) Cytosolic free calcium levels and aldosterone production in isolated bovine adrenal glomerulosa cells: difference in the mode of action of angiotensin II and potassium. J Biol Chem 259:8863–8869

Capponi AM, Lew PD, Wüthrich R, Vallotton MB (1986) Effects of atrial natriuretic peptide on the stimulation by angiotension II of various target cells. J Hypertens 4(suppl 2):S61–S65

Cappuccio FP, Markandu ND, Shore AC, Buckley MG, MacGregor GA (1986) Plasma immunoreactive atrial natriuretic peptide during mineralocorticoid 'escape' in man. J Hypertens 4:790

Caramelo C, Fernandez-Cruz A, Villamediana LM, Sanz E, Rodriguez-Puyol D, Hernando L, Lopez-Novoa JM (1986) Systemic and regional haemodynamic effects of a synthetic atrial natriuretic peptide in conscious rats. Clin Sci 71:323–325

Carrier F, Thibault G, Schiffrin EL, Garcia R, Gutkowska J, Cantin M, Genest J (1985) Partial characterization and solubilization of receptors for atrial natriuretic factor in rat glomeruli. Biochem Biophys Res Commun 132:666–673

Carrier F, Tremblay J, Thibault G (1987) The ANF receptor may be associated to a GTP binding site. Fed Proc 46:1073

Chai SY, Sexton PM, Allen AM, Figdor R, Mendelsohn FAO (1986) In vitro autoradiographic localization of ANP receptors in rat kidney and adrenal gland. Am J Physiol 250,19:F753–F757

Chartier L, Schiffrin EL (1986a) Atrial natriuretic peptide inhibits the stimulation of aldosterone secretion by ACTH in vitro and in vivo. Proc Soc Exp Biol Med 182:132–136

Chartier L, Schiffrin EL (1986b) Evidence that atrial natriuretic factor (ANF) acts by interference with calcium fluxes: comparison with nifedipine and blockade of effects of Bay K 8644 in the rat adrenal glomerulosa. Fed Proc 45:176

Chartier L, Schiffrin EL (1987a) Atrial natriuretic peptide inhibits the effect of endogenous angiotension II on plasma aldosterone in conscious sodium-depleted rats. Clin Sci 72:31–35

Chartier L, Schiffrin EL (1987b) Role of calcium in effects of atrial natriuretic peptide on aldosterone production in adrenal glomerulosa cells. Am J Physiol 252:E485–E491

Chartier L, Schiffrin EL, Thibault G (1984a) Effect of atrial natriuretic factor (ANF)-related peptides on aldosterone secretion by adrenal glomerulosa cells: critical role of the intramolecular disulphide band. Biochem Biophys Res Commun 122:171–174

Chartier L, Schiffrin EL, Thibault G, Garcia R (1984b) Atrial natriuretic factor inhibits the stimulation of aldosterone secretion by angiotensin II, ACTH and potassium in vitro and angiotensin II-induced steroidogenesis in vivo. Endocrinology 115:2026–2028

Chartier L, Thibault G, Schiffrin EL (1984c) Atrial natriuretic factor inhibits the effect of angiotensin II, ACTH and potassium on aldosterone secretion by isolated rat adrenal glomerulosa cells. In: 7th International Congress of Endocrinology, Quebec City, Canada, July 1984

Chimoskey JF, Spielman WS, Brandt MA, Heideman SR (1984) Cardiac atria of B10 14.6 hamsters are different in natriuretic factor. Science 223:820–822

Chino N, Nishiuchi Y, Noda Y, Watanabe TX, Kimura T, Sakakibara S (1985) Structure-activity study of α-human atrial natriuretic polypeptide (α-hANP). In: Deber CM, Hruby VJ, Kopple KD (eds) Peptides: structure and function. Pierce Chemical Company, Rockford, Illinois, pp 945–948

Chiu PJS, Tetzloff G, Syhort EJ (1986) The effects of atriopeptin II on calcium fluxes in rabbit aorta. Eur J Pharmacol 124:277–284

Cody RJ, Atlas SA, Laragh JH, Kubo SH, Covit AB, Ryman KS, Shaknovich A, Pondolfino K, Clark M, Camargo MJF, Scarborough RM, Lewicki JA (1986) Atrial natriuretic factor in normal subjects and heart failure patients. Plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. J Clin Invest 78:1362–1374

Coffey RG (1986) Phosphatidylserine and phorbol myristate acetate stimulation of human lymphocyte guanylate cyclase. Int J Biochem 18:665–670

Cogan E, Debière MF, Pepersack T, Abramson M (1986) High plasma levels of atrial natriuretic factor in SIADH. New Engl J Med 314:1258–1259

Cogan MG (1986) Atrial natriuretic factor can increase renal solute excretion primarily by raising glomerular filtration. Am J Physiol 250:F710–F714

Cohen ML, Schenck KW (1985) Atriopeptin II: differential sensitivity of arteries and veins from the rat. Eur J Pharmacol 108:103–104

Collins RD, Weinberger MH, Dowdy AJ, Nokes GW, Gonzales CM, Luetscher JA (1970) Abnormally sustained aldosterone secretion during salt-loading in patients with various forms of benign hypertension: Relation to plasma renin activity. J Clin Invest 49:1415–1426

Crandall ME, Gregg CM (1986) In vitro evidence for an inhibitory effect of atrial natriuretic peptide on vasopressin release. Neuroendocrinology 44:439–445

Criscione L, Burdet R, Hanni H, Kamber B, Truog A, Hofbauer KG (1987) Systemic and regional hemodynamic effects of atriopeptin II in anesthetized rats. J Cardiovasc Pharmacol 9:135–141

Cromlish JA, Seidah NG, Chrétien M (1986a) A novel serine protease (IRCM-serine protease 1) from porcine neurointermediate and anterior pituitary. Lobes isolation, polypeptide chain structure, inhibitor sensitivity, and substrate specificity. J Biol Chem 261:10850–10858

Cromlish JA, Seidah NG, Chrétien M (1986b) Selective cleavage of human ACTH, beta-lipotropin, and the N-terminal glycopeptide at pairs of basic residues by IRCM-serine protease 1, subcellular localization in small and large vesicles. J Biol Chem 261:10859–10870

Crozier IG, Ikram H, Gomez HJ, Nicholls MG, Espiner EA, Warner NJ (1986) Hemodynamic effects of atrial peptide infusion in heart failure. Lancet ii:1242–1243

Cuneo RC, Espiner EA, Nicholls MG, Yandle TG, Joyce SL, Gilchrist NL (1986) Renal, hemodynamic, and hormonal responses to atrial natriuretic peptide in normal man, and effect of sodium intake. J Clin Endocrinol Metab 63:946–953

Currie MG, Newman WH (1986) Evidence for α_1-adrenergic regulation of atriopeptin release from the isolated rat heart. Biochem Biophys Res Commun 137:94–100

Currie MG, Geller DM, Cole BR, Boylan JG, Yu Sheng W, Holmberg SW, Needleman P (1983) Bioactive cardiac substances: potent vasorelaxant activity in mammalian atria. Science 221:71–73

Currie MG, Geller DM, Cole BR, Siegel NR, Fok KK, Adams SP, Eubanks SR, Galluppi GR, Needleman P (1984a) Purification and sequence analysis of bioactive atrial peptides (atriopeptins). Science 223:67–69

Currie MG, Seekin D, Geller DM, Cole BR, Needleman P (1984b) Atriopeptin release from the isolated perfused rabbit heart. Biochem Biophys Res Commun 124:711–717

Cusson JR, Hamet P, Gutkowska J, Kuchel O, Genest J, Cantin M, Larochelle P (1987) Effects of atrial natriuretic factor on natriuresis and cGMP in patients with essential hypertension. J Hypertens 5:435–443

Debinski W, Gutkowska J, Kuchel O, Racz K, Buu NT, Cantin M, Genest J (1986) ANF-like peptide(s) in the peripheral autonomic nervous system. Biochem Biophys Res Commun 134:279–284

Debinski W, Kuchel O, Buu NT, Nemer M, Cantin M, Genest J (1987a) Atrial natriuretic factor in peripheral sympathetic ganglia originates in preganglionic nerve fibers. Clin Res 35:000 (in press)

Debinski W, Kuchel O, Buu NT, Cantin M, Genest J (1987b) Atrial natriuretic factor partially inhibits the stimulated catecholamine synthesis in superior cervical ganglia of the rat. Neurosci Lett 77: 92–96

De Bold AJ (1979) Heart atria granularity. Effects of changes in water and electrolyte balance. Proc Soc Exp Biol Med 161:508–511

De Bold AJ (1982a) Atrial natriuretic factor of the rat heart. Studies on isolation and properties. Proc Soc Exp Biol Med 179:133–138

De Bold AJ (1982b) Tissue fractionation studies on the relationship between an atrial natriuretic factor and specific atrial granules. Can J Physiol Pharmacol 60:324–330

De Bold AJ, Bencosme SE (1975) Autoradiographic analysis of labelled distribution in mammalian atrial and ventricular cardiocytes after exposure to treated leucine. In: Roy PE, Harris P (eds) The cardiac cytoplasm, vol 8. University Park Press, Baltimore, pp 129–138

De Bold AJ, Flynn TG (1983) Cardionatrin I – a novel heart peptide with potent diuretic and natriuretic properties. Life Sci 33:297–302

De Bold AJ, Salerno TA (1983) Natriuretic activity of extracts obtained from hearts of different species and from various rat tissues. Can J Physiol Pharmacol 61:127–130

De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H (1981) A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 28:89–94

De Léan A (1986) Amiloride potentiates atrial natriuretic factor inhibitory action by increasing receptor binding in bovine adrenal zona glomerulosa. Life Sci 39:1109–1116

De Léan A, Gutkowska J, McNicoll N, Schiller PW, Cantin M, Genest J (1984a) Characterization of specific receptors for atrial natriuretic factor in bovine adrenal zona glomerulosa. Life Sci 35:2311–2318

De Léan A, Racz K, Gutkowska J, Nguyen TT, Cantin M, Genest J (1984b) Specific receptor-mediated inhibition by synthetic atrial natriuretic factor of hormone-stimulated steroidogenesis in cultured bovine adrenal cells. Endocrinology 115:1636–1638

De Léan A, Ong H, McNicoll N, Racz K, Gutkowska J, Cantin M (1985a) Identification of aldosterone secretion inhibitory factor in bovine adrenal medulla. Life Sci 36:2375–2382

De Léan A, Thibault G, Seidah NG, Lazure C, Gutkowska J, Chrétien M, Genest J, Cantin M (1985b) Structure-activity relationships of atrial natriuretic factor (ANF). III. Correlation of receptor affinity with relative potency on aldosterone production in zona glomerulosa cells. Biochem Biophys Res Commun 132:360–367

De Léan A, Vinay P, Cantin M (1985c) Distribution of atrial natriuretic factor receptors in dog kidney fractions. FEBS Lett 193:239–242

Deth R, van Breemen C (1977) Agonist-induced release of intracellular Ca^{2+} in the rabbit aorta. J Membr Biol 30:363–380

Deth RC, Wong K, Fukozawa S, Rocco R, Smart JL, Lynch CJ, Wawad R (1982) Inhibition of rat aorta contractile response by natriuresis-inducing extract of rat atrium (abstract). Fed Proc 41:983A

De Torrente A, Robertson GL, McDonald KM, Schrier RW (1975) Mechanism of diuretic response to increased left atrial pressure in the anesthetized dog. Kidney Int 8:355–361

Dietz JR (1984) Release of natriuretic factor from rat heart-lung preparation by atrial distension. Am J Physiol 147:R1093–R1096

Ding J, Thibault G, Gutkowska J, Garcia R, Karabatsos T, Jasmin G, Genest J, Cantin M (1987) Cardiac and plasma atrial natriuretic factor in experimental congestive heart failure. Endocrinology 121:248–257

Drake AJ, Templeton WW, Stanford SC (1982) Evidence for a role of dopamine in cardiac function. Neurochem Int 4:435–439

Dunn BR, Troy JL, Ichikawa I, Brenner BM (1986) Effect of atrial natriuretic peptide (ANP) on hydraulic pressures in the rat renal papilla: implications for ANP-induced natriuresis. Kidney Int 29:382 (abstract)

Edwards BS, Ackermann DM, Schwaab TR, Heublein DM, Edwards WD, Wolf LE, Burnett JC Jr (1986) The relationship between atrial granularity and circulating atrial natriuretic peptide in hamsters with congestive heart failure. Mayo Clin Proc 61:517–521

Epstein M (1978) Renal effects of head-out water immersion in man: implications for an understanding of volume homeostasis. Physiol Rev 58:529–581

Espiner EA, Crozier IG, Nicholls MG, Cuneo R, Yandle TG, Ikram H (1985) Cardiac secretion of atrial natriuretic peptide. Lancet ii:398–399

Faison EP, Siegl PK, Morgan G, Winquist RJ (1985) Regional vasorelaxant selectivity of atrial natriuretic factor in isolated rabbit vessels. Life Sci 37:1073–1079

Fakunding JL, Catt KJ (1980) Dependence of aldosterone stimulation in adrenal glomerulosa cells on calcium uptake: effects of lanthanum and verapamil. Endocrinology 107:1345–1353

Fakunding JL, Chow R, Catt KJ (1979) The role of calcium in the stimulation of aldosterone production by adrenocorticotropin, angiotensin II and potassium in isolated glomerulosa cells. Endocrinology 105:327–333

Fater DC, Schultz HD, Sundet ND, Mapes JS, Goetz KL (1982) Effects of left atrial stretch in cardiac-denervated and intact conscious dogs. Am J Physiol 242:H1056–H1064

Ferlinz J (1980) Right ventricular performance in essential hypertension. Circulation 61:157–162

Fesenko EE, Kolesnikov SS, Lyubarsky AL (1985) Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. Nature 313:310–313

Fesik SW, Holleman WH, Perun TJ (1985) Two-dimensional ^{1}H NMR studies of rat atrial natriuretic factor (1–23). Biochem Biophys Res Commun 131:517–523

Field M, Graf LH, Laird WJ, Smith PL (1978) Heat-stable enterotoxin of E. coli: *in vitro* effects on GC activity, cGMP concentration and ion transport in small intestine. Proc Natl Acad Sci USA 75:2800–2804

Fiscus RR, Rapoport RM, Waldman SA, Murad F (1985) Atriopeptin II elevates cGMP, activates cGMP-dependent protein kinase and causes relaxation in rat thoracic aorta. Biochim Biophys Acta 846:179–184

Fitts DA, Thunhorst RL, Simpson JB (1985) Diuresis and reduction of salt appetite by lateral ventricular infusions of atriopeptin II. Brain Res 348:118–124

Fluckiger JP, Waeber B, Matsueda G, Delaloye B, Nussberger J, Brunner HR (1986) Effect of atriopeptin III on hematocrit and volemia of nephrectomized rats. Am J Physiol 251:H880–H883

Flynn TG, Davies PL (1985) The biochemistry and molecular biology of atrial natriuretic factor. Biochem J 232:313–321

Flynn TG, de Bold ML, de Bold AJ (1983) The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. Biochem Biophys Res Commun 117:859–865

Flynn TG, Davies PL, Kennedy BP, de Bold ML, de Bold AJ (1985) Alignment of rat cardionatrin sequences with the preprocardionatrin sequence from complementary DNA. Science 228:323–325

Fok KF, Tjoeng FS, Houbion JA, Spear KL, Nugent ST, Eubanks SR, Zupec ME, Olins GM, Blehm DJ, Adams SP, Wakatani K, Needleman P (1985) Structure-activity studies of atriopeptins. In: Deber CM, Hruby VJ, Kopple KD (eds) Peptides: structure and function. Pierce Chemical Company, Rockford, Illinois, pp 953–956

Forssmann WG, Mutt V (1985) Cardiodilatin-immunoreactive neurons in the hypothalamus of Tupaia. Anat Embryol (Berl) 172:1–5

Forssmann WG, Hock D, Lottspeich F, Henschen A, Kreye V, Christmann M, Reinecke M, Metz J, Carlquist M, Mutt V (1983) The right auricle of the heart is an endocrine organ. Anat Embryol (Berl) 168:307–313

Forssmann WG, Birr C, Carlquist M, Christmann M, Finke R, Henschen A, Hock D, Hircheim H, Kreye V, Cottspeich F, Metz J, Mutt V, Reineike M (1984) The auricular myocardiocytes of the heart constitute an endocrine organ. Characterization of a porcine cardiac peptide hormone, cardiodilatin-126. Cell Tissue Res 238:425–430

Franch HA, Callahan LT, Blaine EH (1986) Plasma and atrial content of atrial natriuretic factor in cardiomyopathic hamsters. Life Sci 39:1151–1159

Freeman RH, Davis JO, Vari RC (1985) Renal response to atrial natriuretic factor in conscious dogs with caval constriction. Am J Physiol 248:R495–R500

Fried TA, McCoy RN, Osgood RW, Stein JH (1986) Effect of atriopeptin II on determinants of glomerular filtration rate in the in vitro perfused dog glomerulus. Am J Physiol 250:F1119–F1122

Friedl A, Harmening C, Schuricht B, Hamprecht B (1985) Rat atrial natriuretic peptide elevates the level of cGMP in astroglia-rich brain cell cultures. Eur J Pharmacol 111:141–142

Friedl A, Harmening C, Hamprecht B (1986) Atrial natriuretic hormones raise the level of cyclic GMP in neural cell lines. J Neurochem 46:1522–1527

Frohlich ED, Tarazi RC, Dustan HP (1971) Clinical physiologic correlations in the development of hypertensive heart disease. Circulation 44:446–455

Frossard PM, Gonzales PA, Greenberg B, Fiddes JC, Atlas SA (1986) BglII dimorphism at the human atrial natriuretic peptide (ANP) gene locus. Nucleic Acids Res 14:5121

Fujii K, Ishimatsu T, Kuriyama H (1986) Mechanism of vasodilation induced by alpha-human atrial natriuretic polypeptide in rabbit and guinea-pig renal arteries. J Physiol 377:315–332

Fujio N, Ohashi M, Nawata H, Kato K, Ibayashi H, Kangawa K, Matsuo H (1986) Alpha-human atrial natriuretic polypeptide reduces the plasma arginine vasopressin concentration in human subjects. Clin Endocrinol 25:181–187

Fujioka S, Tamaki T, Fukui K, Okahara T, Abe Y (1985) Effects of a synthetic human atrial natriuretic polypeptide on regional blood flow in rats. Eur J Pharmacol 109:301–304

Fyhrquist F, Tötterman KJ, Tikkanen I (1985) Infusion of atrial natriuretic peptide in liver cirrhosis with ascites. Lancet ii:1439

Garay R, Hannaert P, Rodrigue F, Dunham B, Marche P, Genest J, Braquet P, Bianchi C, Cantin M, Meyer P (1985) Atrial natriuretic factor inhibits Ca^{2+}-dependent K^+ fluxes in cultured vascular smooth muscle cells. J Hypertens 3(suppl 3):S297–S298

Garbers DL, Radany EW (1981) Characteristics of the soluble and particulate forms of guanylate cyclase. In: Dumont JE, Greengard P, Robison GA (eds) Advances in cyclic nucleotide research, vol 14. Raven, New York, pp 241–254

Garcia R, Cantin M, Thibault G, Ong H, Genest J (1982) Relationship of specific granules to the natriuretic and diuretic activity of rat atria. Experientia 38:1071–1073

Garcia R, Thibault G, Genest J (1983) Effect of partially purified atrial natriuretic factor (ANF) on rat and rabbit vascular smooth muscle. Clin Invest Med 6(suppl 2):56A

Garcia R, Thibault G, Cantin M, Genest J (1984a) Effect of a purified atrial natriuretic factor on rat and rabbit vascular strips and vascular beds. Am J Physiol 247:R34–R39

Garcia R, Thibault G, Nutt RF, Cantin M, Genest J (1984b) Comparative vasoactive effects on inactive and synthetic atrial natriuretic factor (ANF). Biochem Biophys Res Commun 119:685–688

Garcia R, Debinski W, Gutkowska J, Kuchel O, Thibault G, Genest J, Cantin M (1985a) Gluco- and mineralocorticoids may regulate the natriuretic effect and the synthesis and release of atrial natriuretic factor by the rat atria *in vivo*. Biochem Biophys Res Commun 131:806–814

Garcia R, Gutkowska J, Genest J, Cantin M, Thibault G (1985b) Reduction of blood pressure and increased diuresis and natriuresis during chronic infusion of atrial natriuretic factor (ANF Arg 101–Tyr 126) in conscious one-kidney, one-clip hypertensive rats. Proc Soc Exp Biol Med 179:539–545

Garcia R, Thibault G, Gutkowska J, Cantin M, Genest J (1985c) Changes in regional blood flow induced by atrial natriuretic factor (ANF) in conscious rats. Life Sci 36:1687–1692

Garcia R, Thibault G, Gutkowska J, Hamet P, Cantin M, Genest J (1985d) Effect of chronic infusion of synthetic atrial natriuretic factor (ANF 8–33) in conscious two-kidney, one-clip hypertensive rats. Proc Soc Exp Biol Med 178:155–159

Garcia R, Thibault G, Gutkowska J, Horky K, Hamet P, Cantin M, Genest J (1985e) Chronic infusion of low doses of atrial natriuretic factor (ANF Arg 101–Tyr 126) reduces blood pressure in conscious SHR without apparent changes in sodium excretion. Proc Soc Exp Biol Med 179:396–401

Garcia R, Thibault G, Hamet P, Gutkowska J, Cantin M, Genest J (1985f) Effect of atrial natriuretic factor [ANF (Arg 101–Tyr 126)] on kallikrein and cyclic GMP in the renovascular hypertensive rat. Clin Exp Hypertens A7:1597–1618

Garcia R, Thibault G, Seidah NG, Lazure C, Cantin M, Genest J, Chrétien M (1985g) Structure-activity relationships of atrial natriuretic factor (ANF). II. Effect of chain-length modifications on vascular reactivity. Biochem Biophys Res Commun 126:178–184

Garcia R, Cantin M, de Léan A, Genest J, Godin J, Gutkowska J, Schiffrin EL, Thibault G (1986a) Comparative biological activities of ANF (Arg 101–Tyr 126) and the synthetic forms of circulating ANF (Ser 99–Tyr 126). Biochem Biophys Res Commun 135:987–993

Garcia R, Lachance D, Thibault G, Cantin M, Gutkowska J (1986b) Mechanisms of release of atrial natriuretic factor. II. Effect of chronic administration of alpha- and beta-adrenergic and cholinergic agonists on plasma and atrial ANF in the rat. Biochem Biophys Res Commun 136:510–520

Garcia R, Thibault G, Gutkowska J, Cantin M (1986c) Effect of chronic infusion of atrial natriuretic factor on plasma and urinary aldosterone, plasma renin activity, blood pressure and sodium excretion in 2-K, 1-C hypertensive rats. Clin Exp Hypertens A8:1127–1147

Garcia R, Cantin M, Genest J, Gutkowska J, Thibault G (1987a) Body fluids and plasma atrial peptide after its chronic infusion in hypertensive rats. Proc Soc Exp Biol Med 185:352–358

Garcia R, Cantin M, Gutkowska J, Thibault G (1987b) Atrial natriuretic factor during development and reversal of one-kidney, one-clip hypertension. Hypertension 9:144–149

Garcia R, Cantin M, Thibault G (1987c) Role of the right and left atria in natriuresis and atrial natriuretic factor release during blood volume changes in the conscious rats. Circ Res 61:99–106

Garcia R, Gutkowska J, Cantin M, Thibault G (1987d) Renin dependency of the effect of chronically administered atrial natriuretic factor in two-kidney, one-clip rats. Hypertension 9:88–95

Garcia R, Thibault G, Cantin M (1987e) Correlation between cardiac hypertrophy and plasma levels of atrial natriuretic factor in non-spontaneous models of hypertension in the rat. Biochem Biophys Res Commun 145:532–541

Gardner DG, Deschepper CF, Ganong WF, Hane S, Fiddes J, Baxter JD, Lewicki J (1986a) Extra-atrial expression of the gene for atrial natriuretic factor. Proc Natl Acad Sci USA 83:6697–6701

Gardner DG, Hane S, Trachewsky D, Schenk D, Baxter JD (1986b) Atrial natriuretic peptide mRNA is regulated by glucocorticoids in vivo. Biochem Biophys Res Commun 139:1047–1054

Gardner DG, Deschepper CF, Baxter JD (1987) The gene for the atrial natriuretic factor is expressed in the aortic arch. Hypertension 9:103–106

Gauer OH, Henry JP (1963) Circulatory basis of fluid volume control. Physiol Rev 43:423–481

Geller DM, Currie MG, Siegel NR, Fok KF, Adams SP, Needleman P (1984a) The sequence of an atriopeptigen: a precursor of the bioactive atrial peptides. Biochem Biophys Res Commun 121:802–807

Geller DM, Currie MG, Waketani K, Cole BR, Adams SP, Fok KF, Siegel NR, Eubanks SR, Gallupi GR, Needleman P (1984b) Atriopeptins: A family of potent biologically active peptides derived from mammalian atria. Biochem Biophys Res Commun 120:333–338

Genest J (1983) Personal views on the mechanisms of essential hypertension. In: Genest J, Kuchel O, Hamet P, Cantin M (eds) Hypertension: physiopathology and treatment, 2nd edn. McGraw-Hill, New York, pp 599–614

Genest J (1984) A new lock at the old mosaic. Cleve Clin Q 51:449–461

Genest J (1986) The atrial natriuretic factor. Br Heart J 56:302–316

Genest J, Koiw E, Nowaczynski W, Sandor T, Biron P (1960) Adrenocortical function in essential hypertension. In: Bock KD, Cottier PT (eds) Essential hypertension, an international symposium. Springer-Verlag, Berlin Heidelberg New York, pp 125–146

Genest J, Nowaczynski W, Kuchel O, Boucher R, Rojo-Ortega JM, Constantopoulos G, Ganten D, Messerli F (1976) The adrenal cortex and essential hypertension. Recent Prog Horm Res 32:377–427

Genest J, Larochelle P, Cusson JR, Gutkowska J, Cantin M, Garcia R, Thibault G, Kuchel O, de Léan A, Hamet P (1987) The atrial natriuretic factor in mild essential hypertension. Transactions, American Clinical and Climatological Association, Colorado Springs Meeting Waverly Press (in press)

George JS, Hagins WA (1983) Control of Ca^{2+} in rod outer segment disks by light and cyclic GMP. Nature 303:344–349

Gerber AL, Arendt RM, Schnizer W, Silz S, Jungst D, Zahreinger J, Paumgartner G (1986) Regulation of atrial natriuretic factor release in man: Effect of water immersion. Klin Wochenschr 64:666–667

Gerzer R, Hoffmann F, Schultz G (1981) Purification of a soluble, sodium-nitroprusside-stimulated guanylate cyclase from bovine lung. Eur J Biochem 116:479–486

Gerzer R, Witzgall H, Tremblay J, Gutkowska J, Hamet P (1985) Rapid increase in plasma and urinary cyclic GMP after bolus injection of atrial natriuretic factor in man. J Clin Endocrinol Metab 61:1217–1219

Gibbs DM (1987) Noncalcium-dependent modulation of in vitro atrial natriuretic factor release by extracellular osmolality. Endocrinology 120:194–197

Gibson TR, Shields PP, Glembotski CC (1987) The conversion of atrial natriuretic peptide (ANF)-(1–126) to ANF (99–126) by rat serum: contribution to ANF cleavage in isolated perfused rat hearts. Endocrinology 120:764–772

Glembotski CC, Gibson TR (1985) Molecular forms of immunoreactive atrial natriuretic peptide released from cultured rat atrial myocytes. Biochem Biophys Res Commun 132:1008–1017

Glembotski CC, Wildey GM, Gibson TR (1985) Molecular forms of immunoactive atrial natriuretic peptide in the rat hypothalamus and atrium. Biochem Biophys Res Commun 129:671–678

Gnädinger MP, Weidmann P, Rascher W, Lang RE, Hellmüller B, Uehlinger DE (1986) Plasma arginine-vasopressin levels during infusion of synthetic atrial natriuretic peptide and different sodium intakes in man. J Hypertens 4:623–629

Goetz KL (1987) Atrial natriuretic peptide and atrial pressure. N Engl J Med 316:485

Goetz KL, Wang BC, Geer PG, Leadley RJ, Reinhardt HW (1986) Atrial stretch increases sodium excretion independently of release of atrial peptides. Am J Physiol 250:R946–R950

Goodfriend TL, Elliott ME, Atlas SA (1984) Actions of synthetic atrial natriuretic factor on bovine adrenal glomerulosa. Life Sci 35:1675–1682

Graham RM, Bloch KD, Delaney VB, Bourke E, Seidman JG (1986) Bartter's syndrome and the atrial natriuretic factor gene. Hypertension 8:549–551

Grammer RT, Fukiemi H, Inagami T, Misono KS (1983) Rat atrial natriuretic factor. Purification and vasorelaxant activity. Biochem Biophys Res Commun 116:696–703

Granger JP, Opgenorth TJ, Salazar J, Romero JC, Burnett JC (1986) Long-term hypotensive and renal effects of atrial natriuretic peptide. Hypertension 8(suppl II):II-112–II-116

Greenberg BD, Bencen GH, Seilhamer JJ, Lewicki JA, Fiddes JC (1984) Nucleotide sequence of the gene encoding human atrial natriuretic factor precursor. Nature 312:656–658

Gruetter CA, Barry BK, McNamara DB, Gruetter DY, Hadowitz PJ, Ignarro LJ (1979) Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. J Cyclic Nucleotide Res 5:211–224

Gruetter CA, Hadowitz PJ, Ignarro LJ (1981) Methylene blue inhibits coronary arterial relaxation and guanylate cyclase activation by nitroglycerin, sodium nitrite and amylnitrite. Can J Physiol Pharmacol 59:150–156

Guazzi M, Fiorentini C, Olivari MP, Polese A (1979) Cardiac load and function in hypertension. Am J Cardiol 44:1007–1012

Gumbiner B, Kelly RB (1982) Two distinct intracellular pathways of transport and secretion of membrane glycoprotein to the surface of pituitary tumor cells. Cell 28:51–59

Gupta BN, Linden RJ, Mary DA, Weatherill D (1982) The diuretic and natriuretic responses to stimulation of left atrial receptors in dogs with different blood volumes. Q J Exp Physiol 67:235–258

Gusella JF, Tanzi RE, Anderson MA, Hobbs W, Gibbons K, Raschtchian R, Gilliam TC, Wallace MR, Wexler NS, Conneally PM (1984) DNA markers for nervous system diseases. Science 225:1320–1326

Gutkowska J, Horky K, Thibault G, Januszewicz P, Cantin M, Genest J (1984a) Atrial natriuretic factor is a circulating hormone. Biochem Biophys Res Commun 125:315–323

Gutkowska J, Thibault G, Januszewicz P, Cantin M, Genest J (1984b) Direct radioimmunoassay of atrial natriuretic factor. Biochem Biophys Res Commun 122:593–601

Gutkowska J, Bourassa M, Roy D, Thibault G, Garcia R, Cantin M, Genest J (1985) Immunoreactive atrial natriuretic factor (IR-ANF) in human plasma. Biochem Biophys Res Commun 128:1350–1357

Gutkowska J, Bonan R, Roy D, Bourassa M, Garcia R, Thibault G, Genest J, Cantin M (1986a) Atrial natriuretic factor in human plasma. Biochem Biophys Res Commun 139:287–295

Gutkowska J, Horky K, Lachance C, Racz K, Garcia R, Thibault G, Kuchel O, Genest J, Cantin M (1986b) Atrial natriuretic factor in spontaneously hypertensive rats. Hypertension 8(suppl I):I-137–I-140

Gutkowska J, Kuchel O, Racz K, Buu NT, Cantin M, Genest J (1986c) Increased plasma immunoreactive atrial natriuretic factor concentrations in salt sensitive Dahl rats. Biochem Biophys Res Commun 136:411–416

Gutkowska J, Racz K, Garcia R, Thibault G, Kuchel O, Genest J, Cantin M (1986d) The morphine effect on plasma ANF. Eur J Pharmacol 131:91–94

Gutkowska J, Baranowska B, Racz K, Thibault G, Garcia R, Cantin M, Genest J (1987a) Effect of opioids on plasma level of immunoreactive atrial natriuretic factor (IR-ANF). In: Proceedings of the Symposium of the 11th Scientific Meeting of ISH 1986. Opioid Peptides and Blood Pressure Control. Springer, Berlin Heidelberg New York

Gutkowska J, Debinski W, Racz K, Kuchel O, Thibault G, Garcia R, Genest J, Cantin M (1987b) Biologically active atrial natriuretic factor-like peptides in peripheral endocrine and neuronal tissues of the rat. In: Brenner BM, Laragh JH (eds) Biologically Active Atrial Peptides, American Society of Hypertension Symposium Series, vol 1, Raven Press, NY, pp 185–187

Gutkowska J, Genest J, Thibault G, Garcia R, Larochelle P, Cusson JR, Kuchel O, Hamet P, de Léan A, Cantin M (1987c) Circulating forms and radioimmunoassay of atrial natriuretic factor. Endocrinol Metab Clin North Am 16:183–198

Guyton AC, Coleman TG, Young DB, Lohmeier TE, DeClue JW (1980) Salt-balance and long-term blood pressure control. Annu Rev Med 13:15–27

Haass M, Zamir N, Zukowska-Grojec Z (1986) Plasma levels of atrial natriuretic peptides in conscious adult spontaneously hypertensive rats. Clin Exp Hypertens A8:277–287

Hamet P, Tremblay J, Thibault G, Garcia R, Cantin M, Genest J (1983) Effect of atrial natriuretic factor on metabolism of cGMP. Endocrinology 112:289

Hamet P, Tremblay J, Pang SC, Garcia R, Thibault G, Gutkowska J, Cantin M, Genest J (1984) Effect of native and synthetic atrial natriuretic factor on cyclic GMP. Biochem Biophys Res Commun 123:515–527

Hamet P, Tremblay J, Pang SC, Skuherska R, Schiffrin EL, Garcia R, Cantin M, Genest J, Palmour R, Ervin FR, Martin S, Goldwater R (1986) Cyclic GMP as mediator and biological marker of atrial natriuretic factor. J Hypertens 4(suppl 2):S49–S56

Hammond TG, Yusufi ANK, Knox FG, Dousa TP (1985) Administration of atrial natiuretic factor inhibits sodium-coupled transport in proximal tubules. J Clin Invest 75:1983–1989

Hang M, Beaulieu S, Barden N (1986) Atrial natriuretic factor gene expression: Modulation of the mRNA levels in the rat brain by salt loading. Soc Neurosci Abstr 12(1):689

Hartle DK, Brody MJ (1984) The angiotensin II pressor system of the rat forebrain. Circ Res 54:355–366

Hartter E, Weissel M, Stummvoll HK, Woloszczuk W, Punzengruber C, Ludvik B (1985) Atrial natriuretic peptide concentrations in blood from right atrial in patients with severe right heart failure. Lancet ii:93–94

Hartter E, Woloszczuk W, Stummvoll HK (1986) Radioimmunoassay of atrial natriuretic peptides in human plasma. Clin Chem 32:441–445

Hasegawa K, Matsushita Y, Inoue T, Morii H, Ishibashi M, Yamaji T (1986) Plasma levels of atrial natriuretic peptide in patients with chronic renal failure. J Clin Endocrinol Metab 63:819–822

Hassid A (1986) Atriopeptin II decreases cystolic free Ca in cultured vascular smooth muscle cells. Am J Physiol 251:C681–C686

Hausler A, Rohr HP, Marbach P, Flückiger E (1978) Changes in prolactin secretion on lactating rat assessed by correlative morphometric and biochemical methods. J Ultrastruct Res 64:74–84

Hayashi Y, Iwasa F, Furuya M, Kanai Y, Minamitake Y, Kubota I, Ohnuma N, Kangawa K, Matsuo H (1986) Structure-activity relationship of atrial natriuretic polypeptide (ANP). In: Kiso Y (ed) Peptide chemistry 1985. Protein Research Foundation, Osaka, pp 27–32

Healey DP, Fanestil DD (1986) Localization of atrial natriuretic peptide binding sites within the rat kidney. Am J Physiol 250:F573–F578

Hedner T, Hedner J, Towle AC, Hartfold M, Ljiungram S, Wikstrand J, Berglund G (1986) Plasma atrial natriuretic peptide (ANP) in patients with mild to moderate hypertension. J Cardiovasc Pharmacol 8:1299

Heisler S, Simard J, Assayag E, Mehri Y, Labrie F (1986) Atrial natriuretic factor does not affect basal, forskolin- and CRF-stimulated adenylate cyclase activity, cAMP formation or ACTH secretion, but does stimulate cGMP synthesis in anterior pituitary. Mol Cell Endocrinol 44:125–131

Henry JP, Gauer OH, Reeves JL (1956a) Evidence of the atrial location of receptors influencing urine flow. Circ Res 41:85–90

Henry JP, Gauer OH, Sicker HO (1956b) The effect of moderate changes in blood volume on left and right atrial pressure. Circ Res 4:91–94

Herzer WA, Deray G, Jackson EK (1987) Effects of atrial natriuretic factor on noradrenergic neurotransmission in vivo in the rat mesentery. J Cardiovasc Pharmacol 9:125–128

Higuchi K, Nawata H, Kato KI, Ibayashi H, Matsuo H (1986a) Alpha-human natriuretic polypeptide (α-hANP) specific binding sites in bovine adrenal gland. Biochem Biophys Res Commun 137:657–663

Higuchi K, Nawata H, Kato KI, Ibayashi H, Matsuo H (1986b) Alpha-human atrial natriuretic polypeptide inhibits steroidogenesis in cultured human adrenal cells. J Clin Endocrinol Metab 62:941–944

Higuchi K, Nawata H, Kato KI, Ibayashi H, Matsuo H (1986c) Lack of inhibitory effect of α-human atrial natriuretic polypeptide on aldosteronogenesis in aldosterone-producing adenoma. J Clin Endocrinol Metab 63:192–196

Hirata Y, Ganguli M, Tobian L, Iwai J (1984a) Dahl S rats have increased natriuretic factor in atria but are markedly hyporesponsive to it. Hypertension 6(suppl I):I-148–I-155

Hirata Y, Tomita M, Yoshimi H, Ikeda M (1984b) Specific receptors for atrial natriuretic factor (ANF) in cultured vascular smooth muscle cells of rat aorta. Biochem Biophys Res Commun 125:562–568

Hirata Y, Ishii M, Sugimoto T, Matsuoka H, Sugimoto T, Kangawa K, Matsuo H (1985a) The effects of human atrial 28-amino acid peptide on systemic and renal hemodynamics in anesthetized rats. Circ Res 57:634–639

Hirata Y, Takata S, Tomita M, Takaichi S (1985b) Binding, internalization and degradation of atrial natriuretic peptide in cultured vascular smooth muscle cells of rat. Biochem Biophys Res Commun 132:976–984

Hirata Y, Tomita M, Takada S, Yoshimi H (1985c) Vascular receptor binding activities and cyclic GMP responses by synthetic human and rat atrial natriuretic peptides (ANP) and receptor down-regulation by ANP. Biochem Biophys Res Commun 128:538–546

Hirata Y, Tomita M, Yoshimi H, Kuramochi M, Ito K, Ikeda M (1985d) Effect of synthetic human atrial natriuretic peptide on aldosterone secretion by dispersed aldosterone-producing adenoma cells in vitro. J Clin Endocrinol Metab 61:677–680

Hirose S, Akiyama F, Shinjo M, Ohno H, Murakami K (1985) Solubilization and molecular weight estimation of atrial natriuretic factor receptor from bovine adrenal cortex. Biochem Biophys Res Commun 130:574–579

Hirth C, Stasch J-P, John A, Kazda S, Morich F, Neuser D, Wohlfeil S (1986) The renal response to acute hypervolemia is caused by atrial natriuretic peptides. J Cardiovasc Pharmacol 8:268–275

Hiruma M, Ikemoto F, Yamamoto K (1986) Rat atrial natriuretic factor stimulates renin release from renal cortical slices. Eur J Pharmacol 125:151–153

Hodsman GP, Tsunoda K, Ogawa K, Johnston CI (1985) Effects of posture on circulating atrial natriuretic peptide. Lancet ii:1427

Hollister AS, Tanaka I, Imada T, Onrot J, Biaggioni I, Robertson D, Inagami T (1986) Sodium loading and posture modulate human atrial natriuretic factor plasma levels. Hypertension (suppl II) 8:II-106–II-111

Holtz J, Stewart DJ, Elsner D, Bassenge E (1986) In vivo peptide-renodilation: minimal potency relative to nitroglycerin in dogs. Life Sci 39:2177–2184

Horiba N, Nomura K, Saito Y, Demura H, Shizume K (1985) Rat atrial natriuretic polypeptide stimulation of adrenal zona glomerulosa cell growth. Biochem Biophys Res Commun 132:261–263

Horky K, Gutkowska J, Garcia R, Thibault G, Genest J, Cantin M (1985) Effect of different anesthetics on immunoreactive atrial natriuretic factor concentrations in rat plasma. Biochem Biophys Res Commun 129:651–657

Horvath J, Ertl T, Schally AV (1986) Effect of atrial natriuretic peptide on gonadotropin release in superfused rat pituitary cells. Proc Natl Acad Sci USA 83:3444–3446

Huang CL, Lewicki J, Johnson LK, Cogan MG (1985) Renal mechanism of action of rat atrial natriuretic factor. J Clin Invest 75:769–773

Huet M, Cantin M (1974) Ultrastructural cytochemistry of atrial muscle cells. II. Characterization of the protein content of specific granules. Lab Invest 30:525–532

Hunter WM, Greenwood FC (1962) Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature 194:495–496

Imada T, Takayanagi R, Inagami T (1985) Changes in the content of atrial natriuretic factor with the progression of hypertension in spontaneously hypertensive rats. Biochem Biophys Res Commun 133:759–765

Inui K, Saito H, Matsukawa Y, Nakao K, Morii N, Imura H, Shimokura M, Kiso Y, Hori R (1985) Specific binding activities and cyclic GMP responses by atrial natriuretic polypeptide in kidney epithelial cell line (LLC-PK_1). Biochem Biophys Res Commun 132:253–260

Ishido M, Fugita T, Hagiwara H, Shimonaka M, Saheki T, Hirata Y, Hirose S (1986) Physical and functional association of the atrial natriuretic peptide receptor with particulate guanylate-cyclase as demonstrated using detergent extracts of bovine lung membranes. Biochem Biophys Res Commun 140:101–106

Jacobowitz DM, Skofitsch G, Keiser HR, Eskay RL, Zamir N (1985) Evidence for the existence of atrial natriuretic factor-containing neurons in the rat brain. Neuroendocrinology 40:92–94

Jaiswal N, Paul AK, Jaiswal RK, Sharma RK (1986) Atrial natriuretic factor regulation of cyclic GMP levels and steroidogenesis in isolated fasciculata cells of rat adrenal cortex. FEBS Lett 199:121–124

Jakobs KH, Johnson RA, Schultz G (1983) Activation of human platelet adenylate cyclase by a bovine sperm component. Biochim Biophys Acta 756:369–375

Jamieson JD, Palade GE (1964) Specific granules in atrial muscle cells. J Cell Biol 23:151–172

Januszewicz P, Gutkowska J, de Léan A, Thibault G, Garcia R, Genest J, Cantin M (1985) Synthetic atrial natriuretic factor induces release (possibly receptor-mediated) of vasopressin from rat posterior pituitary. Proc Soc Exp Biol Med 178:321–325

Januszewicz P, Larose P, Ong H, Gutkowska J, Genest J, Cantin M (1986a) Effect of atrial natriuretic factor on plasma vasopressin in conscious rats. Peptides 7:989–993

Januszewicz P, Thibault G, Garcia R, Gutkowska J, Genest J, Cantin M (1986b) Effect of synthetic atrial natriuretic factor on arginine vasopressin release by the rat hypothalamo-neurohypophysial complex in organ culture. Biochem Biophys Res Commun 134:652–658

Januszewicz P, Thibault G, Gutkowska J, Garcia R, Mercure C, Jolicoeur F, Genest J, Cantin M (1986c) Atrial natriuretic factor and vasopressin during dehydratation and rehydratation in rats. Am J Physiol 251:E497–E501

Jeffrey FE, Wagner R, Abelmann WH (1970) Left and right ventricular pressures in the normal and the cardiomyopathic Syrian hamster (mesocricetus auratus). Proc Soc Exp Biol Med 135:940–943

John A, Stasch JP, Neuser D, Hirth C, Morich FJ (1986) The use of a monoclonal antibody to measure plasma atriopeptins in rat. Life Sci 38:1991–1997

Johnson RA, Jakobs KH, Schultz G (1985) Extraction of the adenylate cyclase-activating factor of bovine sperm and its identification as a trypsin-like protease. J Biol Chem 260:114–121

Jüppner H, Brabant G, Kupteina U, Kirschner M, Klein H, Hesch RD (1986) Direct radioimmunoassay for human atrial natriuretic peptide (hANP) and its clinical evaluation. Biochem Biophys Res Commun 139:1215–1223

Kaczmarczyk G, Drake A, Eisele R, Nohnkaupt R, Noble MIM, Simgen B, Stubbs J, Reinhardt HW (1981) The role of cardiac nerves in the regulation of sodium excretion in conscious dogs. Pflügers Arch 390:125–130

Kaczmarczyk G, Christie W, Nohnkaupt R, Reinhardt HW (1983) An attempt to quantitate the contribution of antidiuretic hormone to the diuresis of left atrial distension in conscious dogs. Pflügers Arch 395:101–105

Kahn JK, Grekin RJ, Shenker Y, Vinik AI (1986) Plasma levels of immunoreactive atrial natriuretic hormone in patients with diabetes mellitus. Regul Pept 15:323–332

Kambayashi Y, Kawabata T, Hara S, Yamauchi A, Ueda A, Kono M, Doteuchi M, Nakamura M, Inouye K (1986) Synthesis and biological properties of two dimeric forms of human α-atrial natriuretic peptide. FEBS Lett 206:313–318

Kangawa K, Matsuo H (1984) Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (alpha h-ANF). Biochem Biophys Res Commun 118:131–139

Kangawa K, Fukuda A, Kubota I, Hayashi Y, Matsuo H (1984a) Identification in rat atrial tissue of multiple forms of natriuretic polypeptides of about 3,000 daltons. Biochem Biophys Res Commun 121:585–591

Kangawa K, Fukuda A, Minamino N, Matsuo H (1984b) Purification and complete amino acid sequence of beta-rat atrial natriuretic peptide (beta-rANP) of 5,000 daltons. Biochem Biophys Res Commun 119:933–940

Kangawa K, Tawaragi Y, Oikawa S, Miziano A, Sakuragawa Y, Nakazato H, Fukuda A, Minamino N, Matsuo H (1984c) Identification of rat gamma atrial natriuretic polypeptide and characterization of the cDNA encoding its precursor. Nature 312:152–155

Kangawa K, Fukuda A, Matsuo H (1985) Structural identification of beta- and gamma-human atrial natriuretic polypeptide. Nature 313:397–400

Kappagoda CT, Linden RJ, Snow HM (1972) The effect of distending the atrial appendages on urine flow in the dog. J Physiol 277:233–242

Kappagoda CT, Linden RJ, Snow HM (1973) Effect of stimulating right atrial receptors on urine flow in the dog. J Physiol 235:493–502

Katada T, Ui M (1982) Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. Proc Natl Acad Sci USA 79:3129–3133

Katoh Y, Kurosawa T, Takeda S, Kurokawa S, Sakamotom H, Marumo F, Kikawada R (1986) Atrial natriuretic peptide levels in treated congestive heart failure. Lancet i:851

Katsube N, Schwartz D, Needleman P (1985a) Release of atriopeptin in the rat by vasoconstrictors or water immersion correlates with changes in right atrial pressure. Biochem Biophys Res Commun 133:937–944

Katsube N, Wakitani K, Fok KF, Tjoeng FS, Zupec ME, Zupec SR, Eubanks SR, Adams SP, Needleman P (1985b) Differential structure-activity relationships of atrial peptides as natriuretics and renal vasodilators in the dog. Biochem Biophys Res Commun 128:325–330

Kawata M, Nakao K, Morii N, Kiso Y, Yamashita H, Imura H, Sano Y (1985a) Atrial natriuretic polypeptide: topographical distribution in the rat brain by radioimmunoassay and immunohistochemistry. Neuroscience 16:521–546

Kawata M, Ueda S, Nakao K, Morii N, Kiso Y, Imura H, Sano Y (1985b) Immunohistochemical demonstration of alpha-atrial natriuretic polypeptide-containing neurons in the rat brain. Histochemistry 83:1–3

Keeler R, Azzarolo AM (1983) Effect of atrial natriuretic factor on renal handling of water and electrolytes in rats. Can J Physiol Pharmacol 61:996–1002

Kern HF (1980) Effect of chronic administration of gastrointestinal hormone on the pancreas. Hepatogastroenterology 27:407–410

Khalil F, Fine BKU, Kuriyama S, Nakamura A, Aviv A (1986) A higher number of vascular smooth muscle cell (VSMC) receptors with a lower affinity to the atrial natriuretic factor (ANF) in the spontaneously hypertensive (SHR) rat. Pediatr Res 20:171A

Kihara M, Nakayama K, Nakao K, Sugawara A, Morii N, Sakamoto M, Suda M, Shimokura M, Kiso Y, Imura H, Yamori Y (1985) Accelerated natriuresis induced by synthetic atrial natriuretic polypeptide in spontaneously hypertensive rats. Clin Exp Hypertens A7:539–551

Kimura H, Murad F (1975) Two forms of guanylate cyclase in mammalian tissues and possible mechanisms for their regulation. Metabolism 24:439–445

Kisch B (1956) Electronmicroscopy of the atrium of the heart. I. Guinea pig. Exp Med Surg 14:99–112

Kiso Y, Shimokura M, Hosoi S, Fujisaki T, Fujiwara Y, Yoshida M (1985) Syntheses and properties of peptides related to atrial natriuretic hormone. In: Deber CM, Hruby VJ, Kopple KD (eds) Peptides: structure and function. Pierce Chemical Company, Rockford, Illinois, pp 949–952

Kleinert HD, Maack T, Atlas SA, Januszewicz A, Sealey JE, Laragh JH (1984) Atrial natriuretic factor inhibits angiotensin-, norepinephrine-, and potassium-induced vascular contractility. Hypertension 6(suppl I):I-143–I-147

Kleinert HD, Volpe M, Odell G, Marion D, Atlas SA, Camargo MJ, Laragh JH, Maack T (1986) Cardiovascular effects of atrial natriuretic factor in anesthetized and conscious dogs. Hypertension 8:312–316

Knorr M, Locher R, Stimpel M, Neyses L, Vetter W (1986) Cyclic GMP and cytosolic free calcium in the interaction between atrial natriuretic polypeptide and angiotensin II in smooth muscle cells. In: Natriuretic Hormones in Hypertension, Satellite Symposium, 11th Scientific Meeting of the International Society of Hypertension, Heidelberg, abstract

Kobrin J, Kardon MB, Trippodo NC, Pegram BL, Frohlich ED (1985) Renal response to acute volume overload in conscious rats with atrial appendectomy. J Hypertens 3:145–148

Koch K-W, Kaupp UB (1985) cGMP directly regulates a cation conductance in membranes of bovine rods by a cooperative mechanism. J Biol Chem 260:6788–6800

Kohno M, Takaori K, Matsuura T, Murakawa K, Kanayama Y, Takeda T (1986a) Atrial natriuretic polypeptide in atria and plasma in experimental hyperthyroidism and hypothyroidism. Biochem Biophys Res Commun 134:178–183

Kohno M, Yasunari K, Matsuura T, Takaori K, Kanayama Y, Takeda T (1986b) Increased circulating atrial natriuretic polypeptide in severe essential hypertension and its decline with antihypertensive therapy. Clin Res 34(No 2) Abstract

Koike H, Sada T, Miyamoto M, Oizumi K, Sugiyama M, Inagami T (1984) Atrial natriuretic factor selectively increases renal blood flow in conscious spontaneously hypertensive rats. Eur J Pharmacol 104:391–392

Kojima I, Kokuma K, Rasmussen H (1985) Characteristics of angiotensin II, K^+- and ACTH-induced calcium influx in adrenal glomerulosa cells. J Biol Chem 260:9171–9176

Kondo K, Kida O, Kangawa K, Matsuo H, Tanaka K (1985) Enhanced natriuretic and hypotensive responsiveness to alpha-human atrial natriuretic polypeptide (alpha-hANP) in SHR. Clin Exp Hypertens A7:1097–1107

Kondo Y, Imai M, Kangawa K, Matsuo H (1986) Lack of direct action of α-human atrial natriuretic polypeptide on the in vitro perfused segments of Henle's loop isolated from rabbit kidney. Pflügers Arch 406:273–278

Koseki C, Hayashi Y, Ohnuma N, Imai M (1986a) Difference in molecular size of receptors for alpha-rat atrial natriuretic polypeptide among the kidney, aorta, and adrenal gland as identified by direct UV-photoaffinity labeling. Biochem Biophys Res Commun 136:200–207

Koseki C, Hayashi Y, Torikai S, Furuya M, Ohnuma N, Imai M (1986b) Localization of binding sites for α-rat atrial natriuretic polypeptide in rat kidney. Am J Physiol 250:F210–F216

Koseki C, Kanai CY, Hayashi Y, Ohnuma N, Imai M (1986c) Intrarenal localization of receptors for α-rat atrial natriuretic polypeptide: an autoradiographic study with ^{125}I-labeled ligand injected in vivo into the rat aorta. Jpn J Pharmacol 42:27–33

Kouz S, Bourassa MG, David PR, Dyrda I, Bonan R, Gutkowska J, Genest J, Cantin M (1986a) Higher IR-ANF plasma levels in patients with idiopathic cardiomyopathies. Abstract, American Heart Association 59th scientific session, Dallas, November 1986

Kouz S, Bourassa MG, Laurier J, Gutkowska J, Genest J, David PR, Dyrda I (1986b) Plasma concentration of immunoreactive atrial natriuretic factor (IR-ANF) in patients with coronary artery disease. J Am Coll Cardiol 7:210A

Koyama S, Nishida Y, Hosomi H, Abe Y (1986) Participation of baroreceptor reflexes in blood pressure and sympathetic nerve responses to a synthetic human atrial natriuretic peptide in anesthetized dogs. Eur J Pharmacol 172:43–48

Kuchel O, Cuche JL, Buu NT, Guthrie GP Jr, Unger T, Nowaczynski W, Boucher R, Genest J (1977) Catecholamine excretion in "idiopathic" edema: Decreased dopamine excretion, a pathogenic factor? J Clin Endocrinol Metab 44:639–646

Kuchel O, Garcia R, Debinski W, Racz K, Buu NT, Cantin M, Genest J (1986) The blood pressure decrease-induced sympathetic discharge following atrial natriuretic factor administration may offset its natriuretic action. Proc Soc Exp Biol Med 182:570–575

Kuchel O, Debinski W, Racz K, Buu NT, de Léan A, Garcia R, Cantin M, Genest J (1987a) Atrial natriuretic factor is an inhibitory modulator of peripheral sympathoadrenomedullary activity. In: Brenner BM, Laragh JH (eds) Biologically Active Atrial Peptides, American Society of Hypertension Symposium Series, vol 1, Raven Press, NY, pp 318–321

Kuchel O, Debinski W, Racz K, Buu NT, Garcia R, Cusson JR, Larochelle P, Cantin M, Genest J (1987b) An emerging relationship between peripheral sympathetic nervous activity and atrial natriuretic factor. Life Sci 40:1545–1551

Kuchel O, Gutkowska J, Buu NT, Cantin M, Genest J (1987c) Responses of plasma immunoreactive atrial natriuretic factor, aldosterone and urinary dopamine to salt-loading in a patient with severe idiopathic edema. Horm Metab Res 19:45–46

Kuchel O, Racz K, Debinski W, Gutkowska J, Buu NT, Cantin M, Genest J (1987d) Catecholamines and atrial natriuretic factor in Dahl and spontaneously hypertensive rats. Can J Physiol Pharmacol 65:1690–1696

Kudo T, Baird A (1984) Inhibition of aldosterone production in the adrenal glomerulosa by atrial natriuretic factor. Nature 312:756–757

Kuno T, Andresen JW, Kamisaki Y, Waldman SA, Chang LY, Saheki S, Leitman DC, Nakane M, Murad F (1986) Co-purification of an atrial natriuretic factor receptor and particulate guanylate cyclase from rat lung. J Biol Chem 261:5817–5823

Kuribayashi T, Nakazato M, Tanaka M, Nagamine M, Kurihara T, Kangawa K, Matsuo H (1985) Renal effects of human alpha-atrial natriuretic polypeptide. N Engl J Med 312:1456–1457

Kurtz A, della Bruna R, Pfeilschifter J, Taugner R, Bauer C (1986) Atrial natriuretic peptide inhibits renin release from juxtaglomerular cells by a cGMP-mediated process. Proc Natl Acad Sci 83:4769–4773

Kyncl J, Bush EN, Buckner SA (1986) Atriopeptin III (ANF) interaction with alpha receptor subtypes and their pre vs. post-synaptic functions. J Cardiovasc Pharmacol 8:1312

Lachance D, Garcia R, Gutkowska J, Cantin M, Thibault G (1986) Mechanisms of release of atrial natriuretic factor. I. Effect of several agonists and steroids on its release by atrial minces. Biochem Biophys Res Commun 135:1090–1098

Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger TH (1985) Atrial natriuretic factor – a circulating hormone stimulated by volume loading. Nature 314:264–266

Lappe RW, Smits JF, Todt JA, Debets JJ, Wendt RL (1985) Failure of atriopeptin II to cause arterial vasodilation in the conscious rat. Circ Res 56:606–612

Larochelle P, Beroniade V, Gutkowska J, Cusson J, Lécrivain A, du Souich P, Cantin M, Genest J (1987a) Influence of hemodialysis on the plasma levels of the atrial natriuretic factor in chronic renal failure. Clin Invest Med 10:350–354

Larochelle P, Cusson JR, Gutkowska J, Genest J, Cantin M (1987b) Mesure du facteur natriurétique de l'oreillette chez des patients avec hypertension essentielle et rénovasculaire. Arch Mal Coeur 80:976–981

Larochelle P, Cusson JR, Gutkowska J, Schiffrin EL, Hamet P, Kuchel O, Genest J, Cantin M (1987c) Plasma atrial natriuretic factor concentrations in essential and renovascular hypertension. Br Med J 294:1249–1252

Larochelle P, Cusson JR, Hamet P, du Souich P, Gutkowska J, Schiffrin EL, Genest J, Cantin M (1987d) Pharmacodynamic effects of bolus administration of atrial natriuretic factor in normal volunteers. In: Brenner BM, Laragh JH (eds) Biologically Active Atrial Peptides, American Society of Hypertension Symposium Series, vol 1, Raven Press, NY, pp 451–454

Larose P, Meloche S, du Souich P, de Léan A, Ong H (1985) Radioimmunoassay of atrial natriuretic factor: human plasma levels. Biochem Biophys Res Commun 130:553–558

Lattion AL, Michel JB, Arnauld E, Corvol P, Soubrier F (1986) Myocardial recruitment during ANF mRNA increase with volume overload in the rat. Am J Physiol 25:890–896

Lazure C, Seidah NG, Pelaprat D, Chrétien M (1983) Proteases and post-translational processing of prohormones: a review. Can J Biochem Cell Biol 61:501–515

Lazure C, Seidah NG, Chrétien M, Thibault G, Garcia R, Cantin M, Genest J (1984) Atrial pronatriodilatin: a precursor for natriuretic factor and cardiodilatin amino acid sequence evidence. FEBS Lett 172:80–86

Ledsome JR, Wilson N (1984) The relationship between plasma vasopressin concentration and urinary excretion during left atrial distension in anesthetized dogs. Pflügers Arch 400:381–387

Ledsome JR, Wilson N, Courneya CA, Rankin AJ (1985) Release of atrial peptide by atrial distension. Can J Physiol Pharmacol 63:739–742

Ledsome JR, Wilson N, Rankin AJ, Courneya CA (1986) Time course of release of atrial natriuretic peptide in the anesthetized dog. Can J Physiol Pharmacol 64:1017–1022

Leitman DC, Murad F (1986) Comparison of binding and cyclic GMP accumulation by atrial natriuretic peptides in endothelial cells. Biochim Biophys Acta 885:74–79

Leitman DC, Andresen JW, Kuno T, Kamisaki Y, Chang JK, Murad F (1986) Identification of multiple binding sites for atrial natriuretic factor by affinity cross-linking in cultured endothelial cells. J Biol Chem 261:11650–11655

Liard JF, Petters G (1973) Role of the retention of water and sodium in two types of experimental renovascular hypertension in the rat. Pflügers Arch 344:93–108

Linden RJ (1979) Atrial reflexes and renal function. Am J Cardiol 44:879–883

London GM, Safar ME, Safar AL, Surion AC (1985) Blood pressure in the "low pressure system" and cardiac performance in essential hypertension. J Hypertens 3:337–342

Luetscher J, Beckerhoff R, Dowdy AJ, Wilkinson R (1972) Incomplete suppression of aldosterone secretion and plasma concentration in hypertensive patients on high sodium intake. In: Genest J, Koiw E (eds) Hypertension '72. Springer, Berlin Heidelberg New York, pp 286–292

Luft FC, Lang RE, Aronoff GR, Ruskoaho H, Toth M, Ganten D, Sterzel RB, Unger T (1986a) Atriopeptin III kinetics and pharmacodynamics in normal and anephric rats. J Pharmacol Exp Ther 236:416–418

Luft FC, Sterzel RB, Lang RE, Trabold EM, Veelken R, Ruskoaho H, Gao Y, Ganten D, Unger T (1986b) Atrial natriuretic factor determinations and chronic sodium homeostasis. Kidney Int 29:1004–1010

Lynch DR, Braas KM, Snyder SH (1986) Atrial natriuretic factor receptors in rat kidney, adrenal gland and brain: autoradiographic localization and fluid balance dependent changes. Proc Natl Acad Sci USA 83:3357–3361

Maack T, Kleinert HD (1986) Renal and cardiovascular effect of atrial natriuretic factor. Biochem Pharmacol 35:2057–2064

Maack T, Marion DN, Camargo MJ, Kleinert HD, Laragh JH, Vaughan ED Jr, Atlas SA (1984) Effects of auriculin (atrial natriuretic factor) on blood pressure, renal function, and the renin-aldosterone system in dogs. Am J Med 77:1069–1075

MacGregor GA, Sagnella ND, Markandu ND, Shore AC (1986) Raised plasma levels of atrial natriuretic peptide in untreated essential hypertension. J Cardiovasc Pharmacol 8:1301

Maki M, Takayanagi R, Misono KS, Pandey KN, Tibbetts C, Inagami T (1984) Structure of rat atrial natriuretic factor precursor deduced from cDNA sequence. Nature 309:722–724

Manning PT, Schwartz D, Katsube NC, Holmberg SW, Needleman P (1985) Vasopressin-stimulated release of atriopeptin: endocrine antagonists in fluid homeostasis. Science 229:395–397

Mantyh CR, Brecha NC, Kruger L, Mantyh PW (1985) Autoradiographic localization of atrial natriuretic factor binding sites in the periphery and central nervous system of the rat and guinea pig. Neuroscience 11:189 (Abstract)

Mantyh CR, Kruger L, Brecha NC, Mantyh PW (1986) Localization of specific binding sites for atrial natriuretic factor in peripheral tissues of the guinea pig, rat, and human. Hypertension 8:712–721

Marie JP, Guillemot H, Hatt PY (1976) Le degré de granulation des cardiocytes auriculaires. Etude planimétrique au cours de différents apports d'eau et de sodium chez le rat. Pathol Biol (Paris) 24:549–554

Marin-Grez M, Briggs JP, Schubert G, Schnermann J (1985) Dopamine receptor antagonists inhibit the natriuretic response to atrial natriuretic factor (ANF). Life Sci 36:2171–2176

Marin-Grez M, Fleming JT, Steinhausen M (1986) Atrial natriuretic peptide causes preglomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. Nature 324:473–476

Marsh EA, Seymour AA, Haley AB, Whinnery MA, Napier MA, Nutt RF, Blaine EH (1985) Renal and blood pressure responses to synthetic atrial natriuretic factor in spontaneously hypertensive rats. Hypertension 7:386–391

Marumo F, Sakamoto H, Ando K, Ishigami T, Kawakami M (1986) A highly sensitive radioimmunoassay of atrial natriuretic peptide (ANP) in human plasma and urine. Biochem Biophys Res Commun 137:231–236

Masters SB, Martin MW, Harden TK, Brown JH (1985) Pertussis toxin does not inhibit muscarinic-receptor-mediated phosphoinositide hydrolysis or calcium mobilization. Biochem J 227:933–937

Matsuo H, Kangawa K, Miyata A (1985) Atrial natriuretic polypeptides (ANP): molecular forms and distribution in mammalian tissues and plasma. In: Deber CM, Hruby VJ, Kopple KD (eds) Peptides: structure and function. Pierce Chemical Company, Rockford, Illinois, pp 925–932

Matsuoka H, Ishii M, Sugimoto T, Hirata Y, Sugimoto T, Kangawa K, Matsuo H (1985) Inhibition of aldosterone production by alpha-human atrial natriuretic polypeptide is associated with an increase in cGMP production. Biochem Biophys Res Commun 127:1052–1056

McKenzie JC, Tanaka I, Misono KS, Inagami T (1985) Immunocytochemical localization of atrial natriuretic factor in the kidney, adrenal medulla, pituitary, and atrium of rat. J Histochem Cytochem 33:828–832

Meisheri KD, Taylor CJ, Saneii H (1986) Synthetic atrial peptide inhibits intracellular calcium release in smooth muscle. Am J Physiol 250:C171–C174

Meloche S, Ong H, Cantin M, de Léan A (1986) Molecular characterization of the solubilized atrial natriuretic factor receptor from bovine adrenal zona glomerulosa. Mol Pharmacol 30:537–543

Mendez RE, Dunn BR, Troy JL, Brenner BM (1986) Modulation of the natriuretic response to atrial natriuretic peptide by alterations in peritubular Starling forces in the rat. Circ Res 59:605–611

Metzler CH, Lee M-E, Thrasher TN, Ramsay DJ (1986) Increased right or left atrial pressure stimulates release of atrial natriuretic peptides in conscious dogs. Endocrinology 119:2396–2398

Meyer-Lehnert H, Tsai P, Schrier RW (1986) Atrial natriuretic factor (ANF) inhibits arginine vasopressin (AVP)-stimulated Ca^{++} fluxes and cell contraction in vascular smooth muscle cells (VSMC). In: Natriuretic Hormones in Hypertension, Satellite Symposium, 11th Scientific meeting of the International Society of Hypertension, Heidelberg. Abstract

Michener ML, Gierse JK, Seatharom R, Fok KF, Olins PO, Mai MS, Needleman P (1986) Proteolytic conversion of atriopeptin prohormone. Mol Pharmacol 30:552–557

Misono KS, Fukumi H, Grammer RT, Inagami T (1984a) Rat atrial natriuretic factor: complete amino acid sequence and disulfide linkage essential for biological activity. Biochem Biophys Res Commun 119:524–529

Misono KS, Grammer RT, Fukumi H, Inagami T (1984b) Rat atrial natriuretic factor: isolation, structure and biological activities of four major peptides. Biochem Biophys Res Commun 123:444–451

Misono KS, Grammer RT, Rigby JW, Inagami T (1985) Photoaffinity labeling of atrial natriuretic factor receptor in bovine and rat adrenal cortical membranes. Biochem Biophys Res Commun 130:994–1001

Miyata A, Kangawa K, Toshimori T, Hatoh T, Matsuo H (1985) Molecular forms of atrial natriuretic polypeptides in mammalian tissues and plasma. Biochem Biophys Res Commun 129:248–255

Mogannam JD, Chang D, Chang JK, Tang J, Xei CW (1986) Synthesis and bioassay of a more potent atriopeptin III analog. Abstracts of the First World Congress on Biologically Active Atrial Peptides, American Society of Hypertension, New York, May 31–June 1, p 108A

Moore HP, Gumbiner B, Kelly RB (1983) Chloroquine diverts ACTH from a regulated to a constitutive secretory pathway in AtT 20 cells. Nature 302:434–436

Morgan DA, Peuler JD, Koepke JP, DiBona GF, Mark AL (1986) The renal sympathetic nerves attenuate the natriuretic effects of atrial peptides. Clin Res 34:937A

Morii N, Nakao K, Sugawara A, Sakamoto M, Suda M, Shimokura M, Kiso Y, Kihara M, Yamori Y, Imura H (1985) Occurrence of atrial natriuretic polypeptide in brain. Biochem Biophys Res Commun 127:413–419

Morii N, Nakao K, Kihara M, Sugawara A, Sakamoto M, Yamori Y, Imura H (1986) Decreased content in left atrium and increased plasma concentration of atrial natriuretic polypeptide in spontaneously hypertensive rats (SHR) and SHR stroke-prone. Biochem Biophys Res Commun 135:74–81

Murphy KMM, McLaughlin LL, Michener ML, Needleman P (1985) Autoradiographic localization of atriopeptin III receptors in rat kidney. Eur J Pharmacol 111:291–292

Murthy KK, Thibault G, Garcia R, Gutkowska J, Genest J, Cantin M (1986a) Degradation of atrial natriuretic factor in the rat. Biochem J 240:461–469

Murthy KK, Thibault G, Schiffrin EL, Garcia R, Chartier L, Gutkowska J, Genest J, Cantin M (1986b) Disappearance of atrial natriuretic factor from circulation in the rat. Peptides 7:241–246

Nakamura M, Inagami T (1986) Atrial natriuretic factor inhibits norepinephrine release evoked by sympathetic nerve stimulation in isolated perfused rat mesenteric arteries. Eur J Pharmacol 123:459–461

Nakao K, Sugawara A, Morii N, Sakamoto M, Suda M, Soneda J, Ban T, Kihara M, Yamori Y, Shimakura M, Kiso Y, Imura H (1984) Radioimmunoassay for alpha-human and rat atrial natriuretic polypeptide. Biochem Biophys Res Commun 124:815–821

Nakao K, Sugawara A, Moni N, Sakamoto M, Yamada T, Itoh H, Shono S, Nishemura K, Bau T, Kikara M, Yamori Y, Shikomura M, Kiso Y, Kangawa K, Matsuo H, Imura H (1985) Pharmacokinetic study on alpha-human atrial natriuretic polypeptide in man. In: Kiso Y (ed) Peptide chemistry. Proceedings of the 23rd Symposium on Peptide Chemistry. Protein Research Foundation, Osaka, Japan, pp 23–26

Nakaoka H, Imataka K, Amano M, Fujii J, Ishibashi M, Yamaji T (1985) Plasma levels of atrial natriuretic factor in patients with congestive heart failure. N Engl J Med 313:892–893

Nakayama K, Ohkubo H, Hirose T, Inayama S, Nakanishi S (1984) mRNA sequence for human cardiodilatin-atrial natriuretic factor precursor and regulation of precursor mRNA in rat atria. Nature 310:699–701

Napier MA, Dewey RS, Albers-Schonberg G, Bennet GD, Rodkey JA, Marsh EA, Whinnerey M, Seymour AA, Blaine EH (1984a) Isolation and sequence determination of peptide components of atrial natriuretic factor. Biochem Biophys Res Commun 120:981–988

Napier MA, Vandlen RL, Albers-Schonberg G, Nutt RF, Brady S, Lyle T, Winquist R, Faison EP, Heinel LA, Blaine EH (1984b) Specific membrane receptors for atrial natriuretic factor in renal and vascular tissues. Proc Natl Acad Sci USA 81:5946–5950

Napier MA, Arcuri KE, Vandlen RL (1986a) Binding and internalization of atrial natriuretic factor by high-affinity receptors in A10 smooth muscle cells. Arch Biochem Biophys 248:516–522

Napier MA, Lipari MT, Vandlen RL (1986b) Tissue and species distribution of receptor subtypes for atrial natriuretic factor. In: Peeters H (ed) Protides of the Biological Fluids. Pergamon, Oxford, vol 34, pp 217–220

Naruse N, Naruse K, Obana K, Kurimoto F, Sakurai H, Honda T, Higashida T, Demura H, Inagami T, Shizume K (1986) Immunoreactive alpha-human atrial natriuretic polypeptide in human plasma. Peptides 7:141–145

Naruse M, Obana K, Naruse K, Yamaguchi H, Demura H, Inagami T, Shizume K (1987) Atrial natriuretic polypeptide inhibits cortisol secretion as well as aldosterone secretion in vitro from human adrenal tissue. J Clin Endocrinol Metab 64:10–16

Natsume T, Kardon MB, Trippodo NC, Januszewicz A, Pegram BL, Frohlich ED (1986) Atriopeptin III does not alter cardiac performance in rats. J Hypertens 4:477–480

Needleman P, Adams SP, Cole BR, Currie MG, Geller DM, Michener ML, Saper CB, Schwartz D, Standaert DG (1985) Atriopeptins as cardiac hormones. Hypertension 7:469–482

Nemeh MN, Gilmore JP (1983) Natriuretic activity of human and monkey atria. Circ Res 53:420–423

Nemer M, Chamberland M, Sirois D, Argentin S, Drouin J, Dixon RAF, Zivin RA, Condra JH (1984) Gene structure of human cardiac hormone precursor, pronatriodilatin. Nature 312:654–656

Nemer M, Antakly T, Sarrieau A, Lavigne JP, Drouin J (1986a) High atrial content of glucocorticoid receptor and glucocorticoid induction of ANF mRNA. J Steroid Biochem 25:1065

Nemer M, Lavigne JP, Drouin J, Thibault G, Gannon M, Antakly T (1986b) Expression of atrial natriuretic factor gene in heart ventricular tissues. Peptides 7:1147–1152

Nemer M, Sirois D, Drouin J (1986c) XhoI polymorphism in the pronatriodilatin gene. Nucleic Acids Res 14:8696

Nemer M, Sirois D, Drouin J (1986d) TaqI polymorphism at the 3′ end of the pronatriodilatin. Nucleic Acids Res 14:8697

Nemer M, Argentin S, Lavigne JP, Chamberland M, Drouin J (1987) Glucocorticoid regulation of pronatriodilatin gene expession. J Cell Biochem 11A:121

Netchitailo P, Feuilloley M, Pelletier G, Cantin M, Leboulanger F, Andersen A, Vaudry H (1986) Localization of atrial natriuretic factor (ANF)-immunoreactive material in the hypothalamo-pituitary complex of the frog. Neurosci Lett 72:141–146

Nicklas JM, DiCarlo LA, Koller PT, Morady F, Diltz EA, Shenker Y, Grekin RJ (1986) Plasma levels of immunoreactive atrial natriuretic factor increase during supraventricular tachycardia. Am Heart J 112:923–928

Nishiuchi T, Saito H, Yanasaki Y, Saito S (1986) Radioimmunoassay for atrial natriuretic peptide: Method and results in normal subjects and patients with various diseases. Clin Chim Acta 159:45–47

Noresson E, Ricksten SE, Thorn P (1979) Left atrial pressure in normotensive and spontaneously hypertensive rats. Acta Physiol Scand 107:9

Nozuki M, Mouri T, Itoi K, Takahashi K, Totsune K, Saito T, Yoshinaga K (1986) Plasma concentrations of atrial natriuretic peptide in various diseases. Tohoku J Exp Med 148:439–447

Nutt RF, Brady JF, Lyle TA, Ciccarone TM, Paleveda WJ, Cotton GD, Veber DF, Winquist RJ (1986) Synthesis of atrial natriuretic factor and highly active analogs. In: Peeters H (ed) Protides of the biological fluids, vol 34. Pergamon, Oxford, pp 55–58

Obana K, Naruse M, Inagami T, Brown AB, Naruse K, Kurimoto F, Sakurai H, Demura H, Shizume K (1985a) Atrial natriuretic factor inhibits vasopressin secretion from rat posterior pituitary. Biochem Biophys Res Commun 132:1088–1094

Obana K, Naruse M, Naruse K, Sakurai A, Demura H, Inagami T, Shizume K (1985b) Synthetic rat atrial natriuretic factor inhibits in vitro and in vivo renin secretion in rats. Endocrinology 117:1282–1284

Ogawa K, Matsuno T, Tsuchiya S, Ban M, Minaguchi K (1981) Plasma levels of norepinephrine, cyclic AMP and cyclic GMP in essential hypertension. Jpn Circ J 45:654–660

Ogawa K, Ito T, Hashimoto H, Ito Y, Ohno O, Tsuboi H, Takasu N, Tanahashi T, Satake T (1986) Plasma atrial natriuretic factor in congestive heart failure. Lancet i:106

Ogihara T, Shima J, Hara H, Kumahara Y, Kangawa K, Matsuo H (1986a) Changes in human plasma atrial natriuretic polypeptide concentration in normal subjects during passive leg raising and whole-body tilting. Clin Sci 71:147–150

Ogihara T, Shima J, Hara H, Tabuchi Y, Hashizume K, Nagano M, Katahira K, Kangawa K, Matsuo H, Kumahara Y (1986b) Significant increase in plasma immunoreactive atrial natriuretic polypeptide concentration during head-out water immersion. Life Sci 38:2413–2418

O'Grady SM, Field M, Nash NT, Rao MC (1985) Atrial natriuretic factor inhibits Na-K-Cl cotransport in teleost intestine. Am J Physiol 249:C531–C534

Ohashi M, Fujio N, Kato K, Nawata H, Ibayashi H, Matsuo H (1986a) Effect of human-alpha-atrial natriuretic polypeptide on adrenocortical function in man. J Endocrinol 110:287–292

Ohashi M, Fujio N, Nawata H, Kato K-I, Ibayashi H (1986b) Alpha-human atrial natriuretic polypeptide-induced rise of plasma and urinary cyclic 3′5′-guanosine monophosphate concentration in human subjects. Clin Exp Hypertens A8:67–73

Ohashi M, Fujio N, Nawata H, Kato K-I, Ibayashi H, Kangawa K, Matsuo H (1987) High plasma concentrations of human atrial natriuretic polypeptide in aged men. J Clin Endocrinol Metab 64:81–85

Ohlstein EH, Berkowitz BA (1985) Cyclic guanosine monophosphate mediates vascular relaxation induced by atrial natriuretic factor. Hypertension 7:306–310

Oikawa S, Imai M, Veno A, Tanak S, Nogushi T, Nakazato H, Kangawa K, Fukuda A, Matsuo H (1984) Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide. Nature 309:724–726

Oikawa S, Imai M, Inuzuka C, Tawaragi Y, Nakazato H, Matsuo H (1985) Structure of dog and rabbit precursors of atrial natriuretic polypeptides deduced from nucleotide sequence of cloned cDNA. Biochem Biophys Res Commun 132:892–899

Olins GM, Palton DR, Tjoeng FS, Blehm DJ (1986) Specific receptors for atriopeptin III in rabbit lung. Biochem Biophys Res Commun 140:302–307

Olivari MT, Fiorentini C, Polese A, Guazzi MD (1978) Pulmonary hemodynamics and right ventricle formation in hypertension. Circulation 57:1185–1190

Ong H, McNicoll N, Lazure C, Seidah NG, Chrétien M, Cantin M, de Léan A (1986) Purification and sequence determination of bovine atrial natriuretic factor. Life Sci 38:1309–1315

Opgenorth TJ, Burnett JC Jr, Granger JP, Scriven TA (1986) Effects of atrial natriuretic peptide on renin secretion in nonfiltering kidney. Am J Physiol 250:F798–F801

Osborn MJ, Hammill SC, Burnett JC (1986) Enhanced levels of circulating atrial natriuretic peptide during ventricular pacing in humans. J Am Coll Cardiol 7:192A

Osol G, Halpern W, Tesfamariam B, Nakayama K, Weinberg D (1986) Synthetic atrial natriuretic factor does not dilate resistance-sized arteries. Hypertension 8:606–610

Pandey KN, Kovacs WJ, Inagami T (1985) The inhibition of progesterone secretion and the regulation of cyclic nucleotides by atrial natriuretic factor in gonadotropin responsive murine Leydig tumor cells. Biochem Biophys Res Commun 133:800–806

Pandey KN, Pavlou SN, Kovacs WJ, Inagami T (1986) Atrial natriuretic factor regulates steroidogenic responsiveness and cyclic nucleotide levels in mouse Leydig cells in vitro. Biochem Biophys Res Commun 138:399–404

Pang SC, Hoang MC, Tremblay J, Cantin M, Garcia R, Genest J, Hamet P (1985) Effect of natural and synthetic atrial natriuretic factor on arterial blood pressure, natriuresis and cyclic GMP excretion in spontaneously hypertensive rats. Clin Sci 69:721–726

Pasternac A, Kouz S, Gutkowska J, Petitclerc R, Vellas B, de Champlain J, Cantin M (1986) Abnormal plasma levels of atrial natriuretic factor (ANF) in patients with symptomatic mitral valve prolapse. J Am Coll Cardiol 7:169A

Pecker MS, Cody RJ, Laragh JH, Sala C, Atlas SA (1987) Atrial natriuretic factor (ANF) response to saline infusion in hypertensive and normotensive subjects. Kidney Int 31:282

Pegram BL, Trippodo NC, Natsume T, Kardon MB, Frohlich ED, Cole FE, MacPhee AA (1986) Hemodynamic effects of atrial natriuretic hormone. Fed Proc 45:2382–2386

Pettersson A, Ricksten SE, Towle AC, Hedner J, Hedner T (1985) Plasma atrial natriuretic peptide and haemodynamics in conscious normotensive and spontaneously hypertensive rats after acute blood volume expansion. J Hypertens 3(suppl 3):S-311–S-313

Quirion R, Dalpé M, de Léan A, Gutkowska J, Cantin M, Genest J (1984) Atrial natriuretic factor (ANF) binding sites in the brain and related structures. Peptides 5:1167–1172

Quirion R, Dalpé M, Dam TV (1986) Characterization and distribution of receptors for the atrial natriuretic peptides in mammalian brain. Proc Natl Acad Sci USA 83:174–178

Racz K, Kuchel O, Cantin M, de Léan A (1985) Atrial natriuretic factor inhibits the early pathway of steroid biosynthesis in bovine adrenal cortex. FEBS Lett 192:19–22

Racz K, Kuchel O, Buu NT, Gutkowska J, Debinski W, Cantin M, Genest J (1986a) Zona glomerulosa cell responses to atrial natriuretic factor in genetically hypertensive rats. Am J Physiol 251:H689–H692

Racz K, Kuchel O, Gutkowska J, Buu NT, Cantin M, Genest J (1986b) Dopaminergic control of the atrial natriuretic factor release? Hypertension 8:825 (abstract)

Raine AE, Erne P, Bürgisser E, Müller FB, Bolli P, Burkart F, Bühler FR (1986) Atrial natriuretic peptide and atrial pressure in patients with congestive heart failure. N Engl J Med 315:533–537

Rankin AJ, Courneya CA, Wilson N, Ledsome JR (1986) Tachycardia releases atrial natriuretic peptide in the anesthetized rabbit. Life Sci 38:1951–1957

Rapoport RM, Draznin MB, Murad F (1983) Endothelium-dependent vasodilator- and nitrovasodilator-induced relaxation may be mediated through cyclic GMP formation and cyclic GMP-dependent protein phosphorylation. Trans Ass Am Physicians 96:19–30

Rapoport RM, Waldman SA, Schwartz K, Winquist RJ, Murad F (1985) Effects of atrial natriuretic factor, sodium nitroprusside, and acetylcholine on cGMP levels and relaxation in rat aorta. Eur J Pharmacol 115:219–229

Rascher W, Tulassay T, Lang RE (1985) Atrial natriuretic peptide in plasma of volume-overloaded children with chronic renal failure. Lancet ii:303–305

Reichlin M, Schnure JJ, Vance VK (1968) Induction of antibodies to porcine ACTH in rabbits with nonsteroidogenic polymers of BSA and ACTH. Proc Soc Exp Biol Med 128:84–85

Richards AM, Ikram H, Yandle TG, Nicholls MG, Webster MWI, Espiner EA (1985a) Renal, haemodynamic, and hormonal effects of human alpha-atrial natriuretic peptide in healthy volunteers. Lancet i:545–549

Richards AM, Nicholls MG, Espiner EA, Ikram H, Yandle TG, Joyce SL, Cullens MM (1985b) Effects of alpha-human atrial natriuretic peptide in essential hypertension. Hypertension 7:812–817

Richards AM, Nicholls MG, Ikram H, Webster MW, Yandle TG, Espiner EA (1985c) Renal, hemodynamic, and hormonal effects of human alpha atrial natriuretic peptide in healthy volunteers. Lancet i:545–549

Richards AM, Tonolo G, Davidson G (1986a) Plasma atrial natriuretic peptide in essential hypertension. Proceedings, Council for High Blood Pressure Research, American Heart Association. Abstract no 46

Richards AM, Tonolo G, Tillman D, Connell JM, Hepburn D, Robertson JIS (1986b) Plasma atrial natriuretic peptide in stable and accelerated essential hypertension. J Hypertens 4:790–791

Ricksten SE, Yao T, Ljunez B, Thoren P (1980) Distensibility of left atrium in normotensive and spontaneously hypertensive rats. Acta Physiol Scand 110:413–418

Riegger GAJ, Kromer EP, Kochsiek K (1986) Human atrial natriuretic peptide: Plasma levels, hemodynamic, hormonal and renal effects in patients with severe congestive heart failure. J Cardiovasc Pharmacol 8:1107–1112

Rodeheffer RJ, Tanaka I, Imada T, Hollister AS, Robertson D, Inagami T (1986) Atrial pressure and secretion of atrial natriuretic factor into the human central circulation. J Am Coll Cardiol 8:18–26

Ruskoaho H, Toth M, Lang RE (1985) Atrial natriuretic peptide secretion: synergistic effect of phorbol ester and A23187. Biochem Biophys Res Commun 133:581–588

Ruskoaho H, Toth M, Ganten D, Unger Th, Lang RE (1986) The phorbol ester induced atrial natriuretic peptide secretion is stimulated by forskolin and bay K8644 and inhibited by 8-bromo-cyclic GMP. Biochem Biophys Res Commun 139:266–274

Saavedra JM, Correa FM, Plunkett LM, Israel A, Kurihara M, Shigematsu K (1986a) Binding of angiotensin and atrial natriuretic peptide in brain of hypertensive rats. Nature 320:758–760

Saavedra JM, Israel A, Kurihara M, Fuchs E (1986b) Decreased number and affinity of rat atrial natriuretic peptide (6–33) binding sites in the subfornical organ of spontaneously hypertensive rats. Circ Res 58:389–392

Safar ME, Weiss YA, London GM, Frackowiak RF, Milliez PL (1974) Cardiopulmonary blood volume in borderline hypertension. Clin Sci Mol Med 47:153–164

Sagnella GA, Markandu ND, Shore AC, MacGregor GA (1985) Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. Lancet ii:1208–1211

Sagnella GA, Markandu ND, Shore AC, MacGregor GA (1986) Raised circulating levels of atrial natriuretic peptides in essential hypertension. Lancet i:179–180

Saito Y, Nakao K, Morii N, Sugawara A, Shiono S, Yamada T, Itoh H, Sakamoto M, Kurahashi K, Fujiwara M, Imura H (1986) Bay K8644, a voltage-sensitive calcium channel agonist, facilitates secretion of atrial natriuretic polypeptide from isolated perfused rat hearts. Biochem Biophys Res Commun 138:1170–1176

Sakamoto H, Inoue K, Marumo F (1986) High plasma atrial natriuretic peptide independent of sodium balance in SIADH. JAMA 256:1293–1294

Sakamoto M, Nakao K, Kihara M, Morii N, Sugawara A, Suda M, Shimokura M, Kiso Y, Yamori Y, Imura H (1985) Existence of atrial natriuretic polypeptide in kidney. Biochem Biophys Res Commun 128:1281–1287

Sakamoto M, Nakao K, Morii N, Sugawara A, Yamada T, Itoh H, Shiono S, Saito Y, Imura H (1986) The lung as a possible target organ for atrial natriuretic polypeptide secreted from the heart. Biochem Biophys Res Commun 135:515–520

Salazar FJ, Fiksen-Olsen MJ, Opgenorth TJ, Granger JP, Burnett JC Jr, Romero JC (1986a) Renal effects of ANP without changes in glomerular filtration rate and blood pressure. Am J Physiol 251:F532–F536

Salazar FJ, Romero JC, Burnett JC Jr, Schryver S, Granger JP (1986b) Atrial natriuretic peptide levels during acute and chronic saline loading in conscious dogs. Am J Physiol 251:R499–R503

Samson WK (1985a) Dehydration-induced alterations in rat brain vasopressin and atrial natriuretic factor immunoreactivity. Endocrinology 117:1279–1281

Samson WK (1985b) Atrial natriuretic factor inhibits dehydration and hemorrhage-induced vasopressin release. Neuroendocrinology 40:277–279

Saper CB, Standaert DG, Currie MG, Schwartz D, Geller DM, Needleman P (1985) Atriopeptin-immunoreactive neurons in the brain: Presence in cardiovascular regulatory areas. Science 227:1047–1049

Sasaki A, Kida O, Kangawa K, Matsuo H, Tanaka K (1985) Cardiosuppressive effect of alpha-human atrial natriuretic polypeptide (alpha-hANP) in spontaneously hypertensive rats. Eur J Pharmacol 115:321–324

Sasaki A, Kida O, Kangawa K, Matsuo H, Tanaka K (1986) Involvement of sympathetic nerves in cardiosuppressive effects of α-human atrial natriuretic polypeptide (α-hANP) in anesthetized rats. Eur J Pharmacol 120:345–349

Sato M, Abe K, Takeuchi K, Yasujima M, Omata K, Hiwatari M, Kasai Y, Tanno M, Kohzuki M, Kudo K, Yoshinaga K, Inagami T (1986) Atrial natriuretic factor and cyclic guanosine-3′,5′-monophosphate in vascular smooth muscle. Hypertension 8:762–771

Scarborough RM, Schenk DB, McEnroe GA, Arfsten A, Kang LL, Schwartz K, Lewicki JA (1986) Truncated atrial natriuretic peptide analogues. Comparison between receptor binding and stimulation of cyclic GMP accumulation in cultured vascular smooth muscle cells. J Biol Chem 261:12960–12964

Schenk DB, Johnson LK, Schwartz K, Sista H, Scarborough RM, Lewicki JA (1985a) Distinct atrial natriuretic factor receptor sites on cultured bovine aortic smooth muscle and endothelial cells. Biochem Biophys Res Commun 127:433–442

Schenk DB, Phelps MN, Porter JG, Scarborough RM, McEnroe GA, Lewicki JA (1985b) Identification of the receptor for atrial natriuretic factor on cultured vascular cells. J Biol Chem 260:14887–14890

Schiebinger RJ, Linden J (1986a) Alpha- and beta-adrenergic agonists directly and distinctively influence atrial natriuretic peptide release. Hypertension 8:825 (abstract)

Schiebinger RJ, Linden J (1986b) Effect of atrial contraction frequency on atrial natriuretic peptide secretion. Am J Physiol 251:H1095–H1099

Schiffrin EL (1986) Down-regulation of binding sites for atrial natriuretic peptide in platelets of patients with congestive heart failure. Circulation 74(suppl II):II-463

Schiffrin EL, St-Louis J (1985) Vascular and adrenal binding sites for atrial natriuretic factor in rats: effects of sodium, mineralocorticoids and hypertension. Clin Invest Med 8:A-127

Schiffrin EL, St-Louis J (1987) Decreased density of vascular receptors for atrial natriuretic peptide in DOCA-salt hypertensive rats. Hypertension 9:504–512

Schiffrin EL, Taillefer R (1986) Correlation of left ventricular ejection fraction and plasma atrial natriuretic peptide in congestive heart failure. N Engl J Med 315:765–766

Schiffrin EL, Lis M, Gutkowska J, Genest J (1981) Role of Ca^{2+} in the response of adrenal glomerulosa cells to angiotensin II, ACTH, K^+ and ouabain. Am J Physiol 241:E42–E46

Schiffrin EL, Chartier L, Thibault G, St-Louis J, Cantin M, Genest J (1985a) Vascular and adrenal receptors for atrial natriuretic factor in the rat. Circ Res 56:801–807

Schiffrin EL, Gutkowska J, Kuchel O, Cantin M, Genest J (1985b) Plasma concentration of atrial natriuretic factor in a patient with paroxysmal atrial tachycardia. N Engl J Med 312:1196–1197

Schiffrin EL, Deslongchamps M, Thibault G (1986a) Platelet binding sites for atrial natriuretic factor in humans. Characterization and effects of sodium intake. Hypertension 8 (suppl II):II-6–II-10

Schiffrin EL, Poissant L, Cantin M, Thibault G (1986b) Receptors for atrial natriuretic factor in cultured vascular smooth muscle cells. Life Sci 38:817–826

Schiffrin EL, St-Louis J, Garcia R, Thibault G, Cantin M, Genest J (1986c) Vascular and adrenal binding sites for atrial natriuretic factor. Effects of sodium and hypertension. Hypertension 8 (suppl I):I-141–I-145

Schiffrin EL, Tremblay J, Hamet P, St-Louis J (1986d) Vascular binding sites for atrial natriuretic peptide in rats: down-regulation in volume expanded states is mediated by ANP. Clin Res 32:486A

Schiller PW, Maziak L, Nguyen TMD, Godin J, Garcia R, de Léan A, Cantin M (1985) Synthesis and biological activity of a linear fragment of the atrial natriuretic factor (ANF). Biochem Biophys Res Commun 131:1056–1062

Schiller PW, Bellini F, Dionne G, Maziak LA, Garcia R, de Léan A, Cantin M (1986) Synthesis and activity profiles of atrial natriuretic peptide (ANP) analogs with reduced ring size. Biochem Biophys Res Commun 138:880–886

Schiller PW, Maziak LA, Nguyen TM-D, Godin J, Garcia R, de Léan A, Cantin M (1987) Superactive analogs of the atrial natriuretic peptide (ANP). Biochem Biophys Res Commun 143:499–505

Schultz KD, Schultz K, Schultz G (1977) Sodium nitroprusside and other smooth muscle-relaxants increase cyclic GMP levels in rat ductus deferens. Nature 265:750–751

Schwartz D, Geller DM, Manning PT, Siegel NR, Fok KF, Smith CF, Needleman P (1985) Ser-Leu-Arg-Arg-atriopeptin III: the major circulating form of atrial peptide. Science 229:397–400

Schwartz D, Katsube NC, Needleman P (1986) Atriopeptin release in conditions of altered salt and water balance in the rat. Biochem Biophys Res Commun 137:922–928

Scivoletto R, Carvalho MH (1984) Cardionatrin causes vasodilation in vitro which is not dependent on the presence of endothelial cells. Eur J Pharmacol 101:143–145

Scriven TA, Burnet JC Jr (1985) Effects of synthetic atrial natriuretic peptide on renal function and renin release in acute experimental heart failure. Circulation 72:892–897

Seidah NG, Lazure C, Chrétien M, Thibault G, Garcia R, Cantin M, Genest J, Nutt RF, Brady SF, Lyle TA, Paleveda WJ, Colton CD, Ciccarone TM, Veber DF (1984) Amino acid sequence of homologous rat atrial peptides: natriuretic activity of native and synthetic forms. Proc Natl Acad Sci USA 81:2640–2644

Seidah NG, Cromlish JA, Hamelin J, Thibault G, Chrétien M (1986) Homologous IRCM-serine protease #1 from pituitary, heart atrium and ventricle: a common prohormone maturation enzyme? Biosci Rep 6:835–844

Seidman CE, Bloch KD, Klein KA, Smith JA, Seidman JG (1984a) Nucleotide sequences of the human and mouse atrial natriuretic factor genes. Science 226:1206–1209

Seidman CE, Duby AD, Choi E, Graham RM, Haber E, Homcy C, Smith JA, Seidman JG (1984b) The structure of rat preproatrial natriuretic factor as defined by a complementary DNA clone. Science 225:324–326

Sen I (1986) Identification and solubilization of atrial natriuretic factor receptors in human placenta. Biochem Biophys Res Commun 135:480–486

Seymour AA, Blaine EH, Mazack EK, Smith SG, Stabilito II, Haley AB, Napier MA, Whinnery MA, Nutt RF (1985a) Renal and systemic effects of synthetic atrial natriuretic factor. Life Sci 36:33–44

Seymour AA, Marsh EA, Mazack EK, Stabilito II, Blaine EH (1985b) Synthetic atrial natriuretic factor in conscious normotensive and hypertensive rats. Hypertension 7(suppl I): I-35–I-42

Seymour AA, Smith SG, Mazack EK, Blaine EH (1986) A comparison of synthetic rat and human atrial natriuretic factor in conscious dogs. Hypertension 8:211–216

Seymour AA, Sweet CS, Stabilito II, Emmert SE (1987) Cardiac and hemodynamic responses to synthetic atrial natriuretic factor in rats. Life Sci 40:511–519

Shapiro JT, de Leonardis VM, Needleman P, Hintze TH (1986) Integrated cardiac and peripheral vascular response to atriopeptin 24 in conscious dogs. Am J Physiol 251:H1292–H1297

Share L, Iitake K, Crofton JT, Brooks DP, Ouchi Y, Blaine E (1986) Central atrial natriuretic factor induces vasopressin release in conscious rats. Fed Proc 45:166

Shenker Y, Sider RS, Ostafin EA, Grekin RJ (1985) Plasma levels of immunoreactive atrial natriuretic factor in healthy subjects and in patients with edema. J Clin Invest 76:1684–1687

Shibasaki T, Naruse M, Yamauchi N, Masuda A, Imaki T, Naruse K, Demura H, Ling N, Inagami T, Shizume K (1986) Rat atrial natriuretic factor suppresses proopiomelanocortin-derived peptides secretion from both anterior and intermediate lobe cells and growth hormone release from anterior lobe cells of rat pituitary in vitro. Biochem Biophys Res Commun 135:1035–1041

Shiono S, Nakao K, Morii N, Yamada T, Itoh H, Sakamoto M, Sugawara A, Saito Y, Katsuura G, Imura H (1986) Nature of atrial natriuretic polypeptide in rat brain. Biochem Biophys Res Commun 135:728–734

Shionoiri H, Kaneko Y (1986) Intranasal administration of alpha-human natriuretic peptide produces a prolonged diuresis in healthy men. Life Sci 38:773–778

Singer DRJ, Shore AC, Markandu ND, Buckley MG, MacGregor GA (1986) Atrial natriuretic peptide levels in treated congestive heart failure. Lancet i:851

Skofitsch G, Jacobowitz DM, Eskay RL, Zamir N (1985) Distribution of atrial natriuretic factor-like immunoreactive neurons in the rat brain. Neuroscience 16:917–948

Skowsky WR, Fisher DS (1972) The use of thyroglobulin to induce antigenicity. J Lab Clin Med 80:134–144

Snajdar RM, Rapp JP (1985) Atrial natriuretic factor in Dahl rats atrial content and renal and aortic responses. Hypertension 7:775–782

Snajdar RM, Rapp JP (1986) Elevated atrial natriuretic polypeptide in plasma of hypertensive Dahl salt-sensitive rats. Biochem Biophys Res Commun 137:876–883

Solomon LR, Atherton JC, Bobinski H, Green R (1986) Effect of posture on plasma immunoreactive atrial natriuretic peptide concentrations in man. Clin Sci 71:299–305

Somers VK, Anderson JV, Conway J, Sleight P, Bloom SR (1986) Atrial natriuretic peptide is released by dynamic exercise in man. Horm Metabol Res 18:871–872

Sonnenberg H (1986) Mechanisms of release and renal tubular action of atrial natriuretic factor. Fed Proc 45:2106–2110

Sonnenberg H, Veress AT (1984) Cellular mechanism of release of atrial natriuretic factor. Biochem Biophys Res Commun 124:443–449

Sonnenberg H, Cupples WA, de Bold AJ, Veress AT (1981) Intracellular localization of the natriuretic effect of cardiac atrial extract. Ann NY Acad Sci 372:213

Sonnenberg H, Milojevic S, Chong CK, Veress AT (1983) Atrial natriuretic factor: reduced cardiac content in spontaneously hypertensive rats. Hypertension 5:672–675

Sonnenberg H, Krebs RF, Veress AT (1984) Release of atrial natriuretic factor from incubated rat heart atria. IRCS Med Sci 12:783–784

Sonnenberg H, Honrath U, Chong CK, Wilson DR (1986) Atrial natriuretic factor inhibits sodium transport in medullary collecting duct. Am J Physiol 250:F963–F966

Sosa RE, Volpe M, Marion DN, Glorioso N, Laragh JH, Vaughan ED Jr, Maack T, Atlas SA (1985a) Effect of atrial natriuretic factor on renin secretion, plasma renin and aldosterone in dogs with acute unilateral renal artery constriction. J Hypertens 3(suppl 3):S299–S302

Sosa RE, Volpe M, Marion D, Vaughan ED Jr, Atlas SA, Laragh JH, Maack T (1985b) Prevention of atrial natriuretic factor (ANF)-induced renal hemodynamic effects abolishes its natriuretic action. Clin Res 33:499A

Sosa RE, Volpe M, Marion DN, Atlas SA, Laragh JH, Vaughan ED Jr, Maack T (1986) Relationship between renal hemodynamic and natriuretic effects of atrial natriuretic factor. Am J Physiol 250:F520–F524

Standaert DG, Needleman P, Saper CB (1986a) Organization of atriopeptin-like immunoreactive neurons in the central nervous system of rat. J Comp Neurol 253:315–341

Standaert DG, Saper CB, Rye DB, Wainer BH (1986b) Colocalization of atriopeptin-like immunoreactivity with choline acetyltransferase- and substance P-like immunoreactivity in the pedunculopontine and laterodorsal tegmental nuclei in the rat. Brain Res 382:163–168

Steardo I, Nathanson JA (1986) Brain barrier tissues: end organs for atriopeptin. Science 235:470–473

Stokes TJ Jr, McConkey CL Jr, Martin KJ (1986) Atriopeptin III increases cGMP in glomeruli but not in proximal tubules of dog kidney. Am J Physiol 250:F27–F31

Stone RA, Glembotski CC (1986) Immunoreactive atrial natriuretic peptide in the rat eye: molecular forms in anterior uvea and retina. Biochem Biophys Res Commun 134:1022–1028

Struthers AD, Anderson JV, Causon RC, Christofides ND, Bloom SR (1985) The effect of human atrial natriuretic peptide on urinary sodium and urinary dopamine excretion in man. J Hypertens 3(suppl 3):S315–S317

Sugawara A, Nekar K, Sakamoto M, Morii N, Yamada T, Itoh H, Shiono S, Imura H (1985) Plasma concentration of atrial natriuretic polypeptide in essential hypertension. Lancet ii:1426

Sugawara A, Nakao K, Morii N, Sakamoto M, Horii K, Shimokura M, Kiso Y, Nishimura K, Ban T, Kihara M, Yamori Y, Kangawa K, Matsuo H, Imura H (1986) Significance of alpha-human atrial natriuretic polypeptide as a hormone in humans. Hypertension 8 (suppl I):I-151–I-155

Sugawara A, Nakao K, Kono T, Morii N, Yamada T, Itoh H, Shiono S, Saito Y, Mukoyama M, Arai H, Imura H (1987) Implication of atrial natriuretic polypeptide in essential hypertension and primary aldosteronism. Abstract, Proceedings of the Inter-American Society of Hypertension, Buenos Aires, May 1987

Sugimoto T, Ishii M, Hirata Y, Matsuoka H, Sugimoto T, Miyata A, Toshimori T, Masuda H, Kangawa K, Matsuo H (1986) Increased release of atrial natriuretic polypeptides in rats with DOCA-salt hypertension. Life Sci 38:1351–1358

Sugrue MF, Viader M-P (1986) Synthetic atrial natriuretic factor lowers rabbit intraocular pressure. Eur J Pharmacol 130:349–350

Sybertz EJ, Desiderio DM (1985) The role of Na^+-K^+-ATPase in the vasorelaxant actions of synthetic atrial natriuretic factor. Arch Int Pharmacodyn Ther 278:142–149

Takagi M, Franco-Saenz R, Mulrow PJ (1986) Effect of atrial natriuretic factor on the plasma aldosterone response to potassium infusion in rats-in vivo study. Life Sci 39:359–364

Takayanagi R, Tanaka I, Maki M, Inagami T (1985) Effects of changes in water-sodium balance on levels of atrial natriuretic factor messenger RNA and peptide in rats. Life Sci 36:1843–1848

Takayanagi R, Grammer RT, Inagami T (1986a) Regional increase of cyclic GMP by atrial natriuretic factor in rat brain: markedly elevated response in SHR. Life Sci 39:573–580

Takayanagi R, Misono KS, Grammer RT, Imada T, Inagami T (1986b) Effect of atrial natriuretic factor on vasorelaxation and secretion of aldosterone in spontaneously hypertensive rats. Fed Proc 45:1023

Tanaka I, Inagami T (1986) Increased concentration of plasma immunoreactive atrial natriuretic factor in Dahl salt sensitive rats with sodium chloride-induced hypertension. J Hypertens 4:109–112

Tanaka I, Misono KS, Inagami T (1984) Atrial natriuretic factor in rat hypothalamus, atria and plasma: determination by specific radioimmunoassay. Biochem Biophys Res Commun 124:663–668

Tanaka H, Shindo M, Gutkowska J, Kinoshita A, Hurata H, Ikeda M, Arakawa K (1986) Effect of acute exercise on plasma immunoreactive atrial natriuretic factor. Life Sci 39:1685–1693

Tarazi RC, Miller A, Frohlich ED, Dustan HP (1966) Electrocardiographic changes reflecting left atrial abnormality in hypertension. Circulation 34:818–822

Tartakoff A, Vassali P, Detraz M (1978) Comparative study on intracellular transport of secretory protein. J Cell Biol 79:694–707

Taylor CJ, Meisheri KD (1986) Inhibitory effects of a synthetic atrial peptide on contractions and 45 Ca fluxes in vascular smooth muscle. J Pharmacol Exp Ther 237:803–808

Thibault G, Garcia R, Cantin M, Genest J (1983a) Atrial natriuretic factor. Characterization and partial purification. Hypertension 5 (suppl I):I-75–I-80

Thibault G, Garcia R, Seidah NG, Lazure C, Cantin M, Chrétien M, Genest J (1983b) Purification of three atrial natriuretic factors and their amino acid composition. FEBS Lett 164:286–290

Thibault G, Carrier F, Garcia R, Gutkowska J, Seidah NG, Chrétien M, Cantin M, Genest J (1984a) The human atrial natriuretic factor (hANF): purification and primary structure. Clin Invest Med 7 (suppl 2):59

Thibault G, Garcia R, Cantin M, Genest J, Lazure C, Seidah NG, Chrétien M (1984b) Primary structure of a high Mr form of rat atrial natriuretic factor. FEBS Lett 167:352–356

Thibault G, Garcia R, Carrier F, Seidah NG, Lazure C, Chrétien M, Cantin M, Genest J (1984c) Structure-activity relationships of atrial natriuretic factor (ANF). I. Natriuretic activity and relaxation of intestinal smooth muscle. Biochem Biophys Res Commun 125:938–946

Thibault G, Lazure C, Schiffrin EL, Gutkowska J, Chartier L, Garcia R, Seidah NG, Chrétien M, Genest J, Cantin M (1985) Identification of a biologically active circulating form of rat atrial natriuretic factor. Biochem Biophys Res Commun 130:981–986

Thibault G, Garcia R, Gutkowska J, Lazure C, Seidah NG, Chrétien M, Genest J, Cantin M (1986) Identification of the released form of atrial natriuretic factor by the perfused rat heart. Proc Soc Exp Biol Med 182:137–141

Thibault G, Garcia R, Gutkowska J, Bilodeau J, Lazure C, Seidah NG, Chrétien M, Genest J, Cantin M (1987a) The propeptide Asn^{1}-Try^{126} is the storage form of rat atrial natriuretic factor. Biochem J 241:265–272

Thibault G, Garcia R, Schiffrin EL, de Léan A, Schiller PW, Gutkowska J, Genest J, Cantin M (1987b) Structure-activity relationships of atrial natriuretic peptides. In: Atrial Hormones and Other Natriuretic Factors. Edited by Mulrow PJ and Schreier R, pp 77–82

Thoren P, Mark AL, Morgan DA, O'Neill TP, Needleman P, Brody MJ (1986) Activation of vagal depressor reflexes by atriopeptins inhibits renal sympathetic nerve activity. Am J Physiol 251:H1252–H1259

Tikkanen I, Fyhrquist F, Metsärinne K, Leidenius R (1985a) Plasma atrial natriuretic peptide in cardiac disease and during infusion in healthy subjects. Lancet ii:66–69

Tikkanen I, Metsärinne K, Fyhrquist F (1985b) Atrial natriuretic peptide in paroxysmal supraventricular tachycardia. Lancet ii:40–41

Tjornhammar ML, Lazaridis G, Bartfai T (1983) Cyclic GMP efflux from liver slices. J Biol Chem 258:6882–6886

Tobian L, Lange J, Magraw S, Coffee K (1974) Body sodium and hypertension, sodium retention by "hypertensive kidneys". Trans Assoc Am Phys 87:225–232

Tobian L, Johnson MA, Lange J, Magraw S (1975) Effect of varying perfusion pressures on the output of sodium and renin and the vascular resistance in kidneys of rats with "post-salt" hypertension and Kyoto spontaneous hypertension. Circ Res 36 (suppl I):I-162–I-170

Tremblay J, Gerzer R, Vinay P, Pang SC, Beliveau R, Hamet P (1985) The increase of cGMP by atrial natriuretic factor correlates with the distribution of particulate guanylate cyclase. FEBS Lett 181:17–22

Tremblay J, Gerzer R, Pang SC, Cantin M, Genest J, Hamet P (1986) ANF stimulation of detergent-dispersed particulate guanylate cyclase from bovine adrenal cortex. FEBS Lett 194:210–214

Tremblay J, Pang SC, Schiffrin EL, Gutkowska J, Cusson JR, Larochelle P, Hamet P (1987) Particulate guanylate cyclase: target enzyme of atrial natriuretic factor. In: Brenner BM, Laragh JH (eds) Biologically Active Atrial Peptides, American Society of Hypertension Symposium Series, vol 1, Raven Press, NY, pp 255–257

Trippodo NC, MacPhee AA, Cole FE, Blakesly HL (1982) Partial chemical characterization of a natriuretic substance in rat atrial heart tissue. Proc Soc Exp Biol Med 170:502–508

Trippodo NC, MacPhee AA, Cole FE (1983) Partially purified human and rat atrial natriuretic factor. Hypertension 5 (suppl I):I-81–I-88

Tsuchiga S, Ogawa K, Sataka K (1980) Plasma levels of cyclic nucleotides in patients with essential hypertension. Jpn Heart J 21:803–815

Tunny TJ, Higgins BA, Gordon RG (1986) Plasma levels of atrial natriuretic peptide in man in primary aldosteronism, in Gordon's syndrome and in Bartter's syndrome. Clin Exp Pharmacol Physiol 13:341–385

Uehlinger DE, Weidmann P, Gnädinger MP, Shaw S (1985) Effects of human atrial natriuretic peptide on the pressor action of noradrenaline and angiotensin II in normal subjects. J Hypertens 3 (suppl 3):S319–S321

Uehlinger DE, Weidmann P, Gnädinger MP, Hasler L, Bachmann C, Shaw S, Hellmüller B, Lang RE (1986a) Increase in circulating insulin induced by atrial natriuretic peptide in normal humans. J Cardiovasc Pharmacol 8:1122–1129

Uehlinger DE, Weidmann P, Gnädinger MP, Shaw S, Lang RE (1986b) Depressor effects and release of atrial natriuretic peptide during norepinephrine or angiotension II infusion in man. J Clin Endocrinol Metab 63:669–674

Van Breemen C, Aaronson PI, Loutzenhiser RD, Meisheri KD (1982) Calcium fluxes in isolated rabbit aorta and guinea-pig tenia coli. Fed Proc 41:2891–2897

Vandlen RL, Arcuri KE, Napier MA (1985) Identification of a receptor for atrial natriuretic factor in rabbit aorta membranes by affinity cross-linking. J Biol Chem 260:10889–10892

Vandlen RL, Arcuri KE, Hupe L, Keegan ME, Napier MA (1986) Molecular characteristics of receptors for atrial natriuretic factor. Fed Proc 45:2366–2370

Vari RC, Freeman RH, Davis JO, Villarreal D, Verburg KM (1986) Effect of synthetic atrial natriuretic factor on aldosterone secretion in the rat. Am J Physiol 251:R48–R52

Verburg KM, Freeman RH, Davis JO, Villarreal D, Vari RC (1986) Control of atrial natriuretic factor release in conscious dogs. Am J Physiol 251:R947–R956

Veress AT, Sonnenberg H (1984) Right atrial appendectomy reduces the renal response to acute hypervolemia in the rat. Am J Physiol 247:R610–R613

Vierhapper H, Nowotny P, Waldhäusl W (1986) Prolonged administration of human atrial natriuretic peptide in healthy men. Reduced aldosteronotropic effect of angiotensin II. Hypertension 8:1040–1043

Villarreal D, Freeman RH, Davis JO, Verburg KM, Vari RC (1986a) Effects of atrial appendectomy on circulating atrial natriuretic factor during volume expansion in the rat. Proc Soc Exp Biol Med 183:54–58

Villarreal D, Freeman RH, Davis JO, Verburg KM, Vari RC (1986b) Renal mechanisms for suppression of renin secretion by atrial natriuretic factor. Hypertension 8 (suppl II):II-28–II-35

Vinay P, Gougoux A, Lemieux G (1981) Isolation of a pure suspension of rat proximal tubules. Am J Physiol 241:F403–F411

Vinay P, Manilier C, Lalonde L, Thibault G, Boulanger Y, Gougoux A, Cantin M (1987) Comparative effect of ANF and various diuretics on isolated nephron segments. Kidney Int 31:946–955

Viquerat CE, Daly P, Swedberg K, Evers C, Curran D, Parmley WW, Chatterjee K (1985) Endogenous catecholamine levels in chronic heart failure: Relation to the severity of hemodynamic abnormalities. Am J Med 78:455–460

Vlasuk GP, Miller J, Bencer GH, Lewicki JA (1986) Structure and analysis of the bovine atrial natriuretic peptide precursor gene. Biochem Biophys Res Commun 136:396–403

Volpe M, Odell G, Kleinert HD, Camargo MJ, Laragh JH, Lewicki JA, Maack T, Vaughan ED Jr, Atlas SA (1984) Antihypertensive and aldosterone-lowering effects of synthetic atrial natriuretic factor in renin-dependent renovascular hypertension. J Hypertens 2:S313–S315

Volpe M, Odell G, Kleinert HD, Muller F, Camargo MJ, Laragh JH, Maack T, Vaughan ED Jr, Atlas SA (1985) Effect of atrial natriuretic factor on blood pressure, renin, and aldosterone in Goldblatt hypertension. Hypertension 7 (suppl I):I-43–I-48

Volpe M, Mele AF, de Luca N, Golino P, Bondiolotti GP, Camargo MJ, Atlas SA, Trimarco B (1986a) Carotid baroreceptor unloading decreases plasma atrial natriuretic factor (ANF) in hypertensive patients. In: Proceedings, 11th Scientific Meeting of the International Society of Hypertension, Heidelberg, abstract 443

Volpe M, Sosa RE, Muller FB, Camargo MJ, Glorioso N, Laragh JH, Maack T, Atlas SA (1986b) Differing hemodynamic responses to atrial natriuretic factor in two models of hypertension. Am J Physiol 250:H871–H878

Von Schroeder HP, Nishimura E, McIntosh CHS, Buchan AMJ, Wilson N, Ledsome JR (1985) Autoradiographic localization of binding sites for atrial natriuretic factor. Can J Physiol Pharmacol 63:1373–1377

Vuolteenaho O, Arganiaa O, Ling N (1985) Atrial natriuretic polypeptides (ANF): rat atria store high molecular weight precursor but secrete processed peptides of 25–35 amino acids. Biochem Biophys Res Commun 129:82–88

Wakitani K, Oshima T, Currie MG, Geller DM, Needleman P (1985a) Vasodilator properties of a family of bioactive atrial peptides in isolated perfused rat kidneys. J Lab Clin Med 105:349–352

Wakitani K, Oshima T, Loewy AD, Holmberg SW, Cole BR, Adams SP, Fok KF, Currie MG, Needleman P (1985b) Comparative vascular pharmacology of the atriopeptins. Circ Res 56:621–627

Waldhäusl W, Vierhapper H, Nowotny P (1986) Prolonged administration of human atrial natriuretic peptide in healthy men: Evanescent effects on diuresis and natriuresis. J Clin Endocrinol Metab 62:956–959

Waldman SA, Rapoport RM, Murad F (1984) Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues. J Biol Chem 259:14332–14334

Waldman SA, Rapoport RM, Fiscus RR, Murad F (1985) Effects of atriopeptin on particulate guanylate cyclase from rat adrenal. Biochim Biophys Acta 845:298–303

Webb RL, della Puca R, Manniello J, Robson RD, Zimmerman MB, Ghai RD (1986) Dopaminergic mediation of the diuretic and natriuretic effects of ANF in the rat. Life Sci 38:2319–2327

Webster PD 3rd, Black O Jr, Mainz DL, Singh M (1977) Pancreatic acinar metabolism and function. Gastroenterology 73:1434–1440

Wei Y, Geller D, Siegel NR, Needleman P (1986) Identification of the cardiac and circulating form of atriopeptin in rabbit. Biochem Biophys Res Commun 138:1263–1268

Weidmann P, Hasler L, Gnädinger MP, Lang RE, Uehlinger DE, Shaw S, Rascher W, Reubi FC (1986a) Blood levels and renal effects of atrial natriuretic peptide in normal man. J Clin Invest 77:734–742

Weidmann P, Hellmueller B, Uehlinger DE, Lang RE, Gnädinger MP, Hasler L, Shaw S, Bachmann C (1986b) Plasma levels and cardiovascular, endocrine, and excretory effects of atrial natriuretic peptide during different sodium intakes in man. J Clin Endocrinol Metab 62:1027–1036

Weil J, Lang RE, Suttmann H, Rampf U, Bidlingmaier F, Gerzer R (1985) Concomitant increase in plasma atrial natriuretic peptide and cyclic GMP during volume loading. Klin Wochenschr 63:1265–1268

Weil J, Bidlingmaier F, Döhlemann C, Kuhnle U, Strom T, Lang RE (1986) Comparison of plasma atrial natiuretic peptide levels in healthy children from birth to adolescence and in children with cardiac diseases. Pediatr Res 20:1328–1331

Whitfield L, Sowers JR, Truck ML, Golub MS (1980) Dopaminergic control of plasma catecholamine and aldosterone responses to acute stimuli in normal man. J Clin Endocrinol Metab 51:724–729

Willingham MC, Pastan I (1984) Endocytosis and exocytosis: current concepts of vesicle traffic in animal cells. Int Rev Cytol 92:51–92

Wilson N, Ledsome JR, Keeler R, Rankin AJ, Wade JP, Courneya CA (1986) Heterologous radioimmunoassay of atrial natriuretic polypeptide in dog and rabbit plasma. J Immunoassay 7:73–96

Winquist RJ, Faison EP, Nutt RF (1984a) Vasodilator profile of synthetic atrial natriuretic factor. Eur J Pharmacol 102:169–173

Winquist RJ, Faison EP, Waldman SA, Schwartz K, Murad F, Rapoport RM (1984b) Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. Proc Natl Acad Sci USA 81:7661–7664

Winquist RJ, Napier MA, Vandlen RL, Arcuri K, Keegan ME, Faison EP, Baskin EP (1985) Pharmacology and receptor binding of atrial natriuretic factor in vascular smooth muscle. Clin Exp Hypertens A7:869–884

Wong M, Samson WK, Dudley CA, Moss RL (1986) Direct, neuronal action of atrial natriuretic factor in rat brain. Neuroendocrinology 44:49–53

Wood P (1963) Polyuria in paroxysmal tachycardia and paroxysmal atrial flutter and fibrillation. Br Heart J 25:273–282

Xu C-B, Jiang B-Q, Wang J-Y, Sun M-L, Chen H, Liu X-R, Tian Y-W, Chen S-J, Li D-G, Xie CW, Tang J (1986) Hemodynamic and renal effects of human alpha-atrial natriuretic peptide in two patients with congestive heart failure. A preliminary report. Chin Med J (Engl) 99:1–8

Yamada K, Tajima K, Moriwaki K, Tarui S, Miyata A, Kangawa K, Matsuo H (1986) Atrial natriuretic peptide in Bartter's syndrome. Lancet i:273

Yamaji T, Ishibashi M, Nakaoka H, Imataka K, Amano M, Fujii J (1985a) Possible role for atrial natriuretic peptide in polyuria associated with paroxysmal atrial arrhythmias. Lancet i:1211

Yamaji T, Ishibashi M, Takaku F (1985b) Atrial natriuretic factor in human blood. J Clin Invest 76:1705–1709

Yamaji T, Hirai N, Ishibashi M, Takaku F, Yanaihara T, Nakayama T (1986a) Atrial natriuretic peptide in umbilical cord blood: evidence for a circulating hormone in human fetus. J Clin Endocrinol Metab 63:1414–1417

Yamaji T, Ishibashi M, Sekihara H, Takabu F, Nakaoka H, Fujii J (1986b) Plasma levels of atrial natriuretic peptide in primary aldosteronism and essential hypertension. J Clin Endocrinol Metab 63:815–818

Yamamoto Y, Higa T, Kitamura K, Tanaka K, Kangawa K, Matsuo H (1985) Plasma concentration of atrial natriuretic polypeptide in chronic hemodialysis patients. Regul Pept (suppl) 4:110–112

Yamamoto K, Kosaki G, Suzuki K, Tanoue K, Yamazaki H (1986) Cleavage site of calcium-dependent protease in human platelet membrane glycoprotein Ib. Thromb Res 43:41–55

Yamanaka M, Greenberg B, Johnson L, Seilhamer J, Brewer M, Friedemann T, Miller J, Atlas S, Laragh J, Lewicki J, Fiddes J (1984) Cloning and sequence analysis of the cDNA for the rat atrial natriuretic factor precursor. Nature 309:719–722

Yandle TG, Espiner EA, Nicholls MG, Duff H (1986a) Radioimmunoassay and characterization of atrial natriuretic peptide in human plasma. J Clin Endocrinol Metab 63:72–79

Yandle TG, Richards AM, Nicholls MG, Cuneo R, Espiner EA, Livisey JH (1986b) Metabolic clearance rate and plasma half-life of alpha-human atrial natriuretic peptide in man. Life Sci 38:1827–1833

Yang-Feng TL, Floyd-Smith G, Nemer M, Drouin J, Francke U (1985) The pronatriodilatin gene is located on the distal short arm of human chromosome 1 and on mouse chromosome 4. Am J Hum Genet 37:1117–1128

Yasujima M, Abe K, Kohzuki M, Tanno M, Kasai Y, Sato M, Omata K, Kudo K, Tsunoda K, Takeuchi K, Yoshinaga K, Inagami T (1985) Atrial natriuretic factor inhibits the hypertension induced by chronic infusion of norepinephrine in conscious rats. Circ Res 57:470–474

Yasujima M, Abe K, Kohzuki M, Tanno M, Kasai Y, Sato M, Omata K, Kudo K, Takeuchi K, Hiwatari M, Kimura T, Yoshinaga K, Inagami T (1986) Effect of atrial natriuretic factor on angiotensin II-induced hypertension in rats. Hypertension 8:748–753

Yip CC, Laing LP, Flynn TG (1985) Photoaffinity labeling of atrial natriuretic factor receptors of rat kidney cortex plasma membranes. J Biol Chem 260:8229–8232

Zachariah PK, Burnett JC, Ritter S, Strong CG (1987) Atrial natriuretic peptide in human essential hypertension. Mayo Clin Proc 62:782–786

Zamir N, Skofitsch G, Eskay RL, Jacobowicz DM (1986) Distribution of immunoreactive atrial natriuretic peptides in the central nervous system of the rat. Brain Res 365:105–111

Zamir N, Haass M, Dave JR, Zukowska-Grojec Z (1987) Anterior pituitary gland modulates the release of atrial natriuretic peptide from cardiac atria. Proc Natl Acad Sci USA 84:541–545

Zeidel ML, Seifter JL, Brenner BM, Silva P (1986a) Atrial peptides inhibit Na^+ entry-dependent oxygen in rabbit inner medullary collecting duct (IMCD) cells. Clin Res 34:731A

Zeidel ML, Seifter JL, Lear S, Brenner BM, Silva P (1986b) Atrial peptides inhibit oxygen consumption in kidney medullary collecting duct cells. Am J Physiol 251:F379–F383

Zimmerman RS, Gharib H, Zimmerman D, Heublein D, Burnett JC Jr (1987) Atrial natriuretic peptide in hypothyroidism. J Clin Endocrinol Metab 64:353–355

Zivin RA, Condra JH, Dixon RAF, Seidah NG, Chrétien M, Nemer M, Chamberland M, Drouin J (1984) Molecular cloning and characterization of DNA sequences encoding rat and human atrial natriuretic factors. Proc Natl Acad Sci USA 81:6325–6329

Rev. Physiol. Biochem. Pharmacol., Vol. 110

The Organisation of Cardiovascular Neurons in the Spinal Cord

JOHN H. COOTE

Contents

Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TJ, England, UK

1 Introduction

The heart and vascular beds throughout the body are regulated by myogenic, metabolic and neural factors. Either directly or indirectly, the neural factors play a key role in the variety and degree of response which is so much a

feature of the cardiovascular system. Many parts of the brain are involved in this control, but directly or indirectly they ultimately influence the spinal cord, wherein lie the preganglionic motoneurons whose axons pass out of the central nervous system to synapse with postganglionic neurons in the peripheral ganglia. With perhaps one major exception (viz. the sacral sympathetic innervation of arterioles in erectile tissue) the sympathetic nerves from the thoracolumbar spinal cord form the main spinal-cord supply to the heart and blood vessels. The sympathetic preganglionic neuron is probably the final site at which major integration occurs, although it is acknowledged that extensive integration also occurs in ganglia (Wood 1975; Skok 1980; Elfin 1983). Therefore, a review of the way in which cardiovascular neurons in the spinal cord are organized must concern itself with the properties and synaptic organisation of sympathetic preganglionic neurons. The extent to which the knowledge gained thereby relates specifically to cardiovascular neurons will depend on how well we can identify characteristics which are specific to cardiovascular-dedicated preganglionic neurons. We will return to this question at the end of our review.

2 Anatomy

2.1 Location

The sympathetic preganglionic neurons lie in the zona intermedia of the grey matter in the thoraco-lumbar cord. Early descriptions based on use of the Golgi stain described them as being located in a triangular region on the lateral border of the grey matter, as they had been identified only in a region of the spinal cord associated with sympathetic white rami communicantes (Clark 1851; Poljak 1924; Bok 1928; Laruelle 1937). The latter region, known as the intermediolateral nucleus, has been described in rat (Navaratnam and Lewis 1970), guinea pig (Bok 1928; Laruelle 1937; Torigoe et al. 1985), rabbit (Laruelle 1937), bat (Poljak 1924), cat (Takahashi 1913; Laruelle 1937; Rexed 1954; Deuschl and Illert 1981), dog (Laruelle 1937; Cummings 1969), fox (Laruelle 1937), monkey (Petras and Cummings 1972; Rao and Bijlani 1980), man (Jacobsohn 1908; Massazza 1923a, b, 1924; Bok 1928; Gagel 1928, 1932; Greving 1928; Laruelle 1937; Riley 1960), frog (Robertson 1987), Terrapin (Leong et al. 1983) and bird (Terni 1923; Huber 1936; MacDonald and Cohen 1970).

Recent work has shown that sympathetic preganglionic neurons are found at other locations in the intermediate grey matter. Petras and Cummings (1972) provide the most comprehensive description of this location in mammals, and their terminology will be used hereafter. In their study of the mon-

key spinal cord following sympathectomy, chromatolysis occurred in cells that were located in several discrete nuclei both medial and lateral to the intermediolateral cell column described by earlier workers. These findings have subsequently been confirmed in other, more recent studies in different animal species using techniques of tracing horseradish peroxidase (HRP). Petras and Cummings (1972) subdivided the intermediolateral cell column into two subnuclei: the nucleus intermediolateralis thoracolumbalis pars principalis (ILp), which is equivalent to the intermediolateral cell column referred to above, and an adjacent nucleus intermediolateralis thoracolumbalis pars funiculus (ILf), which extends into the white matter of the lateral funiculus. Medial to the ILp there are cells in a transverse band across the grey matter forming the nucleus

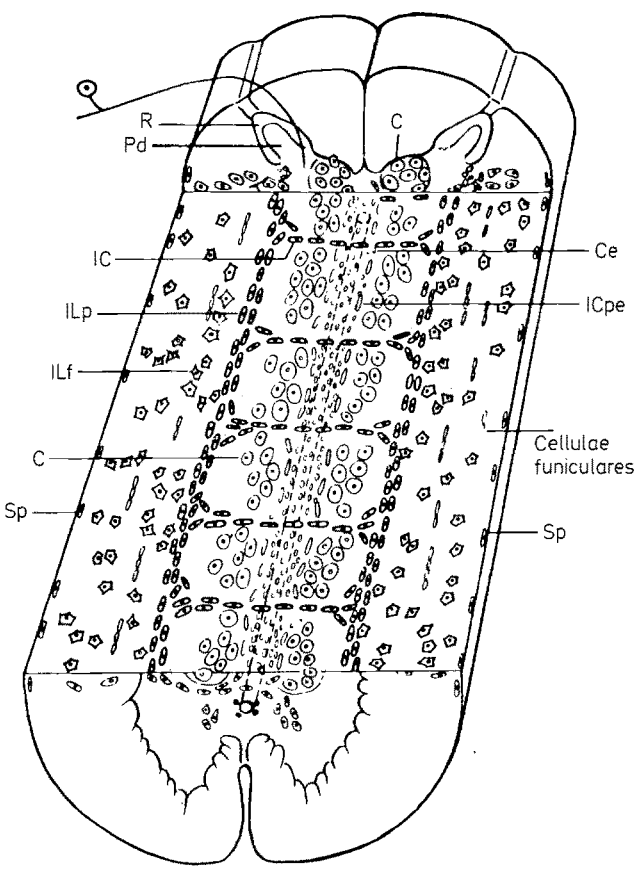


Fig. 1. A schematic illustration, in the transverse and horizontal planes of the topographic relationships of the nuclei intermediolateralis thoracolumbalis pars principalis (*ILp*), pars funicularis (*ILf*), intercalatus spinalis (*IC*) and intercalatus spinalis pars paraependymalis (*ICpe*); *R*, substantia gelatinosa of Rolando; *Pd*, nucleus proprius cornus dorsalis; *Sp*, sub-pial neurons; *C*, Clarke's column; *Ce*, cells of the central grey substance. (From Petras and Cummings 1972)

intercalatus spinalis (IC). The IC joins a paracentral column of cells near the central canal, lying lateral and dorsal to it in most species and called the central autonomic area or nucleus intercalatus spinalis pars paraependymatis (ICpe). The ICpe is the principal location of SPN in the bird (MacDonald and Cohen 1970). There is an additional group of cells, lying ventral and lateral to the central canal, which might be considered part of the sympathetic nervous system but which are not preganglionic neurons because their axons do not pass out of the grey matter but cross transversely to terminate in the ILp (Onuf and Collins 1898; Onuf 1900; Massazza 1922, 1923a, 1924; Poljak 1924; Bok 1928; Laruelle 1937; Petras and Cummings 1972). These cells, known as the intermediomedial cell group (IMM), receive a significant projection of dorsal-root afferents (Petras and Cummings 1972).

The arrangement of these sympathetic cell groups along the longitudinal axis of the spinal cord is illustrated schematically in Fig. 1. The cells of the ILp form tightly knit clusters, giving a distinctly beaded appearance, and the cells of the IC provide a striking ladder-like appearance in the same segments. The distribution of SPN among these sub-nuclei varies at different spinal levels (Petras and Faden 1978; Dalsgaard and Elfvin 1979, 1981; Oldfield and McLachlan 1981; Pardini and Wurster 1984; Torigoe et al. 1985). At each level the majority of neurons are found in the ILp, with the next-largest numbers in the ILf and very few in the IC (although the majority lie here in the terrapin; Leong et al. 1983) and the ICpe (Petras and Cummings 1972; Deuschl and Illert 1978; Chung et al. 1979b; Dalsgaard and Elfvin 1979; Janig and McLachlan 1986; Bennett et al. 1986). In mammals, the sympathetic preganglionic neurons at the third thoracic level are almost exclusively confined to the ILp and ILf (Chung et al. 1979b; Oldfield and McLachlan 1981; Rando et al. 1981; Gilbey et al. 1982b; Murata et al. 1982; Pardini and Wurster 1984).

2.2 Topographical Distribution

There is no convincing evidence at present that sympathetic sub-nuclei selectively innervate particular ganglia or postganglionic neurons to particular end-organs, although evidence does suggest a topographical distribution of preganglionic somata within each sub-nucleus. Several studies have addressed this question. The thoracic ganglia appear to be innervated by cells in the ILp, IC and ICpe (Petras and Faden 1978), as are the cervical sympathetic nerve (Chung et al. 1975; Chung et al. 1979b; Oldfield and McLachlan 1981; Rando et al. 1981; Gilbey et al. 1982b; Murata et al. 1982), the stellate ganglion (Chung et al. 1979b; Oldfield and McLachlan 1981), the lumbar sympathetic trunk (Deuschl and Illert 1978) and the adrenal medulla (Cummings 1969; Petras and Cummings 1972; Schramm et al. 1975). However, Petras and

Faden (1978) considered that ILp neurons innervate paravertebral ganglia more massively than IC neurons or certainly ICpe neurons do, which suggested to them that other, more distally located ganglia may be the principal targets of preganglionic neurons in the IC and ICpe. This possibility was also examined by Deuschl and Illert (1978), who showed it to be unlikely since injection of HRP into peripheral ganglia, such as the coeliac and mesenteric ganglia, still labelled a significant number of neurons in the ILp. This result was strongly supported by the study of Kuo et al. (1980), which showed that exposure of the cat splanchnic nerve to HRP resulted in labelling of neurons in each of the sub-nuclei, although 83% were found in the ILp (see also Dalsgaard and Elfvin 1979).

The clearest indication that sympathetic preganglionic neurons have a topographical distribution comes from recent studies by McLachlan et al. (1985) and Janig and McLachlan (1986). These authors examined their spatial distribution in segments L3–L4 in guinea pig and L2–L5 in cat and found that:

1. Sympathetic preganglionic neurons in the lumbar sympathetic trunk (but not the splanchnic nerves) most likely go to hind limb (the majority to muscle and cutaneous blood vessels), and arise from cell bodies lying close to the lateral edge of the grey matter and amongst the fibre tracts of the white matter.

2. Sympathetic preganglionic neurons in the hypogastric nerve arise from the cell bodies lying medial to the lateral border of the grey matter and spread across medially in a transverse band.

3. Sympathetic preganglionic neurons in the lumbar splanchnic nerves, projecting to the colonic nerves, lie within the grey matter, close to its border (Fig. 2). However, although the principle may be retained in other species, the details may be different, because in the rat, for example, the hypogastric sympathetic preganglionic neurons are in greatest numbers lateral to the mid-line and dorsal to the central canal at L1–L2, corresponding to the ICpe (Hancock and Peveto 1979). There is thus a clear indication that characteristic differences exist between the four sympathetic sub-nuclei with regard to the destination of the axons of associated preganglionic somata. In studies in cat, since the somata of preganglionic neurons in the lumbar splanchnic nerves could be divided into a more laterally located group contributing axons to the colonic nerves and a more medially located group contributing axons to the hypogastric nerve, Janig and McLachlan (1986) proposed that there are restricted columns of preganglionic neurons representing different viscera, organised in a similar way to the motor nuclei of the anterior horn, where columns of cells supply different muscles (Romanes 1951). This is an attractive possibility which is further supported by the evidence of Baron et al. (1985) showing that preganglionic somata supplying the colon and pelvic organs are restricted to discrete groups in the ILp.

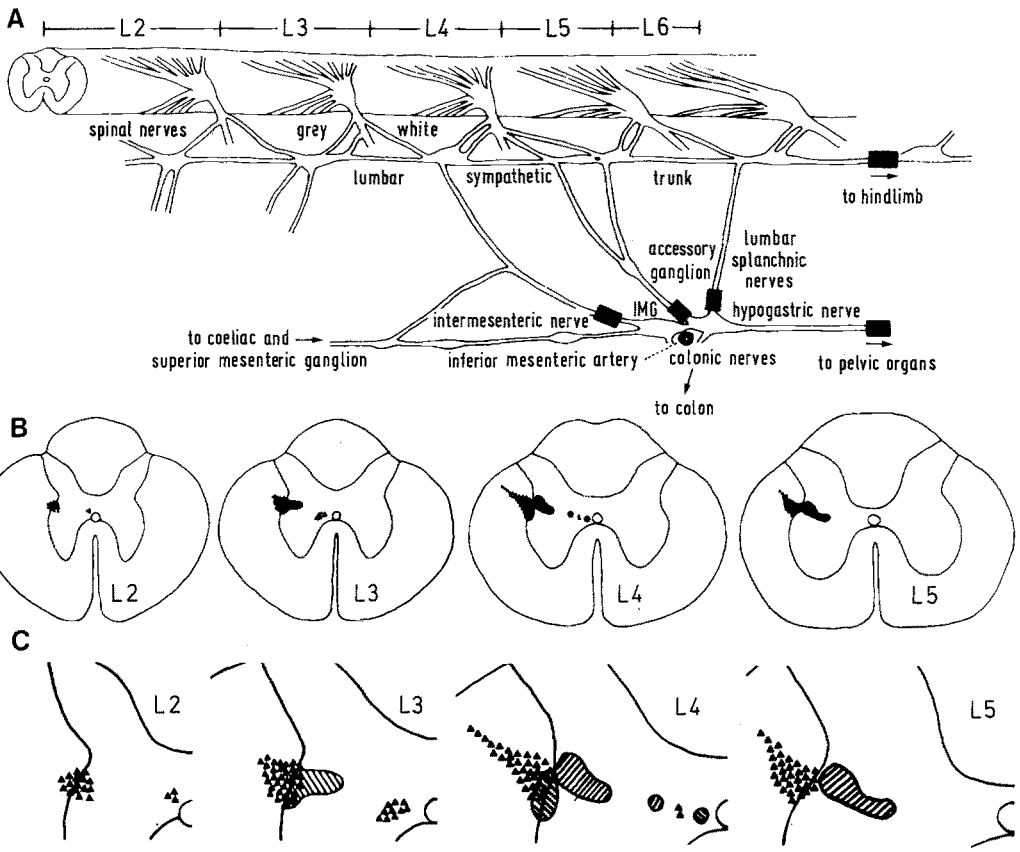


Fig. 2A–C. Location of sympathetic preganglionic neurons projecting in the different peripheral nerve trunks. **A** Anatomy of the lumbar nerve pathways in the cat; *black rectangles* show the various sites at which HRP was applied in different experiments. **B** Transverse sections of spinal cord illustrating the regions containing the highest densities of preganglionic neurons in the most rostral 2 mm of each segment from L2 to L5. **C** Enlargements of the intermediate parts of the spinal cord shown in **B**. Cells labelled are from the lumbar sympathetic trunk (*black triangles*), from the hypogastric nerve (*right hatching*), from the lumbar splanchnic, but not the hypogastric, nerves (*left hatching*); *IMG,* inferior mesenteric ganglion. (From Janig and McLachlan 1986)

2.3 Numbers of Preganglionic Neurons

The total numbers of preganglionic neurons varies according to species although there are many more in mammals than in birds (MacDonald and Cohen 1970), frogs (324; Robertson 1987) or terrapins (541; Leong et al. 1983). In a Nissl study of the cat spinal cord, Henry and Calaresu (1972b) reported a maximum of 53340 neurons in one animal, with the highest density located in T1, T2 and L3–L4 segments. In an HRP study in the rhesus monkey the number of neurons for a similar number of segments was higher;

Rao and Bijlani (1980) reported a maximum of 76219 cells, with highest densities in T2, T3, T4 and L1. There have been no similar studies in man, but two recent studies suggest that the total number of sympathetic preganglionic neurons in man may be greater still. Pearson and Pytel (1978) counted a total of 25150 neurons in the ILp of segments T1–T3, 11100 neurons being in the T3 segment. Such high densities are unlikely to occur at all levels, as is indicated by the study of Low et al. (1977) in which counts of around 5000 cells per segment were made for segments T6–T8. However, assuming a not unreasonable average of 6000 cells per segment for 14 segments (T1–L2) in man, there could be a maximum of 84000 cells.

An interesting observation made by Henry and Calaresu (1972b) was that the number of preganglionic neurons is higher in male (mean 45765) than in female cats (mean 35543). A similar difference has been reported for the rhesus monkey (male 76219; female 67791; Rao and Bijlani 1985) but in man no significant sex difference was found in segments T6, T7 and T8 (Low et al. 1977). The sex difference cannot be related to innervation of genital organs in the male, because there is an uniform difference throughout the segments of the thoraco-lumbar cord. Henry and Calaresu (1972) speculate that it is related to the statistical likelihood of a greater body mass in the fully developed male. In support of this, they cite that the number and size of neurons in sympathetic ganglia may be increased by the administration to new-born mice of nerve growth factor isolated from the submaxillary gland of male mice, and that the amount of nerve growth factor present in the gland is decreased in male rats by ablation of the testes and increased in female rats by the administration of testosterone (Levi-Montalcini and Angeletti 1964).

2.4 Morphology

Preganglionic neurons have cell bodies, which are smaller (15–40 μm) on average than those of ventral-horn somatic motoneurons but larger than those of dorsal-horn cells. The size and shape of the cell body, and the orientation of the proximal dentrites, depends on their location in the spinal cord. Neurons in the ILp are primarily fusiform or spindle-shaped with proximal dendrites orientated cranio-caudally (Fig. 3); small round neurons and large multipolar stellate cells are found here less often (Petras and Cummings 1972; Rethelyi 1972; Chung et al. 1975; Petras and Faden 1978; Hancock and Peveto 1979; Deuschl and Illert 1981; Dalsgaard and Elfvin 1981; Oldfield and McLachlan 1981; Rando et al. 1981; Dembowsky et al. 1985a; Bennett et al. 1986). The latter types of preganglionic neurons are more often seen in the ILf where the predominant orientation is from medial to lateral (Petras and Cummings 1972; Oldfield and McLachlan 1981). Large fusiform and small round cells, which are orientated transversely or obliquely, make up the IC.

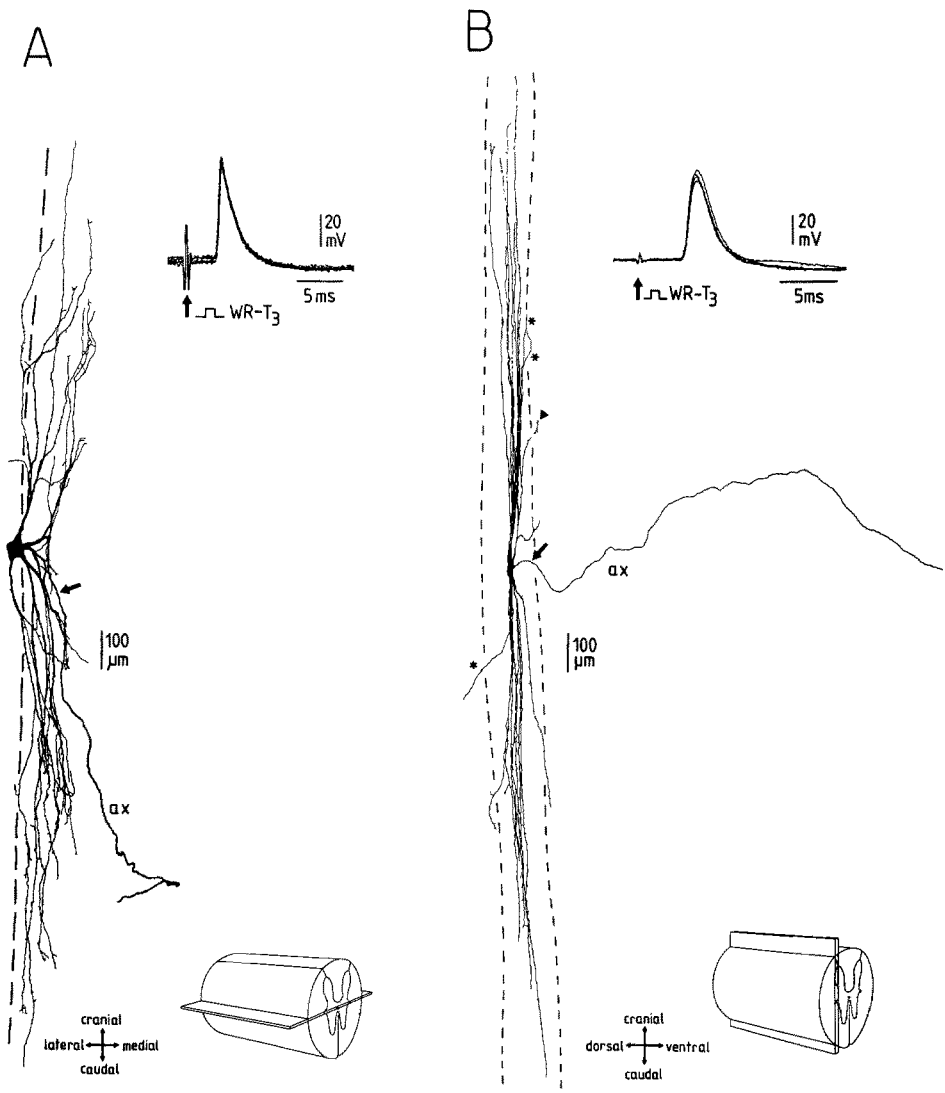


Fig. 3A–B. Camera lucida drawings of two sympathetic preganglionic neurons filled with horseradish peroxidase (HRP) after penetration by micro-electrode shown in the horizontal (**A**) and the parasagittal (**B**) plane. The origin of the axon (*ax*) is marked by an *arrow*. The *dashed lines* mark the border between the ILp and the adjacent white matter at the level of the cell body. Higher-order dendrites leaving the ILp are marked by *asterisks* (ventro- and dorso-lateral projection) and by an *arrowhead* (medial projection). Superimposed sweeps of the antidromic action potential evoked by stimulation of the third thoracic white ramus ($WR\text{-}T_3$) in the cat are also shown for both neurons. (From Dembowsky et al. 1985b)

Small fusiform neurons with their long axis parallel to the central canal form the principal cells of the ICpe (Petras and Cummings 1972; Oldfield and McLachlan 1981; Torigoe et al. 1985).

Three types of preganglionic neurons can also be distinguished from studies of ultrastructure (Rethelyi 1972; Wong and Tan 1980). The larger types, I and II, are distinguishable by differences in the rough endoplasmic reticulum, whilst type-III cells are small, have little endoplasmic reticulum and, unlike cells of types I and II, are only partially covered by astrocytic processes.

In a Golgi study of the ILp, Rethelyi (1972) described the cell bodies and dendrites of preganglionic neurons as being strictly cranio-caudally orientated and practically no dendrites were reported leaving the boundaries of the ILp – an arrangement also elegantly demonstrated in a more recent intracellular study using HRP (Fig. 3; Dembowsky et al. 1985b). Such a characteristic is markedly unlike those of cells in the surrounding grey matter (Petras and Cummings 1972; Rethelyi 1972, 1974; Chung et al. 1975). The dendritic fields of cells in the ILp can extend up to 1330 μm in either the cranial or the caudal direction with a total extent of up to 2540 μm (Dembowsky et al. 1985b). However, primary dendrites of a few neurons in the ILp have been observed to project medio-laterally. Whether this is just a remnant of a transverse orientation of dendrites which predominates in the neonate (Rethelyi 1972; Schramm et al. 1976) is uncertain. Such projections could be important for ensuring repeated axo-dentritic contacts with cells of the IMM, which certainly project axons towards the ILp (Petras and Cummings 1972). The medio-laterally orientated dendrites of some ILp neurons may, together with the transversely orientated dendrites of neurons in the ILf and IC, be responsible for the dense transverse band of acetylcholinesterase staining present in the intermediate regions of the thoracic cord (Silver and Wolstencroft 1971; Galabov and Davidoff 1976). There is no major dorsal dendritic projection along the lateral edge of the dorsal horn, at least in the thoracic region (Rethelyi 1972; Dalsgaard and Elfvin 1981; Rando et al. 1981; Dembowsky et al. 1985b), but such a projection from the ILp in the lower thoracic cord and lumbar cord has been described (Deuschl and Illert 1981; Neuhuber 1982; Torigoe et al. 1985).

At a distance of 132–437 μm from the cell body, the axon arises (Dembowsky et al. 1985b) as a direct extension of a primary or second-order dendrite, with no sign of collateral sprouting (Rethelyi 1972; Petras and Cummings 1972; Dembowsky et al. 1985a). Axons from the ILp neurons can be traced along the lateral border of the ventral horn before they join the ventral rootlets (Poljak 1924; Bok 1928; Rethelyi 1972; Dembowsky et al. 1985a). It has been traditionally accepted that all axons of preganglionic neurons follow this route and exit from the spinal cord at or near the segmental level in which their cell bodies are located. However, this view has been questioned by

several recent investigations using HRP, purporting to show that some, if not many, preganglionic axons descend or ascend for several segments intraspinally before leaving the spinal cord (Deuschl and Illert 1978; Faden and Petras 1978; Petras and Faden 1978; Chung et al. 1979b; Deuschl et al. 1981). Others have disputed this (Kuo et al. 1980; Oldfield and McLachlan 1981; Rubin and Purves 1980; Bennett et al. 1986). A recent study by Pardini and Wurster (1984) shows quite elegantly that these different results are most likely a consequence of the different methods used to apply HRP.

Two basic methods have been used to study the route taken by preganglionic axons. One, employing the application of HRP crystals or solution to the central cut end of a single white ramus, has indicated segmental organisation. Another method, employing injection of HRP into a sympathetic ganglion with one or more white rami transected, has supported the idea of intraspinal pathways for preganglionic neurons. Pardini and Wurster (1984) injected HRP into the stellate ganglion only after the central and peripheral ends of transected rami had been securely tied, thus eliminating the possibility of leakage and non-specific uptake of HRP. When doing this with a single ramus intact, they found labelling in neurons of that one segment only. However, if they carried out this procedure but did not put ligatures on cut rami, labelled cells were found in many segments. In a second protocol they injected the ganglia with all rami intact and showed the number of labelled neurons in segments C8–T9 to be similar in control animals and in animals with a cord hemisection at T4. Therefore, these experiments quite clearly show that preganglionic axons leave the spinal cord at the same segment where their cell body is located and that HRP leakage onto cut ends of white rami most likely led to the earlier misleading data and the opposite conclusion.

2.5 Synapses

Several ultrastructural studies are in general agreement about the morphology of sympathetic preganglionic neurons and the types of synapse to be found on them. Axo-dendritic synapses are common to all three types of neuron referred to earlier; they are common in type-III cells but are only rarely seen in cell-types I and II. The axon terminals in the ILp can be divided into three main classes according to the morphology of their synaptic vesicles. One class of bouton contains round agranular vesicles, another has a mixture of agranular and dense-core vesicles and a third type contains flattened or ellipsoidal agranular vesicles (Rethelyi 1972, 1974; Wong and Tan 1974; Tan and Wong 1975; Wong and Tan 1980; Galabov and Davidoff 1976; Chiba and Murata 1981). Chung et al. (1980) also describe three types of synapse in the cat ILp. However, apart from the one containing flattened vesicles it is not

easy to equate these with the above classification, since granular vesicles were not abundant. Terminals containing flattened vesicles outnumber other types on the soma, whilst there are about equal numbers of all types on proximal dendrites. There is a greater density of synaptic covering on dendrites than on the soma, which apparently reflects an increased number of boutons, not a larger surface area of each bouton (Chung et al. 1980).

3 Segmental Afferent Input

3.1 Synaptic Connections

The excitability of sympathetic preganglionic neurons can be synaptically influenced from a great variety of sources. These sources and inputs can be divided into two main categories: peripheral input from somatic and visceral afferents to the spinal cord and central input from spinally projecting brainstem neurons. The presynaptic fibres of the neuropil are probably all unmyelinated and approach the ILp in small bundles, mainly from its lateral side, although a few converge from the medial regions of the grey matter (Rethelyi 1972). These fibres make repeated contacts with dendrites and cell bodies of preganglionic neurons (Rethelyi 1972, 1974; Tan and Wong 1975; Galabov and Davidoff 1976; Wong and Tan 1980; Kuo et al. 1980; Chung et al. 1980).

3.2 Spinal-Cord Afferents

The type of primary afferent fibre which may influence preganglionic cells is now well established, and the extensive literature has been comprehensively reviewed (Koizumi and Brooks 1972; Sato and Schmidt 1973; Schmidt 1974; Wurster 1977; Coote 1978; Koizumi and Brooks 1984; Janig 1985). All authors are agreed that the relevant afferent fibres are to be found amongst those myelinated fibres conducting at below 40 m/s and the unmyelinated fibres, i. e. the A, B and C fibres of cutaneous nerves, muscle nerve fibres of groups II, III and IV (Coote and Perez-Gonzales 1970; Henry and Calaresu 1974b; Koizumi et al. 1970; Sato et al. 1969; Sato and Schmidt 1966; Schmidt and Schönfuss 1970; Schmidt and Weller 1970) and the small myelinated and unmyelinated afferent fibres of visceral nerves (Coote et al. 1969; Franz et al. 1966; Sato et al. 1969; Kuo et al. 1983; Cervero and Connell 1984; Ammons 1986). There is no anatomical indication that any of these primary afferents make monosynaptic connections with sympathetic preganglionic neurons (Schimert 1939; Szentagothai 1948; Petras and Cummings 1972). Following

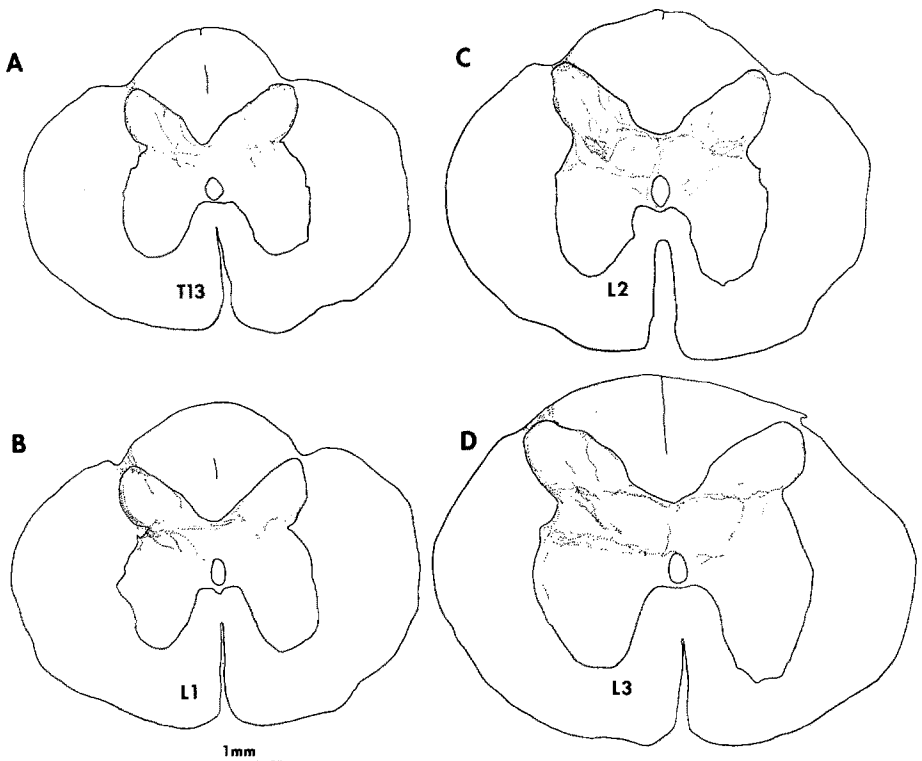


Fig. 4A–D. Camera lucida drawings of terminals of renal afferent fibres retrogradely labelled by the application of HRP to the central cut end of renal nerves in one kitten. Each drawing consists of data from 5–12 transverse sections of the spinal cord at segments T13–L3. (From Kuo et al. 1983)

the more limited degeneration studies of Schimert (1939) and Szentagothai (1948), Petras and Cummings (1972) performed dorsal-root rhizotomies at T4–T8, L1 and L3 segments in the rhesus monkey. Using the Nauta and Fink-Heimer methods, they could find no evidence of degenerating terminals on identified preganglionic cells in the ILp, ILf or IC. The route taken by these afferents immediately on entering the spinal cord is still therefore something of a mystery. The organisation of the thoracic and upper lumbar dorsal horn is only just beginning to undergo detailed investigation (Cervero and Connell 1984; Ammons 1986; Kuo et al. 1983), and the extent of our knowledge is well exemplified in Fig. 4, showing the HRP-filled terminations from renal afferent fibres found in the study by Kuo et al. (1983). Taking account of the limited material from this recent work, together with the more extensive data concerning the organisation of the dorsal horn in the lower lumbar cord, some explanations for the organisation of sympathetic reflexes can be obtained.

On entering the dorsal horn, the small somatic and visceral afferent fibres may synapse directly with neurons in the marginal zone, the tract of Lissauer

(Earle 1952; Kumazawa and Perl 1976; Ranson 1913; Schimert 1939; Szentagothai 1964; Kuo et al. 1983), the fibres of which travel one to two segements before entering the subjacent grey matter. A group of dorsal-root afferent fibres also synapses with cells in the IMM located near the central canal. There is dense degeneration of terminals in this nucleus following dorsal-root rhizotomy (Poljak 1924; Bok 1928; Petras and Cummings 1972). Renal afferent fibres also terminate here (Kuo et al. 1983) and cells in the region of the IMM can be activated by electrical stimulation of afferents in the inferior cardiac nerve (McCall et al. 1977). The axons of cells in the IMM project laterally and divide close to the ILp to send branches cranially and caudally. Evidence concering the synaptic connections made by these terminals is lacking.

In the lower lumbar cord somatic afferent fibres also synapse with cells in the substantia gelatinosa (Kumazawa and Perl 1976), from which fibres pass back into the tract of Lissauer, where they run longitudinally, making synaptic contacts for up to six segments (Szentagothai 1964). There is as yet no evidence that afferent pathways involved in autonomic reflexes have such an organisation. However, if it were so this would be one route enabling the afferent volley to reach up to sex segments on either side of the segmental input, a feature of somato-sympathetic and viscero-sympathetic reflexes (Coote et al. 1969; Franz et al. 1966; Koizumi et al. 1968; Sato and Schmidt 1971; Sato et al. 1965).

The afferent volley may also enter another proprio-spinal system through synapses with cells in laminae IV, V and VI of the dorsal horn, which contribute fibres to a dorso-lateral system ascending and descending via short relays of some two segments (Kerr 1975). Axons of cells in these laminae may be connected to sympathetic preganglionic neurons in the same segment, but as yet there is no information about how this occurs. Cells of laminae V und VI are particularly interesting because a single cell here may receive inputs from small cutaneous fibres and muscle afferents as well as small visceral afferent fibres (Pomeranz et al. 1968; Selzer and Spencer 1969; Fields et al. 1970a; Petras and Cummings 1972; Foreman et al. 1977; Light and Perl 1979; Kuo et al. 1983; Cervero and Connell 1984), the only cells of the dorsal horn too do so, and it is these small visceral afferents which are largely involved in activation of sympathetic reflexes. Many cells of these laminae have radially orientated dendrites confined to the transverse plane (Schiebel and Schiebel 1968; Szentagothai and Rethelyi 1973) and there is a similar arrangement of the presynaptic axons enabling the interneurons to 'collect' the activity of a large number of incoming fibres (Rethelyi 1974); hence they have large receptive fields. Other cells have more restricted receptive fields (Brown 1976). We thus have the basis for discrete and non-specific reflexes, both of which occur in the sympathetic system.

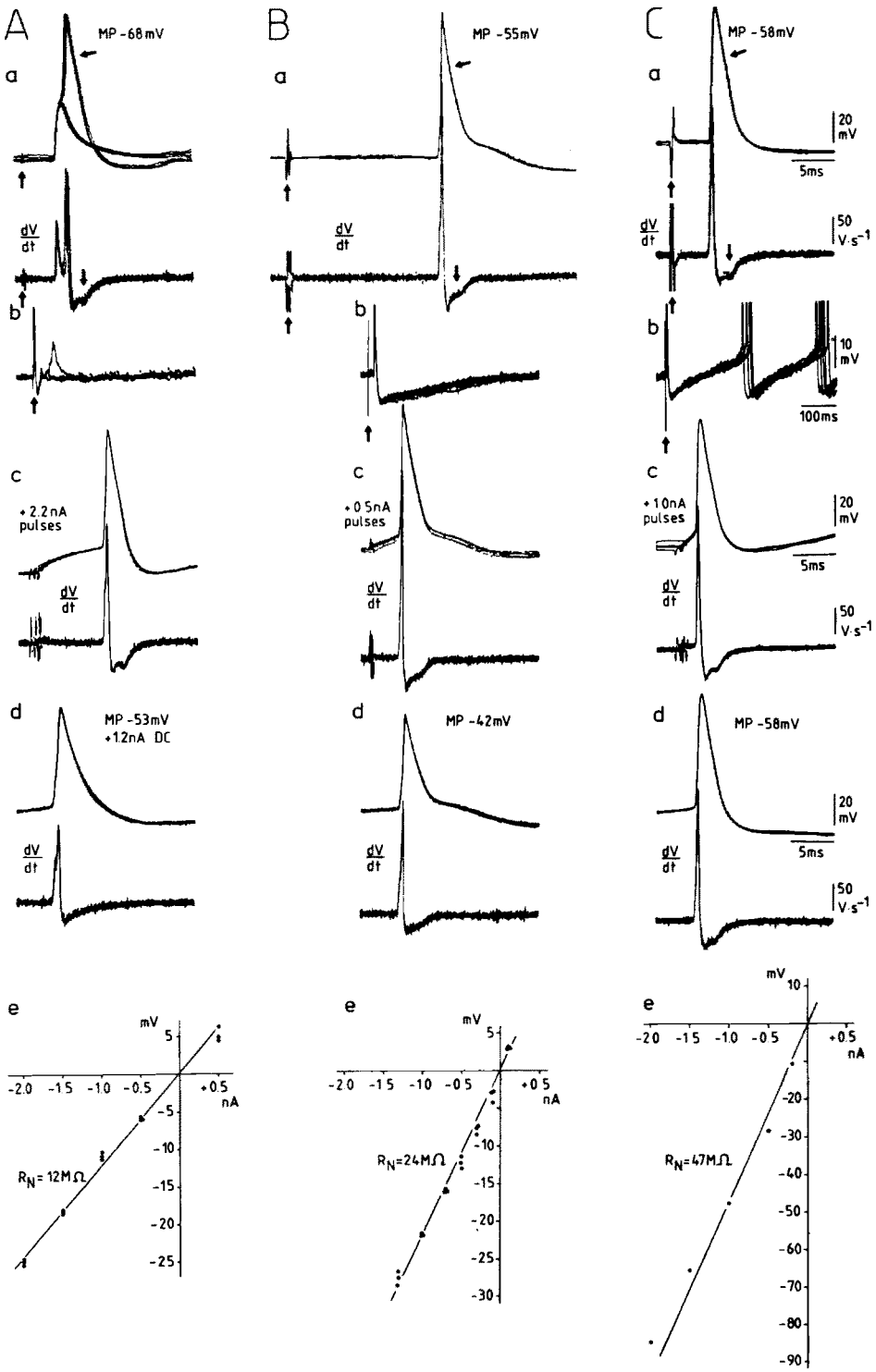
Much of the foregoing description has been acquired from studies which have not specifically addressed the organisation of sympathetic reflex arcs.

However, two recent investigations in which attention has been focused on sympathetic reno-renal reflexes provide important new data as well as confirming some of the above (see Kuo et al. 1983; Ammons 1986). Kuo et al. (1983, Fig. 4), using retrograde and transganglionic transport of horseradish peroxidase from the central cut ends of renal nerves in cats and kittens, showed that the central distribution of these afferents extends from T11 to L6, with greatest concentrations between L1 and L3. Afferents were traced from dorsal roots into Lissauer's tract, where they appeared to occupy the entire medio-lateral extent and were orientated in a rostro-caudal direction. Collateral projections of renal afferent fibres from the marginal zone of the dorsal horn extended along the lateral margins of the dorsal horn, spreading medially into laminae V and VI. Some projections extended towards the dorso-lateral grey matter near the central canal whilst, interestingly, others extended into the grey matter of lamina VII close to the ILp. There was also a distribution of collaterals, albeit less dense, along the medial border of the dorsal horn. A few projections crossed to laminae V, VI and VII of the contralateral grey matter (Kuo et al. 1983). Neurons responding to electrical stimulation of renal afferents have been recorded electrophysiologically in laminae V and VI, thus supporting the anatomical data (Ammons 1986).

In addition to their segmental contribution, cells of laminae IV and V contribute axons to ascending tracts in the ventral funiculus and antero-lateral fasciculus (Fields et al. 1970a, b; Fields and Winter 1970) and to the dorso-lateral funiculus (Hongo et al. 1968; Bryan et al. 1973; Brown 1976). This latter region is very important in the cat since it contains the main ascending afferent pathway for sympathetic reflexes mediated via medullary or supramedullary regions (Ranson and Billingsley 1916; Johansson 1962; Coote and Downman 1966; Chung et al. 1975; Chung and Wurster 1975, 1976; Chung et al. 1979a).

4 Electrophysiology

The behaviour of the sympathetic preganglionic neurons shows several differences from that of other neurons in the central nervous system, and consequently several studies have made intracellular recordings from them (Fig. 5) in order to seek an explanation of their special characteristics (Fernandez de Molena et al. 1965; Coote and Westbury 1979a; McLachlan and Hirst 1980; Yoshimura and Nishi 1982; Dembowsky et al. 1985a, 1986; Yoshimura et al. 1986b; Ma and Dun 1986).


A
MP -68mV
a
dV/dt
b
c
+2.2nA pulses
dV/dt
d
MP -53mV
+1.2nA DC
dV/dt
e
mV
5
-2.0 -1.5 -1.0 -0.5 +0.5 nA
-5
-10
-15
-20
-25
R_N = 12MΩ
B
MP -55mV
a
dV/dt
b
c
+0.5nA pulses
dV/dt
d
MP -42mV
dV/dt
e
mV
5
-2.0 -1.5 -1.0 -0.5 +0.5 nA
-10
-15
-20
-25
-30
R_N = 24MΩ
C
MP -58mV
a
20 mV
5ms
dV/dt
50 V·s^{-1}
b
10 mV
100ms
c
+1.0nA pulses
20 mV
5ms
dV/dt
50 V·s^{-1}
d
MP -58mV
20 mV
5ms
dV/dt
50 V·s^{-1}
e
mV
10
-2.0 -1.5 -1.0 -0.5 +0.5 nA
-20
-30
-40
-50
-60
-70
-80
-90
R_N = 47MΩ

4.1 Membrane Properties

Resting membrane potentials from −40 to −80 mV have been recorded. Even in sympathetic preganglionic neurons, that show a degree of synaptic activity, the highest membrane potentials lie in this range (Dembowsky et al. 1985a; Coote and Westbury 1979a). Membrane input resistances in the range of 12−120 MΩ have been measured with membrane time-constants of 4−26 ms (McLachlan and Hirst 1980; Yoshimura and Nishi 1982; Yoshimura et al. 1986b; Dembowsky et al. 1986; Ma and Dun 1986), which is in the range expected of small neurons.

The current-voltage relationship in these cells is linear with membrane potentials from +5 to −25 mV relative to the resting membrane potential. Some cells show a time-dependent rectification at membrane-potential values in excess of 25−30 mV below the resting membrane potential (Yoshimura et al. 1986b; Dembowsky et al. 1986; McLachlan and Hirst 1980). In many cells the rate of repolarisation following hyperpolarising pulses of more than 10 mV is slower than would be predicted from the measured time-constant of the membrane (Yoshimura et al. 1986b; Dembowsky et al. 1986; Fig. 6), suggesting there is a voltage-sensitive change in conductance reminiscent of the A current described in postganglionic neurons (Adams et al. 1982). The role such a current plays in action-potential repolarisation of these sympathetic preganglionic neurons has not been directly assessed, but since caesium, which is known to block potassium-ion (K+) channels in neuron membranes (Bezanilla and Armstrong 1972), also blocks the spike repolarisation in sympathetic preganglionic neurons, it appears likely that a voltage-sensitive K current is involved (Yoshimura et al. 1986b).

4.2 Action Potential

The action potential shows a rise-time which is slightly slower than that in alpha motoneurons but similar to that in postganglionic neurons (Blackman and Purves 1969). Inflections on the rising phase of the action potential occur, and it can be fractionated into two components, one similar to the initial-segment component and the other similar to the soma-dentritic component

Fig. 5A–C. Some intracellular characteristics of three sympathetic preganglionic neurons. Neurons in **B** and **C** were recorded in cats spinalised at C_3. **a** The resting membrane potential (MP) is given for each neuron. In **a**, **c** and **d**, the first derivative of action potential ($^{dv}/_{dt}$) is also illustrated; **a** shows superimposed sweeps of the antidromic action potential and $^{dv}/_{dt}$. The pronounced "shoulder" is indicated by *arrows*. **b** After-hyperpolarisation following the antidromic spike potential. **c** Action potentials evoked by direct stimulation with short depolarising current pulses. **d** Action potentials generated from depolarisation produced by injection of DC current (**A**), deterioration of recording (**B**) or ongoing synaptic activity (**C**) **e** Current-voltage relationship. R_N, neuronal input resistance. (From Dembowski et al. 1986)

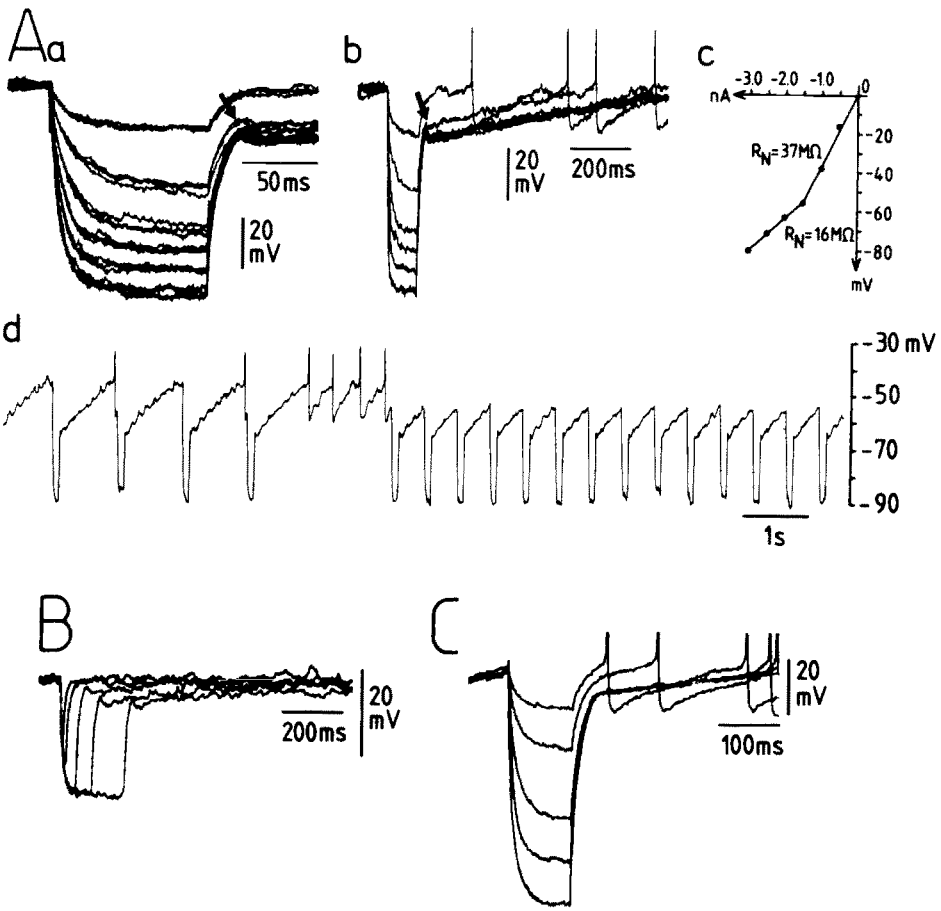


Fig. 6A–C. Inward rectification and evidence for presence of A current (I_A) in preganglionic neurons in spinal cat. **Aa, b** The amplitude of the deflection of steady-state voltage is markedly smaller with large than with small hyperpolarising current pulses (0.5 to 3.0 nA in steps of 0.5 nA). The voltage trajectory at the end of the 100-ms current pulses shows first a notch (*arrow*) and then a slow return to control level. **Ac** The corresponding plot of the current-voltage curve. **Ad** Membrane polarisation and smaller deflection of steady-state voltage following an increase in repetition rate (1 Hz–12 Hz) of the hyperpolarising pulses. Note that the input resistance during the after-hyperpolarisation is markedly reduced. **B** Voltage trajectory typical of an A current observed with long current pulses (2 nA). **C** Another neurone, showing that the discharge of the action potentials is delayed by the voltage trajectory at the end of increasing hyperpolarisation pulses (0.5–2.5 nA, 100 ms). R_N, neuronal input resistance. (From Dembowsky et al. 1986)

(Fernandez de Molena et al. 1965; Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1986; Yoshimura et al. 1986b) of alpha motoneurons (Brock et al. 1952).

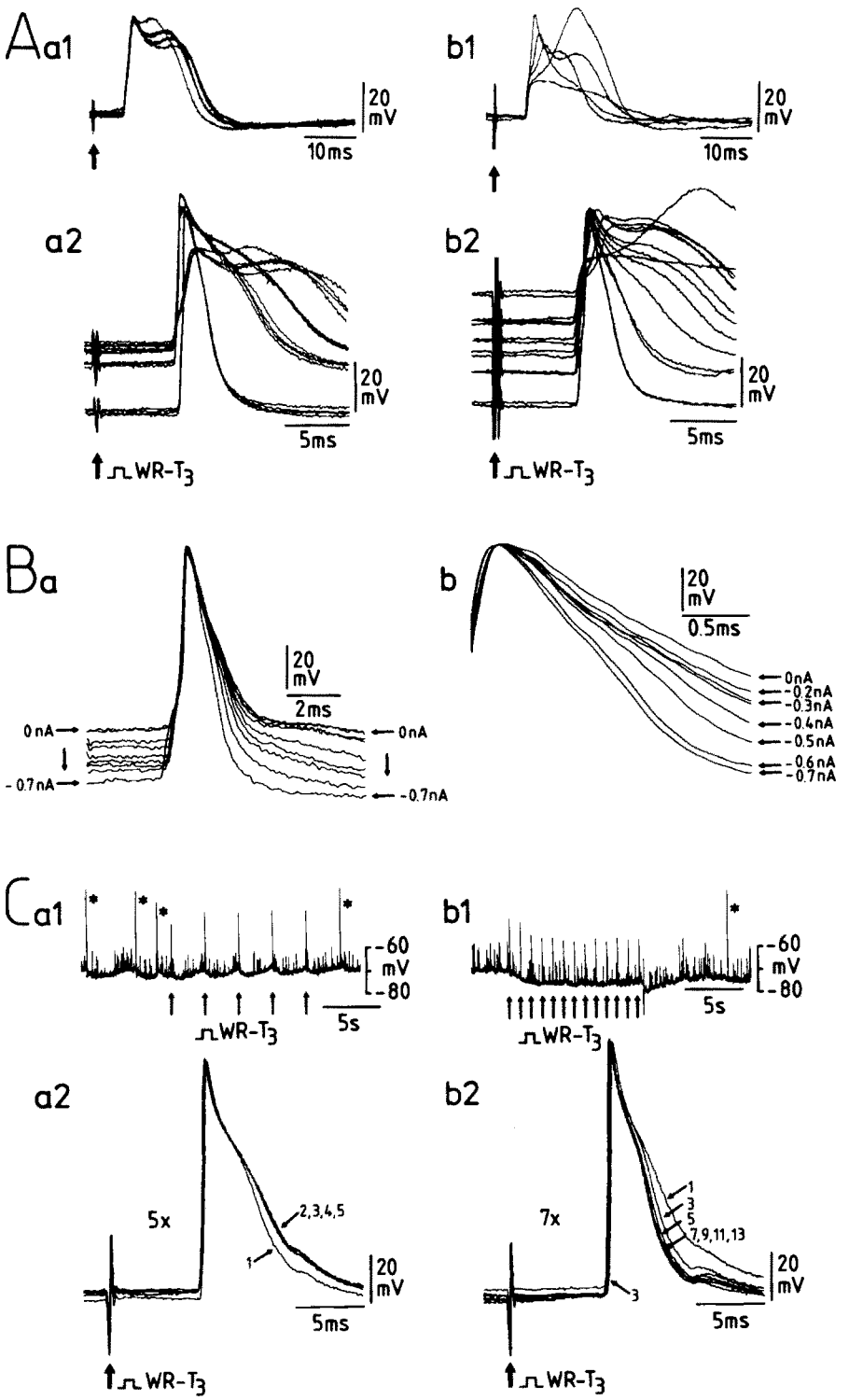
The results of in vitro experiments with channel blockers show that the sympathetic preganglionic neuron spike consists of a tetrodotoxin-sensitive component, presumably due to sodium (Na), and a tetrodotoxin-resistant but

cobalt-sensitive component, presumably due to calcium (Ca). The sympathetic preganglionic neurons, like many other central and peripheral neurons, thus seem capable of generating Ca action potentials in the absence of voltage-dependent Na conductance (Llinas and Hess 1976; Wong and Prince 1978; Gallego 1983; Harada and Takahashi 1983).

A striking feature of the action potential in sympathetic preganglionic neurons is the relatively slow repolarisation, resulting in a long spike duration (Fig. 7). In some neurons spike durations of 1.6–4.0 ms have been observed (Coote and Westbury 1979a; Yoshimura and Nishi 1982; Yoshimura et al. 1986b, c; Dembowsky et al. 1986; Ma and Dun 1986), whilst in others durations of 4–15 ms have been recorded (Fernandez de Molena et al. 1965; Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1986). In the latter neurons, repolarisation shows a prominent 'shoulder' or 'hump'. In vitro studies of the ionic basis for the action potential in identified sympathetic preganglionic neurons of cat spinal-cord slices showed that the hump disappeared and repolarisation was faster when Ca concentrations were low (0.25 mM) whereas it was slower and progressively broadened into a plateau when the voltage-dependent K conductance was blocked with tetraethyl-ammonium (10–20 mM) or caesium. These broad action potentials were significantly shortened by blocking Ca conductance with cobalt (Yoshimura et al. 1986b, c). There is therefore a clear ionic basis for the time course of repolarisation. This is in keeping with the observation of Dembowsky et al. (1986) that the rate of repolarisation is affected by changing the membrane potential with hyperpolarising currents; it confirms the conclusion of McLachlan and Hirst (1980) that this time course could not just result from electronic detection of activity in the dendrites, since these are few and their input resistance relative to the soma is small. The action potential is followed by a hyperpolarisation of up to 16.0 mV with duration ranging from as little as 40 ms (Fernandez de Molena et al. 1965; Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1986) to 4 s or longer (Yoshimura et al. 1986a, b, c; Dembowsky et al. 1986). The ionic basis of this hyperpolarisation is discussed in a later section.

In most sympathetic preganglionic neurons normal ongoing orthodromic synaptic activity takes the form of sub-threshold synaptic potentials. Excitatory postsynaptic potentials greater than 5 mV are required for an action potential to be discharged (Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1985a). No pacemaker-type potentials intrinsic to the neurons have been observed. Intermittent increases in membrane potential occur (Coote and Westbury 1979a; McLachlan and Hirst 1980) and evidence of inhibitory postsynaptic potentials has been provided (Dembowsky et al. 1985a; Yoshimura et al. 1986b).

Dembowsky et al. (1986) have proposed three basic types of sympathetic preganglionic neurons on the basis of passive and active electrophysiological


Aa1
20 mV
10ms
b1
20 mV
10ms
a2
20 mV
5ms
WR-T3
b2
20 mV
5ms
WR-T3
Ba
20 mV
2ms
0 nA
- 0.7 nA
0 nA
- 0.7 nA
b
20 mV
0.5ms
0 nA
- 0.2 nA
- 0.3 nA
- 0.4 nA
- 0.5 nA
- 0.6 nA
- 0.7 nA
Ca1
-60 mV -80
5s
WR-T3
b1
-60 mV -80
5s
WR-T3
a2
5x
2,3,4,5
1
20 mV
5ms
WR-T3
b2
7x
1
3
5
7,9,11,13
3
20 mV
5ms
WR-T3

properties. However, it remains to be established whether their classification has any meaning in terms of functional specificity or morphological differences.

4.3 Somatic and Visceral Afferent Influence

Electrophysiological studies have shown that somatic and visceral afferent fibres have a two-fold excitatory and inhibitory action on sympathetic activity; firstly, a more restricted and circumscribed action through spinal reflex pathways; secondly, a more generalised action involving supraspinal cell groups.

4.4 Excitatory Effects

Following single-shock stimulation of AB, A delta (A_d) and C fibres of cutaneous nerves or group-II, -III and -IV fibres of muscle nerves, at least four waves of reflex excitation in pre- and postganglionic sympathetic nerves have been described:

1. An early reflex with a central delay of 6–15 ms (Beacham and Perl 1964a; Coote and Downman 1966; Coote et al. 1969).
2. An intermediate reflex with a central delay of 20–30 ms (Foreman and Wurster 1975).
3. A late reflex with a central delay of 40–50 ms (Coote and Downman 1966).
4. A very late reflex with a central delay of 300 ms or more (Coote and Perez-Gonzalez 1970; Koizumi et al. 1970; Sato 1972a, 1973; Sato et al. 1969).

In a recent intracellular study, spinal-nerve-evoked excitatory postsynaptic potentials (EPSPs) in preganglionic neurons of the ILp were recorded as having latencies as follows: 2.7–7.5 ms consistent with the central delay of the early reflex; <28 ms, consistent with the intermediate reflex; and 27–55 ms, consistent with the late reflex (Dembowsky et al. 1985a).

Fig. 7A–C. Effects of membrane hyperpolarisation on the action-potential repolarisation of sympathetic preganglionic neurons recorded from the third thoracic segment of the spinal cord in the anaesthetised cat. **A** Antidromic action potential of the two preganglionic neurones (**a, b**) with a plateau-like depolarisation which was continuously reduced with membrane hyperpolarisation by negative DC current (**a2, b2**). **B** Increases in the rate of repolarisation caused by injection of negative DC current shown in two time scales (**a, b**). **C** Continuous records of membrane potential (**a1, b1**) and superimposed sweeps of the antidromic action potential (**a2, b2**) marked by *arrows* in **a1, b1**. The first antidromic action potential in **a2** (repetition rate 1/2.9 s), occurred during the after-hyperpolarisation of a preceding orthodromic spike; the rate of repolarisation is increased. **b** The antidromic repetition rate was increased to 1/0.9 s. The resultant hyperpolarisation due to summation of the after-hyperpolarisation caused a continuous increase in the rate of repolarisation (*1–13*), **Cb2.** (From Dembowsky et al. 1986)

The early reflex persists with unchanged central delay after cervical spinal-cord section, indicating that it is a spinal reflex (Coote and Downman 1965, 1966; Coote et al. 1969; Sato et al. 1967). All the early evidence and that from a recent intracellular study (Dembowsky et al. 1985a) strongly indicate that the spinal-reflex pathway is polysynaptic. However, according to Lebedev et al. (1976a, b), there is an early component which may represent activity evoked monosynaptically in sympathetic preganglionic neurons with axons conducting at greater than 10 m/s, which are located in the white matter lateral to the intermediolateral cell column (ILf). Unlike previous authors (Beacham and Perl 1964a; Coote and Downman 1965, 1966; Coote et al. 1969) Lebedev et al. (1976a, b) calculated a central delay of <1 ms for this early component, based on a decreased intraspinal conduction velocity of the preganglionic axon, which suggested to them that monosynaptic excitation could occur in a small group of preganglionic neurons. There is now clear evidence from in vitro studies using spinal-cord slices that such a slowing of conduction does occur in the sympathetic preganglionic neuron intraspinal pathway (Yoshimura et al. 1986b). Nonetheless, as emphasised earlier, there is still no anatomical evidence of monosynaptic connections between segmental afferents and sympathetic preganglionic neurons, even for those in the ILf (Szentagothai 1948; Petras and Cummings 1972; Rethelyi 1972, 1974).

The intermediate reflex may also be spinally mediated and, in view of the latency, must be dependent on quite long propriospinal pathways (Khayutin and Lukoshkova 1970; Foreman and Wurster 1975; Coote and Sato 1978; Laskey et al. 1979; Weaver et al. 1983).

The late reflex has been found by most investigators to depend on a pathway ascending to and descending from the brainstem (Sell et al. 1958; Sato et al. 1965; Coote and Downman 1966; Sato et al. 1967; Iwamura et al. 1969; Kirchner et al. 1970; Sato and Schmidt 1971). It is well to review the evidence for this in more detail since it has been claimed by Khayutin and Lukoshkova (1970) that the late reflex is primarily dependent on intraspinal pathways. Dembowsky et al. (1985a) observed that stimulation of spinal nerves evoked EPSPs with onset latencies corresponding to early, late and very late reflexes. In spinal cats EPSPs with similar latencies were evoked, but the early EPSPs were slightly shorter and present in all 42 sympathetic preganglionic neurons, whereas late and very late EPSPs were evoked in a markedly lower percentage of sympathetic preganglionic neurons than in the intact cat. Many of these EPSPs were sub-threshold and probably resulted from activity in multisegmental intraspinal pathways (Laskey et al. 1979), which would accord with the observation of Khayutin and Lukoshkova (1970) of small late-reflex responses in spinal animals. However, other evidence suggests that in the cat with an intact central nervous system this proprio-spinal volley may arrive at the preganglionic neurons coincidentally with a volley that is 'long-circuited' to and from the brainstem.

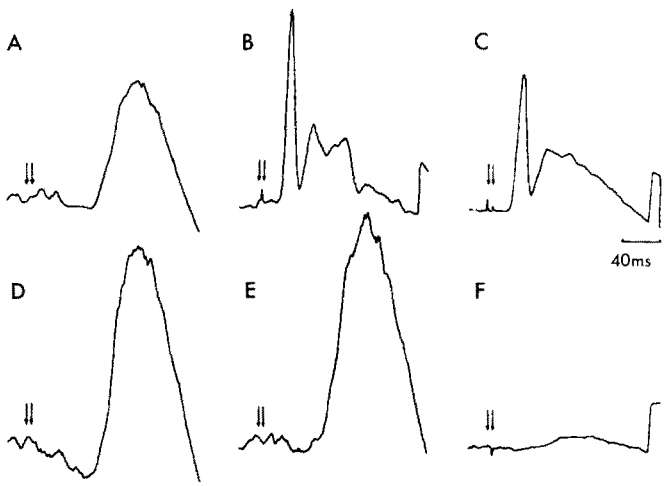


Fig. 8A–F. Reflex responses elicited in the inferior cardiac nerve following double stimulation (*arrows*) of 200 Hz, 0.5 ms duration and 10 V to third intercostal (**A, C**) and lateral popliteal (**D, F**) nerves. **A** and **D** were recorded following contralateral hemisection of the cervical (C_4) spinal cord and a small lesion in the dorsal part of the lateral funiculus on the ipsilateral side. **B** and **E** were recorded 10 min after further lesions at C_4 in the ventral part of the lateral funiculus and the anterior funiculus of the ipsilateral side. **C** and **F** were recorded 10 min after complete spinal-cord section at C4. Anaesthetised cat; amplitude calibration 200 μV. (From Coote and Sato 1978)

Coote and Downman (1966) showed that the central delay of late-reflex responses was always significantly less for cardiac sympathetic nerves than for renal nerves, no matter which of the cervical, thoracic or lumbar dorsal roots was stimulated. Hence, as pointed out originally by Sell et al. (1958), who studied the effects of stimulating fore- and hind-limb nerves, it appears that the latency of the reflex is dependent on the distance of the recording site from the medulla oblongata, not on how far away the afferent input is from the recording site. This is not a characteristic of the early reflexes. Furthermore, the late reflex virtually disappears on complete section of the cervical spinal cord (Fig. 8), and following a lesion restricted to the dorso-lateral funiculus of the cervical spinal cord, or on cooling the medulla oblongata some distance above the obex (Sell et al. 1958; Coote and Downman 1966; Foreman and Wurster 1973, 1975; Coote and Sato 1978). In these situations, the late reflex is replaced by an early reflex and later activity which is temporally dispersed and of low amplitude (Coote and Sato 1978).

Finally, there is a marked shortening of the latency of the spinal volley following transection of pathways in the ventro-lateral funiculus of the cervical spinal cord (Fig. 8; Coote and Sato 1978). Therefore it is most likely that the late high-amplitude reflexes, such a distinctive feature of the cat with an intact neuraxis, depend on facilitation by descending supraspinal pathways

reflexly activated by an ascending spino-medullary volley. This dependence of some sympathetic neurons on supraspinal structures is further emphasised by studies of the sympathetic reflex effects of adequate stimulation of cutaneous receptors. In a review of a thorough and elegant series of papers from the last 15 years, recently published (Janig 1985) in a previous issue of this journal, Janig and his collaborators examined the effects of spinalisation on reflex activity in postganglionic neurons of the lumbar-sympathetic trunk. Reflex discharges elicited by noxious stimulation of the skin were first seen in sudomotor and muscle vasoconstrictor neurons 7–14 days after spinalisation and were not seen until 4 weeks afterwards in cutaneous vasoconstrictor neurons. The recovery of sympathetic reflexes elicited by non-noxious stimulation of the skin (stimulation of hair-follicle receptors and Pacinian corpuscles) takes much longer. Up to 4–6 weeks is required for muscle vasoconstrictor neurons to recover, and up to 7–10 weeks for cutaneous vasoconstrictor neurons and sudomotor neurons (Horeyseck and Janig 1974a; Janig and Spilok 1978).

The very late reflex in the anaesthetised animal is elicited by unmyelinated afferent fibres (Sato et al. 1969; Coote and Perez-Gonzalez 1970; Koizumi et al. 1970; Whitwam et al. 1979). This reflex is mediated supraspinally, since it disappears following acute spinal-cord section (Coote and Downman 1966; Sato 1973). Long-latency reflexes evoked by unmyelinated afferents do reappear in the spinal animal, but not until some 8–12 weeks after spinal-cord section in the cat (Sato 1973), which suggests some growth of synapses is necessary for the mediation of these responses after the ascending and descending limbs of the reflex have been removed.

There is also another very late reflex response, obtainable on stimulation of the small myelinated fibres, which is present only under light chloralose anaesthesia and is mediated via suprapontine structures (Sato 1972a).

Visceral afferent fibres may also elicit spinal and supraspinal sympathetic reflexes. This again applies only to fibres with conduction velocities below 40 m/s, corresponding to A delta and C fibres (Koizumi and Suda 1963; Coote 1964; Kuno 1965; Franz et al. 1966; Fedina et al. 1966; Sato et al. 1969; Calaresu et al. 1978; Khayutin and Lukoshkova 1970; Rogenes 1982; Coote 1984; Szulczyk and Wilk 1985). These reflexes have many characteristics which are similar to those of the somato-sympathetic reflexes (Calaresu et al. 1978; Coote 1984; Szulczyk and Wild 1985). Interestingly, the central delay of spinal viscero-visceral reflexes is longer than that of somato-visceral reflexes (Coote et al. 1969; Franz et al. 1966). Recently EPSPs were recorded in sympathetic preganglionic neurons of the third thoracic segment following electrical stimulation of visceral afferent fibres in either a T3 or a T4 white ramus (Coote and Westbury 1979a; Dembowsky et al. 1985a). The shortest-latency EPSPs observed were 5.6–7.0 ms, which was some 0.6–2 ms longer than for a somatic afferent input, and does not favour the possibility of

monosynaptic connections between visceral afferents and sympathetic preganglionic neurons, at least at these levels of the spinal cord.

4.5 Participation of Various Sympathetic Preganglionic Neurons in Reflexes

It is quite evident from the work of Coote and Downman (1965, 1966) that not all sympathetic neurons can be excited sufficiently to discharge an action potential via each of the reflex pathways. For example, early spinal-reflex discharges are only rarely seen in cardiac and renal sympathetic nerves (Coote and Downman 1966; Coote and Sato 1978), whereas they are commonly observed in cervical sympathetic nerves (Schmidt and Schönfuss 1970), and in mesenteric nerves (Khayutin and Lukoshkova 1970; Koizumi and Suda 1963). They are also observed in a proportion of fibres in all of the white rami (Sato et al. 1965; Coote et al. 1969; Sato and Schmidt 1973) presumably because these contain a mixed population of axons with different end-organ destinations (Coote and Downman 1965). In a study examining the characteristics of individual units in the cervical sympathetic trunk, it was found that out of 534 units only about 30% responded to stimulation of a somatic afferent nerve (Janig and Schmidt 1970).

For those sympathetic neurons that could be reflexly activated to discharge an action potential in the anaesthetised cat, Sato (1972b) and Kaufman and Koizumi (1971) determined to what extent they served as a final common path for the four different somato-sympathetic reflexes. Only 8% showed all four types of reflex discharge; 18% responded only with an early reflex discharge; 34% showed early and late reflex discharges and 48% showed only late or very late reflex discharges. Fernandez de Molena et al. (1965) and De Groat and Ryall (1967) were unable to evoke action potentials by stimulation of somatic afferent nerves in any preganglionic neurons from which they recorded. Hongo and Ryall (1966) found reflexly evoked action potentials in a minority of preganglionic neurons in T3 and T4 segments. Recently, Coote and Westbury (1979b) observed that out of a total of 79 sympathetic preganglionic neurons tested in the third thoracic segment only 22 neurons discharged an action potential on supramaximal stimulation of the third intercostal nerve. Commonly, neurons that were reflexly activated were spontaneously active (Coote and Westbury 1979b; Janig and Schmidt 1970), but the reverse was not true.

Nonetheless, spinal segmental pathways onto most sympathetic preganglionic neurons do exist, since EPSPs related to the input can be recorded in them without discharge of an action potential (Coote and Westbury 1979a, b; Dembowsky et al. 1985a). In the intracellular study of Dembowsky et al. (1985a) many more sympathetic preganglionic neurons

were excited via each of the reflex pathways than is apparent from the single-fibre studies. It was observed in 28 sympathetic preganglionic neurons that early and late EPSPs were evoked in most neurons, and very late EPSPs were evoked in about 50% of cells. Coote and Westbury (1979a) showed that summation of the EPSPs to 4–5 mV was required to fire the cell. This suggests that the reflex firing of a sympathetic preganglionic neuron is dependent on the synchronicity of its excitatory inputs, i. e. the afferent volley must arrive at the neuron at a time when it can summate with other excitatory inputs.

These features of sympathetic preganglionic neurons are important because they show that central synapses in the somato-sympathetic reflex pathways differ in their potency, and that the majority of cells are dependent on a background level of excitation. The latter is most likely provided by supraspinal pathways in view of the lack of reflex discharge in acute spinal animals, despite EPSPs being evoked by segmental afferent stimulation (Dembowsky et al. 1985a).

A supraspinal inhibitory mechanism also plays an important part. As referred to earlier, reflex discharge into cardiac and renal sympathetic nerves is normally of the long-latency variety involving suprasegmental pathways, showing that cardiovascular neurons cannot normally be excited to discharge action potentials via spinal segmental afferent pathways (Kerr 1975; Koizumi and Sato 1972). Nonetheless, such pathways onto these neurons do exist, since spinal somatic afferent reflexes can be occasionally evoked in cardiac and renal nerves, both in animals with an intact neuraxis and after complete cord section (Coote and Downman 1966), and short-latency EPSPs are evoked in the majority of sympathetic preganglionic neurons in both preparations (Dembowsky et al. 1985a). This suggests that in the animal with an intact neuraxis, the potency of the spinal segmental pathway is regulated. This inhibitory regulation can be removed by sectioning a descending sympatho-inhibitory pathway in the ventral quadrant of the spinal cord (Coote and Sato 1978). It is still not clear onto which elements in the reflex pathway the descending control is exerted, but they are likely to be close to the preganglionic neuron, since both 'spontaneous' and reflexly driven discharges are tonically inhibited (Alexander 1946; Coote and Downman 1966, 1969; Coote et al. 1969; Coote and Macleod 1974a, b, 1975; Dembowsky et al. 1980). This selective application of inhibitory regulation to sympathetic preganglionic neurons and their reflex pathways has been further demonstrated by the recent experiments of Rogenes (1982), showing that the reflex response of renal efferent nerves to stimulation of renal chemoreceptors is increased following complete transection of the spinal cord.

Sudomotor reflexes have many features similar to the cardiovascular reflexes, and it is likely that, as in cardiovascular reflexes, electrical stimulation of most somatic afferent nerves normally excites sudomotor neurons only via a suprasegmental pathway (Wang 1964; Wang and Brown 1965a; Wang

et al. 1956). However, Janig and Rath (1977) have now shown that spinal sudomotor reflexes normally mediated by proprio-spinal pathways can be evoked by natural stimulation of receptors in the same limb (Pacinian corpuscles of the pads) in cats with an intact neuraxis. This opens up the possibility that there may be other local sympathetic reflexes that have hither to been obscured by the rather gross electrical stimulation of bundles of afferent nerve fibres.

Two types of sympathetic neuron, the pilomotor neurons and the vasodilator neurons to skeletal muscle, cannot be reflexly excited by somatic afferent fibres in the chloralose-anaesthetised cat (Abrahams et al. 1964; Grosse and Janig 1976), although in the unanaesthetised hypothalamic cat and in the althesin anaesthetised cat (Hilton and Marshall 1982) both piloerection and vasodilatation in skeletal muscle occur as part of a defence reaction in response to stimulation of peripheral receptors (Abrahams et al. 1964). It is likely, therefore, that a suprasegmental pathway mediates this response in these neurons.

Spinal sympathetic reflexes are best represented by the sympathetic outflow to the gastrointestinal tract, in which they are easily elicited by stretch of the viscera or stimulation of mesenteric nerves in the animal with an intact neuraxis (Johansson et al. 1968; Johansson and Langston 1964), pinching of the skin over the abdomen (Sato et al. 1975; Sato and Terui 1976), or by mechanical stimulation of the mucosa and skin of the anus and perianal region and distension of the urinary bladder and colon (Bartel et al. 1986).

These different features of the somatic afferent pathways onto functionally different preganglionic sympathetic neurons suggest a high degree of organisation of the somato-sympathetic and viscero-sympathetic reflexes in the spinal cord.

4.6 Facilitation in the Somato-Sympathetic Reflex Pathway

Marked facilitation of somato-sympathetic reflexes can be produced in the cat with an intact neuraxis, but this is confined to the late and very late reflexes. Repetitive stimulation of the small myelinated afferent fibres at 100–300 Hz augments the reflex discharge recorded from either pre- or postganglionic sympathetic nerve bundles by 200%–300% (Coote and Perez-Gonzalez 1970; Sato and Schmidt 1971; Schmidt and Schönfuss 1970). Such stimulation not only increases the number of neurons firing but also their firing rate (Sato 1972b). The unmyelinated afferent fibres are much more powerful in producing temporal facilitation. The very late group-IV or C-fibre sympathetic reflexes can best be elicited by the application of stimuli to afferent nerves at intervals of less than 8 s, down to 1 s (Fedina et al. 1966; Sato and Schmidt 1966). Under these conditions each consecutive unmyelinated afferent volley

recruits greater numbers of sympathetic units until after about 10–20 stimuli a new stable level is obtained. Such recruitment and facilitation has been demonstrated in renal nerves (Coote and Perez-Gonzalez 1970; Fedina et al. 1966; Koizumi et al. 1970) in lumbar white rami (Sato 1973), in the cervical and lumar sympathetic trunk (Schmidt and Weller 1970) and in sympathetic preganglionic units in muscle and cutaneous nerves (Janig et al. 1972; Koizumi and Sato 1972).

Facilitation seems to be absent or very weak in the spinal animal (Beacham and Perl 1964; Coote et al. 1969; Sato 1972c). This led Beacham and Perl (1964b) to suggest that in the spinal cat the afferent volley excites all the neurons in the sympathetic pool with little subliminal fringe. There was some evidence for this, since following a conditioning volley there was 90% – 100% occlusion of a second test somato-sympathetic reflex. However, we now know that in the acute spinal animal there are many sympathetic preganglionic neurons that cannot be caused to discharge by the segmental afferent volley. Therefore, the interpretation of this observation of Beacham and Perl (1964) must be limited to a specific group of sympathetic preganglionic neurons, and implies that for those neurons, which are especially affected by the spinal segmental pathway, the synaptic connections have a high potency. Recent work on somato-sympathetic reflexes in isolated spinal-cord preparations of the frog reveal more marked spatial facilitation of sympathetic neurons in this animal (Undesser et al. 1981), although in other characteristics the reflexes appear very similar to those observed in mammals.

In cardiac sympathetic neurons the lack of facilitation may be caused by a supraspinal, tonically active inhibitory pathway. A lesion of the ventrolateral quadrant of the cervical spinal cord sparing descending sympatho-excitatory pathways allows marked facilitation (28%) of a spinal reflex occurring in the inferior cardiac nerve in response to repetitive stimulation of the third intercostal nerve (Coote and Sato 1978).

4.7 Inhibitory Action

Stimulation of somatic afferent fibres can inhibit sympathetic activity via spinal and supraspinal pathways. The majority of afferent fibres which may produce sympathetic inhibition are small myelinated fibres of groups II and III or A and B fibres, mostly with conduction velocities above 20 m/s. Numerous experiments have attempted to distinguish these fibres from those eliciting excitatory effects and have encountered difficulties because when stimulating with electrical shocks the threshold of the two sorts of afferent fibres overlaps considerably. What has usually been described, therefore, is a sympathetic reflex followed by a depression of excitability, or 'silent period', lasting 1 s or more (Schmidt and Schönfuss 1970). However, inhibition

without prior excitation has been reported in renal sympathetic nerves (Coote and Perez-Gonzalez 1970), in sympathetic preganglionic neurons of the third thoracic segment of the spinal cord (Wyszogrodski and Polosa 1973) and in postganglionic sympathetic units in muscle nerves (Koizumi and Sato 1972). The latter, however, could have resulted from a summation of after-hyperpolarisation following EPSPs which might even have been below the threshold for discharging an action potential, as described by Dembowsky et al. (1985a). This suggestion finds some support, in that a silent period is present following activity evoked by electrical stimulation in the medulla oblongata (Coote and Downman 1969a, b; Schramm et al. 1979) and following stimulation of the descending tracts in the spinal cord after a complete cord section (Schramm et al. 1979), although the latter effect was not observed for the cardiac sympathetic outflow by Coote and Sato (1978).

From the data presented by Wyszogrodski and Polosa (1973), a mechanism involving summation of after-hyperpolarisation seems unlikely. These authors showed that afferent volleys could even prevent discharge in sympathetic preganglionic neurons that were intensely excited with glutamate. A similar finding was reported by McKenna and Schramm (1985) who recorded from preganglionic neurons in the isolated spinal cord of neonatal rats. These effects were most probably due to inhibitory postsynaptic potentials like those elicited by stimulation of segmental afferent nerves or descending inhibitory pathways in the recent study of Dembowsky et al. (1985a).

Unmyelinated group IV fibres and afferent C fibres can also inhibit sympathetic neurons, but they are very selective. Thus, Janig et al. (1972) reported that group-IV afferents to muscle and C fibres to skin could inhibit a high proportion of postganglionic sympathetic units suppling the skin, but not those supplying muscle. Sato (1972c) showed that early (spinal) reflexes in lumbar white rami were not as profoundly depressed by these afferent fibres as were late (medullary) reflexes.

Such specificity is characteristic of other afferent inputs. For example, the activity in cutaneous vasoconstrictor neurons of the lumbar sympathetic outflow is inhibited by nociceptors and warmth receptors of the skin and viscera as well as vibration mechanoreceptors of the foot pad, whereas activity in muscle vasoconstrictors is only inhibited by cutaneous mechanoreceptors. Sudomotor neurons do not appear to receive any spinal inhibitory inputs (Horeyseck and Janig 1974a, b; Gregor et al. 1976; Grosse and Janig 1976; Janig and Kummel 1977, 1981; Janig 1979).

The potency of this reflex inhibition of the sympathetic preganglionic neurons is greatly dependent on a pathway ascending to and descending from the brainstem, since its duration and magnitude are greatly reduced in acute and chronic spinal animals (Beacham and Perl 1964b; Coote et al. 1969; Coote and Perez-Gonzalez 1970; Kirchner et al. 1971, 1975a; Sato 1972c; Sato et al. 1969; Wyszogrodski and Polosa 1973). The main inhibitory action

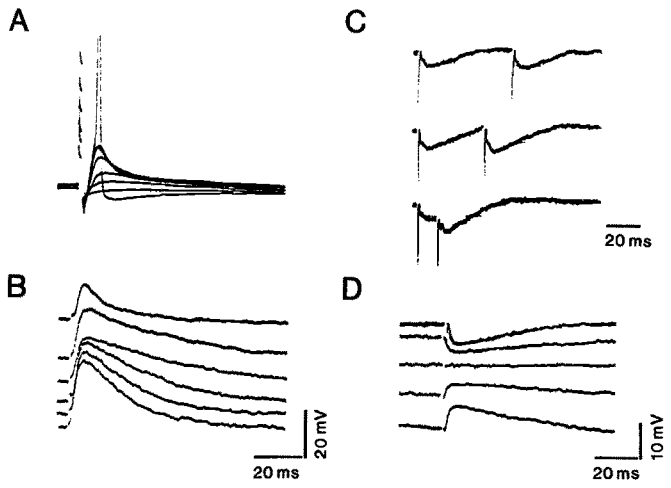


Fig. 9A–D. Properties of synaptic potentials evoked in antidromically identified sympathetic preganlionic neurons in spinal-cord slice (T3 segment) of cat spinal cord in vitro. Data are from four different cells. **A** Excitatory postsynaptic potentials (EPSPs) evoked by stimulation in dorsal horn with gradually increasing strength until an action potential was elicited. **B** Hyperpolarisation of cell membrane causes an increase in amplitude of EPSPs evoked stimulation of the dorsal horn. **C** Inhibitory postsynaptic potentials (IPSPs) evoked by stimulation of dorsal horn. Summation of successive IPSPs occurs when double shocks are given at appropriate intervals. **D** IPSP reversal by membrane hyperpolarisation with injected negative DC current. (From Yoshimura et al. 1986a)

is probably occurring at the spinal level, because (for example) both the late medullary reflex and the early spinal reflex that has occasionally been recorded in cardiac and renal sympathetic nerves are inhibited by it (Coote and Sato 1978).

Inhibitory activity taking place directly on the membrane of sympathetic preganglionic neurons is therefore very likely to occur. This was clearly shown in a study of the cat spinal cord in vivo by Dembowsky et al. (1985a). Other work using thoracic spinal-cord slices has established the existence of this activity beyond all doubt (Fig. 9). Clear synaptically evoked IPSPs that could be reversed on current injection were recorded intracellularly in sympathetic preganglionic neurons in spinal-cord slices of adult cat (Yoshimura et al. 1986a).

In the spinal animal the inhibitory effects seem to be most pronounced at the same segmental level as the somatic afferent input (Beacham and Perl 1964b; Coote et al. 1969; Sato 1972c). However, there are long proprio-spinal inhibitory pathways, since some inhibition of sympathetic activity in the spinal cat can be produced by stimulation of the limb nerves via inputs remote from the sympathetic outflow (Kaufman et al. 1977; Koizumi and Sato 1972; Sato 1972c; Gilbey 1980; Laskey et al. 1979; Kirchner et al. 1975b). Interestingly, quite long-lasting inhibition of sympathetic activity has been demonstrated in the isolated frog spinal cord (Undesser et al. 1981).

4.8 After Hyperpolarisation

Following the discharge of an action potential in a sympathetic preganglionic neuron there is a depression of excitability or 'silent period' (Mannard et al. 1977), which is associated with a hyperpolarisation lasting some 40–500 ms (Fernandez de Molena et al. 1965; Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1985a). This has also been observed in recordings from sympathetic preganglionic neurons in the isolated hemisected spinal cord of the rat (McKenna and Schramm 1985) and is considerably longer (2.5 s) in preganglionic neurons recorded in adult cat spinal cord in vitro (Yoshimura et al. 1986a). A preceding EPSP or action potential is necessary for the manifestation of the depression. It therefore seems most likely that the silent period is due to intrinsic properties of the sympathetic neuron rather than to a synaptic event such as postsynaptic inhibition (Dembowsky et al. 1985a). This conclusion is supported by extracellular studies of sympathetic preganglionic neurons which showed that there is an increase in the axon-soma delay and a decrease in the height of a test antidromic spike for up to 300 ms after a conditioning antidromic spike (Polosa 1968). Also, repetitive antidromic activation is capable of inhibiting glutamate-evoked activity in sympathetic preganglionic neurons (McKenna and Schramm 1985).

The cellular mechanisms underlying the post-impulse depression are now becoming clear. Studies of the after-hyperpolarisation in intracellular recordings of sympathetic preganglionic neurons in vitro show that it is nullified at a membrane potential close to the K-equilibrium potential, reversibly abolished by reducing the amount of Ca in the medium, and blocked by adding cobalt (Co) and barium (Ba) to the medium, all of which suggest that a Ca-dependent increase in K conductance is the underlying mechanism (Fig. 10; Yoshimura and Nishi 1982; Yoshimura et al. 1986a, c). Thus, the sympathetic preganglionic neuron is similar to the lumbar alpha motoneuron (Gustafsson 1974) in that an increase in K conductance is considered to be the cause of after-hyperpolarisation. However, as pointed out by Polosa et al. (1982), it seems unlikely that the prolonged (10–30 s) silent periods reported by some authors (Mannard et al. 1977; McKenna and Schramm 1985) following repetitive antidromic stimulation at 10–40 Hz result from simple summation of post-impulse changes in conductance lasting 500 ms. It has been suggested therefore that these long lasting changes may be due to an additional mechanism extrinsic to the preganglionic neurons, rather like Renshaw inhibition (see later), since they can be produced by ventral-root stimuli that are below the threshold for antidromically discharging a preganglionic neuron (McKenna and Schramm 1985).

Whatever the mechanism, the effect is to make it difficult for preganglionic neurons to discharge at high frequency for any length of time (Pitts and

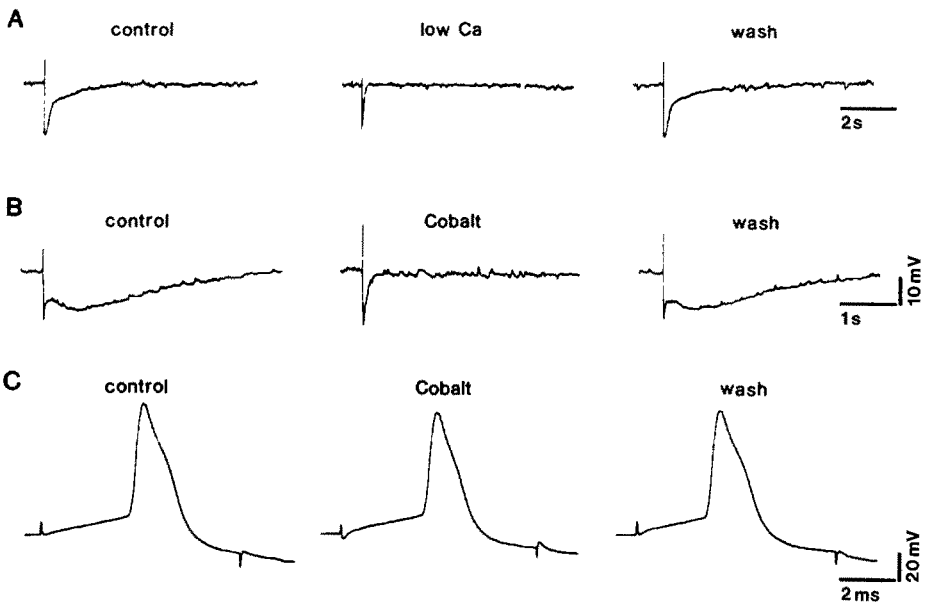


Fig. 10A–C. Records from sympathetic preganglionic neurons antidromically identified in spinal-cord slice (T3 segment) of cat in vitro. Effects of superfusion with Krebs solution containing 0.25 mM Ca (**A**) or 2 mM Co (**B**) on the after-hyperpolarisation. Records were taken from two different cells. **C** Effect of Co on the action potential shown in **B**, shown here on a faster time base. Intracellular stimulation, 2-ms pulses. (From Yoshimura et al. 1986c)

Bronk 1942), and thus to contribute to a stabilisation of their firing frequency within a range of low values.

4.9 Recurrent Inhibition

Another possible cause of prolonged depression of sympathetic preganglionic neurons is that they may be part of a recurrent inhibitory circuit of the type described for alpha motoneurons (Renshaw 1941; Eccles et al. 1954) and for sacral parasympathetic neurons (DeGroat 1976). It has been pointed out by Lebedev et al. (1980) and by Gebber and Barman (1979), that the minimum interstimulus interval at which a full response to a second stimulus occurs without fractionating into initial segment (IS) and soma dendritic (SD) components extends over a wide range, 2–100 ms (see also Fernandez de Molena et al. 1965; Coote and Westbury 1979a). Two broad groups of neurons were identified by Lebedev et al. (1980): a large group that could generate an action potential at an interval of less than 15 ms, the fractionation of the antidromic potential being dependent on refractory mechanisms in the soma (Fernandez de Molena et al. 1965; Coote and Westbury 1979a), and a smaller group that could discharge antidromically only with long intervals between stimuli, rang-

ing from 15 to 100 ms. Since this was so much longer than the refractory period of these neurons, recurrent inhibition was considered a possibility.

Using a similar approach to that of Renshaw (1941), Lebedev et al. (1980) split the T3 or L2 white ramus into two halves and antidromically stimulated the preganglionic axons of one half and their hypothetical collaterals. The other half of the white ramus was used for recording the mass discharge of the remaining preganglionic neurons in response to a stimulus to the central cut ends of the T3 or L2 dorsal root or the intercostal segmental nerve or pathways in the lateral funiculus. Thus, a conditioning antidromic volley could not directly involve the neurons in the other half of the pool that were tested with an orthodromic volley. This eliminated refractoriness as a factor when interpreting the observed effects. The orthodromic discharge of one half of the ramus was depressed by antidromic stimulation of the other half at 20–30 ms, and the inhibitory effect was abolished by administration of intravenous strychnine. Unfortunately, the existence of a cholinergic step in the inhibitory mechanism, which might be expected on the basis of Dale's principle (Eccles 1964) [although this is not without exception (Burnstock 1983)], was not tested in this case. This test seems necessary in order to firmly establish a causal link between the observed inhibition and hypothetical collaterals of preganglionic axons.

A slightly different experiment was performed by Mannard et al. (1977). A filament containing a single tonically active preganglionic neuron was isolated from the cervical sympathetic trunk and the remainder of the nerve stimulated antidromically. Single-shock or repetitive antidromic stimulation of the cervical sympathetic nerve did not influence the firing of the single unit. A similar finding was reported by McKenna and Schramm (1985) whilst recording antidromic action potentials in sympathetic preganglionic neurons and stimulating bundles split from ventral roots in isolated hemisected spinal cord of neonatal rat. However, much of the latter data did suggest that an inhibitory mechanism extrinsic to preganglionic neurons is activated by antidromic activation. McKenna and Schramm showed that elevation of stimulus intensity beyond that necessary to evoke an antidromic spike in preganglionic neurons led to prolongation of the inhibition. This inhibition was not, however, influenced by cholinergic antagonists.

Further support for recurrent inhibitions has come from a study by Gebber and Barman (1979). The soma-dendritic spike of a neuron in segment T3 with a myelinated axon elicited by the second of a pair of antidromic shocks was blocked when the strength of the conditioning shock was increased to intensities which stimulated unmyelinated preganglionic axons. This inhibitory effect was potentiated by the anticholinesterase drug physostigmine given intravenously and attenuated but not abolished by the cholinergic nicotine antagonist dihydro-β-erythroidine given intravenously. These observations of Barman and Gebber (1979) are consistent with an arrangement whereby pre-

ganglionic neurons with unmyelinated axons inhibit those with myelinated axons. The hypothesis, that such an arrangement exists was partially tested by Polosa (1968), but without success.

There is still no further evidence of a cholinergic inhibitory influence on sympathetic preganglionic neurons. Acetylcholine applied iontophoretically in the vicinity of a limited number of preganglionic neurons had little effect on their discharge rate (Coote et al. 1981a). Cholinergic effects have been recorded in preganglionic neurons of adult cat spinal cord slices in vitro (Yoshimura and Nishi 1982), but these effects were excitatory. Furthermore, the existence of recurrent inhibition is not supported by most of the data so far obtained from intracellular recordings (Fernandez de Molena et al. 1965; Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1985a). These show no evidence of hyperpolarisation of the cell membrane (IPSP) when the preganglionic axon bundle containing the axons of the neurons under study was stimulated with intensity below the threshold for the axon of the cell but sufficient to excite some of the other preganglionic axons in the bundle. We should be cautious in making too broad an interpretation from such studies, since only a small sample of neurons is examined in anyone experiment and there is undoubted a bias towards those cells that are most easily and successfully penetrated.

A further worrying feature worth repeating is that no collateral branches have yet been described along the intraspinal course of the preganglionic axons (Rethelyi 1972; Dembowsky et al. 1985b). However, it may be argued that there is no reason to suppose that the effects observed by Lebedev et al. (1980) and Gebber and Barman (1979) require the same anatomical substrate as described for recurrent inhibition of alpha motoneurons.

Gebber and Barman (1979) have suggested that the inhibitory interactions between preganglionic neurons could be mediated by dendro-dendritic connections. The clustering of preganglionic neurons and the close apposition of dendrites, together with the very striking acetylcholinesterase-positive network of fibres between the sympathetic sub-nuclei of the thoracic spinal cord (Silver and Wolstecroft 1971; Galabov and Davidoff 1976) may be significant and relevant to future explanations of this phenomenon.

The experiments of Lebedev et al. (1980), Gebber and Barman (1979) and McKenna and Schramm (1985) still represent the best tests so far of the hypothesis that there is inhibitory interaction between sympathetic preganglionic neurons. It is also worth noting that some extrinsic mechanisms other than after-hyperpolarisation is necessary to explain the naturally low following frequency of sympathetic preganglionic neurons, since intracellular recording from these neurons in vitro shows they can be intracellularly activated at high rates of discharge (Yoshimura et al. 1986a).

5 Thoracic Spinal Interneurons

There is no good anatomical or electrophysiological evidence that somatic and visceral afferent fibres synapse directly on preganglionic neurons in each of the main sympathetic sub-nuclei in the spinal cord (Schimert 1939; Szentagothai 1948; Beacham and Perl 1964a; Franz et al. 1966; Coote and Downman 1966; Coote et al. 1969; Rethelyi 1972, 1974; Petras and Cummings 1972; Coote and Westbury 1979a; Dembowsky et al. 1985a). There must therefore be an interneuron interposed in the spinal-reflex pathways. The only current candidates for this role are neurons in the intermediomedial nucleus (IMM) of the thoracic cord, which in mammals lies just lateral and ventral to the central canal (Petras and Cummings 1972; Massazza 1923a, b, 1924; Laruelle 1937; Bok 1928). The IMM cells send axons towards the ILp (Bok 1928; Petras and Cummings 1972), and dense terminal degeneration occurs close to these cells following dorsal-root rhizotomy (Petras and Cummings 1972). In addition recent HRP-tracing studies have shown that visceral afferents terminate on cells in the IMM (Kuo et al. 1983). Electrophysiological confirmation of these anatomical studies was provided by McCall et al. (1977) who showed that units in the IMM could be excited by both brainstem stimulation and stimulation of visceral afferent fibres in the inferior cardiac nerve.

By contrast, many spinally projecting brainstem neurons involved in sympathetic regulation have terminals in one or another of the sympathetic subnuclei; these often lie close to the dendrites and soma of preganglionic neurons (see earlier section). In the case of the 5-hydroxytryptaminergic (5-HT) projections, axo-somatic and axo-dendritic synapses onto preganglionic neurons have recently been identified (Bacon and Smith 1986). The number of supraspinal afferents that make monosynaptic connections with preganglionic neurons is at present unclear. The intracellular records of Dembowsky et al. (1985a), whilst showing some monosynaptic EPSPs in reponse to stimulation of supraspinal afferents, showed that most EPSPs had a variable latency of onset, suggesting that at least one synapse was interposed.

Gebber and McCall (1976) claimed to have identified sympathetic interneurons, although their evidence is somewhat equivocal. They used the method of post-R-wave time-interval analysis to determine whether non-antidromically activated units in the ILp discharge in phase with the R-wave and similarly to – but leading the discharge of – antidromically activated units. Fifteen such units were studied and it was established that they had a positive post-R-wave relationship. These few units were all that were found in 21 cats and after searching five thoracic spinal-cord segments, suggesting that such units are few in number. Additionally, the only direct evidence that these neurons were interneurons was that they were not antidromically excited by stimulation of the cervical sympathetic nerve. This method would only have

identified a proportion of preganglionic neurons in any of the segments, since considerable numbers synapse in the stellate ganglion and project to thoracic organs (Oldfield and McLachlan 1981; Pardini and Wurster 1984). Nonetheless, the discharge pattern of the 'non-antidromically' activated cells was distinctly different from that of the antidromically activated neurons. They discharged spontaneously in bursts with short interspike intervals (<20 ms). Although this is rather low, there is now evidence from two laboratories that a 'bursting' type of discharge is relatively common amongst preganglionic neurons. Providing there is sufficient central inspiratory activity, interspike intervals can certainly range as low as 25 ms (Preiss et al. 1975; Preiss and Polosa 1977; Polosa et al. 1980; Gilbey et al. 1986). Gebber and McCall (1976) pioneered this field, but more studies of this sort are required to make their results convincing. A recent paper by Barman and Gebber (1984) has gone some way towards eliminating earlier problems. After vagotomy and denervation of the aortic sinus in cats, spontaneous activity of the inferior cardiac nerve was correlated with antidromically identified preganglionic neurons in the third thoracic segment and with non-antidromically excitable units in the vicinity of the ILp. All displayed a 2–6 Hz rhythm and in response to medullary stimulation were excited with nearly identical latencies of onset. However, evidence that the two types of neuron in the ILp are synaptically connected is still lacking.

In a further study (McCall et al. 1977), a search was made for spinal-cord neurons that could be excited by stimulation of the arterial baroreceptors, it being argued by the authors that such a demonstration should identify them as interneurons in the baroreceptor reflex arc. Unfortunately, the effect of bilateral carotid occlusion was tested, but not the effect of a pressure rise in an isolated carotid sinus. Consequently, although 29 units in the region of the IMM stopped discharging, there was no test for direct synaptic input from the arterial baroreceptors. Since sympatho-inhibitory inputs from cardiac, thoracic and intestinal receptors have all been described, and would all have been stimulated by the pressor response following carotid occlusion, it cannot be ruled out that activation of these receptors inhibited units in the IMM (Sarnoff and Yamada 1959; Heymans et al. 1936; Pagani et al. 1974; Malliani et al. 1981). The identification of baroreceptor spinal interneurons would have been more convincing if the histograms of the time interval after the R-wave had been similar for each unit. In fact, 10 of the 19 units which were examined in detail had a discharge correlated with the R-wave, but the histograms were of three types: multimodal with four or more peaks, in three neurons, indicating that the input could be only indirectly related to the baroreceptor input; unimodal in five neurons (Fig. 11); and bimodal in two neurons. McCall et al. (1977) therefore concluded that the pattern of R-wave locking of unit discharge in the medial regions of the spinal grey matter was not solely dependent on the level of baroreceptor input.

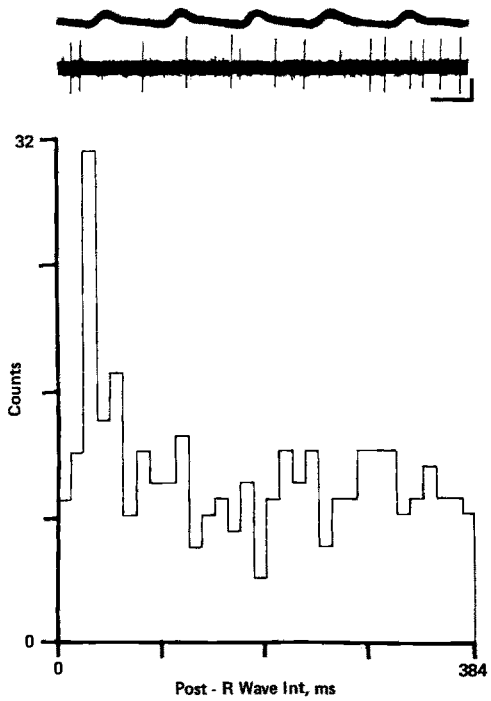


Fig. 11. Unimodal post-R-wave time-interval histogram of a spinal unit recorded extracellularly in segments T1–T4 of cat spinal cord. The number of computer sweeps was 250. Address bin 9 ms, oscillographic traces of blood pressure (145/95 mmHg) and unitary discharge are shown *above histogram.* Horizontal calibration, 200 ms; vertical calibration, 100 μV. (From McCall et al. 1977)

McCall et al. (1977) also recorded from units in the nucleus tractus solitarius (NTS). Such units showed post-R-wave time-interval histogram patterns of the multimodal and unimodal types but the post-R-wave peaks were later than those generated by the spinal units, which is puzzling if the authors are suggesting that the activity of the NTS units is partly responsible for the activity of the spinal units. Indeed, if the pathway is a direct one of the sort McCall et al. (1977) demonstrated by stimulating a depressor site in the NTS, some 8 or more milliseconds should be added to the activity peak of the spinal units. It is therefore still not clear whether the cells in the IMM recorded by McCall et al. (1977) really are interneurons in the pathway through which baroreceptor inhibition of sympathetic preganglionic neurons occurs. The key pieces of information that are missing are whether or not the spinal neurons recorded by McCall et al. (1977) can be excited on baroreceptor activation and whether or not they project onto sympathetic preganglionic neurons. Nevertheless, it cannot be denied that neurons in the ventrolateral nucleus of the NTS with relatively rapidly conducting axons (15 m/s) project to the spinal cord. This has also been shown by Lipski and Trzebski (1975) and by Trzebski et al. (1975). It is possible that these are axons of R_B inspiratory neurons which also project to the spinal cord (Nakayama and von Baumgarten 1964; Bianchi and Barillot 1975; Lipski and Trzebski 1975). The R_B neurons are excited by pulmonary inflation, which is known to inhibit

some sympathetic preganglionic neurons, and it has been tentatively suggested that the R_B neurons are involved in this inhibitory action (Lipski et al. 1977).

6 Spinal Afferent Input and Specific Types of Preganglionic Neuron

Important functional connections exist between spinal afferent fibres from particular kinds of visceral or somatic receptors and cardiovascular neurons. The innervation of the kidney, heart, hind-limb skin and tail skin are well-studied examples of this. Electrical stimulation of some renal afferent fibres may cause an increase in blood pressure (Calaresu et al. 1976; Stella et al. 1984); stimulation of other renal afferent fibres may cause a decrease in blood pressure (Aars and Akre 1970; Ueda et al. 1967) and affect activity in renal sympathetic efferent fibres, hence the term 'reno-renal reflexes' (Astrom and Crafoord 1968; Aars and Akre 1970; Calaresu et al. 1978; Simon and Schramm 1984). Similar reflexes have been obtained by more-natural activation of receptor endings within the kidney (Beacham and Kunze 1969; Fransisco et al. 1980; Kostreva et al. 1981; Recordati et al. 1982; Rogenes 1982; Katholi et al. 1983; Smits and Brody 1984; Gilmore and Tomamatsu 1985). Two classes of renal receptors have been identified in cat, and are probably connected with cells in the dorsal horn of segments T11–L4 via A and C fibres (Kuo et al. 1983; Ammons 1986). Renal mechanoreceptors activated by renal-vein occlusion (Astrom and Crafoord 1967, 1968; Beacham and Kunze 1969; Kostreva et al. 1981; Uchida et al. 1971), ureteral occlusion (Astrom and Crafoord 1967; Beacham and Kunze 1971), ureteral occlusion (Astrom and Crafoord 1967; Beacham and Kunze 1969; Fransisco et al. 1980) and increased renal perfusion pressure (Astrom and Crafoord 1968; Nijima 1971) have been identified. In addition, renal chemoreceptors responding to renal ischaemia induced by prolonged reductions in renal blood flow, and others responding to changes in their chemical environment induced by back-flow of non-diuretic urine into the renal pelvis (Fig. 12), have been documented (Recordati et al. 1978, 1980; Rogenes 1982). The latter may well encompass some receptors previously classified as mechanoreceptors (Recordati et al. 1980; Rogenes 1982).

Studies in dog and rat have provided strong evidence that renal reflexes are of physiological importance. Denervation of one kidney results in an increase in efferent activity of the renal nerve to the contralateral intact kidney, together with a decrease in urine flow and Na excretion (Colindres et al. 1980; DiBona and Rios 1980). Apparently some species differences exist, since in dog stimulation of renal mechanoreceptors elicits an increase in contralateral efferent renal-nerve activity and renal vasoconstriction, whereas in rat there

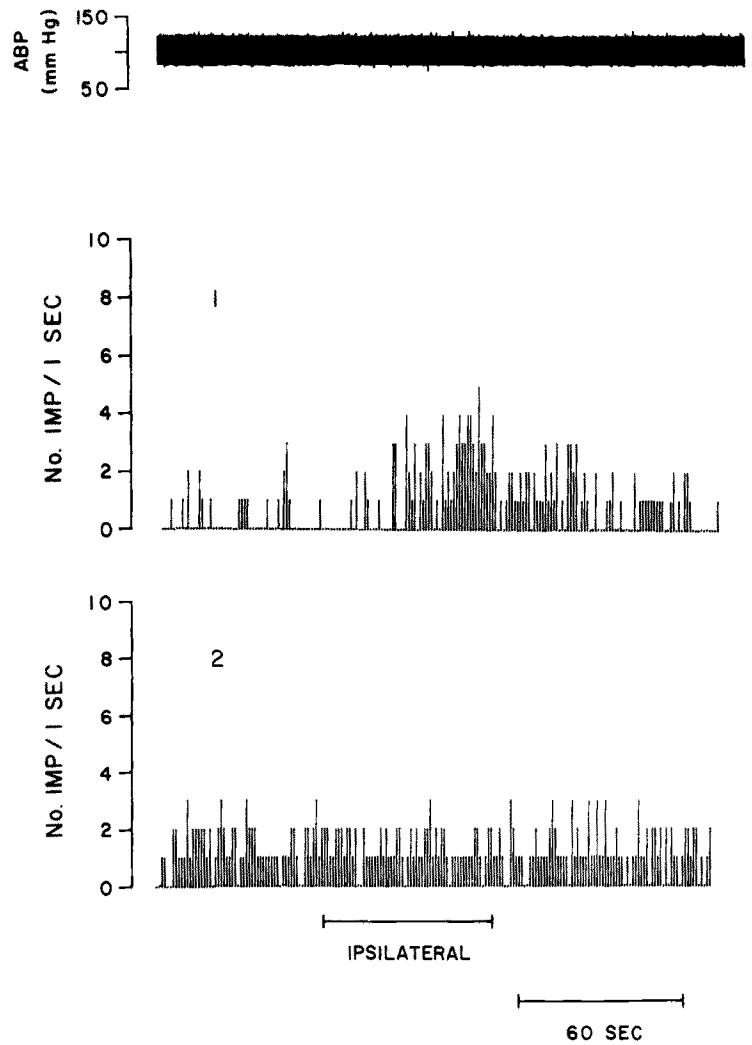


Fig. 12. Spike counts of two single units recorded simultaneously from a single filament dissected from a renal nerve bundle in anaesthetised rat. Responses are shown during stimulation of the ipsilateral chemoreceptors (*bar*) in the kidney by increasing the back-flow of urine (60 µl) into the renal pelvis at a pressure of 22 mmHg. *Upper trace,* arterial blood pressure (ABP); *lower tracees* show spike counts in efferent renal nerves (1 and 2) expressed as number of impulses per second. (From Rogenes 1982)

is a decrease in efferent renal-nerve activity associated with a diuresis and natriuresis (Kopp et al. 1984).

Both spinal and supraspinal pathways are involved in these reno-renal reflexes (Calaresu et al. 1978; Kopp et al. 1985). Apparently though, at least in the rat, spinal segmental pathways are most important in the response of renal efferent nerves to renal chemoreceptor stimulation since the response is

increased following transection of the spinal cord at C3 (Rogenes 1982), suggesting also that these spinal pathways are under tonic supraspinal inhibitory control.

Reflexes initiated by renal receptors are not, however, restricted to the kidneys (Calaresu et al. 1976, 1978; Stella et al. 1984; Aars and Akre 1970; Ueda et al. 1967). Renal mechanoreceptor stimulation also decreases the activity of sympathetic efferent nerves to the heart, in association with a decrease in contractile force of the right ventricle (Kostreva et al. 1981).

Recent evidence suggests, however, that synaptic potency is often maximal between afferents and sympathetic efferents of the same organ. Stimulation of mesenteric afferent nerves by serosal application of bradykinin causes greater excitation of mesenteric sympathetic nerves than of renal or splenic sympathetic nerves (Stein et al. 1986). Additionally, chemical stimulation of splenic receptors causes greater excitation of activity in splenic sympathetic nerves than in renal sympathetic nerves (Calaresu et al. 1984).

There are afferents running in the cardiac sympathetic nerves to the spinal cord that can evoke spinal reflexes in the same (cardiac sympathetic) nerves (Armour 1973; Malliani et al. 1981). Afferent fibres from the heart and aorta, stimulated by increasing pressure, hypoxia or chemical stimulation, have converging and opposite influences on single preganglionic neurons of the upper thoracic region (Pagani et al. 1974). Fibres from the heart increase preganglionic neuronal activity, and those from the aorta decrease activity. Stimulation of similar receptors by stretch of the ventricles or aorta increases activity in renal sympathetic fibres (Weaver 1977; Weaver et al. 1979). Although at first sight this suggests that the aortic mechanoreceptors have reciprocal influences on cardiothoracic and renal outflow, it is by no means clear that they do, because simultaneous recordings have not been made from cardiac and renal efferents. Increases in pulmonary vascular pressure can inhibit renal sympathetic activity via cardiothoracic spinal afferents (Weaver et al. 1979).

The cardiac, aortic and pulmonary receptors eliciting these spinal reflexes possibly participate in tonic control of the cardiovascular system. Apparently, stimulation of these receptors in conscious dogs does not produce pain. Homoeostasis is maintained by interaction with supraspinal inhibitory regulating mechanisms (Malliani et al. 1983).

Substantial information about the relationship between specific afferent inputs and different types of sympathetic preganglionic neurons is also available from the exhaustive studies of Janig and his collaborators on the lumbar sympathetic outflow in the cat (Janig 1985). Non-noxious stimulation of the skin anywhere on the body produces an increase in firing of sympathetic postganglionic units supplying the hind-limb cutaneous vascular bed, but a decrease in the firing of sympathetic postganglionic units supplying the vascular bed of hind-limb muscle. By contrast, noxious stimulation of the skin

produces the opposite effects on the two sympathetic efferents (Horeyseck and Janig 1974a, b). Noxious mechanical stimulation of the toes of a hind foot produces an inhibition of sympathetic activity confined to the cutaneous vasoconstrictor units of the same limb. Similar stimulation of the contralateral foot or fore-limb has little effect (Horeyseck and Janig 1974b). Noxious stimulation of the tail skin inhibits vasoconstrictor units to the same skin region, whereas similar stimulation of the skin elsewhere on the body excites the cutaneous vasoconstrictor units of the tail (Grosse and Janig 1976).

This principle of selective regulation is seen in other sympathetic cardiovascular outflows. Stimulation of cutaneous warmth receptors leads to a decrease in sympathetic vasoconstrictor activity to skin and an increase in vasoconstrictor activity to the gastrointestinal tract. Stimulation of cold receptors leads to the opposite effects (Riedel et al. 1972). Cardiac sympathetic neurons are excited by activation of both types of temperature receptor (Kaufman et al. 1977). Non-noxious stimulation of visceral receptors in the urinary bladder and colon by distension, and of receptors in the anus and vagina by mechanical stimulation, leads to excitation of muscle vasoconstrictor neurons but inhibition of cutaneous vasoconstrictor neurons. Interestingly, visceral (splanchnic) vasoconstrictor neurons are not greatly affected by these stimuli (Bahr et al. 1986a–c). If bladder afferents are activated electrically or by distension of the bladder, one group of units in the cervical sympathetic nerve is excited whilst another group of units is inhibited (Schondorf et al. 1983).

These characteristic patterns of reaction are partially preserved in acute and chronic spinal animals. However, significant changes do occur, suggesting that supraspinal influences as well as propriospinal pathways are important for their normal manifestation (Janig 1985; Schondorf et al. 1983; Calaresu et al. 1984).

7 Central Inputs

Both inhibitory and excitatory pathways descend from the brainstem in the lateral and ventral funiculi (Coote and Macleod 1974a, 1984).

7.1 Inhibitory Pathways

The sympatho-inhibitory pathways lie in two main regions, one in the dorsal part of the lateral funiculus (Illert and Gabriel 1972; Coote and Macleod 1972, 1974a, 1975; Henry and Calaresu 1974a; Lipski and Trzebski 1975) and the other just lateral to the ventral horn (Eh and Huan-Ji 1964; Illert and Seller 1969; Illert and Gabriel 1972; Coote and Macleod 1974a, 1975;

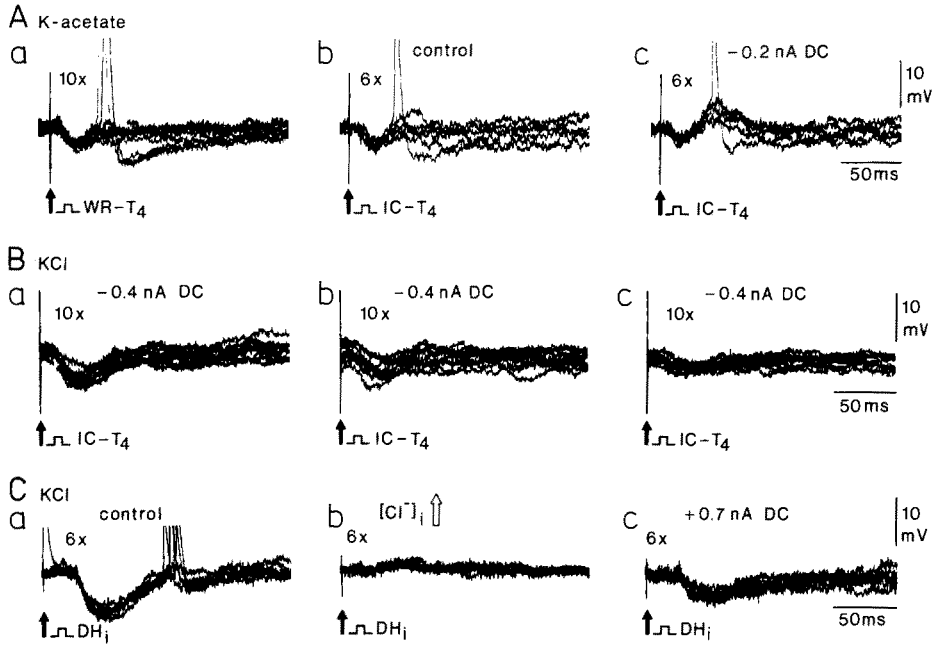


Fig. 13A–C. Identification of IPSPs evoked in three different antidromically identified sympathetic preganglionic neurons in the third thoracic segment of the spinal cord in anaesthetised cat. The neuron in **A** was recorded using a micro-electrodes filled with potassium acetate, whereas in **B** and **C** micro-electrode filled with potassium chloride were used. **Aa–c** IPSPs preceding an action potential, produced in **a** by stimulation of the fourth thoracic white ramus ($WR\text{-}T_4$) and in **b** and **c** by stimulation of the fourth intercostal nerve ($IC\text{-}T_4$). In **c**, injection of negative DC current reduced the size of the IPSP compared to **b**. **Ba–c** IPSPs evoked by stimulation of the fourth intercostal nerve, showing the effect of progressive injection (**b**, **c**) of chloride ions (Cl^-) into the cell by negative DC current (−0.4 nA). **Ca–c** IPSPs evoked by stimulation in the dorso-lateral funiculus (Ph_i). **a** Control IPSP. **b** Effect of passive diffusion of Cl^- into cell. **c** Subsequent injection of positive DC current (+0.7 nA). The shifts in the membrane potential due to current injection have not been reproduced. (From Dembowsky et al. 1985)

Dorokhova et al. 1974; Henry and Calaresu 1974a). Coote and Macleod (1974a, 1975) have shown that inhibition evoked by stimulating points in the dorso-lateral region has a characteristic long latency to onset, whereas that arising from stimulating ventral points has a short latency to onset. Several pieces of evidence favour the idea that the latency of these inhibitory effects is not a consequence of long polysynaptic intersegmental or intrasegmental connections. Firstly, the responses are all or none, the dorso-lateral effects appearing at high stimulus currents and the ventral effects appearing at lower stimulus currents. Secondly, there was no shortening of latency with either an increase in the stimulus current or an increase in the number of shocks at high frequency. Thirdly, stimulation of brainstem inhibitory sites, whose efficacy is dependent on the integrity of either the dorso-lateral or the ventral region,

elicited inhibition of latencies that were remarkably consistent with a slowly conducting pathway (1–2.5 m/s) or a more rapidly conducting pathway (20 m/s) and identical to those calculated for each of the spinal-cord regions (Coote and Macleod 1975). Whether any of these pathways terminates directly on sympathetic preganglionic neurons is unresolved, but there are early indications that some might (see later). Recently, Dembowsky et al. (1985 a) recorded IPSPs of relatively constant latency in two preganglionic neurons following stimulation of a dorso-lateral descending inhibitory pathway. They calculated conduction velocities of 2.2 and 2.6 m/s (Fig. 13).

7.2 Excitatory Pathways

Most authors agree that the reticulo-spinal excitatory fibres to sympathetic preganglionic neurons descend in the dorsal part of the lateral funiculus (where they seemingly intermingle with sympatho-inhibitory 'axons') and in the medial parts of the lateral funiculus of the cervical spinal cord (Fig. 14;

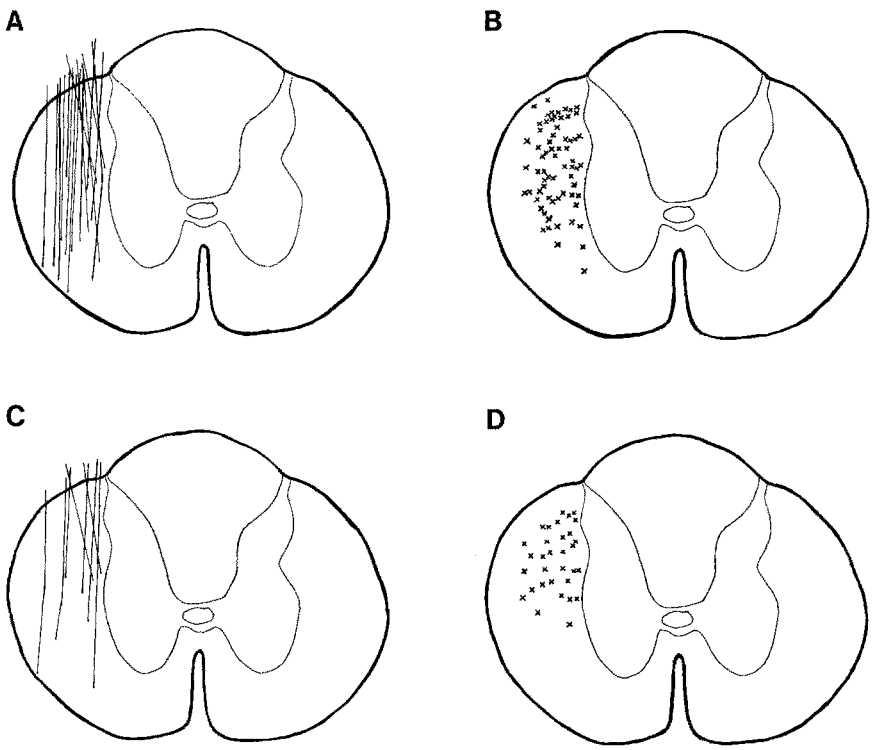


Fig. 14A–D. Transverse sections of cat spinal cord at the fourth cervical segment. **a** Electrode tracks explored in five animals; **b** Sites (*x*) from which responses were evoked in third thoracic white ramus. **c** Electrode tracks in three cats in which recordings were also made from the tenth thoracic white ramus. **d** Sites (*x*) from which responses were evoked in the tenth thoracic white ramus. (From Coote and Macleod 1984)

Kell and Hoff 1952; Cicardo and Garcia 1958; Kerr and Alexander 1964; Coote and Downman 1966; Illert and Seller 1969; Illert and Gabriel 1972; Foreman and Wurster 1973; Coote and Macleod 1974a; Henry and Calaresu 1974a). If the rather approximate estimates of conduction velocity are assumed to be correct, the fibres contributing to these descending tracts in the cat spinal cord are likely to be small myelinated ones. The majority of reports give estimates of 3–7 m/s based on measurements of the overall latency of sympathetic responses evoked either by stimulation of the brainstem and spinal cord (Coote and Downman 1966; Gootman and Cohen 1971; Kirchner 1971; Illert and Gabriel 1972; Foreman and Wurster 1973; Gebber et al. 1973; Seller 1973; Taylor and Gebber 1973; Coote and Macleod 1974a; Neumayr et al. 1974; Szulczyk and Trzebski 1979) or by stimulation of somatic and visceral afferent fibres (Kell and Hoff 1952; DeGroat and Lalley 1974; Janig and Szulczyk 1979). Most of these investigations did not take into account the possibility that the measured latency of responses following activation of descending axons was increased by delay in long chains of interneurons at the segmental level. In the few cases in which an attempt was made to overcome this problem (Gootman and Cohen 1971; Foreman and Wurster 1973) no detailed search of the spinal cord was performed, so faster pathways could have been missed.

These criticisms are particularly relevant since Henry and Calaresu (1974a–c), in a series of papers, concluded that bulbo-spinal cardiac sympatho-excitatory axons have a high conduction velocity. They reported a mean of 60 m/s following either antidromic activation of reticulo-spinal neurons of presumably cardiac dedication or orthodromic activation of presumed sympathetic units in the thoracic spinal cord on stimulation of certain sites in the medulla. The question was reinvestigated in a recent study of cats. The lateral white matter of the cervical spinal cord and regions of the medulla were systematically explored for sympatho-excitatory points whilst recording from two sympathetic outflows simultaneously. Regions that were a suitable distance apart were chosen, in order to give a clear difference in latency so that the velocity of axonal conduction between the two outflows could be calculated precisely. In addition, in a few cases, conduction velocity was also measured by determining the latency shift of evoked responses resulting from moving the stimulating electrode a known distance closer to the recording site. These two procedures showed sympatho-excitatory axons in the spinal cord to have conduction velocities of 1.6–7.9 m/s (Fig. 15; Coote and Macleod 1984). A recent intracellular study calculated a similar range of conduction velocities for descending axons in the dorso-lateral funiculus. When stimulated, these axons evoked EPSPs in preganglionic neurons of the third thoracic segment; most conduction velocities were from less than 1.0 m/s to 10 m/s with a few faster than this, in one neuron the conduction velocity was 25 m/s (Dembowsky et al. 1985a). None of the faster group of fibres were able to evoke EPSPs large enough to discharge cells.

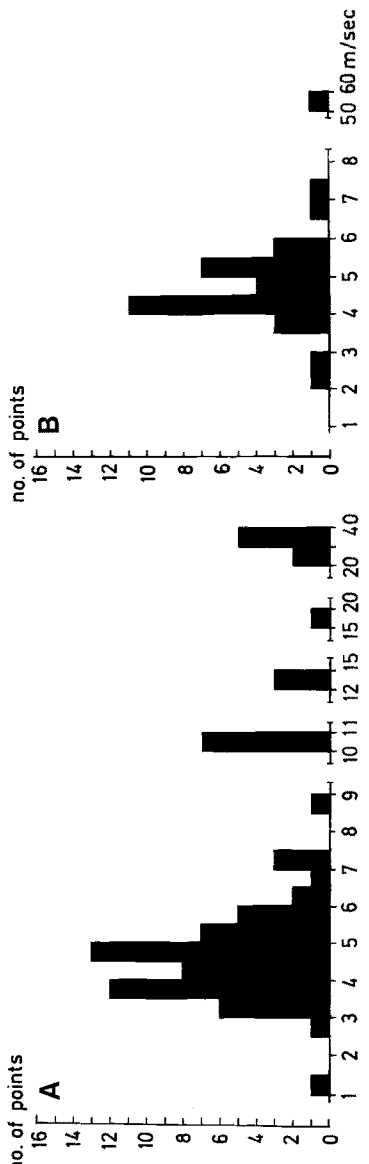


Fig. 15. A, B Conduction velocity of descending excitatory pathway for (**A**) 60 points in fourth cervical segment (C4) evoking responses in the third thoracic white ramus (T3WR) and (**B**) 32 points in C4 evoking responses in tenth thoracic white ramus (T10WR). **C** Conduction velocity between T3WR and T10WR for 21 points in spinal cord. (**D** Conduction velocity between T3WR and T10WR for stimulation of sites in the medulla. (From Coote and Macleod 1984)

This range of conduction velocities places the sympatho-excitatory fibres in a rather select group of reticulo-spinal axons. Earlier observations have indicated that the range of conduction velocities in myelinated reticulo-spinal fibres originating from the medulla is 11–150 m/s (Wolstencroft 1964; Wilson and Peterson 1981). It seems that these fibres innervate limb motoneurons or respiratory motoneurons (Grillner and Lund 1968; Lund and Pompeiano 1968; Grillner et al. 1970; Bianchi 1971; Peterson et al. 1975, 1979; Hilaire and Monteau 1976). It is unclear how much reliance can be placed on the failure to find slower fibres in these studies. However, a recent investigation was designed to test precisely the extent to which different spinal-cord motoneurons are innervated by different types of descending excitatory axons (Khimonidi et al. 1980). It was concluded that descending fibres conducting at 4.0–8.9 m/s specifically activate only sympathetic vasomotor neurons. Respiratory motoneurons and sacral parasympathetic preganglionic neurons were activated by different groups of descending fibres with greater conduction velocities. It therefore seems probable that the slowly conducting bulbo-spinal fibres form a specific and functionally dedicated group.

7.3 Numbers of Synapses

The electrophysiological experiments described have provided little evidence of monosynaptic inputs to preganglionic neurons. A calculation based on fairly precise estimates of conduction velocity for some 21 points between T3 and T10 enabled Coote and Macleod (1984) to determine the total time involved in conduction from a stimulating site in the cervical spinal cord to the segment of origin of the preganglionic neurons. This time was compared to the difference between the experimentally determined overall latency to the segment and the latency from the ventral root to the recording site. The difference should reflect synaptic delay and conduction along the intraspinal part of the preganglionic axon. The segmental delay so calculated was 1.7 (SK 0.6) ms for T10 and 1.6 (SD 0.5) ms for T3. Since this also includes time for conduction along the preganglionic axon before it leaves the cord, it does suggest that only one or, at the most, two synapses are involved. The possibility that monosynaptic connections are made by at least some descending excitatory pathways is given more support by the observations of Dembowsky et al. (1985a) that in a few preganglionic neurons EPSPs with a constant latency were evoked on repetitive stimulation of a descending tract in the dorso-lateral funiculus (Fig. 16). Nonetheless, in most of their experiments, EPSPs of rather variable latency occurred, which might suggest that pathways with more than one synapse are more common. However, monosynaptic connections made by terminals of brainstem neurons are more in keeping with the anatomical data. Many terminals in the ILp and in the more medial sym-

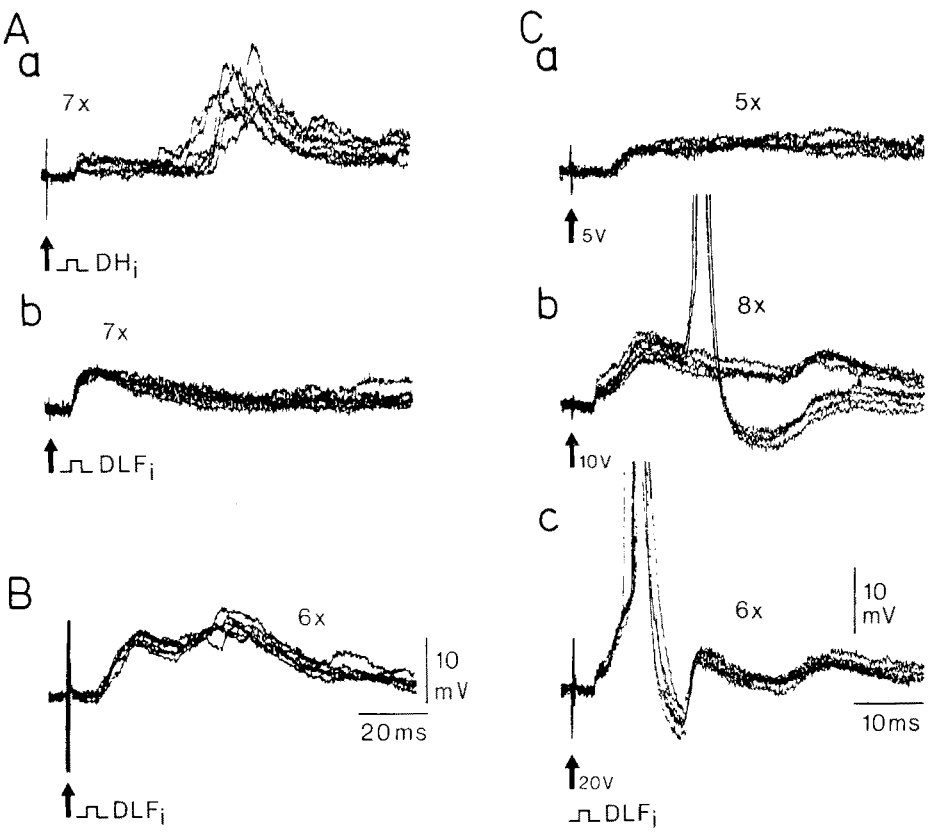


Fig. 16A–C. EPSPs evoked by stimulation of spinal pathways (*DLF*, dorso-lateral funiculus; *DH*, superficial part of the DLF) in three different preganglionic neurons in two intact anaesthetised cats (**A, B**) and in one cat spinalised at C3 (**C**). Early and late EPSPs (**Aa** and **B**) or only early EPSPs (**Ab**) could be recorded. **C** shows the effect of increasing stimulus voltage (**a–c**) on amplitude of early EPSP and initiation of action potential. (From Dembowsky et al. 1985a)

pathetic sub-nuclei have been described. These arise from axons in the lateral funiculus (Smith 1965; Rethelyi 1972, 1974; Wong and Tan 1974; Saper et al. 1976; Basbaum et al. 1978; Martin et al. 1979; Mizukawa 1980; Holstege and Kuypers 1982) but details of their synaptic connections are lacking.

7.4 Origin of Terminals

The axons of these terminals arise from spinally projecting neurons located in several regions of the brainstem and hypothalamus. The regions include:

1. The raphe pallidus.

2. The raphe obscurus (Dahlström and Fuxe 1964, 1965; Basbaum and Fields 1979; Amendt et al. 1979; Loewy et al. 1981; Loewy 1981; Loewy and McKellar 1981).

3. A region lying lateral to the raphe pallidus called either the arcuate group or the nucleus interfascicularis hypoglossi (Loewy and McKellar 1981).

4. The nucleus raphe magnus (Basbaum et al. 1978; Martin et al. 1979; Holstege and Kuypers 1982).

5. A region surrounding the lateral reticular nucleus at the level of the exit of the hypoglossal nerve rootlets (Dahlström and Fuxe 1964; Satoh et al. 1977; Amendt et al. 1979; Smolen et al. 1979; Loewy et al. 1981; Loewy and McKellar 1981; Blessing et al. 1981).

6. A ventro-lateral region in the rostral medulla known as the nucleus paragigantocellularis pars lateralis (Amendt et al. 1979; Helke et al. 1982).

7. A region in the ventro-lateral pons between the facial nerve exit and the superior olive (Loewy et al. 1979b).

8. The lateral pontine reticular formation at the level of the facial nucleus (Basbaum et al. 1978).

9. A region ventro-lateral to the brachium conjunctivum in the dorso-lateral pons (Saper and Loewy 1980; Holstege and Kuypers 1982).

10. The paraventricular nucleus of the hypothalamus (Saper et al. 1976; Swanson and McKellar 1979).

11. A region in the dorsal and lateral hypothalamus (Saper et al. 1976; Björklund and Skagerberg 1979; Blessing and Chalmers 1979).

12. A medial region and a lateral region in the posterior hypothalamus (Beattie et al. 1930; Ciriello and Calaresu 1977; Hosoya 1980).

13. Possibly, a retro-chiasmatic area in the ventral hypothalamus (Hosoya 1980).

7.5 Synaptic Morphology

Studies of ultrastructure have shown that terminals in the ILp can be divided into three classes according to their synaptic morphologies. One type contains round agranular vesicles, another a mixture of agranular and dense-cored vesicles and the third type contains flattened or ellipsoidal vesicles (Rethelyi 1972; Wong and Tan 1980; Chung et al. 1980). In one study it was shown that synapses with flattened vesicles were quite prominent on the soma of preganglionic neurons outnumbering synapses with only round agranular vesicles, whilst there was only an approximately equal distribution of each class of synapse on dendrites. Furthermore, it was shown that the total surface area covered by boutons was greater on the proximal dendrites (40%) than on the soma (12%; Chung et al. 1980). The possibility that these synapses represent the terminals of supraspinal axons has been tackled in

three studies so far, with varying degrees of success. In one study, Rethelyi (1974) performed some preliminary ultrastructural experiments in which he was unable to demonstrate degenerating terminals in the ILp 48 h after destruction of the lateral funiculus at C2 (it is not clear whether this was done uni- or bilaterally). It is possible that this failure was due to the very short survival times employed. On the other hand, Wong and Tan (1974) observed degenerating terminals on dendrites of preganglionic neurons in the ILp following systemic administration of the neurotoxin 6-hydroxydopamine. The neurotoxin was given so as to specifically destroy catecholamine-containing neurons and, assuming it did this, the result suggests that the terminals of catecholaminergic perikarya, which are present only in the brainstem, form axodendritic synapses with preganglionic neurons. However, the experimental protocol does raise some doubts as to whether the drug destroyed only catecholaminergic neurons, since it was administered into the systemic circulation, from which its access to the central nervous system would have been limited and its action possibly non-specific.

A recent ultrastructural study (Bacon and Smith 1986) published in preliminary form similarly suggests that some bulbo-spinal neurons make monosynaptic connections with preganglionic neurons in the ILp. These authors labelled preganglionic neurons supplying the adrenal medulla with HRP. They subsequently identified, using immunohistochemistry, boutons containing 5-hydroxytryptamine (5-HT) ending on the preganglionic neurons. Two types of 5-HT-containing boutons were recognised, terminating on perikarya and dendrites. One type contained agranular vesicles; the other type contained large unlabelled vesicles together with the labelled agranular vesicles. This work, although at an early stage, seems also to confirm the idea that bulbo-spinal 5-HT neurons projecting to the ILp may be of two types, one containing only the indoleamine and another containing both 5-HT and a peptide.

7.6 Chemical Coding of Terminals

Catecholamine and 5-HT are not the only chemicals that may be associated with descending brainstem projections to autonomic nuclei in the spinal cord. Terminals in the region of the ILp, containing substance P, oxytocin, vasopressin, angiotensin II, methionine encephalin, thyrotropin-releasing hormone, somatostatin, vasoactive intestinal polypeptide and avian pancreatic polypeptide, have been demonstrated (see reviews by Emson 1979; Holets and Elde 1982; Gilbert et al. 1982a). However, it is probably an oversimplification to consider that each of these endogenous chemicals is solely associated with a separate pathway. We know that perikarya with axonal projections to sympathetic preganglionic nuclei in the spinal cord can contain peptides as well

as a classical neurotransmitter such as noradrenaline or 5-HT. Spinal projections from some neurons in the raphe nuclei may contain only 5-HT (Loewy and McKellar 1981) whilst others may have leucine or methionine encephalin and still others thyrotropin-releasing hormone or substance P (Hokfelt et al. 1978; Bowker et al. 1983; Helke et al. 1982; Lovick and Hunt 1983; Johansson et al. 1981; Helke et al. 1985). It is also possible that in some spinally projecting neurons opioid peptides coexist with catecholamines (Hokfelt et al. 1980) and substance P with adrenaline (Marson and Loewy 1985; Lorenz et al. 1985). In addition, avian pancreatic polypeptide has been demonstrated in terminals around the autonomic sub-nuclei of the thoracic cord. These may be the same terminals as those thought to contain adrenaline (Lundberg et al. 1980).

8 Origin and Influence of Chemically Coded Afferents

There have been numerous attempts to relate the location of spinally projecting and chemically coded neurons in the brainstem and hypothalamus to the sympatho-excitatory and sympatho-inhibitory pathways established electrophysiologically (see reviews by Loewy and McKellar 1980; Loewy and Neil 1981; Dampney et al. 1985; Coote 1985, 1986a, b). The data concerning the anatomy and physiology of these pathways have been surrounded by controversy but recent experiments are helping to reconcile the earlier differences and are leading to a better appreciation of the role that bulbo-spinal pathways play in cardiovascular control. Many investigators have been possessed by the idea that a particular chemical could have only one action on preganglionic neurons no matter what their end-organ destination and therefore that similarly chemically coded neurons from several locations in the brain would have only one action. This now seems less likely, as will become apparent when we consider each chemically coded system.

8.1 5-Hydroxytryptamine

8.1.1 Anatomy

The terminals of nerve-cell bodies containing 5-hydroxtryptamine (5-HT) are probably present in most parts of the central nervous system of mammals, birds, reptiles and amphibia (Broughton 1972; Fuxe and Jonsson 1973; Saavedra et al. 1974; Parent 1975, 1978; Tauber 1974; Kuypers and Martin 1982; Bobillier et al. 1976). The cell bodies are only found in the brainstem, localised to mid-line nuclei or closely associated regions extending from the

decussation of the pyramids to the mesencephalon. Similar groups of neurons have been identified in birds, opossum, mouse, rat, rabbit, cat, monkey and man (Nobin and Björklund 1973; Hubbard and di Carlo 1974b; Lackner 1980; Cabot et al. 1982; Kuypers and Martin 1982). These have been labelled $B_1 - B_9$ neurons (Dahlström and Fuxe 1964). Spinally projecting 5-HT neurons lie mainly in the $B_1 - B_3$ cell group and an associated ventral medullary group sometimes referred to as the arcuate group or the nucleus interfascicularis hypoglossi (Dahlström and Fuxe 1965; Loewy 1981; Loewy and McKellar 1981; Steinbusch 1981; Bowker et al. 1982a). According to Bowker et al. (1982a), some 5-HT neurons in the pontine cell group B_5 and midbrain group B_7 also project to the spinal cord, the latter rather interestingly only to the cervical spinal cord.

Axons of these spinally projecting neurons are small, $1-2$ μm in diameter, and probably unmyelinated (Dahlström and Fuxe 1965; Loizou 1972; Coote and Macleod, unpublished observations). They descend in the lateral and ventral funiculi, concentrated towards the outer margin of the spinal cord, at least from the thoracic level onwards (Dahlström and Fuxe 1964; Coote and Macleod 1974a; Mizukawa 1980; Loewy 1981; Kojima et al. 1982). It has been shown in the opossum, rat, cat, dog, monkey and bird, that the sympathetic cell groups in the ILp throughout the thoraco-lumbar spinal cord receive a dense 5-HT innervation (Dahlström and Fuxe 1965; Konishi 1968; Coote and Macleod 1974a; Coote et al. 1978; Crutcher and Bingham 1978; Cabot et al. 1979; Mizukawa 1980; Loewy 1981; Loewy and McKellar 1981; Martin et al. 1982; Kojima et al. 1982). However, whether the terminals end pre- or postsynaptically on sympathetic preganglionic neurons or on interneurons is unclear. According to Mizukawa (1980), the 5-HT terminals form a plexus which is most dense in the dorsal part of the ILp. This raises the possibility that the dendrites are more richly contacted than the soma in preganglionic neurons.

This innervation arises, at least in part, from the nucleus raphe pallidus and nucleus raphe obscurus of the medulla. Dahlström and Fuxe (1965) treated rats with nialamide and were able to trace 5-HT-containing neurons from the nuclei raphe pallidus (B_1) and raphe obscurus (B_2) via the dorsal and lateral funiculi to their termination in the ILp. Lesions in the dorso-lateral funiculus abolished formaldehyde-induced 5-HT fluorescence in the ILp.

More substantive evidence for this 5-HT projection to the ILp from the B_1 and B_2 groups of cells has come from recent double-labelling studies in rat, which also confirmed that 5-HT axons generally travel in the lateral and dorso-lateral funiculi (Loewy 1981; Loewy and McKellar 1981). In the cat and the opossum 5-HT axons innervating the ILp also descend in the dorso-lateral funiculus, since lesions here (either local injection of 5,6-dihydroxytryptamine or sectioning) abolish 5-HT fluorescence in the ILp (Coote et al. 1978; Martin et al. 1982). Some of these descending axons may well originate from cell

bodies in the nuclei raphe pallidus and obscurus, since following injection of HRP more or less confined to the ILp, HRP is found in the cells of these brainstem nuclei (Amendt et al. 1979), and since electrical stimulation in the dorsal quadrant of the spinal cord in cats antidromically excites slowly conducting axons with cell bodies in these nuclei (Morrison and Gebber 1984).

There is also clear evidence, from studies in rat, of a 5-HT projection from a ventral medullary group of cells lying lateral to B_1 (Loewy and McKellar 1981; Helke et al. 1986) and close to the surface of the ventral medulla. These have been termed the arcuate group of 5-HT neurons or nucleus interfascicularis hyperglossi, although it is difficult to consider them as a nucleus because the cells are so scattered and diffuse.

Neurons in the nucleus raphe magnus also project to the ILp, but there is no clear evidence as yet that they contain 5-HT (nevertheless, see also Helke et al. 1986). In cat and opossum there is dense labelling of terminals in the ILp following injections of [^{3}H]-leucine in the region of the nucleus raphe magnus (Fig. 10; Holstege and Kuypers 1982; Martin et al. 1979). In the rat, Bowker et al. (1982a) have shown that may 5-HT-containing cells in the nucleus raphe magnus project to the spinal cord, the majority sending their axons via the lateral and dorso-lateral funiculi. Since the 5-HT innervation of the ILp appears to come mainly from fibres in the lateral funiculus, it was natural for Bowker et al. to conclude that many of the 5-HT cells in the nucleus raphe magnus (b_3) were destined for the ILp. This was reinforced because Basbaum and Fields (1979) confirmed Bowker's findings and also reported that many neurons in cat nuclei raphe pallidus and obscurus send spinal projections via the ventro-lateral and ventral funiculi. However, on closer examination the data presented by Bowker et al. (1982a) are not so conclusive. They found that the number of 5-HT cell bodies in the nucleus raphe magnus, which took up HRP was considerably reduced (by 81%) following section of the dorsal quadrant of the spinal cord above the level of the injection site. However, there was nearly as marked a reduction in the number of HRP-stained 5-HT neurons in the nucleus raphe obscurus (by 71%) and nucleus raphe pallidus (by 41%). These percentages refer to my own calculations, based on data shown in Fig. 9 of Bowker et al. (1982a).

8.1.2 Actions of 5-HT Pathways

Progress in establishing the role of spinally projecting 5-HT neurons in sympathetic regulation has been slow. Inadequacies of experimental design and technique have been partly to blame for this. For example, recording blood pressure and heart rate whilst electrically stimulating with electrodes placed in the raphe nuclei is too imprecise to allow the conclusion that spinally projecting neurons are involved. Similarly, in most cases the actions of drugs

which deplete brain 5-HT have not been confined to the spinally projecting neurons. As a consequence, the data conflict (see Khun et al. 1980a, b). Nonetheless, such approaches have quite clearly indicated that the raphe nuclei are not homogeneous in function. There are distinct regions from which pressor responses and increases in heart rate can be obtained, whereas at other locations in the raphe nuclei depressor responses and heart-rate decreases predominate (Coote and Macleod 1974a; Neumayr et al. 1974; Gillis et al. 1976; Adair et al. 1977; Smits et al. 1978; Cabot et al. 1979; Yen et al. 1983).

By far, the most compelling evidence regarding the influence on sympathetic activity of spinally projecting 5-HT neurons has come from electrophysiological studies. It has been shown in cat and bird, that both inhibition of sympathetic activity – recorded in either thoracic white rami (Fig. 17)

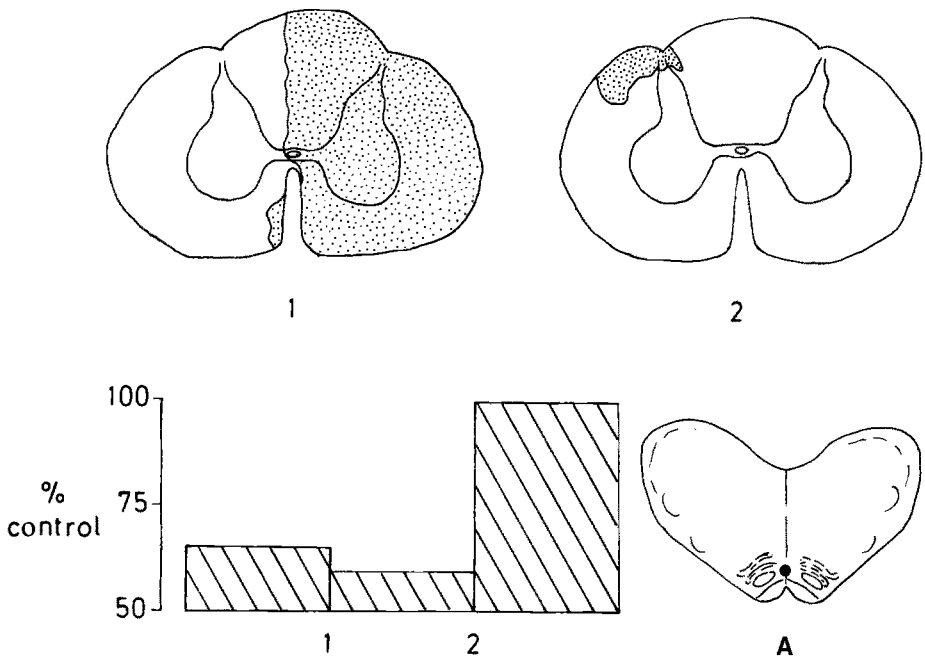


Fig. 17. Effect of spinal-cord lesions on the inhibition of spinal sympathetic reflex activity in the tenth thoracic white ramus (T10WR) produced by stimulation in the nucleus raphe pallidus at the point shown in cross-section of medulla (**A**). A short train of stimuli (1 ms pulse duration, 100 Hz for 100 ms) delivered to this point 175 ms before eliciting the spinal reflex in T10WR depressed the sympathetic reflex. In the histogram the degree of this inhibition is shown as the size of the T10WR reflex response preceded by brain stimulation expressed as a percentage of control reflex responses before and after each of two lesions (*1* and *2*) were made in the spinal cord at the fourth cervical level. The extent of these lesions is represented by the *stippled areas* in the transverse section of spinal cord in *1* and *2*. Note that after lesion *2* medullary stimulation no longer had any effect on the T10WR reflex. Anaesthetised cat. (From Coote and Macleod 1975)

or postganglionic nerves to the heart and kidney – and decreases in blood pressure result from stimulation of the nucleus raphe pallidus, possibly including cells of the nucleus interfascicularis hyperglossi (Coote and Macleod 1974a; Neumayr et al. 1974; Cabot et al. 1979). The inhibition is likely to occur at a spinal site, since spinal sympathetic reflexes are depressed together with supraspinal sympathetic reflexes and spontaneous sympathetic activity (Fig. 17; Coote and Macleod 1974a; Neumayr et al. 1974). The effect on spinal reflexes could not have been due to disfacilitation, because removal of all descending excitatory inputs to sympathetic neurons by spinal-cord transection had little effect on the size or threshold of the particular sympathetic reflex studied (Coote and Macleod 1974a). It was argued by Coote and Macleod (1974a; 1975) that small indolamine neurons were activated in these experiments, because the stimulus threshold for eliciting the sympatho-inhibitory effects was markedly higher than that needed to elicit sympatho-inhibitory or excitatory effects from the classical pressor and depressor regions of the medulla. Furthermore, the fact that sympatho-inhibitory effects correlated well with electrode placements close to 5-HT neurons appears to support the conclusion.

Other circumstantial evidence suggesting that small spinally projecting 5-HT neurons are involved in sympatho-inhibition is difficult to disregard. The inhibitory effect is preferentially abolished by small cuts in the dorso-lateral cervical spinal cord (Fig. 17; Coote and Macleod 1975) sparing the descending sympatho-excitatory pathways and ventro-medial sympatho-inhibitory pathways. The lesions were placed in the region of spinal cord from which inhibition of sympathetic activity can be obtained in spinal cats (Illert and Gabriel 1970, 1972; Coote and Macleod 1974a, 1975), suggesting that an inhibitory pathway descends in this region. A comparison of the latency to onset of sympatho-inhibition obtained from the dorso-lateral funiculus in the mid-cervical spinal cord (>40 ms) with that from the nucleus raphe pallidus of the medulla (>100 ms) suggests that neurons from both regions with similar axonal conduction velocities are involved – that is, of course, assuming a fairly direct influence on the sympathetic cell groups (Coote and Macleod 1974a, 1975).

Clear evidence that the inhibitory effect of stimulating elements in the nucleus raphe pallidus is exerted close to or on sympathetic preganglionic neurons was provided in a further paper by Gilbey et al. (1981). In this study, the excitability of sympathetic neurons was tested by intraspinal stimulation of descending excitatory pathways as well as by reflex activation of the sympathetic neurons. Stimulation in the nucleus raphe pallidus inhibited activity elicited by either means, so it is unlikely that the effect of the raphe stimulation was mediated via an action on dorsal-horn transmission. Recently, Morrison and Gebber (1984) recorded neurons in the nucleus raphe pallidus which were antidromically activated following stimulation in the dorso-lateral

funiculus of the upper thoracic spinal cord. Many of these had latencies greater than 100 ms, supporting the idea that the axons of raphe spinal sympatho-inhibitory neurons are located in this region of the spinal cord. Sympatho-inhibitory effects elicited from some regions of the nucleus raphe obscurus are also probably mediated via small spinally projecting neurons travelling in the dorso-lateral funiculus (Coote and Mcleod 1974a; Gilbey et al. 1981).

8.1.3 Pharmacological Studies

Pharmacological studies also suggest that the inhibition of sympathetic activity elicited by stimulating sites in the nuclei raphe pallidus and obscurus is mediated by spinally projecting 5-HT neurons. The first piece of such evidence concerns the effects on sympathetic activity of systemically administered 5-hydroxytryptophan (5-HTP), the precursor of 5-HT. The 5-HTP passes into the central nervous system, where it is metabolised and is presumed to cause overflow of transmitter from 5-HT nerve terminals (Anderson and Shibuya 1966; Baker and Anderson 1970). In spinal cats, this treatment profoundly inhibits activity in pre- and postganglionic sympathetic nerves (Hare et al. 1972; Neumayr et al. 1974; Coote and Macleod 1974a). It is likely that 5-HT, rather than its precursor 5-HTP, mediates this inhibitory effect for the following reasons: firstly, the onset and time course of the inhibition are consistent with the kinetics of precursor uptake, followed by synthesis and release of 5-HT; secondly, the effect of 5-HT is prevented by blocking the synthesis of 5-HT with a central decarboxylase inhibitor RO 4-4602 (Franz et al. 1978); thirdly, tricyclic antidepressants whose action selectively involves 5-HT, amitriptyline and chlorimipramine, rapidly and markedly enhance the depression produced by 5-HTP (Sangdee and Franz 1979).

The involvement of spinally projecting 5-HT neurons in inhibiting sympathetic neurons was tested more directly in anaesthetised cats by examining the effect of a variety of 5-HT antagonists on raphe-spinal inhibition with long latency to onset (Fig. 18; Gilbey et al. 1981). Sympathetic responses were evoked in T3 or T10 white rami, either by stimulating a spinal nerve or by intraspinal stimulation at the mid-cervical level. In seven cats, lysergic acid diethylamide (LSD) in a dose range of 20–50 μg/kg i.v. or 0.6 μg applied topically to a spinal-cord segment reversibly reduced raphe-spinal inhibition by 40%–100%, topical application being more effective than intravenous administration. In five of these cats, stimulation within the classical inhibitory region of the ventro-medial reticular formation at sites unlikely to contain 5-HT neurons produced a short latency to onset of inhibition of sympathetic activity, which was unaffected by LSD. The relatively low concentration of LSD and its selective action in antagonising the raphe-spinal inhibition argue

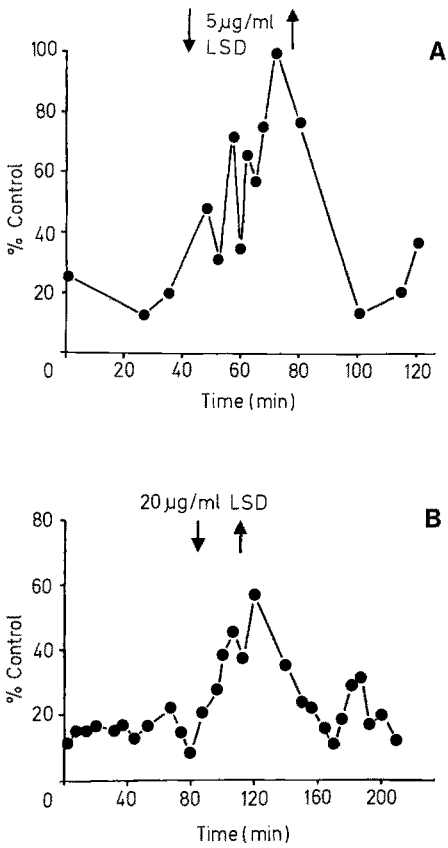


Fig. 18A, B. Effect of topically applied lysergic acid diethylamide (LSD) on raphe spinal inhibition of intraspinally evoked sympathetic discharge. Results from two different experiments are illustrated. **A** Maximum antagonism was obtained with 5 µg/ml LSD. **B** A concentration of 20 µg/ml LSD was required to produce an effect (vol. 20 µl). *Filled circles* show the size of intraspinally (C4) evoked sympathetic discharge in the third thoracic white ramus (average×8) obtained following raphe stimulation and plotted as a percentage of the mean value of the bracketing (average×8) intraspinally evoked sympathetic discharge otained during the absence of raphe stimulation (*ordinate*) at various times (*abscissa*) during the pre-drug, drug and post-drug periods. Time of drug application is indicated by *distance between arrows*. Raphe stimulation commenced 400 ms prior to spinal-cord stimulation. (From Gilbey et al. 1981)

against LSD acting as a 5-HT agonist, since this characteristic of the drug is only manifest at high concentration (0.5–2.0 mg/kg) and is accompanied by non-specificity (Haigler and Aghajanian 1974). The putative antagonists of 5-HT, methysergide, cinanserin and cyproheptadine, all depressed sympathetic discharge in the absence of brain stimulation, in both intact and spinal cat. This excludes the possibility that the effect of these drugs in the intact cat was due to their antagonism of a tonically active 5-HT-excitatory system.

An inhibitory role for a bulbo-spinal 5-HT pathway is also supported by studies in which raphe stimulation inhibited sympathetic preganglionic neurons in the pigeon (Cabot et al. 1979). However, the results of the latter study should be interpreted cautiously. It is unclear, whether stimulating electrodes located rostrally in the medullary raphe were in the raphe magnus or pallidus and this could have some bearing on the meaning of the rather fast conduction velocities (4–12 m/s) reported for the inhibitory fibres. It seems unlikely that the small unmyelinated axons of 5-HT neurons would conduct at such speeds. In cat, as well as the more numerous, larger and faster-con-

ducting neurons which are in the raphe magnus, there is also a population of slow, small neurons (Wessendorf and Anderson 1983; Morrison and Gebber 1984) which are likely to contain 5-HT. Unfortunately, Cabot et al. (1979) presented no further evidence that the raphe neurons contained 5-HT, nor was there evidence indicating that the sympatho-inhibitory effects were taking place at a spinal as opposed to a medullary site.

In contrast to the foregoing, direct excitatory actions of raphe (particulary raphe magnus) stimulation have been claimed (Howe et al. 1983; McCall 1984). However, whether the observed excitatory effects were due to release of 5-HT and its actions on specific receptors on preganglionic neurons was not clearly established. McCall (1984) showed that stimulation at various sites in nuclei raphe magnus, pallidus and obscurus excited or inhibited activity in the inferior cardiac nerve of anaesthetised cats. Excitation was blocked by intravenous methysergide and by metergoline and very slightly potentiated by the uptake-inhibitor chlorimipramine. By contrast, inhibition was unaffected by these drugs. Similarly, an excitatory response elicited by stimulation of a lateral pressor area was unaffected by drug treatment. This evidence again needs cautious interpretation, since unfortunately no evidence was provided that the raphe stimulus was exciting spinally projecting pathways. Even if it had been, the calculated conduction velocities of 3.3 m/s, a figure which contains a number of assumptions and approximations, still seems somewhat fast for small unmyelinated 5-HT axons.

The axons of raphe spinal neurons reported by Wessendorf et al. (1981) and by Morrison and Gebber (1984), can be divided into three groups: those axons conducting between 0.7 and 1.0 m/s, those conducting between 3.1 and 6.0 m/s and those conducting between 6.1 and 32.0 m/s. Wessendorf et al. (1981) considered that the first two groups were 5-HT neurons, since the number of units located for each electrode penetration was markedly reduced following chronic treatment with 5,7-dihydroxytryptamine. However, there was also a marked lack of units with considerably higher conduction velocities, which might make one suspect the specificity of the 5-HT neurotoxin. An additional problem with the study by McCall (1984) was that 5-HT antagonists were administered intravenously and therefore there is no way of knowing whether their ability to block raphe-sympathetic responses was due to the action of a 5-HT excitatory synapse in the brainstem or spinal cord. Nonetheless, an excitatory role for a defined 5-HT pathway is supported by other data from more recent experiments. An important development in the last few years has been that of selective stimulation of cell bodies, rather than fibres, of neurons, using micro-injection of excitatory amino acids. Such chemical stimulation (with kainic acid) of bulbo-spinal neurons in the region of the B_3 cell group of the rat caused an increased release of 5-HT in the thoracic spinal cord (Pilowsky et al. 1986). The fact that this was associated with a pressor response led these authors to suggest that this particular group

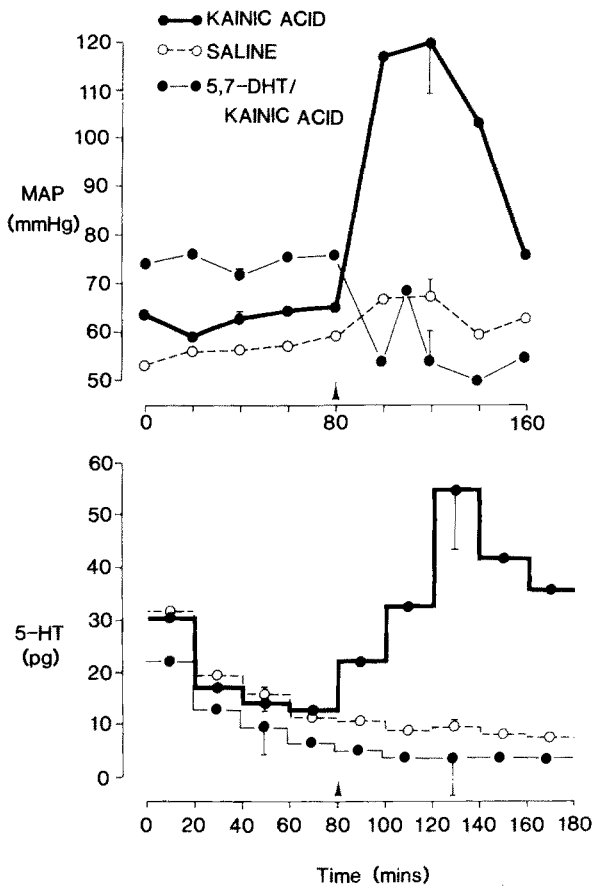


Fig. 19. The effect of brainstem kainic acid injections on blood pressure and release of 5-hydroxytryptamine (5-HT) from spinal cord. The *upper panel* shows the effect on mean arterial blood pressure (mmHg) of bilateral micro-injections of kainic acid into the region of the lateral B3 5-HT cells of five normal Wistar Kyoto rats (*black circles and thick black lines*) and of five Wistar Kyoto rats pretreated with 5,7-dihydroxytryptamine 2 weeks earlier (*black circles and thin black lines*). Vehicle-injected animals received micro-injections of vehicle alone (*open circles and dotted line*). Micro-injections are indicated by *arrow* at 80 min. Standard errors of the means are shown. The *lower panel* shows the effect on 5-HT efflux (pg/20 min collection period) of the same bilateral micro-injections of kainic acid. Efflux of 5-HT was measured using a dialysis probe inserted into the spinal cord in the lower thoracic region. (From Pilowsky et al. 1986)

of spinally projecting 5-HT neurons had a sympatho-excitatory action. Their conclusion was supported by a subsequent study showing that both the release of 5-HT and the pressor response were absent after intracisternal treatment with 5,7-dihydroxytryptamine (Fig. 19; Chalmers et al. 1985).

8.1.4 Microiontophoresis

In studies employing the techniques of microiontophoresis, 5-HT has been found to excite the majority of sympathetic preganglionic neurons (Fig. 20; DeGroat and Ryall 1967; Coote et al. 1981a; Kadzielawa 1983b; McCall 1983). Excitation occurs irrespective of whether the neurons are quiescent, spontaneously active or induced to discharge with excitatory amino acids such as L-glutamate or DL-homocysteic acid. Recent studies on rat and cat spinal-cord slices in vitro show that the excitatory actions of 5-HT on sympathetic preganglionic neurons are associated with a slow depolarisation and an in-

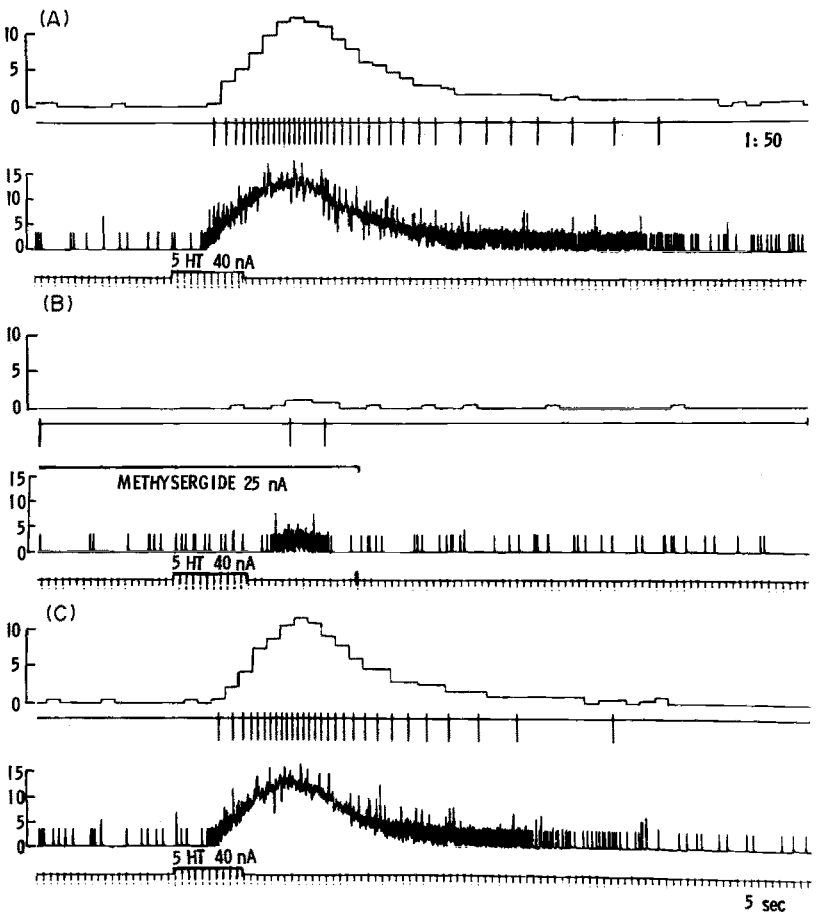


Fig. 20A–C. Example of the excitatory effect of iontophoretically applied 5-HT with a current of 40 nA on a spontaneously active antidromically identified sympathetic preganglionic neuron in the third thoracic segment of the anaesthetised cat **A** before and **B** during and following an 8-min ejection (*bottom trace*) of methysergide (25 nA). **C** Recording taken 45 min after methysergide. *Upper trace,* average rate of discharge integrated over 10 s; *third trace in each panel,* rate of firing; both rates were calibrated in spikes/s. (From Kadzielawa (1983b)

crease in membrane resistance, which may cause repetitive firing of the cell (Yoshimura and Nishi 1982; Ma and Dun 1986). The slow depolarisation is still present with tetrodotoxin or low Ca/high Mg, but is abolished at a membrane potential which nullifies the spike after hyperpolarisation. It is therefore probably caused mainly by a decrease in a voltage-dependent K conductance (Yoshimura and Nishi 1982; Ma and Dun 1986). This is different from the relatively 'fast' EPSP associated with natural firing of sympathetic preganglionic neurons, which is linked to a decrease in input resistance and appears to be dependent on changes in Na and Ca conductance (Yoshimura and Nishi 1982; Yoshimura et al. 1986a).

The 5-HT slow depolarisation can be blocked by methysergide and cyproheptadine (Ma and Dun 1982). It is noteworthy that the depolarisation evoked by 5-HT in sympathetic preganglionic neurons is in many respects similar to that elicited in guinea pig (Wood and Mayer 1979) and cells of the coeliac ganglion (Dun and Ma 1984). In these ganglia, 5-HT appears to mediate a slow excitatory potential, which enhances the responsiveness of the ganglion cells to incoming 'fast' synaptic potentials, for seconds to minutes.

Unfortunately, these in vitro experiments only go a little way towards resolving the controversy regarding the action of descending 5-HT pathways. Whereas it seems clear that synapses on some or most sympathetic preganglionic neurons at which 5-HT is released mediate a slow depolarisation, the possibility exists that other actions of 5-HT are lost simply as a consequence of preparing a slice, which may remove dendritic synapses that are more remote.

In two studies, 5-HT applied microiontophoretically inhibited 14% of the thoracic preganglionic neurons tested (Coote et al. 1981a; Kadzielawa 1983b). Inhibition was usually limited to only the period of ejection, after which firing increased rapidly to reach or even exceed the previous control level. Coote et al. (1981a) concluded that neuronal inhibition was most likely due to an action of creatinine which was ejected along with the 5-HT, since inhibition was never observed when the bimaleate salt of 5-HT was used. However, this argument would seem to be dismissed because Kadzielawa (1983b) observed inhibition in a precisely similar proportion of sympathetic preganglionic neurons when using the hydrochloride salt of 5-HT.

If the results of McCall (1984) and Pilowsky et al. (1986) can be more firmly substantiated, then there is a possibility that some spinally projecting 5-HT neurons (possibly synapsing directly with sympathetic preganglionic neurons) are excitatory, whilst other spinally projecting 5-HT neurons are inhibitory to sympathetic activity. For the majority of preganglionic neurons, this latter effect requires either an inhibitory interneuron or another type of 5-HT receptor on the preganglionic neuron, to which the 5-HT boutons are tightly coupled. A recent study in this laboratory has provided evidence for these possibilities.

The actions of 5-HT on different sympathetic vasoconstrictor neurons were examined in rat by recording activity in postganglionic neurons to the kidney and to blood vessels of hind-limb skeletal muscle. Several drugs including 5-HT were administered to restricted regions of the spinal cord through a catheter passed down the sub-arachnoid space via a slit in the atlanto-occipital membrane until its tip lay at an appropriate segmental level (Yusof 1986; Yusof and Coote 1986a). There were two actions of 5-HT on the sympathetic outflow to the kidney, but only one action on that to the skeletal muscle of the hind limb. Intrathecal 5-HT in low doses (20–100 μg) increased activity in the renal nerve (mean change 157% ± 39% for a 50 μg dose). At higher doses of 5-HT, the initial excitatory response in the renal nerve was followed by inhibition (65%). By contrast, intrathecal 5-HT at all dose levels (20–800 μg) caused only inhibition of activity in the vasoconstrictor fibres to skeletal muscle. This suggests the possibility that the sub-population of preganglionic neurons which were inhibited by microiontophoretic application of 5-HT in previous studies (Coote et al. 1981a; Kadzielawa 1983b) were muscle vasoconstrictor neurons. A further observation made in the study by Yusof and Coote (1986a) was that the inhibitory effects of 5-HT were mediated quite clearly by a 5-HT_2-like receptor, whereas the excitatory effect of 5-HT was probably mediated via a receptor of sub-type 5-HT_1: a conclusion also, supported by the somewhat more indirect data of Ramage (1985). His findings supported by iontophoretic studies, showed that the excitatory actions of 5-HT are all attenuated by methysergide, metergoline and cinanserin, all of which are more likely to affect a receptor of sub-type 5-HT_1 (Fig. 20; Kadzielawa 1983b; McCall 1983; Fozard 1984; Bradley et al. 1986). Dahlström and Fuxe (1965) implied that 5-HT terminals could end directly on sympathetic preganglionic neurons, and there is now good evidence for this from ultrastructure studies (Bacon and Smith 1986). In view of the data presented above, it is therefore possible that the two types of 5-HT receptor are present on the membrane of at least a proportion of the preganglionic neurons. Consistent with such an idea is the observation that sympathetic preganglionic neurons innervating the adrenal medulla receive two types of 5-HT afferent fibre, one with a homogeneous population of vesicles in the terminal boutons and the other with a mixture of large and small vesicles (Bacon and Smith 1986). Clearly, further data is required before these exciting observations can be correctly interpreted. However, it is difficult to escape the conclusion, even at this stage, that the spinally projecting 5-HT system of neurons in the brainstem is both anatomically and functionally coded.

8.2 Catecholamines

8.2.1 Anatomy

There is a wealth of anatomical evidence demonstrating that catecholamine-containing neurons project to the spinal cord (Dahlström and Fuxe 1964; Westlund et al. 1982). These neurons are arranged in groups located in quite different regions of the brainstem. They were originally identified in the rat and classified as A_1-A_{13} (Dahlström and Fuxe 1964; Palkovits and Jacobowitz 1974). Similar groups are present in cat (Dahlström and Fuxe 1964; Pin et al. 1968; Coote and Macleod 1974a; Poitras and Parent 1978; Lackner 1980), dog (Ishikawa et al. 1975), rabbit (Blessing et al. 1978), primates (Hubbard and di Carlo 1974a; Felten et al. 1974; Garver and Sladek 1975; Demirjian et al. 1976), man (Nobin and Björklund 1973), birds (Fuxe and Ljunggren 1965; Ikeda and Gotah 1971; Dube and Parent 1981) and probably in reptiles and amphibia (Parent 1973, 1975, 1978). Although terminals of these neurons are to be found throughout the grey matter at all levels of the spinal cord, the sympathetic lateral cell column, the ILp and the more medial regions of grey matter in the thoracic cord receive a particularly dense innervation. In a study in cat by Mizukawa (1980), using the glyoxilic-acid method for the demonstration of monoamines, the ILp appeared to be more richly innervated in its ventral part than in its dorsal part. Light-microscopic examination suggests that the catecholamine terminals are varicose and synapse with the soma of sympathetic preganglionic neurons (Dahlström and Fuxe 1965; Chiba and Murata 1981) as well as the dendrites (Dahlström and Fuxe 1965; Smolen, Glazer and Ross 1979; Glazer and Ros 1980; Chiba and Murata 1981).

Several studies have attempted to identify the groups of catecholamine neurons which contribute to this innervation. Dahlström and Fuxe (1965) noted that following spinal-cord transection, neurons in the A_1 and A_2 cell groups displayed swelling and accumulation of fluorescent material, indicating that spinally projecting axons had been cut, a finding which was seemingly confirmed by more recent studies by Nygren and Olsen (1977). A surprising feature, however, was that Dahlström and Fuxe (1965) could find no evidence for descending projections from other catecholamine cell groups which recent experiments have shown quite clearly (Satoh et al. 1977; Loewy et al. 1979b; Westlund et al. 1983).

In the experiments of Loewy et al. (1979b) injections of [^{3}H]-amino acid restricted to the A_5 region of the pons resulted in quite dense labelling of terminals in the ILp. Labelling was not present if these injections had been preceded by intraventricular administration of the catecholamine neurotoxin 6-hydroxydopamine (6 OHDA). Cells in the A_5 region containing catecholamine could also be labelled following injections of HRP into the thoracic

spinal cord. Westlund et al. (1983) injected HRP into the thoracic spinal cord at unidentified locations and convincingly showed that cells in the A_7 group were doubly labelled for HRP and catecholamine. However, in contrast to Satoh et al. (1977) and Loewy et al. (1979b), there were very few labelled cells in A_5 and there were no doubly labelled cells in A_1. This latter observation has been interpreted as indicating that the A_1 catecholamine neurons in the rat do not project to the spinal cord. Such a dogmatic conclusion should be looked at with caution, since the anatomical techniques are not foolproof (Nauta et al. 1974; Heuser and Reese 1973; Jones 1975; Hedreen and McGrath 1977). It is also clearly incorrect according to the data presented in Figs. 2, 4 and 5 of McKellar and Loewy (1982).

Hence, anatomical evidence leads us to conclude that in the rat there are spinally projecting catecholamine neurons in areas A_7, A_6, A_5, A_2 and A_1, but there is clear evidence of innervation of the ILp from only A_5 and perhaps to a lesser extent from A_1.

In general, electrophysiological experiments are in agreement with this. Small spinally projecting neurons in the A_1, A_5 and A_6 regions have been identified by antidromic activation of their axons in the spinal cord, and their location correlated with the position of catecholamine-containing cells (Fig. 21; Guyenet 1980; Andrade and Aghajanian 1982; Fleetwood-Walker et al. 1983; Byrum et al. 1984; Coote and West, unpublished observations). No similar studies have so far been conducted on the A_7 group.

In the rabbit, catecholamine-fluorescent cells containing retrogradely transported HRP from the spinal cord are located mainly in areas A_7, A_6, A_5 and the rostral part of A_1 in the medulla (Blessing et al. 1981). Similar double-labelling studies in the chick show that A_6 and A_1 project to the thoracic spinal cord but that A_1 provides the main catecholamine innervation of the grey matter dorsal to the central canal (that is, the ICpe), presumably because this autonomic nucleus dominates in the bird (Smolen et al. 1979).

Fleetwood-Walker and Coote (1981a) used a somewhat different approach in cat to determine which group of spinally projecting catecholamine neurons innervates the ILp. Following electrolyte lesions placed to destroy separate catecholamine cell groups in the medulla and pons of different cats, biochemical analysis of discrete regions in the third thoracic segment of the spinal cord was performed. Bilateral lesions in the ventro-lateral medulla, to destroy A_1 neurons, caused a marked and very selective depletion of noradrenaline in the ILp; whereas lesions of A_2, A_5 or A_6 had no effect or only a small effect on catecholamine levels in this nucleus. A contribution from A_7 cannot be excluded, because the ventro-lateral medullary lesion was quite extensive and could have destroyed A_7 fibres passing through this region on their way to the spinal cord (Fleetwood-Walker and Coote 1981a).

Electrophysiological studies in cat have shown that spinally projecting neurons with slowly conducting axons are located in the region occupied by

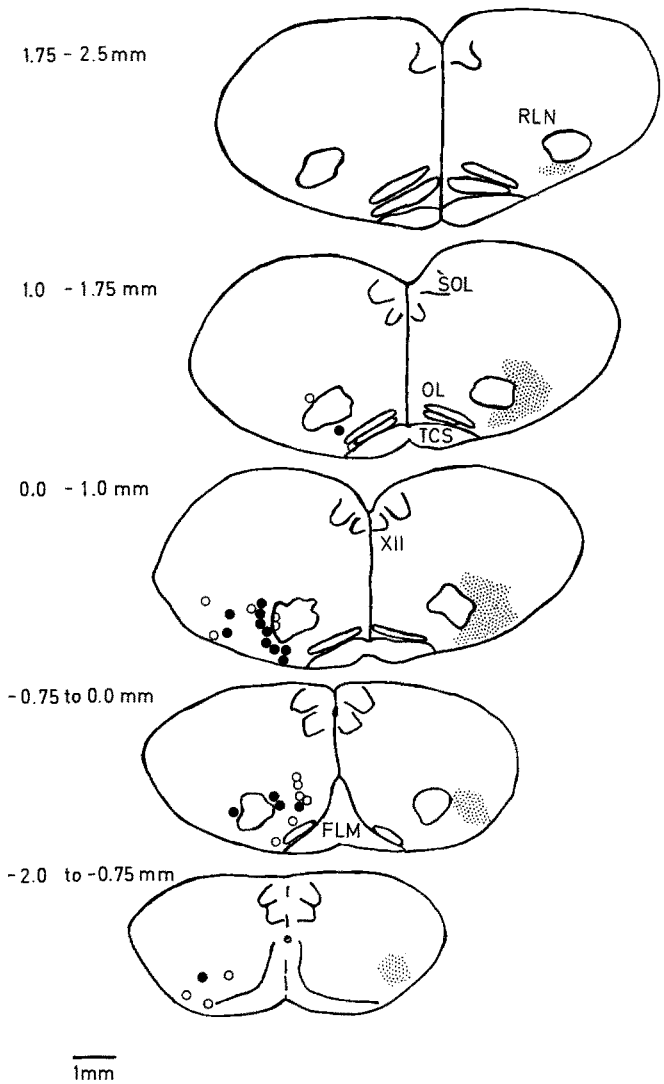


Fig. 21. Representative transverse sections through various levels of the rat medulla, cut along the plane of the electrode tracks. Rostro-caudal positions are indicated in millimetres along an axis perpendicular to the vertical axis, measured from the zero vertical plane running through the obex. The sites of the recorded antidromic neurons are shown on the *left, Black circles* represent cells with conduction velocities below 1.5 m/s, open circles show cells with conduction velocities above 1.5 m/s. The division at 1.5 m/s was chosen because this was found to be the upper limit including all units correlating with catecholamine cells. On the *right,* the *hatching* shows the extent of the A_1 catecholamine cell group around the inferior olivary nucleus; RLN lateral reticular nucleus; *FLM,* medial-longitudinal fasciculis; *OL,* lateral reticular nucleus; *SOL,* solitary nucleus; *TCS,* corticospinal tract; *XII,* hypoglossal nucleus. (From Fleetwood-Walker et al. 1983)

A_1 cells (Caverson et al. 1983; Coote, unpublished observations), but evidence that these are catecholamine cells is lacking.

In summary, the ILp in the third thoracic segment of the cat receives a dense and highly specific noradrenaline innervation, which originates from cell bodies in the A_1 region or from axons passing through this ventro-lateral medullary area, possibly from cells in A_7.

An ultrastructural study has shown that terminals of these spinally projecting noradrenaline cells make axo-dendritic synaptic contact with sympathetic preganglionic neurons (Wong and Tan 1974). The latter authors also showed there was degeneration of nerve terminals in the ILp following 6-OHDA given systemically. Some caution is required in interpreting this interesting result because of possible non-specific action of the neurotoxin particularly when given by the systemic route. It would be highly important to repeat this ultrastructural study, perhaps using gold-labelled antibodies directed against catecholamines.

8.2.2 Adrenaline-Containing Neurons

There have been some suggestions that the ILp receives a specific innervation from spinally projecting adrenaline-containing neurons located at the rostral end of A_1 (Blessing et al. 1981; McKellar and Loewy 1982; Lorenz et al. 1985). This group of neurons, designated as C_1, was originally described by Hokfelt et al. (1974) using an immunohistofluorescence technique based on an antibody to phenylethanolamine *n*-methyl-transferase (PNMT), the adrenaline-synthesizing enzyme. Some of these neurons may also contain substance P (Lorenz et al. 1985). Terminal projections were identified in the ILp of the rat.

Quantitative biochemical methods of estimating adrenaline levels in the ILp have been less convincing. Reid et al. (1975), using mass fragmentography, showed that adrenaline was present in rat spinal cord in small amounts, but van der Guyten et al. (1976) could not demonstrate its presence in the ILp. In the rabbit, the presence of adrenaline in the ILp has been inferred only indirectly in terms of the difference between the total catecholamine content and the amounts of noradrenaline and dopamine present (Zivin et al. 1975). In the cat, adrenaline levels in the T3 segment of the thoracic cord were too low to allow a reliable interpretation of the data obtained from a highly sensitive radio-enzymatic assay (Fleetwood-Walker and Coote 1981 a). However, if there is a very high rate of adrenaline turnover, biochemical methods might give a spuriously low concentration, whereas a radio-immunoassay of the enzyme PNMT would not be influenced by this and hence would be a more reliable indicator.

8.2.3 Dopamine

Quantitative measurements have shown that dopamine (DA) is present in the spinal cord in a number of animal species but in much lower amounts than noradrenaline, 75–100 ng/100 mg protein, compared to at least 950 g/100 mg protein for noradrenaline in the cat (Fleetwood-Walker and Coote 1981 b). The identification of DA in spinal-cord tissue raises the question as to whether it is part of an independent nerve supply or is present solely as a precursor of noradrenaline or adrenaline. There are now several pieces of evidence suggesting the former. It has been shown that the concentration of DA in certain regions of the spinal grey matter at different segmental levels has little correspondence to the levels of noradrenaline. Also, the time course of the disappearance of DA caudal to a transection of the spinal cord is markedly different to that for noradrenaline. Finally, DA levels can be altered by lesions in the brainstem or pharmacologically manipulated independently of noradrenaline levels (Magnusson 1973; Commissiong 1978, 1979; Fleetwood-Walker and Coote 1981 a, b).

Furthermore, there is evidence for a nigro-spinal DA pathway (Commissiong et al. 1979), and double-labelling histochemical studies have indicated that DA-containing cell bodies in the dorsal and posterior hypothalamus of rabbit (Blessing and Chalmers 1979) and rat (Björklund and Skagerberg 1979) can be retrogradely labelled by substances injected into the spinal cord.

However, none of these data indicates that there is a specific DA innervation of the sympathetic sub-nuclei in the spinal cord. In addition, binding studies on specific regions of cat thoracic spinal cord have failed to reveal DA receptors in the ILp (Coote et al. 1979).

8.2.4 Actions of Catecholamine Pathways

The major influence of catecholamine innervation on sympathetic activity is likely to be inhibitory, although this is another area surrounded by controversy. Electrical stimulation of fibres of passage or cells via electrodes accurately located amongst catecholamine neurons in the ventro-lateral medulla (as judged by fluorescence histochemistry of the brain in experimental animals) produced inhibition of sympathetic activity in white rami or in vasomotor postganglionic fibres in renal nerve (Coote and Macleod 1974 a). This inhibition was abolished after a limited region of the dorso-lateral funiculus of the cervical spinal cord was damaged, whereas inhibition or excitation from other brainstem sites was unaffected (Fig. 22; Coote and Macleod 1975). This suggests the inhibition was produced by stimulating a descending pathway to the IML, an assumption which appeared to be supported by the demonstration in spinal animals of sympatho-inhibitory sites confined to that part of the

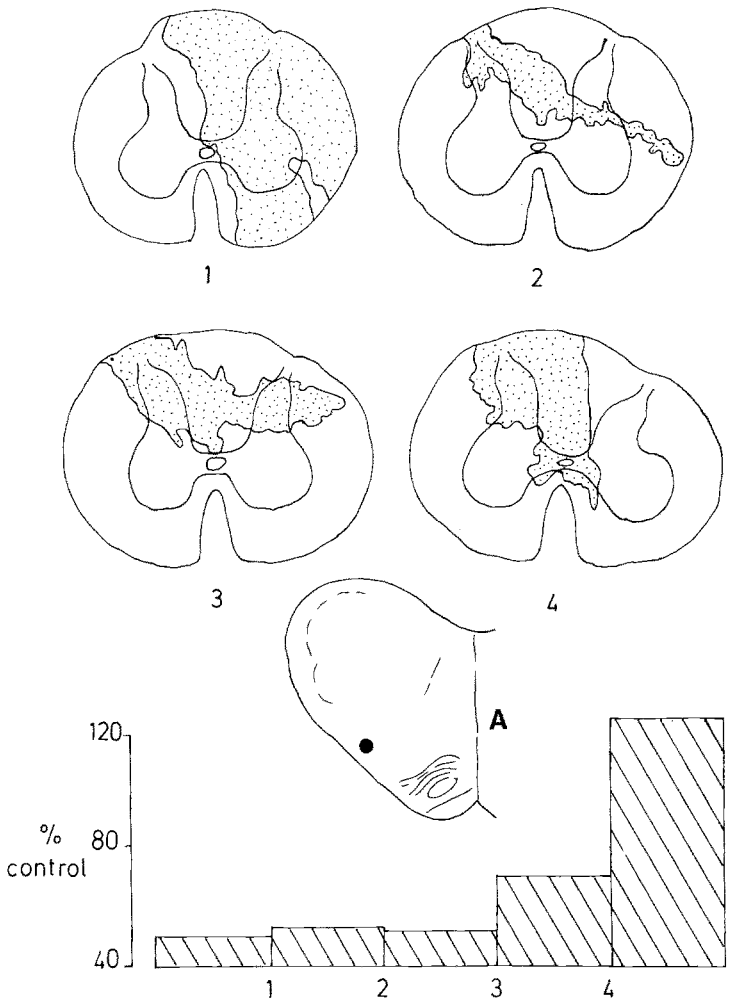


Fig. 22. Effect of spinal-cord lesions on the inhibition of spinal reflex activity in the tenth thoracic white ramus (T10WR) produced by electrical stimulation in the ventro-lateral medulla at the point marked by the *black circle in* (**A**). In the *bar graph* the size of the T10WR reflex response elicited 250 ms after medullary stimulation (1 ms pulse duration, 100 Hz for 150 ms), expressed as a percentage of the size of control reflex responses, is shown after four lesions (*stippled areas in 1, 2, 3 and 4*) had been made in the spinal cord at C3. Anaesthetised cat. (From Coote and Macleod 1975)

dorso-lateral funiculus where lesions abolished the effects of stimulating the ventro-lateral medulla. In addition, the calculated conduction velocities to sympathetic outflow for medullary sites and spinal sites were similar, having a mean of less than 2 m/s (Coote and Macleod 1974a, 1975), which is consistent with the effect being mediated by small unmyelinated catecholamine fibres. Further support for such a conclusion comes from the observation that

the alpha$_2$-adrenoreceptor antagonist piperoxane (at <2 mg/kg) given intravenously reduced the inhibitory effect of stimulating in the ventro-lateral medulla by some 70% in three animals (Coote and Macleod, unpublished observations). However, it is conceded that great weight cannot be placed on this result, since the drug given by the intravenous route may be acting at many sites in the nervous system to produce its effect. Another study claimed that stimulation of the A_1 region of the cat medulla caused excitation of sympathetic preganglionic neurons (Franz et al. 1975). It is difficult to take this claim seriously, since the published location of tested sites shows they were far too dorsal in the medulla and no evidence was provided that catecholamine neurons were present in this region. In any case, the latency to onset of the effect was far too short to be dependent on a slowly conducting catecholamine pathway. The authors then presumed that the depressant action of chlorpromazine on these responses was due to its antagonism of alpha adrenoreceptors, despite the drug's having several non-adrenergic actions.

A sympatho-inhibitory region located in the caudal ventro-lateral medulla has since been confirmed by several studies in the rabbit (Blessing and Reis 1982, 1983; Pilowski et al. 1985; Blessing and Willoughby 1985) and in the rat (Day et al. 1983), although the authors are not in favour of a spinally projecting A_1 or C_1 involvement.

The results of experiments on the physiological role of the A_5 group of cells are confusing. Loewy et al. (1979a) first contended that this group of cells constitutes a vasomotor centre, since they observed in rat, that electrical stimulation via electrodes in this region produced a pressor response after fibres of passage from regions above the midbrain had been eliminated by hemisection of the midbrain 4 weeks previously. This pressor response was absent in animals that had also been treated with 6-hydroxydopamine intraventricularly 1 month before the study. However, such correlations are tenuous, as was subsequently shown by the same laboratory (Neil and Loewy 1982; Stanek et al. 1984). These further studies re-examined the role of A_5 by activating cells of the region by applying the excitatory amino acid L-glutamate. This stimulation produced decreases in blood pressure and heart rate and a differential change in regional blood flow consisting of vasodilatation in skeletal muscle and vasoconstriction in the splanchnic area and skin. However, in a further study it was shown that this response is not controlled by descending noradrenaline-containing neurons but by some other spinally projecting cells (Loewy et al. 1986). The physiological role of the catecholamine neurons innervating the sympathetic preganglionic neurons is therefore still very much an open question.

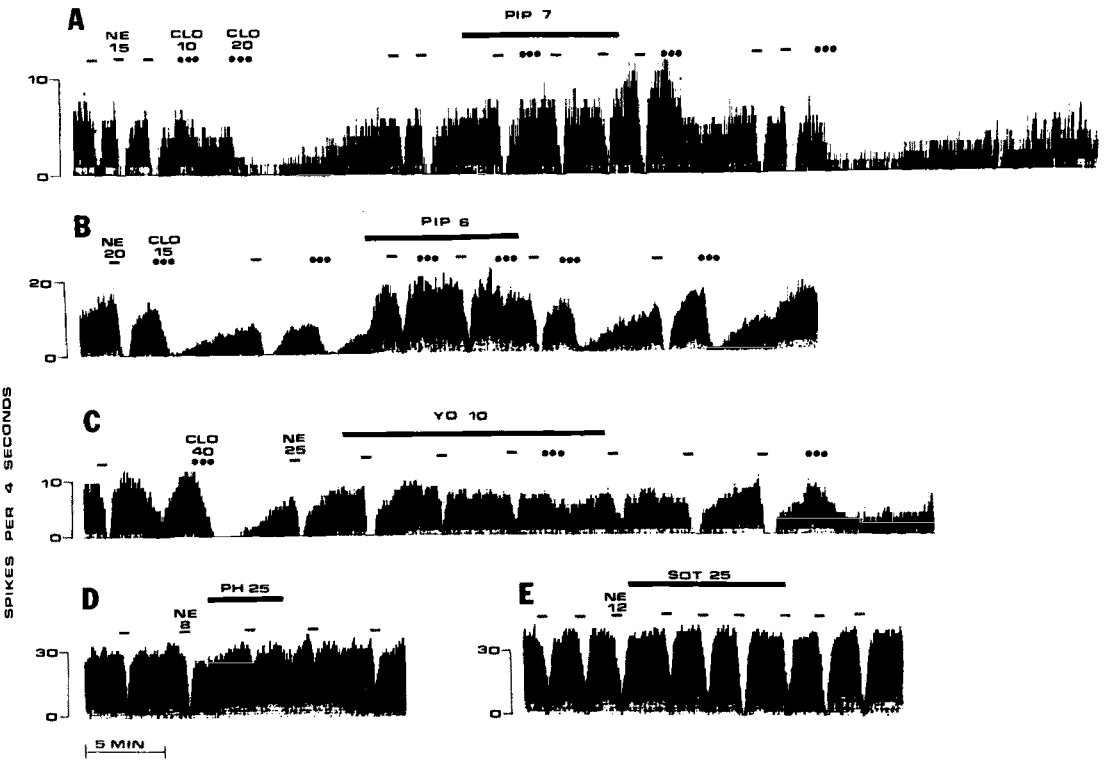


Fig. 23A–E. Pharmacological characterisation of the receptor-mediated inhibition of sympathetic preganglionic neuronal discharge following iontophoresis of noradrenaline (*NE*) and clonidine (*CLO*). **A–D** Integrated rate histograms (bin width, 4 s) illustrating the antagonism of NE- and CLO-induced inhibition of spontaneous discharge by the alpha-adrenergic antagonists, piperoxan (*PIP*), yohimbine (*YO*) and phentolamine (*PH*). E Lack of effect of the beta-antagonist Sotalol (*SOT*) on the inhibitory effect of NE. The duration of iontophoretic current applications is indicated by the *horizontal solid or dotted lines* above the histograms; the *associated numbers* present the current (nA) applied. Anaesthetised pigeon. (From Guyenet and Cabot 1981)

8.2.5 Microiontophoresis

Iontophoretic administration of the catecholamines noradrenaline, dopamine and adrenaline into the vicinity of sympathetic preganglionic neurons or antecedent neurons inhibits their activity (DeGroat and Ryall 1967; Coote et al. 1981a; Kadzielawa 1983a), an effect which is blocked by alpha$_2$-adrenoreceptor antagonists such as yohimbine or piperoxane, in cat (Kadzielawa 1983a), and bird (Fig. 23; Guyenet and Cabot 1981; Guyenet and Stornetta 1982). Complete interpretation of this result is of course dependent on whether the catecholamine fibres terminate directly on sympathetic neurons, and at present this is still not proven (but see Wong and Tan 1974). However, it is quite clear from these studies that each of the catecholamines

has a similar action, i.e. inhibition, and that the adrenoreceptors in the IML are predominantly of the alpha$_2$ type (Coote et al. 1979; Unnerstall et al. 1984; Dashwood et al. 1985); these facts must limit the scope of any interpretation.

8.2.6 In Vitro Studies

Another twist to the story has come from recent in vitro studies of sympathetic preganglionic neurons in spinal-cord slices. In these preparations, noradrenaline induces slow depolarisation in most cells of the spinal cord in neonatal rat and adult cat (Yoshimura and Nishi 1982; Yoshimura et al. 1986b; Ma and Dun 1985a) and hyperpolarisation in about 14% of cells in adult cat spinal cord (Yoshimura and Nishi 1982; Yoshimura et al. 1986b). Depolarisation is associated with an increase in membrane input resistance, is little affected by tetrodotoxin or low Ca/high Mg and can be prevented by hyperpolarising the membrane to a level which abolishes the after-hyperpolarisation. It is also prevented by pharmacological blockade with prazosin, an alpha$_1$-adrenoreceptor antagonist. This suggests it is due to direct action of noradrenaline on the sympathetic preganglionic neurons at alpha$_1$ receptors which decrease voltage-dependent K conductance (Ma and Dun 1985a; Yoshimura et al. 1986b).

The action of noradrenaline is exerted mainly on the repolarising phase of the spike and on the spike after hyperpolarisation. The repolarisation 'hump', a characteristic of some types of sympathetic preganglionic neuron (Dembowsky et al. 1986), is abolished by noradrenaline. An after-depolarisation replaces the fast phase of the after-hyperpolarisation, and the slow phase of the after-hyperpolarisation is suppressed. These effects are Ca- and voltage-dependent, suggesting that noradrenaline produces an increase in excitability of the sympathetic preganglionic neuron by decreasing the voltage-dependent, Ca-activated outward K current. Thus, the catecholamine controls the neuron discharge independently of its effects on membrane potential, although this controlling action depends on the presence of an ongoing excitatory input to the cell.

Despite the clear analysis of membrane events provided by these well-designed in vitro experiments, unfortunately they do little to help resolve the controversy regarding the actions of synaptically released catecholamines in vivo. So far, it is quite clear that iontophoretic administration of catecholamines in the vicinity of identified sympathetic preganglionic neurons in vivo does not excite them but inhibits their activity, whether it be natural ongoing activity, glutamate-evoked activity (DeGroat and Ryall 1967; Coote et al. 1981a; Kadzielawa 1983a; Guyenet and Cabot 1981; Guyenet and Stornetta 1982) or synaptically evoked activity (Coote et al. 1981a). Furthermore, the

inhibitory effects are prevented by alpha$_2$-adrenoreceptor antagonists (Kadzielawa 1983a; Guyenet and Stornetta 1982). Although both alpha$_1$-binding sites (Coote et al. 1979), and alpha$_2$-binding sites (Unnerstall et al. 1984; Dashwood et al. 1985) are present in the ILp, the alpha$_2$ site is overwhelmingly more abundant (see also Jones et al. 1982), so it is something of a puzzle that alpha$_2$-mediated actions have not so far been manifest in the in vitro studies. A sympathetic preganglionic neuron in a tissue slice lacks the ongoing synaptic drive present in intact spinal cord, but this is unlikely to have affected the outcome, since noradrenaline has similar effects in intact and spinal preparations (DeGroat and Ryall 1967). Differences related to species and age are also unlikely contributory factors, although the studies by Ma and Dun (1985) were performed on neonatal rats 8–15 days old and the iontophoretic studies were carried out on adult cats and birds; Yoshimura et al. (1986b) used spinal-cord slices from adult cats. Possibly important, for neurons whose dentritic arborisations can extend for 2.5 mm (Dembowsky et al. 1985a) is the likelihood that alpha$_2$ sites on dendritic synapses are removed in slices that are only 500 μm thick.

8.2.7 Pharmacological Studies

Again, pharmacological studies are more strongly supportive of an inhibitory role for the catecholamine pathways. Perfusion of the subarachnoid space of the thoracic spinal cord with noradrenaline (0.30 μmol) in rat inhibits sympathetic nerve activity (Lo Pachin and Rudy 1983). Intravenous administration of L-dihydroxyphenylalanine (L-dopa, 40–100 mg/kg) in a majority of laboratories causes a decrease in both pre- and postganglionic sympathetic activity (Sinha et al. 1973; Coote and Macleod 1974a). This effect occurs in spinal animals and is presumed to be due to uptake of L-dopa by monoamine nerve terminals in spinal cord and subsequent release of endogenous transmitter. Although there is little doubt that such an action occurs, the result should be viewed with some caution because L-dopa still has similar effects on spinal reflexes, although to a lesser degree, even following degeneration of descending monoaminergic fibres in chronically spinalised cats (Baker and Anderson 1970; Kirchner et al. 1975). Also, L-dopa may exert a direct action on certain central neurons (Ohye et al. 1970).

Other pharmacological evidence supports a sympatho-inhibitory role for the descending catecholamine pathway. Haeusler (1977) demonstrated in the cat that splanchnic-nerve discharges evoked by intraspinal stimulation are reduced by clonidine (< 25 mg/kg) and alpha-methyl-dopa (< 100 mg/g), this effect being blocked by the alpha-adrenoreceptor antagonists phentolamine and piperoxane, which had no effect on responses evoked by stimulating descending excitatory pathways in the spinal cord. Clonidine (10–25 mg/kg

i.v.) also blocks somato-sympathetic reflexes in white rami or ongoing activity of renal nerves in spinal animals (Franz et al. 1975; Coote and Macleod, unpublished observations). Franz et al. (1975) attributed the depression of sympathetic outflow by clonidine to stimulation of 5-HT receptors on sympathetic preganglionic neurons, mainly because of the evidence that its effect was antagonised by tolazoline. Yet this drug, as well having some antagonism to 5-HT, is a more effective antagonist of alpha-adrenoreceptors. This also seems to be the conclusion made by Franz from later experiments in which the inhibitory effect of clonidine was prevented by yohimbine the alpha$_2$-adrenoreceptor antagonist (Franz and Madsen 1982).

The conclusion that the bulbo-spinal catecholamine pathway is inhibitory is further reinforced by the enhancement of the spinal component of the somato-sympathetic reflex in T3 white ramus following intravenous administration of yohimbine (0.45 mg/kg), the alpha$_2$-adrenoreceptor antagonist. This enhancement indicates that the bulbo-spinal catecholamine neurons to the IML are tonically active. Either cooling the dorso-lateral funiculus or producing a small lesion in the dorso-lateral funiculus of the cervical cord where catecholamine fibres descend (which abolishes the inhibitory effect of stimulating the ventro-lateral medulla, from or through which catecholamine neurons destined for the ILp descend, see earlier section) causes enhancement of somato-sympathetic reflexes (Coote and Macleod 1975; Dembowsky et al. 1980). This observation supports the suggestion that neurons of this type are tonically active. An inhibitory influence exerted by noradrenaline or adrenaline was also indicated by initial studies with p-methoxyphenylethylamine, an agent which seems to act by releasing monoamines at central synapses even when given intravenously. DeGroat and Lalley (1973) showed that a sympathetic reflex evoked in a splanchnic nerve by pelvic-nerve stimulation in spinal cats was strongly depressed by this drug and the effect was prevented by prior administration of phenoxybenzamine but not by haloperidol or the 5-HT-antagonists methysergide and cyproheptadine.

At variance with these results is the interesting observation by Hare et al. (1972) that intravenous L-dopa in spinal cats has a biphasic effect on somato-sympathetic reflexes recorded in the upper thoracic white rami. An initial decrease is followed by prolonged, marked enhancement. Surprisingly, intraspinal evoked responses in the white rami were only depressed by L-dopa. Pretreatment with *p*-chlorophenylaniline (pCPA) eliminated the depresant action, as did tolazoline. The assumption made by these investigators was that pCPA and tolazoline were having specific actions on a 5-HT system in the ILp, but no evidence was provided for this, and they carried out no controls. The fact that tolazoline by itself enhanced both reflex-evoked and intraspinally evoked sympathetic responses and that L-dopa enhancement of the somato-sympathetic reflexes was reduced following pCPA treatment would appear to justify this criticism.

In contrast to the conclusion that noradrenaline is a sympatho-excitatory transmitter in the spinal cord, Franz and collaborators provided evidence that adrenaline may be a sympatho-inhibitory transmitter (Franz et al. 1982b; Sangdee and Franz 1983). It was shown that inhibition of adrenaline synthesis using selective inhibitors of the enzyme phenylethanolamine-*N*-methyl transferase caused gradual enhancement of the sympathetic response to intraspinal stimulation, with no effect on a segmental afferent sympathetic reflex.

Taylor and Brody (1976) also concluded that the descending noradrenergic pathway was excitatory to sympathetic preganglionic neurons. They electrically stimulated the lateral funiculus in spinal cats caudal to a transection and found that the evoked pressor response or vasoconstriction in a perfused hind limb was attenuated by infusing large doses (up to 900 mg) of the alpha-adrenoreceptor antagonist phentolamine into the sub-arachnoid space. No controls for the specificity of the antagonist's action were performed.

Others have considered that adrenaline-synthesising neurons in the rostral ventro-lateral medulla (Howe et al. 1980) project selectively to sympathetic preganglionic neurons and carry the important tonic excitatory drive to these cells (Ross et al. 1983). However, although these authors stimulated cell bodies in the rostral ventro-lateral medulla, no evidence was provided that the sympatho-excitatory effects were mediated by adrenaline. In fact, in the same region there are spinally projecting substance-P-containing cells thought by others to cause sympatho-excitation by releasing substance P into the spinal cord (Helke et al. 1982; Loewy and Sawyer 1982; Takano et al. 1984; Lorenz et al. 1985), although this also needs substantiating more rigorously.

There is also conflicting evidence on the action of DA. In contrast to the iontophoretic data showing an inhibitory effect of this amine (Coote et al. 1981a), Simon and Schramm (1983) found that in acutely spinalised rats, DA caused excitation of activity in renal sympathetic nerves when applied by intrathecal superfusion to the surface of the thoracic spinal cord. Furthermore, reduction of spinal-cord levels of each of the catecholamines with the superfusion of alpha-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase, reduced the magnitude of increases in sympathetic nerve activity evoked by stimulation of the spinal cord by 40%. Additionally, reduction of the concentrations of noradrenaline and adrenaline by superfusion with disulfiram, an inhibitor of dopamine hydroxylase, had no effect on the evoked responses, but the responses to DA were increased. The authors concluded that DA was acting at specific receptors, since its action was attenuated by intrathecal haloperidol. However, it is difficult to take this claim seriously since haloperidol alone produced a marked reduction in ongoing renal-nerve activity in the spinal animal. Since there are no DA somata in the spinal cord, this suggests that haloperidol was having a direct depresant action on sympathetic preganglionic neurons rather like that of the other DA antagonist chlor-

promazine (Hare et al. 1972; Neumayr et al. 1974; Bernthal and Koss 1979). Therefore, the influence of an endogenous DA innervation on sympathetic vasoconstrictor neurons is unclear. This is a neglected area and is deserving of further study.

8.3 Substance P

A dense plexus of substance-P-containing fibres and terminals is found in the principal sympathetic cell nucleus (ILp) in the spinal cord of all vertebrates studied so far including pigeon (Davis and Cabot 1984), oppossum (DiTirro et al. 1981), rat (Ciriello and Kanazawa 1978; Ljungdahl et al. 1978; Holets and Elde 1982; Helke et al. 1982; Davis et al. 1984; Schroder 1985), cat (Holets and Elde 1983; Krukoff et al. 1985a, b; Stock et al. 1983), hamster (Hancock 1982) and man (de Lanerolle and LaMotte 1982). There also appears to be a light innervation of the ICpe sub-nucleus close to the central canal (Davis et al. 1984; Schroder 1985; Krukoff et al. 1985a, b).

Some of these substance-P-containing terminals arise from spinally projecting neurons in the ventro-lateral medulla (Helke et al. 1982; Lovick and Hunt 1983; Marson and Loewy 1985), an area of the brain which is also thought to contain cells from which terminals in the ILp containing 5-HT and thyrotropin-releasing hormone arise (Bowker et al. 1981; Bowker et al. 1982b; Chan-Palay 1979; Chan-Palay et al. 1978; Johansson et al. 1981; Ljungdahl et al. 1978; Helke et al. 1985). Furthermore, it is probable that one or the other of these peptides coexists with 5-HT (Bowker et al. 1981, 1982b; Gilbert et al. 1982a, b; Marson and Loewy 1985) or adrenaline (Lorenz et al. 1985) in sub-populations of these spinally projecting neurons which terminate in the ILp.

There is also, in the ventro-lateral medulla (in the nucleus interfascicularis hypoglossi and perhaps the nucleus paragigantocellularis pars lateralis) a group of neurons containing substance P but not 5-HT, which innervate the ILp (Helke et al. 1982, 1986). There is also a group of neurons in the ventro-lateral part of areas B_1 and B_3, containing 5-HT but not substance P, which also innervate the ILp (Loewy and McKellar 1981). However, not all of the substance P in the ILp is in bulbo-spinal afferent terminals; some (around 30%) in rat, and more (<80%) in pigeon, is in terminals of intraspinal neurons, since a degree of substance-P-like immunoreactivity remains in the ILp following total transection of the cervical cord (Davis et al. 1984; Davis and Cabot 1984). Afferent fibres containing substance P appear to pass from the marginal zone of the dorsal horn along its lateral border to end in lamina VII of the spinal-cord grey matter (Davis et al. 1984) rather reminiscent of the projection of renal afferent fibres reported by Kuo et al. (1983). Radioimmunoassay has confirmed the presence of a substance in the spinal cord

which is indistinguishable from synthetic substance P (Davis and Cabot 1984; Davis et al. 1984).

It has been suggested that substance P is a chemical mediator of excitatory input to sympathetic preganglionic neurons. Applied iontophoretically near preganglionic neurons of the upper thoracic cord in cat or rat, the peptide excites neurons that are spontaneously active or have been already excited by glutamate, although it is ineffective on silent neurons (Gilbey et al. 1983; Backman and Henry 1984b). On the other hand, splanchnic sympathetic neurons recorded in the lower thoracic cord of the cat are either weakly excited or weakly inhibited by substance P (Felpel and Hoffman 1986). Substance P applied to the spinal cord through an intrathecal catheter excites preganglionic neurons projecting to the adrenal medulla (Yashpal et al. 1985) or to the splanchnic area and kidney (Yusof and Coote 1986b; Coote 1986).

Evidence of this nature supports the idea that the substance-P pathway from the ventral medulla to the ILp is an excitatory one. Although stimulation in the general area occupied by substance-P neurons in the ventral medulla elicits pronounced increases in sympathetic nerve activity, there has so far been no rigorous experimental proof that this is a consequence of the release of substance P onto sympathetic preganglionic neurons, because of the lack of specific antagonists (Yusof 1986; Yusof and Coote 1986b, 1987). There have, though, been several attempts to demonstrate that substance-P neurons are involved in generating the tonic activity of sympathetic vasoconstrictor neurons which gives rise to the characteristic vasomotor tone in the periphery. Intrathecal administration of substance-P antagonists into the thoracic cord lowers blood pressure in normotensive and hypertensive rats (Keeler and Helke 1985; Loewy and Sawyer 1982; Takano et al. 1985), which suggested to these authors a tonic sympatho-excitatory role for substance P. Pressor effects elicited by application onto the ventral medullary surface of kainic acid (an excito-toxic drug) or bicuculline, which presumably antagonised inhibition of cells mediated by gamma-amino butyric acid, can be prevented at the spinal-cord level by intrathecal injection of substance-P antagonists (Keeler and Helke 1985; Loewy and Sawyer 1982). Furthermore, the increases in blood pressure induced by ventro-medullary kainic acid were correlated with increased substance-P release from the spinal cord (Takano et al. 1984). High-affinity binding sites for substance P are present in the thoracic cord (Charlton and Helke 1985; Maurin et al. 1984; Takano et al. 1986; Niwa et al. 1986), most probably on sympathetic preganglionic neurons (Takano and Loewy 1985), which also have a high affinity for the substance-P antagonist (D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11})-sub P, the hypotensive action of which can be countered by a specific substance-P agonist pGLu^{5}, MePhe^{8}, Sar^{9}-SP$_{5-11}$ (DiME-SP or DiMe-C$_{7}$) (Keeler et al. 1985). Although this supports the idea that substance P may mediate the actions of tonically active descending vasomotor pathways, it by no means proves it. The interaction

may not have been at specific receptors, and it could have been the consequence of algebraic summation of opposing non-specific effects. In a more critical examination of this problem, Yusof and Coote (1986a, 1987) carried out a series of experiments on anaesthetised rats using the method of subarachnoid perfusion to administer drugs to the central nervous system (Yaksh and Rudy 1976; Coote and Suter 1985; Suter and Coote 1987). Recordings were made of activity in pre- and postganglionic sympathetic nerves to the splanchnic area. The excitatory effect of intrathecal substance P (10 µl, 5–10 µg), 5-HT (10 µl, 70–220 µg), glutamate (10 µl, 0.85 mg) and of electrical stimulation (30 Hz, 100 µA, 10 s) of the ventral medulla were compared during and after the antagonist (D-Arg^{1}, D-Pro^{2}, D-Trp^{79}, Leu^{11})-sub P (10 µl, 1–40 µg) given intrathecally. In eight rats the spontaneous sympathetic activity had virtually disappeared 20 min after the antagonist was given, and all the test responses were markedly decreased. At 75 min there was no response to any of the test stimuli, and blood pressure had fallen to 50 mmHg. This strongly indicates that the action of the substance-P antagonist was non-specific. We should therefore look cautiously at evidence based on the actions of the antagonist unless controls for specificity are carried out.

There are a number of other features of substance P that lead us to question whether it has a role as the major excitatory transmitter mediating the tonic drive to sympathetic vasomotor neurons. Its inability to excite 'silent' neurons unless they are first activated by glutamate, the slow time course of onset of excitation, the slow build-up of excitation to a peak, the prolonged effects after the end of application (Gilbey et al. 1983; Backman and Henry 1984b; Felpel and Hoffman 1986), are all properties quite the opposite to those expected for a mediator of the tonic drive to sympathetic neurons. Furthermore, the location of the caudal substance-P group of spinally projecting cells in the medulla is not coincident with the retro-facial group of spinally projecting neurons that most evidence implicates as the source of the tonic excitatory drive to sympathetic vasomotor neurons (Brown and Guyenet 1984; Barman and Gebber 1985; McAllen et al. 1982; Lebedev et al. 1986; Hilton et al. 1983; Pilowsky et al. 1985; Lovick 1987). It is therefore fair to conclude that although substance P excites sympathetic preganglionic neurons and is released following selective activation of spinally projecting neurons in the ventral medulla, the identity of the central neurotransmitter of vasomotor tone is still unresolved. The substance-P neurons could, however, still exert an important influence on the excitability of sympathetic preganglionic neurons by inducing a long-lasting sub-threshold depolarisation so that cells are more easily excited by another stimulus, as has been reported for neurons in the dorsal and ventral horns of the lumbar spinal cord (Otsuka and Takahashi 1977; Zieglgänsberger and Tulloch 1979).

8.4 Thyrotropin-Releasing Hormone

Radio-immunoassay and immuno-histochemistry have established that thyrotropin-releasing hormone (TRH) is widely distributed in the mammalian brain (Hokfelt et al. 1975; Jackson and Reichlin 1974; Kubeck et al. 1977; Oliver et al. 1974; Spindel and Wurtman 1980; Bowker et al. 1983) and spinal cord (Kardon et al. 1977; Gilbert et al. 1982a; Bowker et al. 1983; Hokfelt et al. 1978; Johansson et al. 1981). The identity of TRH in these regions has been confirmed using thin-layer chromatography and high-pressure liquid chromatography (Krieder 1979; Spindel and Wurtman 1980; Jackson 1980). Specific binding of TRH to brain-tissue membranes has been demonstrated in the monkey, with high levels in the cerebral cortex, hypothalamus, interpeduncular nucleus and periaqueductal grey matter, but low levels in the cervical and lumbar spinal cord (Ogawa et al. 1981). Spinally projecting TRH neurons are found in the ventro-medullary reticular nuclei, nucleus interfascicularis hypoglossi (NIH) and nucleus paragigantocellularis lateralis (PGCL) as well as throughout the caudal raphe nuclei (Johansson et al. 1981; Lechan et al. 1983; Bowker et al. 1983; Helke et al. 1986). Antisera to TRH stain networks of fibres around neurons of the ILp in rat (Hokfelt et al. 1978; Gilbert et al. 1982b; Bowker et al. 1983; Lechan et al. 1984). These fibres are terminals derived exclusively from supraspinal sources (Jackson and Reichlin 1974; Kardon et al. 1977). Part of the TRH innervation of the ILp comes from neurons located in the NIH and PGCL, the latter forming a quite discrete innervation (Helke et al. 1986). About half of the TRH fibres also contain 5-HT, since 5,7-dihydroxytryptamine given intracisternally to destroy 5-HT neurons reduces TRH levels in the ILp region of the thoracic cord by 45% (Helke et al. 1986). Thus, the spinally projecting TRH-containing neurons form at least three sub-groups which innervate the ILp.

Iontophoretic application of TRH near sympathetic preganglionic neurons in the T1–T3 segments of cat spinal cord has a weak excitatory effect, but only on spontaneously discharging cells (Backman and Henry 1984b). In the rat, application of TRH to the lower thoracic spinal cord via an intrathecal catheter excites sympathetic neurons contributing to the splanchnic outflow, an effect which is similar to that of low doses of 5-HT (Coote 1986; Yusof and Coote 1986a). In contrast, TRH applied intrathecally to the lumbar sympathetic vasoconstrictor neurons supplying hind-limb skeletal muscle inhibits their activity, an effect which again is similar to the action of 5-HT on these neurons. This suggests that the chemical coding may reflect functional coding, the TRH and 5-HT neurons being responsible for a pattern of sympathetic response.

8.5 Oxytocin and Vasopressin

There is abundant neuro-anatomical evidence indicating that in several primates and in calf, cat and rat there is a major projection from the parvocellular cells in the dorso-medial paraventricular nucleus (PVN) of the hypothalamus to the sympathetic nuclei in the intermediate grey region of the thoracic and lumbar spinal cord (Swanson and Kuypers 1980; Armstrong et al. 1980; Luiten et al. 1985). Kuypers and Maisky (1975) demonstrated that neurons in cat PVN were retrogradely labelled following horseradish-peroxidase injections into the spinal cord. Similar findings were reported for the rat (Hancock 1976). These results were confirmed by Saper et al. (1976), who also studied the anterograde transport of tritiated amino acids from the PVN to the spinal cord in monkey, cat and rat, and found labelled fibre terminals in the ILp, ILf, IC and ICpe. Immuno-histochemical studies in the above species and in man indicate that many (>20%) spinally projecting PVN contain oxytocin, vasopression and the associated neurophysins, with apparent predominance of the neurons containing oxytocin and neurophysin I (Buijs 1978; Swanson and McKellar 1979; Swanson and Sawchenko 1980; Nilaver et al. 1980; Sofroniew 1980; Armstrong et al. 1980; Gibson et al. 1981; Glick and Brownstein 1980; Holets and Elde 1982; Lang et al. 1983; Sofroniew and Weindl 1981; Yamaji et al. 1981; Millan et al. 1984a).

The spinally projecting neurons probably do not project to the neurohypophysis (Ono et al. 1978; Armstrong et al. 1980; Swanson and Kuypers 1980; Millan et al. 1984a; Yamashita et al. 1984). On the basis of the way in which the innervation is distributed within the thoracic segments of the rat (Swanson and McKellar 1979) and cat (Krukoff et al. 1985a), it was suggested that specific visceral organs may be preferentially influenced by the PVN-spinal neurons containing oxytocin. It was shown in rat that the ILp was most densely innervated in segments T1–T3, T9–T11 and T13–L2, whereas in cat it was T2–T8, T13, L2–L3, and that the innervation within a particular spinal segment occurred at irregularly spaced intervals correlated with clusters of preganglionic neurons in the ILp (Gilbey et al. 1982b; Rando et al. 1981). A similar situation exists in the human spinal cord, although the distribution favours segments T4–T5, T7–T10 and T12 (Jenkins et al. 1984). There is even a differenial distribution of neurophysin- and oxytocin-immunoreactive fibres to functionally different pre-ganglionic neurones. Holets and Elde (1982, 1983) used true blue or fast blue to retrogradely label sympatho-adrenal preganglionic neurons in the ILp of thoracic-cord segments T1–T13 and lumbar cord (L1). Oxytocin or neurophysin terminals did not appear to impinge on the labelled cells, although they did converge on other unlabelled cells in the ILp.

The influence of this peptide innervation has been studied in anaesthetised rat (Gilbey et al. 1982a) and cat (Backman and Henry 1984a; Yamashita et

al. 1984). In rat oxytocin applied by microiontophoresis was observed to consistently produce a decrease in firing of the preganglionic neurons (16/23 neurons), only 1 neuron being excited. This depressant action exerted on the preganglionic neurons by oxytocin was mimicked by low-intensity electrical stimulation confined to the paraventricular nucleus (Fig. 24). Arginine vasopressin (AVP) applied microiontophoretically was also predominantly inhibitory, 8/14 neurons being inhibited and 4/14 being excited (Gilbey et al. 1982a). Gilbey et al. (1982a) argued that inhibition of the sympathetic preganglionic neurons by both the paraventricular nucleus and the peptides was effected at the cell membrane, since glutamate-evoked firing of the cells was also inhibited. This argument assumes that the neuronal firing produced by the release of glutamate from the micropipette is the result of its direct action on the soma-dendritic membrane of the preganglionic neuron. If such is the case, it is unlikely that suppression of glutamate-evoked activity is due to the removal of the sub-threshold tonic activity, as this is likely to be minimal relative to the glutamate-evoked depolarisation which causes the cell to discharge. The more inconsistent action of AVP may reflect the paucity of innervation of preganglionic neurons by vasopressin terminals (Sofroniew 1980). On the other hand, it may represent a true difference in response of different sympathetic preganglionic neurons to AVP.

Other, less direct evidence supports an excitatory role for AVP in the rat spinal cord. Sub-arachnoid perfusion of the rat thoracic cord with AVP causes an increase in blood pressure which is not prevented by intravenous administration of the antagonist $A(Ch_2)_5$D-Tyr-AVP, suggesting that AVP was exciting sympathetic preganglionic neurons in the spinal cord (Riphagen and Pittman 1985). This excitatory action was apparently restricted to sympathetic vasoconstrictor outflows, since in a further series of experiments the increase in blood pressure was shown to occur without activation of the adrenal medulla (Riphagen et al. 1986). In addition, increases in mesenteric, renal and hind-quarter vascular resistance were noted after intrathecal AVP application in the experiments of Porter and Brody (1985). Recent work on isolated spinal-cord slices from neonatal rats, showing that vasopressin depolarises lateral-horn cells (Ma and Dun 1985b) supports this conclusion. However, caution must be exercised, since all substances so far applied to the spinal cord in vitro by these authors seem to have an excitatory effect, often unlike the in vivo action of the substance.

Studies in the cat have emphasised an excitatory influence of oxytocin and AVP on sympathetic preganglionic neurons. Backman and Henry (1984a) obtained little effect on neurons in segments T1–T3 during iontophoretic application of the peptides, but in some cases activity increased on cessation of the iontophoretic current. This led to the conclusion that oxytocin and AVP excited the sympathetic preganglionic neurons. This is so unlike the action of these peptides as observed in the rat, with respect to the time course as well

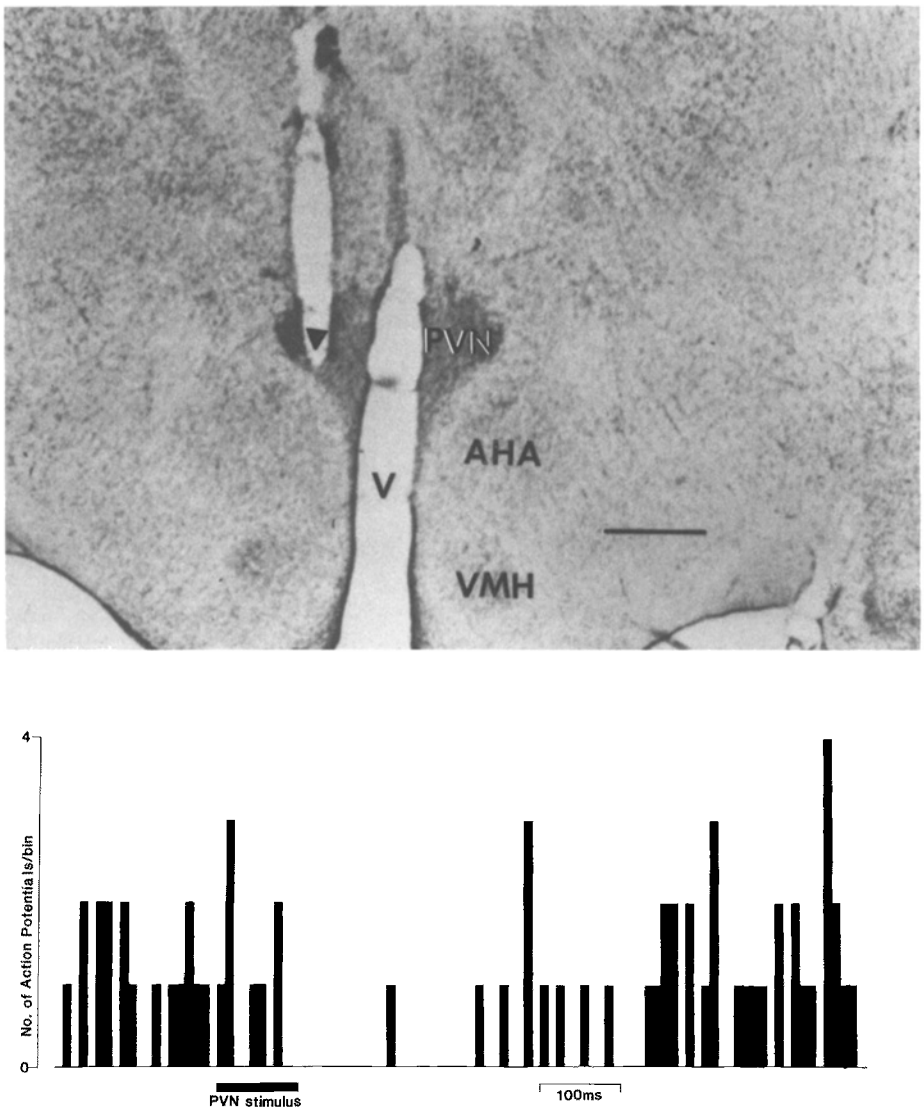


Fig. 24. Effect of a brief stimulus (ten pulses, 1 ms duration, 100 ms at 100 Hz and 100 μA) applied at electrode tip placed as indicated by the *solid triangle* in the track left by the electrode in the paraventricular nucleus (PVN) in the transverse section through the anterior hypothalamus (AHA) shown *above*; *VHM*, ventromedial nucleus; *V*, third ventricle; *bar*, 500 μm. *Below:* peri-stimulus histogram illustrating the inhibitory effect produced by this brain stimulation (PVN stimulus) on the discharge of a glutamate-activated sympathetic preganglionic neuron antidromically identified in the second thoracic segment of the spinal cord. The histogram shows 16 trials, with 3 s between each trial. Bin width, 10 ms; Anaesthetised rat. (From Gilbey et al. 1982)

as direction of effect, that it suggests the recording electrodes were some distance from the soma or dendrites of the preganglionic neurons and the effects observed were due to peptides acting on antecedent neurons or terminals. However, Yamashita et al. (1984) have also argued that a paraventricular spinal pathway is likely to be excitatory. They were unable to inhibit ongoing activity in a thoracic white ramus (T3) when electrically stimulating the paraventricular nucleus. It is conceivable that in the study by Yamashita et al., inhibitory effects were masked by preferential stimulation of fibres passing through the nucleus.

Additionally, inhibitory effects on a few preganglionic neurons may not have shown up clearly in a functionally mixed, multifibre recording of activity in the white ramus, where the signal-to-noise ratio was low. The study by Yamashita et al. is also notable because few units that were antidromically excited by stimulation of the spinal cord were located in the paraventricular nucleus, yet tracing studies show that spinally projecting neurons are relatively dense in this nucleus (>1500 neurons; Kuypers and Maisky 1975; Saper et al. 1976; Swanson and Kuypers 1980). Further studies are needed to clarify the influence and functional role of what is clearly an important innervation of sympathetic preganglionic neurons. With regard to function, it is interesting that the spinal-cord pools of oxytocin and AVP are generally independent of their neurohypophyseal counterparts, not only anatomically but also biochemically. Hence, each of the peptides in the spinal cord is insensitive to physiological manipulations which affect their counterparts in neurohypophyseal terminals. Furthermore, there is no close inter-relationship of spinal-cord vasopressin with dynorphin-related peptides or oxytocin with methionine-encephalin, in contrast to the peptides of the neurohypophysis (Millan et al. 1984a, b).

8.6 Angiotensin II

Using immuno-histochemical techniques, Fuxe et al. (1976), Stock et al. (1983) and Lind et al. (1985) have demonstrated the presence of material resembling angiotensin II (AII) in varicose nerve terminals in various regions of the brain and the spinal cord. In particular they have shown that there is a high density of such terminals in the ILp of the thoracic spinal cord. Fuxe et al. (1976) found no evidence of cell bodies containing AII in the spinal cord, but demonstrated AII-like material in axons of the lateral funiculus projecting to the IML, suggesting that the AII innervation is of supraspinal origin. Further support for this innervation comes from studies using ^{125}I-labelled angiotensin analogues, which have demonstrated the presence of specific binding sites for angiotensin in the grey matter of the spinal cord (Mendelsohn et al. 1984).

Angiotensin II has been shown to excite neurons in various parts of the central nervous system (CNS), including the supra-optic region (Nicholl and Barker 1971), sub-fornical organ (Felix and Akert 1974; Felix and Schlegel 1978; Nicholaidis et al. 1984), hypothalamus (Wayner et al. 1973) and cerebral cortex (Phillips and Linacher 1974; Sudakov et al. 1976). It also excites neurons in nervous tissue in vitro, including hypothalamic and hippocampal slices (Hass et al. 1980; Knowles and Phillips 1980) and hypothalamic and spinal neurons in culture (Gawhiler and Dreifuss 1980; Legendre et al. 1984; Phillips et al. 1980).

These results raise the possibility that AII is involved in regulating the level of excitation of sympathetic preganglionic neurons. This was tested in a recent study (Coote and Suter 1985; Suter and Coote 1987) by administering AII and the AII-antagonist saralasin intrathecally (i.t.) to the spinal cord of rats, whilst monitoring blood pressure and activity in pre- and postganglionic nerves. Intrathecal AII (20 µl, 10^{-5} M) caused a significant increase in blood pressure and sympathetic nerve activity. These responses were shown to depend on an action of AII in the spinal cord. The magnitude of the response was dependent on the location of the catheter tip within the sub-arachnoid space, T9–T10 being best for the above changes. Preceding i.t. AII with saralasin (20 µl, 10^{-3} M) prevented the changes, and the action of AII was not blocked by ketanserin (Coote, unpublished observations), suggesting the effect was mediated by specific AII receptors (Suter and Coote 1987).

These data do not show that AII has a direct excitatory action on sympathetic preganglionic neurons. However, since Fuxe et al. (1976) have obtained evidence of AII-like material in nerve terminals near sympathetic preganglionic neurons, projecting from the lateral funiculus, it is likely that AII acts at the level of these neurons. It is unclear, however, whether it acts directly as a conventional transmitter, or modulates the action of another substance, which may be excitatory or inhibitory. An interesting observation by Suter and Coote (1987) was that the application of saralasin alone to the spinal sub-arachnoid space did not inhibit ongoing sympathetic nerve activity to any marked extent, possibly due to the AII-agonist properties of saralasin (Knowles and Phillips 1980) or perhaps because the concentration of antagonist reaching the AII receptors was insufficient to block ongoing activity, although it was sufficient to block the action of exogenous AII which presumably had to overcome the same obstacles to reach the receptors.

It is also possible that AII does not significantly contribute to maintaining the resting level of activity in sympathetic nerves, but is involved only when sympathetic reflexes controlling the cardiovascular system are initiated. Supporting this proposal is the observation that i.t. saralasin did reduce the increase in sympathetic activity evoked reflexly by reducing the blood pressure with i.v. acetylcholine. Interestingly, Ganten et al. (1978) and Crofton et al. (1981) have shown that intracerebroventricular administration of saralasin

and converting-enzyme inhibitors (which block AII biosynthesis) has no effect on the blood pressure of normal rats, but causes a fall in the blood pressure of spontaneously hypertensive rats (SHR). It is possible, therefore, that AII is released as a transmitter onto sympathetic preganglionic neurons from nerve terminals which are only active when activity in the sympathetic pathways controlling the cardiovascular system needs to be markedly elevated. Alternatively, AII may be a modulator which is released during sympathetic activation and may alter the action on sympathetic preganglionic neurons of a conventional transmitter, thereby increasing the level of activity in sympathetic outflow from the spinal cord. AII acting in this way may be released either from purely angiotensinergic nerve terminals or as a co-transmitter with a conventional transmitter. In either case, at resting levels of activity, insufficient AII may be released to affect the action of the conventional transmitter, but during sympathetic activation the amount in the region of the synaptic cleft may build up, either through the release of more AII per unit of presynaptic activity or through saturation of the processes terminating the action of the peptide, so that it enhances the effect of the increased postsynaptic activity.

8.7 Methionine Encephalin

Fibres and terminals containing methionine encephalin (met-encephalin) within the principal sympathetic nucleus of the spinal cord have been described (Stock et al. 1983; Holets and Elde 1982, 1983; Hancock 1982). These disappear after transection of the spinal cord, suggesting that they originate from supraspinal nuclei (Holets and Elde 1982). A descending met-encephalin pathway to the sympathetic nucleus in the spinal cord has been described (Hokfelt et al. 1979), and cell bodies containing met-encephalin have been localised in the raphe nuclear group (Bowker 1981). These could be the source of the terminals in the spinal cord. However, met-encephalin neurons must be distinct from neurons containing 5-HT, because treatment with the 5-HT neurotoxin 5,7-dihydroxytryptamine does not deplete the neuropeptide (Gilbert et al. 1982b). Encephalins are known to modify the release of neurotransmitters, hormones and other secretions and to induce metabolic changes (Loh and Li 1977; Ipp et al. 1978; Konturek et al. 1978; Meites et al. 1978; Miller and Cuatrecasas 1978). It is therefore possible that this innervation of the ILp plays a similar role. Intravenous administration to spinal cats of opiates, including morphine, fentanyl, methadone and meperidine, which might mimic the actions of endogenous encephalins, depresses intraspinal and reflex-evoked spinal sympathetic activity in white rami. This action is rapidly and completely reversed by small doses of the specific antagonist naloxone (Franz et al. 1982a). The encephalin innervation is therefore likely to be an inhibitory one.

8.8 Somatostatin

Somatostatin-immunoreactive fibres have been identified in the ILp of rat and other species (Forssmann 1978; Stock et al. 1983; Holets and Elde 1982, 1983; Hancock 1982; Schroder 1985; Krukoff et al. 1985b). At least in rat, these fibres in the lower thoracic segments preferentially surround the preganglionic neurons destined for the adrenal medulla (Holets and Elde 1982, 1983). In cat, somatostatin terminals are most heavily distributed to segments C8–T1, and T5 (Krukoff et al. 1985b), being sparsely distributed at other levels. Since they disappear from the ILp after cervical-cord section, somatostatin fibres do not originate from primary afferents, but from a descending supraspinal pathway, although as yet there are no clues as to the location of their cell bodies. The peptide is not co-located with 5-HT in spinally projecting neurons, since it is not depleted after destruction of the 5-HT neurons with 5,7-dihydroxytryptamine (Holets and Elde 1982). Somatostatin has inhibitory effects when given iontophoretically to neurons in the dorsal horn (Randic and Miletic 1978; Renaud et al. 1978) but there have been no similar studies of sympathetic preganglionic neurons.

8.9 Other Peptides

Terminals immunoreactive for vasoactive intestinal polypeptide and avian pancreatic polypeptide have been demonstrated in the ILp of the thoracic cord in cat and rat (Stock et al. 1983; Gilbert et al. 1982a). There is also evidence that reasonable numbers of terminals immunoreact with neurotensin-like substances in each of the sympathetic sub-nuclei throughout the thoracic and lumbar segments of the cat spinal cord (Krukoff et al. 1985b). In addition, terminals that are immunoreactive for cholecystokinin (CCK)-like substances have been demonstrated in the ILp of cats at L1 (Schroder 1985). Little more is known about these innervations.

8.10 Excitatory and Inhibitory Amino Acids

There are several amino acids that have gained recognition as neurotransmitter candidates in the mammalian nervous system, but until recently none has seriously been considered as a mediator of synaptic influences on the sympathetic preganglionic neuron. The excitatory amino acids, glutamate and aspartate, are of particular interest because they show an unequal regional distribution in the thoracic spinal cord and they occur in high concentrations in the region occupied by the ILp. This suggests they may have another role apart from that in intermediary metabolism. Our own studies on two cat

spinal cords (Fleetwood-Walker and Coote, unpublished observations) have shown that in the ILp of segments T1–T2, aspartate concentration was as high as 57 nmol/mg protein compared to 44 nmol/mg protein in the dorsal horn and 64 nmol/mg protein in the ventral horn. The corresponding figures for glutamate were: 76 nmol/mg protein in the ILp, 86 nmol/mg protein in the dorsal horn and 81 nmol/mg protein in the ventral horn. The values for the dorsal and ventral grey matter of these two segments of the thoracic cord were not very different from those reported by others for the corresponding regions in the lumbar cord (Graham et al. 1967; Rizzoli 1968; Lane et al. 1978), in which these amino acids have been investigated as likely transmitter candidates (Watkins and Evans 1981).

With respect to sympathetic preganglionic neurons, several authors have shown that iontophoretic application of glutamate in the vicinity of the soma increases their rate of discharge up to a maximum of 20–30 Hz, or causes silent cells to commence firing (Wyszogrodski and Polosa 1973; Coote et al. 1981a, b; Kadzielawa 1983a, b; Gilbey et al. 1983; Backman and Henry 1983b). There are only a few cells that fail to discharge an action potential when glutamate is applied. In such cases, the height of the antidromic action potential is decreased, suggesting that there is depolarisation or an increase in membrane conductance (Backman and Henry 1983b). Such an effect was clearly shown in intracellular recordings from cells in the ILp from a slice preparation of cat spinal cord (Yoshimura and Nishi 1982). Aspartate applied in a similar manner to the preganglionic neurons is about equipotent with glutamate in the majority of cells, although in 4/16 neurons where careful comparison was made, aspartate was more powerful and in 2 out of the 16 cells glutamate was more potent (Backman and Henry 1983b). In the spinal-cord slice preparation, cells of the ILp show depolarisation with increasing membrane resistance when aspartate is applied (Yoshimura and Nishi 1982). Generally, preganglionic neurons are very sensitive to both of these amino acids: in many cases merely removing the retaining current is sufficient to cause excitation (Coote et al. 1981a; Backman and Henry 1983b).

Despite this start, the roles of glutamate and aspartate as excitatory transmitters in the spinal sympathetic circuits have not been established, because no comprehensive examination using agonists and antagonists has so far been conducted. More extensive studies have been conducted on the role of the inhibitory amino acids glycine and gamma-amino butyric acid (GABA) in the spinal sympathetic circuits. In both cat and rat spinal cord, the concentrations of GABA (18 nmol/mg protein) and its biosynthetic enzyme glutamic-acid decarboxylase (GAD) in the intermediate grey region, including the ILp, is about 63%–86% of that in the dorsal-horn grey matter, where their concentrations are the highest attained in the spinal cord (Tappaz et al. 1976; Fleetwood-Walker and Coote, unpublished observations). The level of GABA or GAD in the ILp is considerably higher than in the ventral grey matter.

Glycine is also present in considerable quantities in the ILp. A neurochemical study of the cat T1–T2 segments, conducted in my own laboratory (Fleetwood-Walker and Coote, unpublished observations), found values as high as 64 nmol/mg protein for ILp grey matter and 75 nmol/mg protein for ventral grey matter, which in the lumbar spinal cord also contains the highest concentrations of glycine (Graham et al. 1967; Rizzoli 1968; Aprison et al. 1969; Lane et al. 1978).

Both GABA and glycine, applied iontophoretically near single preganglionic neurons in the ILp of segments T1–T3 in cat spinal cord, inhibit ongoing activity of the neurons and also prevent antidromic invasion of the soma-dendritic region, suggesting that they both act at a postsynaptic site. These effects are rapid in onset and recovery, and can be selectively blocked by iontophoretic application of bicuculline and strychnine (Backman and Henry 1983a).

The membrane events associated with the inhibitory actions of glycine on cells in the ILp have been studied in slice preparations of rat spinal cord (Yoshimura and Nishi 1982; Mo and Dun 1987). Intracellular recordings showed that glycine caused a hyperpolarisation which had a reversal potential of −67 mV and was similar to IPSPs occurring spontaneously or elicited by stimulation of dorsal rootlets. These IPSPs were reversibly abolished by strychnine but not by bicuculline. These experiments clearly establish, for the first time, the presence of endogeneous glycine-mediated inhibition in the spinal synaptic control of cells that may well be sympathetic preganglionic neurons. A further interesting feature of the study by Mo and Dun (1987) was that in a few ILp cells glycine caused a depolarisation with a reversal potential of −40 mV quite distinct from that of the IPSP, which persisted in low Ca^+ concentrations or in solutions containing tetrodotoxin, suggesting it was due to a direct postsynaptic action of glycine. Moreover, this effect was also antagonised by strychnine and not by bicuculline.

Numerous other experiments have suggested the possibility of a GABA-mediated as well as glycine-mediated inhibitory action at synapses on sympathetic preganglionic neurons. In preparations of spinal-cord slices, all the neurons examined in the ILp were susceptible to the hyperpolarisation and shunting actions of GABA (Yoshimura and Nishi 1982). Gootman and Cohen (1981) showed that the activity of sympathetic preganglionic neurons in spinal cats is increased more by strychnine given intravenously than by asphyxia. Strychnine nitrate (0.2–0.3 mg/kg i.v.) in the cat abolishes the inhibitory effect of antidromically activating one pool of preganglionic neurons on the orthodromic activation of another group of preganglionic neurons, so-called recurrent inhibition (Lebedev et al. 1980).

The GABA antagonist, picrotoxin, given intravenously in relatively high doses (8–10 mg/kg) causes oscillations in blood pressure and sympathetic nerve activity in spinal cats (Polosa et al. 1969). Extracellular recordings of sympathetic preganglionic neurons show that following picrotoxin most, if

not all, neurons become active as compared to about 20% under normal conditions (Polosa 1968; Polosa et al. 1969). In addition, the discharge becomes characteristically phasic, consisting of bursts of action potentials separated by a period of inactivity lasting up to 30 s. The frequency of firing in each burst may reach 40 Hz, a rate far greater than the maximum rate at which these neurons can be driven synaptically.

According to Polosa et al. (1969), depressor responses produced by stimulation of somatic afferent nerves are diminished or abolished by sub-convulsive doses of picrotoxin, whereas baroreceptor-elicited and vagal falls in blood pressure are unaffected. The latter results are interesting in view of the recent observations indicating that baroreceptor inhibition of spinally projecting ventromedullary neurons is mediated by GABA (Sun and Guyenet 1985). If the latter has anything to do with blood-pressure regulation, the most probable reason for the failure of Polosa et al. (1969) to antagonise this system may be an inadequate dose of picrotoxin.

The duration of inhibition of sympathetic preganglionic neurons produced by somatic afferent stimulation in the spinal cat is reduced by picrotoxin (0.1–0.4 mg/kg i.v.), but not affected by strychnine (0.1–0.2 mg/kg i.v.; Wyszogrodski 1972; Polosa et al. 1982). This contrasts with the observation made by Mo and Dun (1987) using the isolated spinal-cord slice preparation referred to above.

Despite the demonstration of these numerous effects, the roles of spinal-cord GABA and glycine in regulating the activity of sympathetic preganglionic neurons does not appear to be dominant. None of the powerful descending inhibitory pathways from the ventro-lateral medulla, ventromedial reticular formation or mid-line nuclei utilise them, since inhibition produced by stimulating these regions is unaffected by strychnine, picrotoxin or bicuculline. Furthermore, they are not involved in preventing a somatic afferent volley from exciting preganglionic neurons via a short segmental pathway (Coote and Sato 1978).

9 Ongoing Activity in Sympathetic Preganglionic Neurons

It is generally accepted that various kinds of synaptic input continously converge onto sympathetic preganglionic neurons. Action potentials are generated from this ongoing synaptic activity, mostly at irregular intervals and low rates (Polosa 1968; Mannard and Polosa 1973; Seller 1973; Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembovsky et al. 1985a). The rate of resting ongoing activity probably depends on the end-organ destination of the preganglionic neuron (see Janig 1985), but it lies in the range of 0.1–7 impulses/s for neurons in the upper thoracic and lumbar segments

(Polosa 1968; Janig and Schmidt 1970; Sato 1972b; Kaufman and Koizumi 1971; Mannard and Polosa 1973; Seller 1973; Coote and Westbury 1979a, b; McLachlan and Hirst 1980; Janig and Szulczyk 1980; Dembowsky et al. 1985a). In addition, many neurons may not be discharging even though there is convergent ongoing synaptic activity onto the cell membrane (Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1985a). This synaptic input is carried predominantly by supraspinal pathways, since transection of the cervical spinal cord reduces it to low levels (Dembowsky et al. 1985a). Similarly, discharge frequency is greatly reduced in the spinal animal (Alexander 1945, 1946; Beacham and Perl 1964b; Polosa 1968; Mannard and Polosa 1973).

Oscillations of sympathetic activity with periodicities in the 2–6 Hz range, around 10 Hz, and at a frequency related to the much slower respiratory rhythm, are common features of the discharge of the sympathetic neuron pools (Weidinger and Huber 1964; Cohen and Gootman 1969, 1970; Koizumi et al. 1971; Gootman and Cohen 1973; Gootman et al. 1980; Barman and Gebber 1980). This tendency for the discharge of sympathetic neuron pools to oscillate in synchrony at distinct frequencies even though any individual neuron may be discharging irregularly, is also predominantly dependent on brainstem structures (Camerer et al. 1977; Gootman and Cohen 1980, 1981; Barman and Gebber 1980; Gebber and Barman 1980; Ardell et al. 1982). The 2–6-Hz rhythm in sympathetic-nerve discharge is largely dependent upon the fundamental organisation of neurons in the brainstem which drive sympathetic preganglionic cardiovascular neurons, since it is little changed in the decerebrate cat (Fig. 25) and disappears in most sympathetic outflows following section of the connection between the brainstem and spinal cord (Barman and Gebber 1980; Gebber and Barman 1980; Gebber 1980; Ardell et al. 1982). However, there may be a very weak tendency for some of the sympathetic spinal circuits to oscillate at 2–6-Hz (e.g. splanchnic outflow) but the synchrony between sympathetic neuron pools at different segmental levels is lost (Gootman and Cohen 1980, 1981; Ardell et al. 1982). The 2–6-Hz rhythm is still present following baroreceptor denervation, although the phase relationship between the sympathetic discharge and the blood-pressure pulse disappears in cat (Fig. 25; Barman and Gebber 1980). It appears that the cardiac-related baroreceptor input entrains this 2–6 Hz rhythm and also governs the occurrence of large and more frequent slow waves (5–6 Hz) in sympathetic-nerve discharge following strong sensory stimulation (Barman and Gebber 1980; Gebber 1980).

9.1 Baroreceptor Inputs

The baroreceptor entrainment of the 2–6-Hz rhythm occurs at two levels at least. It is now quite clear that a group of spinally projecting neurons in the

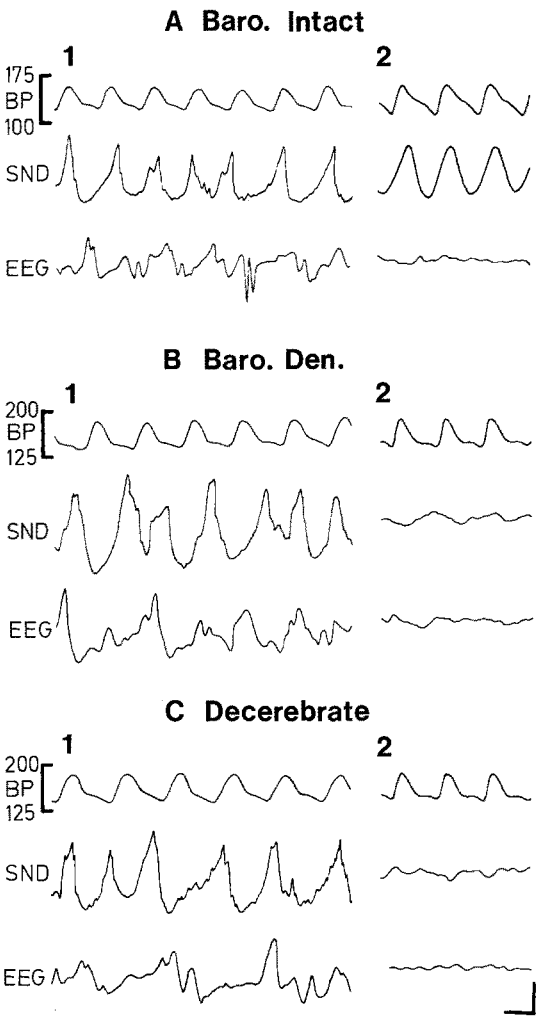


Fig. 25 A–C. Splanchnic activity patterns (*SND*) and electroencephalogram (*EEG*) before and after baroreceptor denervation and after decerebration. **A** Baroreceptor reflexes intact: *1* oscillographic record of arterial blood pressure (BP), in mmHg, SND (*middle trace*) and parietal-frontal EEG (*bottom trace*), *2* R-wave-triggered, computer-averaged records (64 trials) with sequence of traces as in **A**; address bin, 1 ms. **B1, B2** same as in **A** but after baroreceptor denervation. *C1, C2* same as in **B** but after mid-collicular decerebration. Horizontal calibration, 20 ms; vertical calibration, 100 μV for SND and 50 μV for EEG. (From Barman and Gebber 1980)

sub-retro-facial region of the ventro-lateral medulla, just rostral to the exit of the twelfth cranial nerve, are critically important for the tonic excitatory drive to sympathetic preganglionic vasoconstrictor neurons in the spinal cord of the anaesthetised animal (Schlafke and Loeschcke 1967; Guertzenstein and Silver 1974; Feldberg and Guertzenstein 1976; Schlafke and See 1980; McAllen et al. 1982; Yamada et al. 1982; Hilton et al. 1983; Brown and Guyenet 1984; Dampney et al. 1985; Blessing and Willoughby 1985; Lovick and Hilton 1985; Lebedev et al. 1986; McAllen 1986a, b; Lovick 1987a,b). It is also clear that these neurons have a cardiac-related pattern of activity and can be inhibited after a relatively long delay (>200 ms) by activation of baroreceptors, either by raising pressure in a blind-sac preparation of the carotid sinus in cat (Yamada et al. 1984; Sun and Guyenet 1985; Brown and Guyenet 1985;

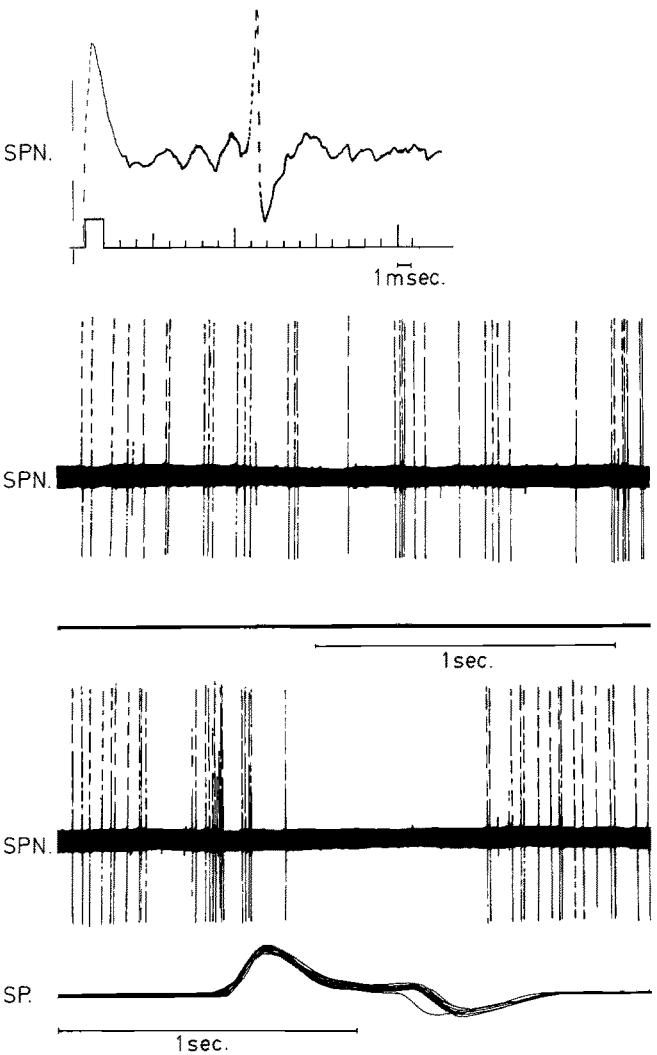


Fig. 26. Recordings of a single antidromically identified sympathetic preganlionic neuron (*SPN, top trace*) in the third thoracic segment, caused to discharge by micro-electrophoretic application of glutamate (*middle SPN* showing 12 superimposed consecutive periods of recording). The *bottom recordings* show the effect of an abrupt rise in carotid-sinus pressure (*SP*) of one side (blind-sac preparation) repeated 12 times at 5-s intervals. Twelve superimposed records are shown. Anaesthetised cat. (From Coote et al. 1981b)

McAllen 1985, 1986) or by electrical stimulation of the aortic nerve in rabbit (Terui et al. 1986).

There is also strong evidence that sympathetic activity can be powerfully influenced by the baroreceptors via a bulbo-spinal inhibitory pathway acting at a spinal site (Fig. 26; Gebber et al. 1973; Coote and Westbury 1974; Taylor and Gebber 1973; Coote and Macleod 1974b; Taylor and Gebber 1975;

McCall et al. 1977; Coote and Macleod 1977; Barman and Wurster 1978; Coote 1980; Coote et al. 1981 b; Fedorka et al. 1985). There are therefore two routes by which the baroreceptors reduce the activity of sympathetic preganglionic neurons. In view of the fundamental importance of this it is well to review the evidence in more detail. Coote and Macleod (1974 b) showed that following activation of carotid-sinus baroreceptors somatic afferent reflexes in the cardiac sympathetic nerves were inhibited some 20 ms before similar reflexes in the renal sympathetic nerves, suggesting that the inhibition reached cardiac sympathetic preganglionic neurons before renal sympathetic preganglionic neurons, possibly due to the different conduction distances in the spinal cord of descending inhibitory axons to the two pools of sympathetic neurons. This explanation presupposed that the tonic excitatory drive to the two outflows arose from a common pool of neurons in the brainstem, where there would be little difference in the delay if the baroreceptor afferents acted directly on them. However, in view of recent evidence indicating that tonically active neurons in the ventro-medulla are functionally dedicated and located at quite separate sites (Krasyukov et al. 1981; Darlington and Ward 1985 a, b; Lovick and Hilton 1985; Lovick 1986, 1987 a,b; Dampney and McAllen 1986, McAllen 1986 b), the previous argument may not be valid, although it is still difficult to account for all of the 20-ms difference referred to earlier.

Stronger evidence, however, comes from the finding that spinal segmental reflexes in the thoracic white rami are inhibited by baroreceptor activation (Coote and Macleod 1974 b; Barman and Wurster 1978), as is activity evoked in sympathetic pre- and postganglionic neurons by stimulating descending excitatory fibres in the spinal cord and brainstem (Gebber et al. 1973; Taylor and Gebber 1973; Barman and Wurster 1978; Lebedev et al. 1979; Coote 1980; Coote et al. 1981 b; Chiang 1980). Several pieces of evidence indicate that the baroreceptor spinal inhibitory axons act directly on the sympathetic preganglionic neuron. Firstly, maximal baroreceptor activation (200 mmHg pressure rise) can inhibit glutamate-evoked activity in antidromically identified preganglionic neurons even when this activity has been increased from zero to 31 Hz. Such an effect is unlikely to be due to disfacilitation caused by the baroreceptors blocking the tonic excitatory input to the neurons, since it is improbable that such a high discharge rate induced by glutamate depends on summation with subliminal excitatory drive. Also, the induced activity of the preganglionic neurons was much more regular than either the synaptic acitvity or the action-potential output normally observed in sympathetic preganglionic neurons (Coote and Westbury 1979 a; McLachlan and Hirst 1980; Dembowsky et al. 1985 a). A second piece of evidence is that increases in membrane potential reminiscent of IPSPs can be elicited by raising the pressure in an isolated carotid sinus (McLachlan and Hirst 1980) or by electrical stimulation of the aortic nerve of the cat (Fedorka et al. 1985).

Although the coincidence of these increases in membrane potential is remarkable and they have a time course and form rather similar to the IPSPs observed in parasympathetic preganglionic neurons following pelvic-nerve stimulation (DeGroat and Ryall 1968), this evidence will always be equivocal until conductance measurements during baroreceptor stimulation are forthcoming.

Gebber and his collaborators consider that the spinal component of baroreceptor inhibition takes place on a sympathetic antecedent neuron (Gebber et al. 1973; Taylor and Gebber 1973; McCall et al. 1977). Their experiments differentiated two sympatho-excitatory pathways, a faster one (conduction velocity 4–5 m/s) which evoked responses in pre- and postganglionic neurons that were not inhibited by baroreceptor stimulation and a slower one (conduction velocity 2–3 m/s) that evoked responses that were inhibited by the baroreceptors. Both types of response could be recorded in the same preganglionic neuron (although only two examples were examined). It was therefore reasonable for them to conclude that baroreceptor inhibition of the longer-latency response occurred on antecedent neurons in the spinal cord that were not common to both slow and fast pathways (Taylor and Gebber 1973). This interpretation is, however, open to question because the baroreceptor stimulation was not precisely controlled, either in its magnitude or timing, and inhibitory effects on excitation with a 'short' latency might well have been missed; the effect on the later response could have been a combination of disfacilitation and direct inhibition. On the other hand, the results of Gebber et al. (1973) and Taylor and Gebber (1973) may relate to a single discrete population of preganglionic neurons, since similar dual excitatory inputs have not been reported for the splanchnic outflows (Taylor and Gebber 1975). Others have reported that in the majority of preganglionic neurons in the T3 or T2 segment the responses evoked by stimulation of descending pathways of the 'fast' typ (5 m/s) or 'slow' type (2.5 m/s) are powerfully and equally inhibited by baroreceptor activation (Barman and Wurster 1978; Coote et al. 1981 b).

Two bulbo-spinal baroreceptor inhibitory pathways have been described. One characteristically has a long latency to onset (>70 ms); in its time course and sensitivity it closely parallels the baroreceptor system of blood-pressure control (Barman and Wurster 1978; Coote 1980; Coote et al. 1981 b). This can be perferentially blocked by sectioning a small region in the dorso-lateral funiculus of the cervical spinal cord (Coote and Macleod 1974 b). The other pathway has a short latency to onset (mean, 18 ms) and is weak and of short duration (Taylor and Gebber 1975). On the questionable basis of its resemblance to inhibition evoked from the so-called paramedian reticular formation of the medulla, its origin was presumed to be from this medullary site. However, there is no evidence that the paramedian region is involved in baroreceptor inhibition of vasomotor activity (see review by Spyer 1981).

Even Barman and Gebber (1978) showed that its total destruction left baroreceptor reflexes little impaired. This raises some serious problems with the previous interpretation of the data published by Taylor and Gebber (1973) and Gebber (1976), since much of the evidence was based on the response to stimulation of the paramedian region.

There is every reason to suppose that baroreceptor activation will have varying effects on different sympathetic preganglionic neurons, depending on their functional dedication. Baroreceptors have more powerful effects on splenic sympathetic neurons than on cardiac sympathetic neurons, which likewise are more affected than renal sympathetic neurons (Ninomiya et al. 1971). Renal sympathetic neurons are more influenced than are gastric sympathetic neurons (Nisimaru 1971) and muscle sympathetic vasoconstrictor neurons are very powerfully inhibited whereas cutaneous vasoconstrictor neurons are only weakly inhibited (Janig 1985). Some sympathetic neurons, like sudomotor neurons, are not affected at all (Prout et al. 1964; Janig 1985). It is therefore not too surprising that there has been a failure to clearly discern baroreceptor-related IPSPs in some intracellular studies (Dembowsky et al. 1985a). It is also possible that IPSPs may be difficult to detect using intracellular recordings from the soma of preganglionic neurons because in some neurons baroreceptor inhibition may be remote, out on the dendrites, a possibility that is not too unlikely in view of the dendritic inhibitory synapses demonstrated on spinal ventral-horn neurons (Cook and Cangiano 1972) and on Mauthner's neurons in fish (Diamond 1968).

9.2 The 10-Hz Rhythm

Green and Heffron (1967) and Cohen and Gootman (1969, 1970) were the first to document a 10-Hz rhythm in the discharge of sympathetic nerve bundles. They noticed that it was usually unrelated to the phases of the cardiac cycle. McCall and Gebber (1975) concluded that the 10-Hz rhythm is generated in the spinal sympathetic network, because they observed a 10-Hz periodicity in autocorrelograms of renal sympathetic discharge during asphyxia in a few high-spinal cats. This was not confirmed by Gootman and Cohen (1981), despite challenging the spinal networks with asphyxia and strychnine. It seems fair to conclude that, as with the periodicity of 2–6 Hz, the 10-Hz rhythm is predominantly generated in the brainstem networks, although it may also be a fundamental but subtle characteristic of the way in which sympathetic networks in the spinal cord are organised.

9.3 Respiratory-Related Inputs

The discharge of sympathetic neurons also oscillates in phase with the respiratory cycle, oscillation being greatest during the mid-inspiratory phase

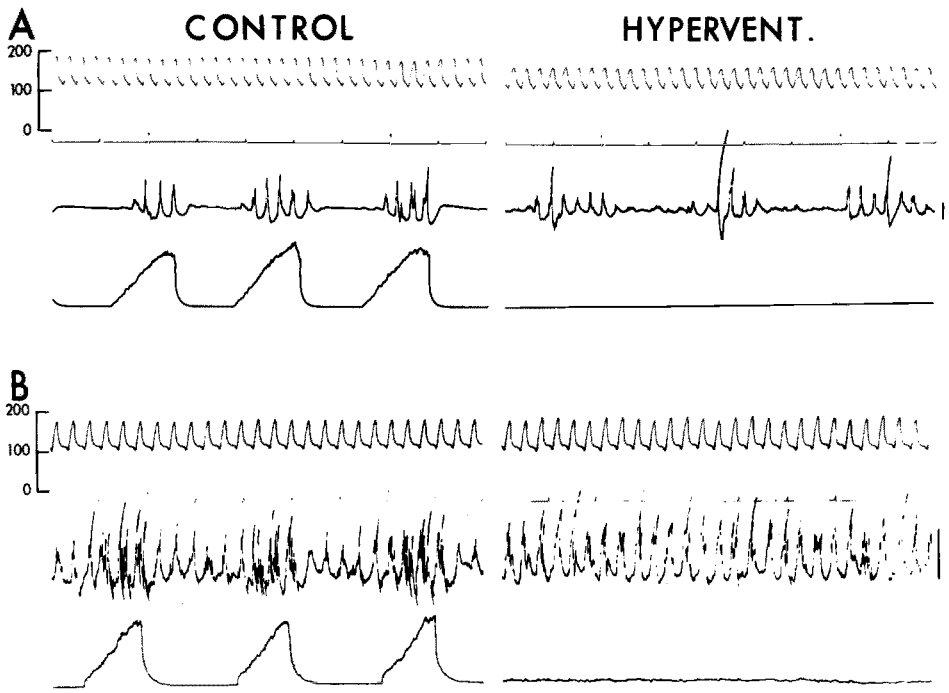


Fig. 27A,B. Effect of hyperventilation (shown on *right*) on slow rhythmic components in sympathetic and phrenic nerve discharges in two vagotomised cats (**A** and **B**). Traces represent arterial blood pressure (mmHg) time marker (1 s), sympathetic nerve activity (external carotid nerve) and integrated record of phrenic nerve discharge. Anaesthetised cat. (From Barman and Gebber 1976)

and least in the early expiratory phase (Adrian et al. 1932; Barman and Gebber 1976; Bronk et al. 1936; Downing and Siegel 1963; Cohen and Gootman 1970; Gootman and Cohen 1974; Gootman et al. 1975; Gootman 1980; Green and Heffron 1967; Koizumi et al. 1971; Lipski et al. 1977; Okada and Fox 1967; Preiss et al. 1975; Preiss and Polosa 1977; Mannard and Polosa 1973; Gerber and Polosa 1978, 1979; Gilbey et al. 1985, 1986). This phase relationship normally disappears following vagotomy and abolition of the central inspiratory activity during hyperventilation. However, an oscillation with similar frequency persists in some experiments and is not present in the spinal animal (Fig. 27; Barman and Gebber 1976). This suggests that a brainstem sympathetic system has an intrinsic slow oscillation which becomes entrained to the central respiratory oscillator (Gebber 1980). This entrainment mainly results from the respiratory inputs, an excitatory one from central chemoreceptors operating via central neurons that control inspiratory drive and a predominantly inhibitory input from stretch receptors in the lung (Cohen and Gootman 1970; Gootman and Cohen 1974; Preiss et al. 1975; Preiss and Polosa 1977; Gerber and Polosa 1978, 1979; Lipski et al. 1977;

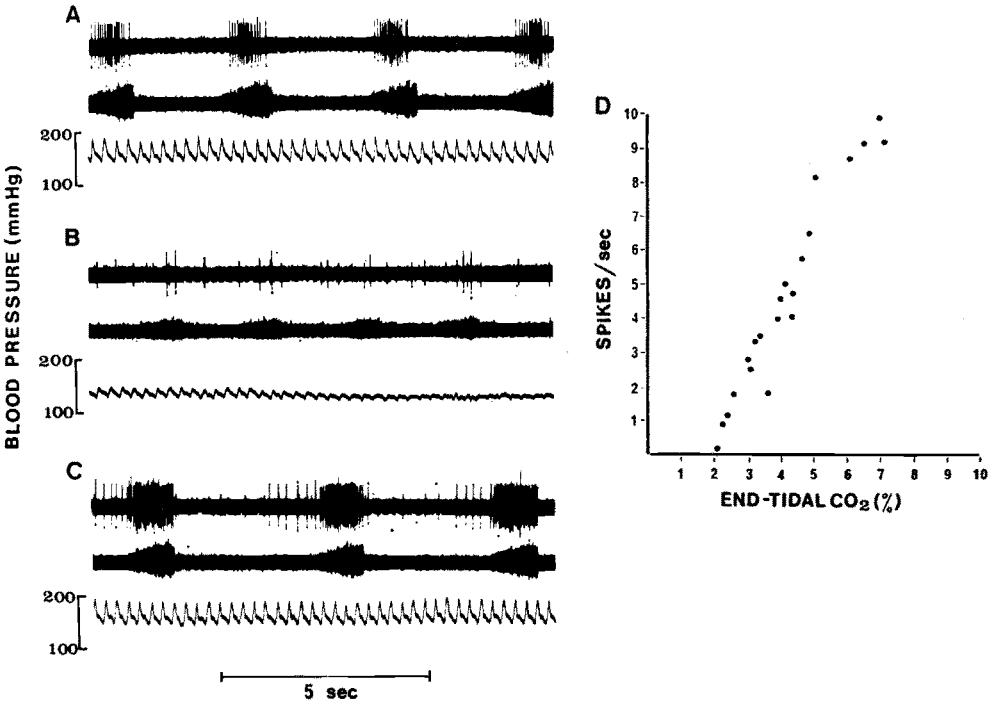


Fig. 28A–D. Cervical sympathetic preganglionic unit with inspiratory-burst firing pattern and response to inhaled CO_2. **A–C** recordings from (*top to bottom*) sympathetic preganglionic unit, phrenic neurogram, arterial blood pressure. End-tidal CO_2 was 4.5% in **A**, 2.3% in **B** and 7% in **C**. **D** Mean firing rate of the sympathetic unit versus end-tidal CO_2 concentration at which a given firing rate was observed. A loss of pulsation in the blood-pressure record in **B** was due to a clot in the catheter tip. Anaesthetised cat. (From Preiss and Polosa 1977)

Polosa et al. 1980; Cohen et al. 1980; Gootman et al. 1980; Bainton et al. 1985).

Some sympathetic preganglionic neurons are dominated by the central input related to inspiratory drive, so that they display a respiratory-linked burst of activity during normocapnia (Fig. 28; Preiss et al. 1975; Preiss and Polosa 1977; Polosa et al. 1980; Gilbey et al. 1985, 1986). Others apparently receive a very weak central input related to inspiratory drive so that at physiological arterial pressures of CO_2 and O_2 ($PaCO_2$ and PaO_2) they discharge in response to other inputs but show no respiratory periodicity. However, at high $PaCO_2$ and during asphyxia, when central inspiratory drive is likely to have increased, they aquire an inspiration-synchronous component in their firing (Preiss et al. 1975; Preiss and Polosa 1977). Still other neurons that have membrane potentials below the threshold for firing, so that they are 'silent', also receive a synaptic input related to central inspiratory drive. When made to discharge by the release of glutamate near the cell bodies from glass micropipettes, neurons of this type begin to fire in a respiratory-modulated

pattern (Preiss and Polosa 1977; Gilbey et al. 1985, 1986). In 'silent' preganglionic neurons, shifts in antidromic latency indicative of shifts in membrane potential, correlated with increases or decreases in central inspiratory drive, have been demonstrated (Lipski et al. 1977). These 'silent' neurons may become active with a respiratory rhythm during hypercapnia (Preiss and Polosa 1977).

Extracellular studies suggest that respiratory modulation of the activity of sympathetic preganglionic neurons is a consequence of the direct action of a central inspiratory-related input on the membrane of these cells (Fig. 29, Lipski et al. 1977; Preiss and Polosa 1977; Gilbey et al. 1986). In one study (Fig. 29) a decrease in antidromic latency measured to the soma-dendritic spike, which is indicative of depolarisation, was associated with central inspiratory activity recorded in a phrenic nerve. This decrease was even greater during hypercapnia when central inspiratory activity was greater (Lipski et al. 1977). Shifts of up to 0.15 ms were recorded in the latency from stimulus to soma-dendritic spike in preganglionic neurons. The shifts indicate decreases in membrane conductance and membrane depolarisations of up to 7 mV (Gustafsson and Lipski 1980; Lipski 1981), which is certainly large enough to strongly influence the discharge pattern of the neurons (Coote and Westbury 1979a, b; McLachlan and Hirst 1980; Dembowsky et al. 1985a). Another interesting study showed that glutamate failed to disrupt the basic discharge pattern of respiratory-modulated neurons. Gilbey et al. (1986) showed that both silent neurons, caused to discharge with phrenic-related bursts and spontaneously active neurons discharging in a similar manner had their discharge related to central inspiratory activity altered in a stereotyped manner by step-wise increases in the amount of glutamate injected by iontophoretic current. The onset of the inspiratory synchronous discharge began progressively earlier in relation to the beginning of the phrenic discharge, and there was an increase in the spike frequency between bursts until a plateau was reached. The time at which the burst was switched off was relatively unaffected.

It therefore appears as though the synaptic input to these neurons is similar to that received by thoracic inspiratory motoneurons and gives rise to slowly increasing EPSPs (Sears 1964; Berger 1979). Unfortunately however, this picture has not been confirmed by any of the intracellular studies to date, which have shown EPSPs occurring irregularly and only occasionally reaching the threshold required to discharge the cell. The latter characteristics led Coote and Westbury (1979a) to consider that the preganglionic neuron was a detector of the coincident arrival of EPSPs. This is somewhat puzzling in view of the data of Gilbey et al. (1986) and others. However, the intracellular data may be misleading because in order to give greater stability for the recording in upper thoracic segments, intracellular studies have been conducted under conditions of hypocapnia, that is, low tidal volume and high respiratory fre-

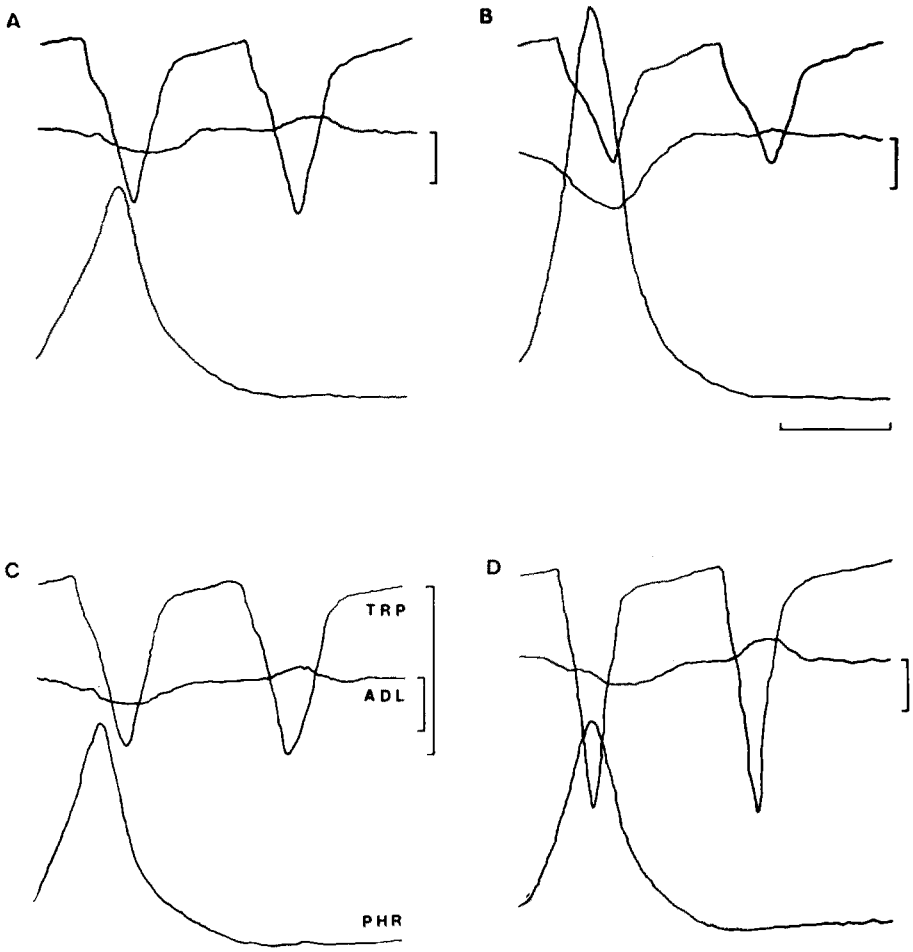


Fig. 29A–D. Computer display of temporal patterns of antidromic invasion of sympathetic preganglionic neurons recorded in the third thoracic segment of the anaesthetised cat. In each panel the *top two traces* show tracheal pressure (TRP, downward, displacement lung inflation, calibration shown in **C** is 5 mmHg); antidromic latency of the soma-dendritic spike of a preganglionic neuron (*ADL*) in which shortening of latency is indicated by downward displacement of the trace [calibration bar, 0.1 ms]. The *third trace* shows integrated phrenic-nerve activity (*PHR*). **A** Control record. **B** Effect of hypoventilation evoked by turning down tidal volume of respirator. **C** Second control run. **D** Effect of increasing the rate and depth of lung inflation. Each record is averaged over 64 respiratory cycles; *horizontal bar,* 1 s. (From Lipski et al. 1977)

quency, which would have minimised respiratory influences (Coote and Westbury 1979a; McLachlan and Hirst 1980; Dembowsky et al. 1985a). Therefore, the important question as to whether or not activity from respiratory sources is received at the sympathetic preganglionic neuronal membrane remains to be answered.

An inspiratory influence on the preganglionic neurons is unlikely to be carried by the bulbo-spinal inspiratory afferents, since the conduction velocities of these fibres are around 30 m/s (Bianchi 1971; Hilaire and Monteau 1976; Khimonidi et al. 1980), whereas it is clear that sympatho-excitatory axons are smaller (conduction velocity 1.6–8.0 m/s) although a few larger fibres (<25 m/s) have been observed (Khimonidi et al. 1980; Coote and Macleod 1984; Dembowsky et al. 1985 a). It is also possible that central inspiratory activity is injected into the sympathetic systems via the ventro-medullary neurons responsible for vasomotor tone. There is no information regarding this possibility as yet, but it does appear that central inspiratory activity is an important generator of activity in sympathetic preganglionic neurons and this may explain why the cardiovascular-generating region in the ventral medulla is closely associated with structures which others have considered to have a mainly respiratory function. Estimates based on neurophysiological observations show that in normocapnia 60% of the action-potential output of the preganglionic-neuron pool projecting into the cervical sympathetic nerve is generated by the central inspiratory activity (Preiss and Polosa 1977; Gilbey et al. 1986). Also, as much as 70% of the neurons in the T3 segment – many probably destined for the heart and blood vessels of the thorax – have an inspiratory-related synaptic input (Lipski et al. 1977).

Polosa et al. (1980) also made an indirect estimate, based on measurements of hind-limb vascular resistance in an earlier study (Lioy et al. 1978), that approximately 33% of hind-limb neurogenic vascular tone in normocapnia is generated by central inspiratory activity. It was suggested by Polosa et al. (1980) that the central inspiratory oscillator, in addition to providing an input for the generation of action potentials in individual sympathetic neurons, also has the important functional property of entraining and synchronising the activities of all the (presumably cardiovascular) preganglionic neurons it drives. This synchronisation in turn is likely to result in enhancement of transmission at the ganglionic synapses by the process of spatial summation. Enhancement of transmission at the neuro-effector junctions by spatio-temporal summation is also likely to result.

Another important respiratory input influencing the cardiovascular system is that associated with lung inflation (Daly et al. 1967; Daly and Robinson 1968; Glick et al. 1969). Lung inflation stimulates pulmonary stretch receptors that evoke vagal afferent activity, which produces inhibition of sympathetic discharge (Bronk et al. 1936; Downing and Siegel 1963; Okada and Fox 1967; Przybyla and Wang 1967; Gootman and Cohen 1974; Gerber and Polosa 1978; Cohen, Gootman and Feldman 1980). These inhibitory effects are likely to occur on the membrane of the sympathetic preganglionic neuron (Fig. 29). Lipski et al. (1977) observed changes in antidromic latency associated with lung inflation in 13 out of 27 preganglionic neurons tested.

To overcome the problem of interaction between vagal stretch-receptor input and central inspiratory activity, the changes in excitability were examined during a second inflation which was added during each phrenic-driven respiratory cycle,

so that inflation occurred when no inspiratory activity was present and therefore the changes observed could not have been due to changes in central inspiratory activity. In eight neurons, an increase of antidromic latency to lung inflation occurred, suggesting hyperpolarisation and an increase in conductance (Gustafsson and Lipski 1980; Lipski 1981). In five neurons a decrease in antidromic latency occurred, indicating depolarisation. Increasing the rate of inflation within each stroke of the pump increased the shift in antidromic latency. In one neuron (tested repeatedly), which showed a decrease in antidromic latency related to inflation, bilateral vagotomy abolished the pump-related latency shift. There is no evidence concerning the location or type of bulbo-spinal neurons mediating either the inhibitory or excitatory effect of lung inflation on the membrane of preganglionic neurons, although Lipski et al. (1977) speculated that the R β inspiratory neurons in the ventrolateral part of the nucleus tractus solitarius are involved. An inhibitory effect exerted at a spinal locus on splanchnic neurons in the cat following electrical stimulation of the vagus nerve has been demonstrated during intraspinal stimulation of descending sympatho-excitatory pathways (Chiang 1980).

The demonstration of two populations of preganglionic neurons identified by their differing response to lung inflation is probably related to their end-organ destination. It is to be expected that preganglionic neurons projecting to the heart would be excited during normal lung inflation, since this causes a tachycardia, albeit mainly due to vagal inhibition (see review by Daly 1986), whereas those neurons projecting to systemic blood vessels would be inhibited during lung inflation, since this causes a reduction in vascular resistance (Salisbury et al. 1960; Angell-James and Daly 1969; Daly et al. 1967; Daly and Robinson 1968; Daly et al. 1983; Daly et al. 1984).

These influences are mainly carried by pulmonary afferent fibres travelling in the vagus nerve (see review, Daly 1986; Lipski et al. 1977). However, there is an inspiratory-related reflex, the afferent and efferent limbs of which involve only pathways to and from the spinal cord, deep inspiration producing cutaneous vasoconstriction in the fingers or toes of spinal man (Gilliatt et al. 1948; Gilliatt 1948). Therefore a relatively high-threshold, inspiratory-related excitatory influence on some preganglionic vasoconstrictor neurons is to be expected in vagotomised or spinal animals, although this possibility has not been explored further.

Another respiratory-related input is that from the arterial chemoreceptors. The effect on the respiratory and cardiovascular systems of stimulating these receptors has recently been very comprehensively reviewed by Daly (1986). Stimulation of the carotid bodies causes bradycardia and an increase in systemic vascular resistance in anaesthetised animals, but only when other known sensory inputs to the nervous system, such as pulmonary ventilation, are controlled (Daly 1986). In some spontaneously breathing animals the heart-rate response is usually tachycardia largely attributable to inspiratory-

related gating of the primary excitation of cardiac vagal inhibitory neurons by chemoreceptors (Daly 1986). With regard to sympathetic outflow, the primary chemoreceptor response is interesting because it consists of inhibition of sympathetic activity in nerves to the heart (Daly and Scott 1962; Downing et al. 1962; Kollai and Kozumi 1977; Davis et al. 1977) together with excitation of sympathetic vasoconstrictor activity in nerves to the skin, muscle, splanchnic area and kidney (Iriki and Kozawa 1975; Gregor and Janig 1977; Dean and Coote 1986; Yusof 1986). Whether this differential response occurs as a consequence of selective inhibition and excitation of relevant end-organ-coded sympathetic cardiovascular neurons in the brainstem or spinal cord has not been resolved.

9.4 Hypoxia

Sympathetic preganglionic neurons also display changes in activity in response to relatively severe systemic hypoxia or hypercapnia in sino-aortic-denervated animals and spinal animals (Kaya and Starling 1909; Mathison 1910; Gregor and Janig 1977; Iriki and Kozawa 1975; Alexander 1945; Rohlicek and Polosa 1981, 1983; Kehrel et al. 1962; Lioy et al. 1978; Johnson et al. 1969; Szulczyk and Trzebski 1976). Rohlicek and Polosa (1981, 1983) recorded from single units in strands dissected from the cervical sympathetic nerve in cat. The firing rate of the neurons was independent of PaO_2 from normoxia down to a PaO_2 of 40 mmHg, but graded reductions below this level caused first a reduction in sympathetic activity and then an increase in some neurons. The depressant action of hypoxia was absent in the spinal cat. In view of the low levels of PaO_2 necessary to provoke these effects, Rohlicek and Polosa (1983) suggested they were unlikely to represent responses to oxygen sensors in the nervous system, but directly resulted from anoxia reducing the activity of the Na pump in the neuronal membrane. Inhibition of preganglionic neurons as well as excitation can also be explained in this way by assuming that the same process occurs on bulbo-spinal sympatho-inhibitory neurons in the brainstem, resulting in their activation and a consequent inhibitory effect on preganglionic neurons in the spinal cord (Rohlicek and Polosa 1983).

9.5 Response to CO_2

In cats in which afferents from peripheral arterial chemoreceptors have been cut, there is a significant vasoconstrictor response to systemic hypercapnia (Lioy et al. 1978). This response is greatly attenuated, but not abolished, by cold block of the superficial areas of the ventral medulla (Hanna et al. 1979).

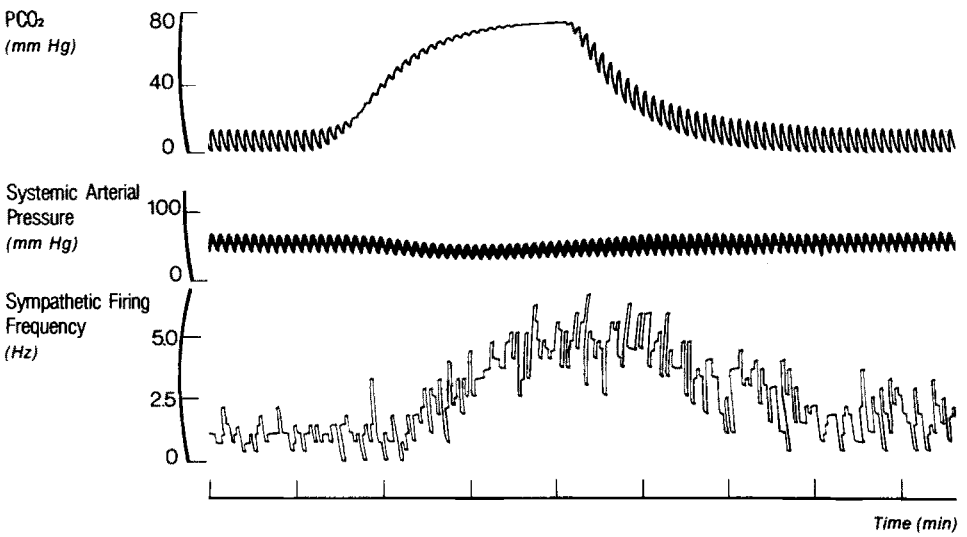


Fig. 30. Response of a cervical sympathetic preganglionic unit, in an anaesthetised acute C4-spinal cat, to ventilation with 10% CO_2 and 90% O_2. From the *top*, traces show tidal variations in PCO_2, arterial blood pressure and rate-meter record of unity activity. (From Zhang et al. 1982)

Polosa and his colleagues (Zhang et al. 1982) investigated the source of the residual CO_2 sensitivity of sympathetic preganglionic neurons by recording from single units in the cervical sympathetic trunk of spinal cats (Fig. 30). The firing rate recorded in the majority of tonically active units increased some 3.7 times during hypercapnia with end-tidal PCO_2 between 30 mmHg and 70 mmHg. This CO_2 sensitivity is one order of magnitude smaller than that found in the cat with intact connections between the brainstem and the spinal cord. Similar findings were reported by Szulczyk and Trzebski (1976), who observed increased activity of sympathetic postganglionic fibres during superfusion of the dorsal aspects of the thoracic spinal cord with hypercapnic acid artificial CSF. Whether this action of CO_2 or H^+ is on the membrane of preganglionic neurons or on some antecedent neuron in the spinal cord is unclear. Whichever is the case, the response is the opposite to that observed for cortical neurons (Krnjevic et al. 1965), brainstem respiratory neurons (Marion and Lamb 1975; Mitchell and Herbert 1974) and the majority of alpha motoneurons (Papajewksi et al. 1969), which are depressed by systemic hypercapnia.

9.6 Mayer-Type Oscillations

Slower rhythms in sympathetic discharge with repetition rates of 3 cycles/min have been observed in experimental animals (Koepchen 1962; Weidinger and

Leschhorn 1964; Fernandez de Molena and Perl 1965; Kaminski et al. 1970; Preiss and Polosa 1974). These oscillations are much slower than respiratory rhythms, and the accompanying blood-pressure oscillations have been referred to as third-order vasomotor or Mayer waves (Mayer 1876). The oscillations of sympathetic activity can be initiated by haemorrhage but are independent of cyclic changes in sensory feedback from cardiovascular receptors, since sino-aortic deafferentation does not prevent them (Mayer 1876; Andersson et al. 1950; Armstrong and Irby 1962; Guyton and Harris 1951). They are similarly unaffected by preventing the blood-pressure or Mayer waves with an adrenergic antagonist or arterial pressure stabilisation (Preiss and Polosa 1974). They can be initiated in sympathetic preganglionic neurons in spinal cat or dog (Fernandez de Molena and Perl 1965; Kaminski et al. 1970) and therefore must be a property of the spinal sympathetic circuits. This does not rule out the possibility that brainstem networks have a similar characteristic.

The Mayer-type oscillations in sympathetic-neuron discharge triggered by haemorrhage or increasing cerebro-spinal fluid pressure have a fairly synchronous phase relationship with sympathetic neuron pools at different spinal-cord levels (Fernandez de Molena and Perl 1965; Kaminski et al. 1970), indicating that proprio-spinal neurons are involved in coupling the networks of sympathetic preganglionic neurons throughout the thoraco-lumbar spinal cord.

10 Organisation of Cardiovascular Preganglionic Neurons

It is evident from the large body of anatomical and electrophysiological data reviewed above that sympathetic preganglionic neurons have unique electrophysiological as well as morphological characteristics. Attempts have been made to relate these features to the target-cell specificity of the neurons. Clearly, the discretely localised clusters of cells in the four autonomic subnuclei contain functionally different types of neurons. In fact, three types of preganglionic neuron can be distinguished morphologically (see Sect. 2.4) and recently Dembowsky et al. (1986) provided evidence that three types of cell could also be distinguished electrophysiologically. However, there is no information as to whether there is a correlation between the physical and electrical characteristics of the cells. In any case, more than three types of neuron would be necessary to fit the many types of effector innervated by sympathetic nerves unless certain generalisations are assumed; perhaps this could be fruitfully explored.

Nonetheless, consideration has been given to the possibility that those somata with axons destined for cardiovascular effectors are morphologically

distinguishable (see Janig 1985). This has been alluded to in a number of studies by considering such properties as axon-conduction velocity, which reflects axon size and possibly soma size, either alone (Bishop and Heinbecker 1932; Eccles 1935; Folkow et al. 1958) or together with reflex and spontaneous firing patterns (Coote and Westbury 1979b; McLachlan and Hirst 1980; Janig and Szulczyk 1980; Bahr et al. 1986a–c). However, although many of these authors concluded that fibre size may be a good indicator of cardiovascular function, this may not be entirely justified. The experiments performed have relied upon the threshold of a response to stimulating bundles of preganglionic fibres, which would not have detected the subliminal effects of different-sized fibres converging onto the same ganglion cell. Since this is a feature of postganglionic somata, at least in the superior cervical ganglion (Perri et al. 1970; Brimble et al. 1972; Wallis and North 1978; Skok and Ivanov 1983), it might be expected that somata specified for a particular end organ would have a range of sizes, as would their axons.

This was the conclusion reached by Coote and Westbury (1979b) on the basis of recordings from the somata of preganglionic neurons of the third thoracic segment in cat. The neurons could be grouped on the basis of whether or not they were discharging action potentials, whether or not they had a cardiac rhythmicity in their discharge pattern and whether or not they were inhibited by stimulation of the carotid baroreceptors. In each group there was a similar spread of conduction velocities. Therefore the idea of a morphology of somata linked to specific target cells of the sort indicated by Janig (1985) is too broad a concept. It seems more likely that the three cell types emphasised in a number of studies indicated a more subtle functional dedication. What is clearly an important clue to function, however, is the location of somata in the intermediate grey matter. Janig and McLachlan (1986; see Sect. 1) proposed a viscero-topic organisation of preganglionic neurons based on longitudinal columns of neurons with like formation, similar to the way in which motor nuclei of the anterior horn supplying individual muscles are organised (Romanes 1951). For the preganglionic neurons some modification to this suggestion is necessary to take account of the ladder-like arrangement of the autonomic sub-nuclei. In any case, the evidence goes no further than suggesting that the medio-lateral arrangement of preganglionic somata indicates regional distribution rather than the function of cells. Nonetheless, this is an important and stimulating idea. Another attractive probability highlighted by Janig and McLachlan (1986) was that preganglionic somata with like function are restricted to specific clusters of cells within each column. Direct evidence for this is available in the data of Holets and Elde (1982, 1983) which show that preganglionic neurons supplying chromaffin cells of the adrenal medulla, when retrogradely labelled with fast blue, appear in clusters at the border of the ILp and ILf of each segment from T1 to L2.

Electrophysiological recordings from somata of preganglionic neurons support the application of these data to other functional types of neuron. In particular, 'cardiovascular-type' neurons have been identified in several ways thanks to the extensive and thorough investigations performed on lumbar postganglionic neurons by Janig and his collaborators (Janig 1985). Some authors have used the cardiac- and respiratory-related occurrence of action potentials (Polosa 1968; Lebedev et al. 1974; Coote and Westbury 1979b; Gilbey et al. 1982a, b, 1986) or similar related changes in membrane potential (Lipski et al. 1978). Other studies have looked for powerful effects of baroreceptor stimulation on firing pattern or membrane potential (Gebber et al. 1973; Coote and Westbury 1974, 1979b; McLachlan and Hurst 1980; Fedorka et al. 1985) together with the characteristics of the response to somatic afferent stimulation (Coote and Westbury 1979b). These studies restricted exploration to the lateral autonomic nuclei, the ILp and ILf, of the upper thoracic segments of cat and rat spinal cord; consequently, it may be misleading to generalise. However, in the third thoracic segment, Coote and Westbury (1979b) found that about 25% of neurons were of the 'cardiovascular type'. In addition, using the above criteria, it is the clear impression, when performing micro-electrode penetrations, that preganglionic neurons occur in clusters and that the somata encountered in each cluster have similar functional characteristics. The somata of the sympathetic columns have relatively extensive dendritic arborisations. These are mainly, orientated in the rostro-caudal axis, and there is a high density of afferent terminations on the soma and dendrites, all of which suggests that these are not simply relay neurons. This is reinforced by the finding that at least three types of synaptic ending can be distinguished at the ultrastructural level.

Numerous investigations referred to in the foregoing account have shown that extensive interaction occurs between several spinal segmental and supraspinal excitatory and inhibitory pathways, some of which undoubtedly make direct monosynaptic connections with dendrites or the soma of preganglionic neurons (see Sect. 7). Intracellular studies have shown that the membrane potential of these neurons fluctuates in both depolarising and hyperpolarising directions (Coote and Westbury 1979a; Coote 1980; McLachlan and Hirst 1980; Dembowsky et al. 1985a). Moreover, in vivo and in vitro studies have established that EPSPs and IPSPs can be evoked in these preganglionic neurons on stimulating axons in the lateral funiculus (Dembowsky et al. 1985a; Yoshimura et al. 1986a). This confirms the conclusion from more indirect extracellular studies showing that the discharge of preganglionic neurons produced by the iontophoretic application of glutamate was prevented by stimulation of spinal afferents (Wyzogrodski and Polosa 1973) or descending pathways (Coote et al. 1981b; Gilbey et al. 1982a).

The functional and anatomical specificity manifest by sympathetic preganglionic neurons is also recognisable in the terminations of the supraspinal

pathways which descend to the thoracic cord in three main regions. Mainly, inhibitory axons of small diameter pass down in the dorso-lateral funiculus, whilst inhibitory axons of large diameter descend in the ventro-lateral funiculus. Predominantly, excitatory axons descend in the intermediate and dorsal regions of the lateral funiculus, originating from groups of small spinally projecting neurons quite distinct from those supplying other types of motoneuron (see Sect. 7.2).

The supraspinal pathways projecting from many regions of the brain appear to preferentially innervate specific segments (see Barman and Gebber 1985; Tucker and Saper 1985) and specific clusters of preganglionic neurons, suggesting that spinally projecting brainstem neurons are specified not only for the system, but possibly also for the end-organ supplied (see Sects. 7.4, 8.4, 8.5 and 8.9).

Several pieces of evidence suggest this. Lovick and Hilton (1985, 1986) and Lovick (1987a, b) observed that micro-injection of DL-homocysteic acid (DLH) into selected sites in the rostral ventro-lateral medulla caused changes in mesenteric blood flow without changes in blood flow in hind-limb skeletal muscle, and vice versa at other sites. In addition, vasoconstriction in hind-limb muscle was elicited from locations quite different to those in which vasodilatation was elicited. McAllen (1985, 1986a, b) confirmed these observations for the activity of sympathetic vasoconstrictor nerves to different vascular beds. These effects do not appear to be simply due to the distribution of descending efferents to different segments of the spinal cord, but appear to result from selective activation of anatomically distinct sub-groups of neurons in the ventro-lateral medulla, each of which is dedicated to controlling a particular vasomotor outflow (Lovick 1987). In a test of these possibilities, Dampney and McAllen (1986) recorded from postganglionic vasoconstrictor fibres to the hind limb which have preganglionic neurons in similar segments of the lumbar spinal cord. One group innervated the blood vessels of skeletal muscle, the other those of skin. It was clearly shown that DLH injected into the ventro-lateral medulla selectively affected one or other of these outflows. Such results also help explain the recent observations of Dean and Coote (1986) that, following the application of the inhibitory amino acid glycine to the surface of the ventral medulla, tonic activity in postganglionic nerve fibres to the vascular bed of hind-limb muscle disappeared substantially earlier than the same activity in nerves to the renal or splanchnic vascular bed.

It is interesting that Brodal (1956) suggested that reticulo-spinal neurons might be functionally coded, an idea that was taken up and tested experimentally by Downman whilst I was a student with him (Coote et al. 1962). The results of the latter work were never published in full because the methods available did not allow an unequivocal interpretation. Later work demonstrating independent central control of vascular and sweat-gland responses in the cat (Prout et al. 1965) suggested that Brodal was right.

Numerous anatomical data concerning the innervation of the sympathetic nuclei in the spinal cord also suggest target-cell specificity of the descending fibre terminals. Holstege and Kuypers (1982) demonstrated a striking innervation of the ILp in the thoracic cord following [^{3}H]-leucine injection into the lateral part (but not the medial part) of the dorso-lateral pontine tegmentum (see Sect. 7.4). Examination of the functional significance of this pathway is only just beginning. Experiments suggest that multiple pathways for control of blood pressure and vasoconstriction in different vascular beds may synapse in a discrete region of the dorso-lateral pons close to the brachium conjunctivum. There is also some indication of selective control of different vascular beds from this region (Darlington and Ward 1985a, b). In other anatomical studies, spinally projecting neurons in the paraventricular nucleus of the hypothalamus were shown to preferentially innervate the ILp of particular segments and, moreover, to be absent in the vicinity of preganglionic neurons supplying the adrenal medulla (Swanson and McKellar 1979; Holets and Elde 1982, 1983; Jenkins et al. 1984; Krukoff et al. 1985b; see Sect. 8.5).

Target-cell specificity of central afferent pathways to sympathetic preganglionic neurons should not come as too much of a surprise in a system that displays such a variety of differential patterns of response. It would also explain one particularly striking response, the Bainbridge reflex, in which stimulation of atrial receptors causes a reflex increase in heart rate, mediated specifically by the right sympathetic cardiac nerves to the sino-atrial node, and a renal vasodilatation from inhibition of renal vasoconstrictor nerve activity (Linden 1979), without obvious involvement of other sympathetic outflows.

The central inputs to preganglionic neurons are also chemically coded. There are terminals in the sympathetic nuclei of the intermediate grey matter of the thoraco-lumbar cord that contain one or more of the amines (noradrenaline, adrenaline, dopamine and 5-HT) and immunoreactive material like one of the peptides (substance P, thyrotropin-releasing hormone, oxytocin, arginine vasopressin, angiotensin II, met-encephalin, somatostatin, vasoactive intestinal polypeptide, avian pancreatic polypeptide, neurotensin and cholecystokinin). The presence of noradrenaline, adrenaline, dopamine, 5-HT, substance P, oxytocin and arginine vasopressin seems clearly established. In the case of the amines, direct synaptic contacts with preganglionic neurons are apparent. The extent to which other chemically coded terminals enter into true synaptic contact with the perikarya of preganglionic neurons or with sympathetic antedecent neurons is unclear. This is an important question worthy of investigation.

These chemically coded neurons are distributed selectively to different segments of the spinal cord and probably to different preganglionic neurons. Target-cell specificity is, however, best exemplified by the noradrenaline, oxytocin and arginine-vasopressin terminals (see Sects. 8.2 and 8.5), but also

particularly well by those containing a somatostatin-like immunoreactive material, which apparently are found only in the vicinity of preganglionic somata in the adrenal medulla (Holets and Elde 1982). It would be interesting to know the origin of such terminals.

In addition to target-cell specificity, it is evident that chemically coded terminals of several of the pathways are associated with particular sub-types of postsynaptic receptor so that there is a receptor specificity also encoded in the connections onto preganglionic neurons. As a consequence, identity of chemical transmitter does not necessarily identify action in any anatomically distinct group of neurons. This principle, although recognised in the peripheral nervous system, has received little attention from those of us involved in the central-nervous regulation of the cardiovascular system. Hence, implicit in many early investigations, including my own, has been the assumption that a chemically recognisable system of neurons will have a similar action on each preganglionic neuron irrespective of its end-organ destination. This is clearly unacceptable for the raphe spinal system of 5-HT-containing neurons, and probably also for the spinally projecting catecholamine neurons.

The recent experiments of Howe et al. (1983), McCall (1984), Pilowsky et al. (1986) and Chalmers et al. (1985) have indicated that spinally projecting 5-HT neurons in the raphe magnus are likely to excite some cardiovascular preganglionic neurons, whereas earlier evidence indicated that spinally projecting 5-HT neurons inhibited certain types of cardiovascular neuron. However, the latter were located in the caudal raphe nuclei pallidus and obscurus (Coote and Macleod 1974a, 1975; Cabot et al. 1979; Gilbey et al. 1981; see Sect. 8.1). There is now a sound experimental basis for understanding these two actions of 5-HT-releasing neurons. Excitatory actions are likely to be produced via 5-HT_1 receptors, whereas inhibitory actions are produced via 5-HT_2 receptors (Yusof and Coote 1986b).

An even more exciting possibility now emerging is that the identity of a co-located substance may indicate a functional specificity. Thus, thyrotropin-releasing hormone, which is co-located in a sub-group of 5-HT neurons in the rostral ventro-lateral medulla (Helke et al. 1986), when applied to the spinal cord itself, mimics the action of 5-HT on cardiovascular neurons, being excitatory to those controlling the splanchnic and renal areas but inhibitory – as is 5-HT – to lumbar vasoconstrictor neurons regulating the vasculature of hind-limb muscle (Yusof and Coote 1986b, 1987; see Sect. 8.4). It is therefore possible that this subgroup of neurons is responsable for the pattern of response associated with arousal. Some support for this idea comes from the recent work of Lovick (1987b) showing that selective activation of neurons in the ventralateral medulla of cats elicits splanchnic vasoconstriction with muscle vasodilatation.

A further indication that some neurons are functionally coded is provided by the data of Futuro-Neto and Coote (1982a, b) which showed that stimula-

tion of spinally projecting neurons in a circumscribed region of the raphe obscurus elicits a pattern of response in cardiovascular outflows which is similar to that seen during the tonic stage of paradoxical sleep. Thus, it would appear that each population of brainstem neurons, with its different complement of chemical messengers, has different actions on cardiovascular target cells in the spinal cord. More specifically, raphe neurons with different chemical messengers might each project to different target cells, or, alternatively, different raphe neurons may control the activity of the same cardiovascular preganglionic neuron, each in a different fashion.

The excitability of the cardiovascular preganglionic neurons in the spinal cord is largely dependent on the influence of supraspinal inputs, tonic activity reaching low levels in animals days or weeks after spinal-cord section (see Sect. 9). The potency of spinal segmental afferents is also greatly dependent on the level of activity in the central descending inputs, dramatic areflexia occurring immediately after their removal by spinal-cord section. However, reflex activity does eventually return; cardio-cardiac, cardio-renal, reno-renal, reno-cardiac, mesenterico-renal and somato-cardiovascular reflexes have been well described (see Sect. 6). Such local reflexes may also be observed in animals with an intact neuraxis (see Sect. 4.4). Thus, the spinal cord contains within itself the neural machinery necessary to generate cardiovascular reflex action, which is sufficient to regulate individual vascular beds so as to supply the needs of individual organs.

These local spinal circuits are, however, constantly modulated (both facilitated and inhibited; see Coote and Sato 1978; Malliani et al. 1983 and Sects. 4.5 and 6) by descending pathways to enable local needs to be subordinated to whole-body needs. This is why there is a baroreceptor inhibitory pathway mediating its effect on the cardiovascular neurons at the spinal level.

Spinal afferent volleys also get long-circuited to the brainstem. This leads to changes in the character of cardiovascular reflexes. The discharge of different groups of cardiovascular neurons is more synchronised as a result, not fractionated or dispersed in time as it is in the spinal animal (see Sect. 4.4). This ensures that there is co-ordinated and simultaneous control of the many vascular beds. Behavioural responses might otherwise be severely limited. Another feature related to this is that the long-circuited volley may result in unequal excitation of neuron pools in the spinal cord, some even being inhibited, so that a pattern of response is elicited in the various pools of vasomotor neurons. The type of response would then depend on the type of receptor activated and the intensity of the stimulus. The efferent volley is long-circuited because the brainstem contains neural systems that are necessary for elaborating complex patterns of response of the sort accompanying goal-directed behaviour.

In conclusion, we can imagine that the numerous groups of cardiac and vasomotor preganglionic neurons in the thoraco-lumbar spinal cord are in-

volved in the moment-by-moment regulation of individual organs via spinal circuitry. In addition, they have the role of initiating and co-ordinating, via supraspinal and spinal afferents, all of the responses, in different vascular beds as well as that of the heart, that contribute to realising behavioural goals.

References

Aars H, Akre S (1970) Reflex changes in sympathetic activity and arterial blood pressure evoked by afferent stimulation of the renal nerve. Acta Physiol Scand 78:184–188

Abrahams VC, Hilton SM, Zbrozyna AW (1964) The role of active muscle vasodilation in the alerting stage of the defence reaction. J Physiol (Lond) 171:189–202

Adair JR, Hamilton BL, Scappaticci KA, Helke CJ, Gillis RA (1977) Cardiovascular responses to electrical stimulation of the medullary raphe area of the cat. Brain Res 128:141–145

Adams PR, Brown DA, Constanti A (1982) M-currents and other potassium currents in bullfrog sympathetic neurones. J Physiol (Lond) 330:537–572

Adrian ED, Bronk DW, Phillips G (1932) Discharges in mammalian sympathetic nerves. J Physiol (Lond) 74:115–133

Alexander RS (1945) The effects of blood flow and anoxia on spinal cardiovascular centres. Am J Physiol 143:698–708

Alexander RS (1946) Tonic and reflex functions of medullary sympathetic cardiovascular centres. J Neurophysiol 9:205–217

Amendt K, Czachurski J, Dembowsky K, Seller H (1979) Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. J Auton Nerv Syst 1:103–117

Ammons WS (1986) Renal afferent input to thoracolumbar neurones of the cat. Am J Physiol 250:R435–R443

Anderson EG, Shibuya T (1966) The effects of 5-hydroxytryptophan and L-tryptophan on spinal synaptic activity. J Pharmacol Exp Ther 153:352–360

Andersson B, Kenney RA, Neil E (1950) The role of the chemoreceptor of the carotid and aortic regions in the production of Mayer waves. Acta Physiol Scand 20:203–220

Andrade R, Aghajanian GK (1982) Single cell activity in the noradrenergic A_5 region. Responses to peripheral manipulations of blood pressure. Brain Res 242:125–135

Angell-James JE, Daly, M de Burgh (1969) Cardiovascular responses in asphyxia: role of arterial chemoreceptors and the modification of their effects by a pulmonary vagal inflation reflex. J Physiol (Lond) 201:87–104

Aprison MH, Shank RP, Davidoff RA (1969) A comparison of the concentration of glycine, a transmitter suspect, in different areas of the brain and spinal cord in seven different vertebrates. Comp Biochem Physiol 28:1345–1355

Ardell JL, Barman SM, Gebber GL (1982) Sympathetic nerve discharge in chronic spinal cat. Am J Physiol 243:H463–H470

Armour JA (1973) Physiological behavior of thoracic cardiovascular receptors. Am J Physiol 225:177–185

Armstrong GG, Irby LS (1962) Arterial blood pressure waves in the absence of functioning aortic and carotid chemoreceptors. Am J Physiol 202:963–966

Armstrong WE, Warach S, Hatton GI, McNeill TH (1980) Subnuclei in the rat hypothalamic paraventricular nucleus: a cytoarchitectural, horseradish peroxidase and immunocytochemical study. Neuroscience 5:1931–1958

Astrom A, Crafoord J (1967) Afferent activity recorded in the kidney nerves of rat. Acta Physiol Scand 70:10–15

Astrom A, Crafoord J (1968) Afferent and efferent activity in the renal nerves of cats. Acta Physiol Scand 74:69–78

Backman SB, Henry JL (1983a) Effects of GABA and glycine on sympathetic preganglionic neurons in the upper thoracic intermediolateral nucleus of the cat. Brain Res 277:365–369

Backman SB, Henry JL (1983b) Effects of glutamate and aspartate on sympathetic preganglionic neurons in the upper thoracic intermediolateral nucleus of the cat. Brain Res 277:370–374

Backman SB, Henry JL (1984a) Effects of oxytocin and vasopressin on thoracic sympathetic preganglionic neurones in the cat. Brain Res Bull 13:679–684

Backman SB, Henry JL (1984b) Effects of substance P and thyrotrophin releasing hormone on sympathetic preganglionic neurones in the upper thoracic intermediolateral nucleus of the cat. Can J Physiol Pharmacol 62:248–251

Bacon SJ, Smith AD (1986) 5-hydroxytryptamine immunoreactive boutons in synaptic contact with identified sympathetic preganglionic neurones in the rat spinal cord. Neurosci Lett [Suppl] 24:S7

Bahr R, Bartel B, Blumberg H, Janig W (1986a) Functional characterisation of preganglionic neurones projecting in the lumbar splanchnic nerves: neurones regulating motility. J Auton Nerv Syst 15:109–130

Bahr R, Bartel B, Blumberg H, Janig W (1986b) Functional characterisation of preganglionic neurones projecting in the lumbar splanchnic nerves: vasoconstrictor neurones. J Auton Nerv Syst 15:130–140

Bahr R, Bartel B, Blumberg H (1986c) Functional characterisation of preganglionic neurones projecting in the lumbar splanchnic nerves: secondary functional properties of lumbar visceral preganglionic neurones. J Auton Nerv Syst 15:141–152

Bainton CR, Richter DW, Seller H, Ballantyne D, Klein JP (1985) Respiratory modulation of sympathetic activity. J Auton Nerv Syst 12:77–90

Baker RG, Anderson EG (1970) The effects of 1–3,4-dihydroxy-phenylamine on spinal reflex activity. J Pharmacol Exp Ther 173:212–223

Barman SM, Gebber GL (1976) Basis for synchronization of sympathetic and phrenic nerve discharges. Am J Physiol 231:1601–1607

Barman SM, Gebber GL (1978) Tonic sympathoinhibition in the baroreceptor denervated cat. Proc Soc Exp Biol Med 157:648–655

Barman SM, Gebber GL (1980) Sympathetic nerve rhythm of brain stem origin. Am J Physiol 239:R42–R47

Barman SM, Gebber GL (1984) Spinal interneurones with sympathetic nerve activity. Am J Physiol 247:R761–R767

Barman SM, Gebber GL (1985) Axonal projection patterns of ventrolateral medullospinal sympatho-excitatory neurones. J Neurophysiol 53:1551–1556

Barman SM, Wurster RD (1978) Interactions of decending spinal sympathetic pathways and afferent nerves. Am J Physiol 234:H223–H229

Baron R, Janig W, McLachlan EM (1985) The afferent and sympathetic components of the lumbar spinal outflow to the colon and pelvic organs in the cat. III. The colonic nerves, incorporating an analysis of the components of the whole lumbar prevertebral outflow. J Comp Neurol 238:158–167

Bartel B, Blumberg H, Janig W (1986) Discharge patterns of motility-regulating neurons projecting in the lumbar splanchnic nerves to visceral stimuli in spinal cats. J Auton Nerv Syst 15:153–163

Basbaum AI, Fields HL (1979) The origin of descending pathways in the dorsolateral funiculus of the spinal cord of cat and rat: further studies on the anatomy of pain modulation. J Comp Neurol 187:513–532

Basbaum AI, Clanton CH, Fields HL (1978) Three bulbospinal pathways from the rostral medulla of the cat: an autoradiographic study of pain modulating systems. J Comp Neurol 178:209–224

Beacham WS, Kunze DL (1969) Renal receptors evoking a spinal vasomotor reflex. J Physiol (Lond) 201:73–85

Beacham WS, Perl ER (1964a) Background and reflex discharge of sympathetic preganglionic neurones in the spinal cat. J Physiol (Lond) 172:400–416

Beacham WS, Perl ER (1964b) Characteristics of a spinal sympathetic reflex. J Physiol (Lond) 173:431–448

Beattie J, Brow GR, Lang CNH (1930) Physiological and anatomical evidence for the existence of nerve tracts connecting the hypothalamus with spinal sympathetic centres. Proc R Soc Lond [Biol] 105:253–275

Bennett JA, Goodchild CS, Kidd C, McWilliam PN (1986) The location and characteristics of sympathetic preganglionic neurones in the lower thoracic spinal cord of dog and cat. J Exp Physiol 71:79–92

Berger AJ (1979) Phrenic motoneurones in the cat: subpopulations and nature of respiratory drive potentials. J Neurophysiol 42:76–90

Bernthal PJ, Koss MC (1979) A spinal sympatho-inhibitory action of chlorpromazine and haloperidol in the cat. Neuropharm 18:697–700

Bezanilla D, Armstrong CM (1972) Negative conductance by entry of sodium and cesium ions into the potassium channels of squid axons. J Gen Physiol 60:588–608

Bianchi AL (1971) Localisation et étude des neurones respiratoires bulbaires. Mise en jeu antidromique par stimulation spinale ou vagale. J Physiol (Paris) 63:5–40

Bianchi AL, Barillot JC (1975) Activity of medullary respiratory neurones during reflexes from the lungs in cats. Respir Physiol 25:335–352

Bishop GH, Heinbecker P (1932) A functional analysis of the cervical sympathetic nerve supply to the eye. Am J Physiol 100:519–532

Björklund A, Skagerberg G (1979) Evidence for a major spinal cord projection from the diencephalic AII dopamine cell group in the rat using transmitter-specific retrograde tracing. Brain Res 177:170–175

Blackman JG, Purves RD (1969) Intracellular recordings from the ganglia of the thoracic sympathetic chain of the guinea pig. J Physiol (Lond) 203:173–198

Blessing WW, Chalmers JP (1979) Direct projections of catecholamine (presumably dopamine) containing neurones from hypothalamus to spinal cord. Neurosci Lett 11:35–40

Blessing WW, Reis DJ (1982) Inhibitory cardiovascular function of the caudal ventrolateral medulla of the rabbit. Brain Res 253:61–71

Blessing WW, Reis DJ (1983) Evidence that GABA and glycine like inputs inhibit vasodepressor neurones in the caudal ventrolateral medulla of the rabbit. Neurosci Lett 37:57–62

Blessing WW, Willoughby JO (1985) Tetrodotoxin elevates arterial pressure but not plasma vasopressin when injected into the caudal ventrolateral medulla of the rabbit. Neurosci Lett 53:259–262

Blessing WW, Chalmers JP, Howe PRC (1978) Distribution of catecholamine containing cell bodies in the rabbit central nervous system. J Comp Neurol 179:407–424

Blessing WW, Goodchild AK, Dampney RAL, Chalmers JP (1981) Cell croups in the lower brainstem of the rabbit projecting to the spinal cord, with special reference to catecholamine containing neurones. Brain Res 221:35–55

Bobillier P, Sequin S, Pititjean F, Salvert D, Touret M, Jouvet M (1976) The raphi nuclei of the cat brainstem: a topographic atlas of their afferent projections as revealed by autoradiography. Brain Res 113:449–486

Bok ST (1928) Das Rückenmark. In: Von Mollendorf W (ed) Handbuch der mikroskopischen Anatomie des Menschen, vol 4. Springer, Berlin Heidelberg New York, pp 478–578

Bowker RM, Steinbusch HWM, Coulter JD (1981) Serotonergic and peptidergic projections to spinal cord demonstrated by a combined retrograte HRP histochemistry and immunocytochemical staining method. Brain Res 211:412–417

Bowker RM, Westlund KN, Sullivan MC, Coulter JD (1982a) Organisation of decending serotonin projections to the spinal cord. Prog Brain Res 57:239–265

Bowker RM, Westlund KN, Sullivan MC, Wilber JF, Coulter JD (1982b) Transmitters of raphespinal complex: immunocytochemical studies. Peptides (Fayetteville) 3:291–298

Bowker RM, Westlund KN, Sullivan MC, Wilber JF, Coulter JD (1983) Descending serotonergic peptidergic and cholinergic pathways from the raphe nuclei: a multiple transmitter complex. Brain Res 288:33–48

Bradley PB, Engel G, Feniuk W, Fozard JR, Humphrey PPA, Middlemiss DN, Mylecharane EJ, Richardson BP, Saxena PR (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytrypamine. Neuropharmacology 25:563–576

Brimble MJ, Wallis DI, Woodward B (1972) Facilitation and inhibition of cells groups within the superior cervical ganglion of the rabbit. J Physiol (Lond) 226:629–652

Brock LG, Coombs JS, Eccles JC (1952) The recording of potentials from motoneurones with an intracellular electrode. J Physiol (Lond) 117:431–460

Brodal A (1956) The reticular formation of the brainstem – anatomical aspects and functional correlations. Thomas, Springfield, I11

Brodwick MS, Junge D (1972) Post stimulus hyperpolarisation and slow potassium conductance increase in *Aplysia* giant neurone. J Physiol (Lond) 223:549–570

Bronk DW, Ferguson LK, Margaria R, Solandt DY (1936) The activity of the cardiac sympathetic centers. Am J Physiol 117:237–249

Broughton R (1972) Phylogenetic evolution of sleep systems. In: Chase MH (ed) The sleeping brain. Brain information service, Brain Research Institute, University of California, Los Angeles, pp 2–7 (Perspectives in the brain sciences, no. 1)

Brown AG (1976) The spinocervical tract: organisation and neuronal morphology. In: Zotterman Y (ed) International symposium on sensory functions of the skin in primates. Pergamon, Oxford, pp 91–103

Brown DL, Guyenet PG (1984) Cardiovascular neurones of the brain stem with projections to the spinal cord. Am J Physiol 247:R1009–R1016

Brown DL, Guyenet PG (1985) Electrophysical study of cardiovascular neurones in the rostral ventrolateral medulla in rats. Circ Res 56:359–369

Bryan RN, Trevino DL, Coulter JD, Willis WD (1973) Location and somatotopic organisation of the cells of origin of the spinocervical tract. Exp Brain Res 17:177–189

Buijs RM (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Pathways to the limbic system, medulla oblongata and spinal cord. Cell Tissue Res 192:423–435

Burnstock G (1983) Recent concepts of chemical communication between excitable cells. In: Osborne NN (ed) Dale's principle and communication between neurones. Pergamon, New York, pp 7–35

Byrum CE, Stornetta R, Guyenet PG (1984) Electrophysiological properties of spinally projecting A_5 noradrenergic neurones. Brain Res 303:15–29

Cabot JB, Wild JM, Cohen DH (1979) Raphe inhibition of sympathetic preganglionic neurones. Science 203:184–186

Cabot JB, Reiner A, Bogan N (1982) Avian bulbospinal pathways: anterograde and retrograde studies of cells of origin, funicular trajectories and laminar terminations. Prog Brain Res 57:79–108

Calaresu FR, Stella A, Zanchetti A (1976) Haemodynamic responses and renin release during stimulation of afferent renal nerves in the cat. J Physiol (Lond) 255:687–700

Calaresu FR, Kim P, Nakamura H, Sato A (1978) Electrophysiological characteristics of renorenal reflexes in the cat. J Physiol (Lond) 283:141–154

Calaresu FR, Tobey JC, Hademann SR, Weaver LC (1984) Splenic and renal sympathetic responses to stimulation of splenic receptors in cats. Am J Physiol 247:R856–R865

Camerer H, Stroh-Werz M, Krienke B, Langhorst P (1977) Postganglionic sympathetic activity with correlation to heart and central cortical rhythms. Pflugers Arch 370:221–225

Caverson, Ciriello J, Calaresu FR (1983) Cardiovascular afferent input to neurons in the ventrolateral medulla projecting to the central autonomic area of the thoracic cord in the cat. Brain Res 274:354–358

Cervero F, Connell LA (1984) Distribution of somatic and visceral primary afferent fibres within the thoracic spinal cord of the cat. J Comp Neurol 230:88–98

Chalmers JP, Kapoor V, Macrae IM, Minson JB, Pilowsky P, West MJ (1985) New approaches to the study of bulbospinal (B_3) serotonergic neurones in the control of blood pressure. J Hypertension 3 [Suppl] 4:S5–S9

Charlton CG, Helke CJ (1985) Autoradiographic localisation and characterisation of spinal cord substance P binding sites: high densities in sensory, autonomic, phrenic and Onuf's motor nuclei. J Neurosci 5:1653–1661

Chan-Palay V (1979) Combined immunohistochemistry and autoradiography after in vivo injections of monoclonal antibody to substance P and ^{3}H-serotonin: coexistence of two putative transmitters in single raphe cells and fiber plexuses. Anat Embryol (Berl) 156:241–245

Chan-Palay V, Jonsson G, Palay SL (1978) Serotonin and substance P coexist in neurones of the rat central nervous system. Proc Natl Acad Sci USA 75:1582–1586

Chiang CY (1980) Central inhibition of depressor reflex at spinal level. Brain Res 192:267–271

Chiba T, Murata Y (1981) Architecture and synaptic relationships in the intermediolateral nucleus of the thoracic spinal cord of the rat: HRP labelling, catecholamine histochemistry and electromicroscopic studies. J Neurocytol 10:315–329

Chung JM, Wurster RD (1975) Localisation of ascending depressor pathways in the cat spinal cord. Fed Proc 34:407

Chung JM, Wurster RD (1976) Ascending pressor and depressor pathways in the cat spinal cord. Am J Physiol 231:786–792

Chung JM, Chung K, Wurster RD (1975) Sympathetic preganglionic neurones of the cat spinal cord: horseradish peroxidase study. Brain Res 91:126–131

Chung JM, Webber CL, Wurster RD (1979a) Ascending spinal pathways for the somato-sympathetic A and C reflexes. Am J Physiol 237:H342–H347

Chung K, Chung JM, Lavelle FW, Wurster RD (1979b) Sympathetic preganglionic neurones in the cat spinal cord projecting to the stellate ganglion. J Comp Neurol 185:23–30

Chung K, Lavelle FW, Wurster RD (1980) Ultrastructure of HRP identified sympathetic preganglionic neurones in cats. J Comp Neurol 190:147–155

Cicardo VH, Garcia JC (1958) Neurogenic arterial hypertension by the cortical-spinal pathway. Arch Int Physiol Biochim 66:309–317

Ciriello AC, Kanazawa I (1978) The distribution of substance P immunoreactive fibres in the rat central nervous system. J Comp Neurol 178:129–156

Ciriello J, Calaresu FR (1977) Descending hypothalamic pathways with cardiovascular function in the cat: a silver impregnation study. Exp Neurol 57:561–580

Clarke J (1851) Researches into the structure of the spinal cord. Philos Trans Soc Lond part 1:607–621

Cohen MI, Gootman PM (1969) Spontaneous and evoked oscillations in respiratory and sympathetic discharge. Brain Res 16:265–268

Cohen MI, Gootman PM (1970) Periodicities in afferent discharge of splanchnic nerve of the cat. Am J Physiol 218:1092–1101

Cohen MI, Gootman PM, Feldman JL (1980) Inhibition of sympathetic discharge by lung inflation. In: Sleight P (ed) Arterial baroreceptors and hypertension. Oxford University Press, Oxford, pp 161–166

Colindres RE, Spielman WS, Moss NG, Harrington WW, Gottschalk CW (1980) Functional evidence for reno-renal reflexes in the rat. Am J Physiol 239:F265–F270

Commissiong JW, Galli CL, Neff NH (1978) Differentiation of dopaminergic and noradrenergic neurons in rat spinal cord. J Neurochem 30:1095–1099

Commissiong JW, Gentleman S, Neff NH (1979) Spinal cord dopaminergic neurons: evidence for an uncrossed nigrospinal pathway. Neuropharmacology 18:565–568

Cook WA, Cangiano A (1972) Presynaptic and post synaptic inhibition of spinal motoneurones. J Neurophysiol 35:389–402

Coote JH (1964) Properties of spinal autonomic reflex arcs and their comparison with somatic arcs. Ph. D. thesis. Royal Free Hospital School of Medicine, London, UK

Coote JH (1978) Somatic sources of afferent input as factors in aberrant autonomic, sensory and motor function. In: Korr IM (ed) The neurobiologic mechanisms in manipulative therapy. Plenum, New York, pp 91–127

Coote JH (1980) Bulbospinal baroreceptor inhibitory pathway to sympathetic preganglionic neurones. In: Sleight P (ed) Arterial baroreceptors and hypertension. Oxford University Press, pp 263–267

Coote JH (1984) Spinal and supraspinal reflex pathways of cardio-cardiac sympathetic reflexes. Neurosci Lett 46:243–247

Coote JH (1985) Noradrenergic projections to the spinal cord and their role in cardiovascular control. J Auton Nerv Syst 14:255–262

Coote JH (1986) Peptidergic projections to the spinal cord and blood pressure control. In: Nakamura K (ed) Brain and blood pressure control. Elsevier, Amsterdam, pp 181–186

Coote JH (1987) The functional role of noradrenaline and 5-hydroxytryptamine terminals in the thoracic spinal cord. In: Taylor EW (ed) Neurobiolgy of the cardiorespiratory system. Manchester University Press, Manchester

Coote JH, Downman CBB (1965) Comparison of reflex volleys in white ramus and postganglionic pathways. J Physiol (Lond) 181:37–38

Coote JH, Downman CBB (1966) Central pathways of some autonomic reflex discharges. J Physiol (Lond) 183:714–729

Coote JH, Downman CBB (1969a) Supraspinal control of reflex activity in renal nerves. J Physiol (Lond) 202:147–160

Coote JH, Downman CBB (1969b) Supraspinal control of reflex activity in renal nerves. J Physiol (Lond) 202:161–170

Coote JH, Macleod VH (1972) The possibility that noradrenaline is a sympathoinhibitory transmitter in the spinal cord. J Physiol (Lond) 225:44–46

Coote JH, Macleod VH (1974a) Evidence for the involvement in the baroreceptor reflex of a descending inhibitory pathway. J Physiol (Lond) 241:477–496

Coote JH, Macleod VH (1974b) The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. J Physiol (Lond) 241:453–457

Coote JH, Macleod VH (1975) The spinal route of sympatho-inhibitory pathways descending from the medulla oblongata. Pflügers Arch 359:335–347

Coote JH, Macleod VH (1977) The effect of intraspinal microinjections of 6-hydroxydopamine on the inhibitory influence exerted on spinal sympathetic activity by the baroreceptors. Pflügers Arch 371:271–277

Coote JH, Macleod VH (1984) Estimation of conduction velocity in bulbospinal excitatory pathways to sympathetic outflows in cat spinal cord. Brain Res 311:97–107

Coote JH, Perez-Gonzales JF (1970) The response of some sympathetic neurones to volleys in various afferent nerves. J Physiol (Lond) 208:261–278

Coote JH, Sato A (1978) Supraspinal regulation of spinal reflex discharge into cardiac sympathetic nerves. Brain Res 142:425–437

Coote JH, Suter C (1985) Excitatory actions of angiotensin II on sympathetic preganglionic neurones in the rat. J Physiol (Lond) 367:49

Coote JH, Westbury DR (1974) The influence of the carotid sinus baroreceptors on activity in single sympathetic preganglionic neurones. J Physiol (Lond) 241:22–23

Coote JH, Westbury DR (1979a) Intracellular recordings from sympathetic preganglionic neurones. Neurosci Lett 15:171–175

Coote JH, Westbury DR (1979b) Functional grouping of sympathetic preganglionic neurones in the third thoracic segment of the spinal cord. Brain Res 179:367–372

Coote JH, Downman CBB, Prout BJ (1962) Threshold mapping of medullary centres in the anaesthetised cat. J Physiol (Lond) 162:12

Coote JH, Downman CBB, Weber WV (1969) Reflex discharges into thoracic white rami elicited by somatic and visceral afferent excitation. J Physiol (Lond) 202:147–160

Coote JH, Macleod VH, Martin IL (1978) A search for the role of bulbospinal tryptaminergic neurones in the control of sympathetic activity. Pflügers Arch 379:109–116

Coote JH, Fleetwood-Walker SM, Mitchell PR (1979) Catecholamine receptors in thoracic spinal cord. Br J Pharmacol 68:137

Coote JH, Macleod VH, Fleetwood-Walker SM, Gilbey MP (1981 a) The response of individual sympathetic preganglionic neurones to microelectrophoretically applied endogenous monoamines. Brain Res 215:135–145

Coote JH, Macleod VH, Fleetwood-Walker SM, Gilbey MP (1981 b) Baroreceptor inhibition of sympathetic activity at a spinal site. Brain Res 220:81–93

Crofton JT, Rockhold RW, Share L, Wang BC, Horovitz ZP, Manning M, Sawyer WH (1981) Effect of intracerebroventricular captopril on vasopressin and blood pressure in spontaneously hypertensive rats. Hypertension 11:71–74

Crutcher KA, Bingham WG (1978) Descending monoaminergic pathways in the primate spinal cord. Am J Anat 153:159–164

Cummings JF (1969) Thoracolumbar preganglionic neurones and adrenal innervation in the dog. Acta Anat (Basel) 73:27–37

Dahlström A, Fuxe K (1964) Evidence for the existance of monoamine containing neurones in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurones. Acta Physiol Scand 62 [Suppl 232]:1–55

Dahlström A, Fuxe K (1965) Evidence for the existence of monoamine containing neurones in the CNS. II. Experimentally induced changes in the intraneuronal amine levels of the bulbospinal neurone system. Acta Physiol Scand 64 [Suppl 247]:1–36

Dalsgaard CJ, Elfvin LG (1979) Spinal origin of preganglionic fibres projecting onto the superior cervical and inferior mesenteric ganglion of the guinea pig as demonstrated by the horseradish peroxidase technique. Brain Res 172:139–143

Dalsgaard CJ, Elfvin LG (1981) The distribution of the sympathetic preganglionic neurones projecting onto the stellate ganglion of the guinea pig. A horseradish peroxidasse study. J Auton Nerv Syst 4:327–337

Daly, M de Burgh (1986) Interactions between respiration and circulation. In: Handbook of physiology. The respiratory system. Vol II. Cherniack NS, Widdicombe JG (eds) American Physiological Society Williams and Wilkins. Baltimore, pp 529–594

Daly, M de Burgh, Robinson BH (1968) An analysis of the reflex systemic vasodilator response elicited by lung inflation in the dog. J Physiol (Lond) 195:387–406

Daly, M de Burgh, Scott MJ (1962) An analysis of the primary cardiovascular reflex of stimulation of the carotid body chemoreceptors in the dog. J Physiol (Lond) 162:555–573

Daly, M de Burgh, Hazzledine JL, Ungar A (1967) The reflex effects of alterations in the lung volume on systemic vascular resistance in the dog. J Physiol (Lond) 188:331–351

Daly, M de Burgh, Litherland AS, Wood LM (1983) The reflex effects of inflation of the lungs on heart rate and hind limb vascular resistance in the cat. IRCS Med Sci Libr Compend 11:859–860

Daly, M de Burgh, Ward J, Wood LM (1984) Comparison of the effects of lungs inflation on the hind limb vascular responses to stimulation of the carotid chemoreceptors and baroreceptors and to urinary bladder distension in the anaesthetised dog. J Physiol (Lond) 353: 115*P*

Dampney RAL, Goodchild AK, Tan E (1985) Vasopressor neurones in the rostral ventrolateral medulla of the rabbit. J Auton Nerv Syst 14:239–254

Dampney RAL, McAllen RM (1986) Functional specificity of ventral medullary pre-sympathetic neurones in the cat. J Physiol (Lond) 377:58*P*

Darlington DN, Ward DG (1985 a) Rostral pontine and caudal mesencephalic control of arterial pressure and iliac, celiac and renal vascular resistance I. Anatomic regions. Brain Res 361:284–300

Darlington DN, Ward DG (1985 b) Rostral pontine and caudal mesencephalic control of arterial pressure and iliac, celiac and renal vascular resistance II. Separate control and topographic organisation. Brain Res 361:301–308

Dashwood MR, Gilbey MP, Spyer KM (1985) The localisation of adrenoreceptors and opiate receptors in regions of the cat central nervous system involved in cardiovascular control. Neuroscience 15:537–551

Davis AL, McCloskey DI, Potter EK (1977) Respiratory modulation of baroreceptor and chemoreceptor reflexes affecting heart rate through the sympathetic nervous system. J Physiol (Lond) 272:691–703

Davis BM, Cabot JB (1984) Substance P containing pathway to avian sympathetic preganglionic neurones: evidence for a major spinal-spinal circuitry. J Neurosci Methods 4:2145–2159

Davis BM, Krause JE, McKelvy JF, Cabot JB (1984) Effects of spinal lesions on substance P levels in the rat sympathetic preganglionic cell column: evidence for local spinal regulation. Neuroscience 13:1311–1326

Day TA, Ro A, Renaud LP (1983) Depressor area within caudal ventrolateral medulla of the rat does not correspond to the A_1 catecholamine cell group. Brain Res 279:299–302

Dean C, Coote JH (1986) A ventromedullary relay involved in the hypothalamus and chemoreceptor activation of sympathetic preganglionic neurones to skeletal muscle. Brain Res 377:279–285

DeGroat WC (1976) Mechanisms underlying recurrent inhibition in the sacral parasympathetic outflow to the urinary bladder. J Physiol (Lond) 257:503–513

DeGroat WC, Lalley PM (1973) Depression by p-methoxyphenylethylamine of sympathetic reflex firing elicited by electrical stimulation of the carotid sinus nerve or pelvic nerve. Brain Res 64:460–465

DeGroat WC, Lalley PM (1974) Reflex sympathetic firing in response to electrical stimulation of the carotid sinus nerve in the cat. Brain Res 80:14–40

DeGroat WC, Ryall RW (1967) An exitatory action of 5-hydroxytryptamine on sympathetic preganglionic neurones. Exp Brain Res 3:299–303

DeGroat WC, Ryall RW (1968) The identification and characteristics of sacral parasympathetic preganglionic neurones. J Physiol (Lond) 196:563–577

DeLanerolle NC, LaMotte CC (1982) The human spinal cord: substance P and methionine-enkephalin immunoreactivity. J Neurosci Methods 2:1369–1386

Dembowsky K, Czachurski J, Amendt K, Seller H (1980) Tonic descending inhibition of the spinal somato-sympathetic reflex from the lower brainstem. J Auton Nerv Syst 2:157–182

Dembowsky K, Czachurski J, Seller H (1985a) An intracellular study of the synaptic input to sympathetic preganglionic neurones of the third thoracic segment of the cat. J Auton Nerv Syst 13:201–244

Dembowsky K, Czachurski J, Seller H (1985b) Morphology of sympathetic preganglionic neurones in the thoracic spinal cord of the cat: an intracellular horseradish peroxidase study. J Comp Neurol 238:453–465

Dembowsky K, Czachurski J, Seller H (1986) Three types of sympathetic preganglionic neurones with different electrophysiological properties are identified by intracellular recordings in the cat. Pflügers Arch 406:112–120

Demirjian CR, Grossman R, Meyer R, Katzman R (1976) The catecholamine pontine cellular groups locus coeruleus, A_4, subcoeruleus in the primate *Cebus apella*. Brain Res 115:395–411

Deuschl G, Illert M (1978) Location of lumbar preganglionic sympathetic neurones in the cat. Neurosci Lett 10:49–54

Deuschl G, Illert M (1981) Cytoarchitectonic organisation of lumbar preganglionic sympathetic neurones in the cat. J Auton Nerv Syst 3:193–213

Deuschl G, Illert M, Aschoff A, Hollander H (1981) Single preganglionic neurones of the cat branch intraspinally and project through different rami communicantes albi. A retrograde double labelling study with fluorescent tracers. Neurosci Lett 21:1–5

Diamond J (1968) The activation and distribution of GABA and L-glutamate receptors on goldfish Mauthner neurones: an analysis of dendritic remote inhibition (with appendix by A. F. Huxley) J Physiol (Lond) 194:669–723

DiBona GF, Rios LL (1980) Renal nerves in compensatory renal responses to contralateral renal denervation. Am J Physiol 238:F26–F30

DiTirro FJ, Ho RH, Martin GF (1981) Immunohistochemical localisation of substance P, somatostatin and methionine-enkephalin in the spinal cord and dorsal root ganglion of the North American opossum, *Didelphus virginia*. J Comp Physiol 198:351–363

Dorokhova MI, Medvedev OS, Reznikova YA, Tsyrlin VA (1974) Some data on the presence of inhibitory vasomotor control at the spinal level. Bull Eksp Biol Med (Rus) 77:3–6
Downing SE, Siegel JH (1963) Baroreceptor and chemoreceptor influences on sympathetic discharge to the heart. Am J Physiol 204:471–479
Downing SE, Remensnyder JP, Mitchell JH (1962) Cardiovascular responses to hypoxic stimulation of the carotid bodies. Circ Res 10:676–685
Dube L, Parent A (1981) The monoamine containing neurones in the avian brain: I. A study of the brainstem of the chicken (*Gallus domesticus*) by means of fluorescence and acetylcholinesterase histochemistry. J Comp Neurol 196:695–708
Dun NJ, Ma RC (1984) Slow non-cholinergic excitatory potentials in neurones of the guinea pig coeliac ganglia. J Physiol (Lond) 351:47–60
Earle KM (1952) The tract of Lissauer and its possible relation to the pain pathway. J Comp Neurol 96:93–111
Eccles JC (1935) The action potential of the superior cervical ganglion. J Physiol (Lond) 85:179–206
Eccles JC (1964) The physiology of nerve cells. Oxford University Press, Oxford
Eccles JC, Fatt P, Koketsu K (1954) Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. J Physiol (Lond) 126:524–562
Eh S, Huan-Ji D (1964) The descending pathways of the bulbar cardiovascular centre. Acta Physiol Sinica 27:108–114
Elfvin L (1983) Autonomic ganglia. Wiley, New York
Emson PC (1979) Peptides as neurotransmitter candidates in the mammalian CNS. Prog Neurobiol 13:61–116
Faden AI, Petras JM (1978) An intraspinal sympathetic preganglionic pathway: anatomic evidence in the dog. Brain Res 144:358–362
Fedina L, Katunskii AY, Khayutin VM, Mitsayni A (1966) Responses of renal sympathetic nerves to stimulation of afferent A and C fibres of tibial and mesenterial nerves. Acta Physiol Hung 29:157–176
Fedorka L, Lioy F, Trzebski A (1985) Synaptic inhibition of sympathetic preganglionic neurones induced by stimulation of the aortic nerve in the cat. J Physiol (Lond) 360:45*P*
Feldberg W, Guertzenstein PG (1976) Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. J Physiol (Lond) 258:337–355
Felix D, Akert K (1974) The effects of angiotensin II on neurones of the cat subfornical organ. Brain Res 76:350–353
Felix D, Schlegel W (1978) Angiotensin receptive neurones in the subfornical organ. Brain Res 149:107–116
Felpel LP, Hoffman RD (1986) Responses of splanchnic driven neurones to substance P and eledoisin related peptides. J Auton Nerv Syst 15:269–274
Felten DP, Laities AM, Carpenter MB (1974) Monoamine containing cell bodies in the squirrel monkey brain. Am J Anat 139:153–166
Fernandez de Molena A, Perl ER (1965) Sympathetic activity and the systemic circulation in the spinal cat. J Physiol (Lond) 181:82–102
Fernandez de Molena A, Kuno M, Perl ER (1965) Antidromically evoked responses from sympathetic preganglionic neurones. J Physiol (Lond) 180:321–335
Fields HL, Winter DL (1970) Somato visceral pathway: rapidly conducting fibres in the spinal cord. Science 167:1729–1730
Fields HL, Meyer GA, Partridge LD (1970a) Convergence of visceral and somatic input onto spinal neurones. Exp Neurol 26:36–52
Fields HL, Partridge LD, Winter DL (1970b) Somatic and visceral receptive field properties of fibres in the ventral quadrant white matter of the cat spinal cord. J Neurophysiol 33:827–837
Fleetwood-Walker SM, Coote JH (1981a). The contribution of brainstem catecholamine cell groups to the innervation of the sympathetic lateral cell column. Brain Res 205:141–155
Fleetwood-Walker SM, Coote JH (1981b). Contribution of noradrenaline, dopamine and adrenaline-containing axons to the innervation of different regions of the spinal cord of the cat. Brain Res 206:95–106

Fleetwood-Walker SM, Coote JH, Gilbey MP (1983) Identification of spinally projecting neurones in the A_1 catecholamine cell group of the ventrolateral medulla. Brain Res 273:25–33
Folkow B, Johansson B, Oberg B (1958) The stimulation threshold of different sympathetic fibre groups as correlated to their functional differentiation. Acta Physiol Scand 44:146–156
Foreman RD, Wurster RD (1973) Localisation and functional characteristics of descending sympathetic spinal pathways. Am J Physiol 225:212–217
Foreman RD, Wurster RD (1975) Conduction in descending spinal pathways initiated by somato-sympathetic reflexes. Am J Physiol 228:905–908
Foreman RD, Schmidt RF, Willis WD (1977) Convergence of muscle and cutaneous input onto primate spinothalamic tract neurones. Brain Res 124:555–560
Forssmann WG (1978) A new somatostatinergic system in the mammalian spinal cord. Neurosci Lett 10:293–297
Fozard JR (1984) Neuronal 5-HT receptors in the periphery. Neuropharmacology 23: 1473–1486
Fransisco LL, Hoversten LG, DiBona GF (1980) Renal nerves in the compensatory adaptation to ureteral occlusion. Am J Physiol 238:F229–F234
Franz DN, Madsen PW (1982) Differential sensitivity of four central sympathetic pathways to depression by clonidine. Eur J Pharmacol 78:53–59
Franz DN, Evans MH, Perl ER (1966) Characteristics of viscero-sympathetic reflexes in the spinal cat. Am J Physiol 211:1292–1298
Franz DN, Hare BD, Neumayr RJ (1975) Reciprocal control of sympathetic preganglionic neurones by monoaminergic bulbospinal pathways and a selective effect of clonidine. In: Milliez P, Safar M (eds) Recent advances in hypertension, vol 1. Boehringer, Ingelheim, pp 85–96
Franz DN, Hare BD, Neumayr RJ (1978) Depression of sympathetic preganglionic neurones by clonidine: evidence for stimulation of 5-HT receptors. Clin Exp Hypertens 1:115–140
Franz DN, Hare BD, McCloskey KL (1982a) Spinal sympathetic neurones: possible sites of opiate withdrawal suppression by clonidine. Science 215:1643–1645
Franz DN, Madsen PW, Peterson RG, Sangdee C (1982b) Functional role of monoaminergic pathways to sympathetic preganglionic neurones. Clin Exp Hypertens [A4] (4,5):543–562
Futuro-Neto HA, Coote JH (1982a) Changes in sympathetic activity to heart and blood vessels during desynchronized sleep. Brain Res 252:259–268
Futuro-Neto HA, Coote JH (1982b) Desynchronized sleep-like pattern of sympathetic activity elicited by electrical stimulation of sites in the brainstem. Brain Res 252:269–276
Fuxe K, Jonsson G (1973) Further mapping of central 5-hydroxytryptamine. Adv Biochem Pharmacol 10:1–12
Fuxe K, Ljunggren L (1965) Cellular localisation of monoamines in the upper brainstem of the pigeon. J Comp Neurol 148:61–90
Fuxe K, Ganten D, Hokfelt T, Bolme P (1976) Immunohistochemical evidence for the existence of angiotensin II-containing nerve terminals in the brain and spinal cord of the rat. Neurosci Lett 2:229–234
Gage PW, Hubbard JI (1966) The origin of the post-tetanic hyperpolarisation of mammalian motor nerve terminals. J Physiol (Lond) 184:335–352
Gagel O (1928) Zur Histologie und Topographie der vegetativen Zentren in Rückenmark. Z Anat Entwicklungsgesch 85:213–250
Gagel O (1932) Die vegetativen Anteile des Rückenmarks. Z Gesamte Neurol Psychiat 138:263–315
Galabov P, Davidoff M (1976) On the vegetative network of guinea pig thoracic spinal cord. Histochemistry 47:247–255
Gallego R (1983) The ionic basis of action potentials in the petrosal ganglion cells of the cat. J Physiol (Lond) 342:591–602
Ganten D, Fuxe K, Phillips MI, Mann JFE, Ganten U (1978) The brain isorenin-angiotensin system: biochemistry, localisation and possible role in drinking and blood pressure regulation. In: Ganong WF, Martini L, (eds) Frontiers in neuroendocrinology, vol 5. Raven, New York, pp 61–100

Garver DL, Sladek JR (1975) Monoamine distribution in primate brain. I. Catecholamine containing perikarya in the brain stem of *Macaca speciosa*. J Comp Neurol 159:289–304

Gawhiler BH, Dreifuss JJ (1980) Transition from random to phasic firing induced in neurones cultured from the hypothalamic supraoptic area. Brain Res 193:415–425

Gebber GL (1976) Basis for phase relations between baroreceptor and sympathetic nervous discharge. Am J Physiol 230:263–270

Gebber GL (1980) Central oscillators responsible for sympathetic nerve discharge. Am J Physiol 239:H143–H155

Gebber GL, Barman SM (1979) Inhibitory interactions between preganglionic sympathetic neurones. In: Meyer P, Schmitt H (eds) Nervous systems and hypertension. Wiley, New York, pp 137–145

Gebber GL, Barman SM (1980) Basis for 2–6 cycle/s rhythm in sympathetic nerve discharge. Am J Physiol 239:R48–R56

Gebber GL, McCall RB (1976) Identification and discharge patterns of spinal sympathetic interneurones. Am J Physiol 231:722–733

Gebber GL, Taylor DG, Weaver LC (1973) Electrophysiological studies on organisation of central vasopressor pathways. Am J Physiol 224:470–481

Gerber U, Polosa C (1978) Effects of pulmonary stretch receptor afferent stimulation on sympathetic preganglionic neurone firing. Can J Physiol Pharmacol 56:191–198

Gerber U, Polosa C (1979) Some effects of superior laryngeal nerve stimulation on sympathetic preganglionic neurone firing. Can J Physiol Pharmacol 57:1073–1081

Gibson SJ, Polak JM, Bloom SR, Wall PD (1981) The distribution of nine peptides in the rat spinal cord with special emphasis on the substantia gelatinosa and on the area around the central canal (Lamina x). J Comp Neurol 201:65–79

Gilbert RFT, Emson PC, Hunt SP, Bennett GW, Marsden CA (1982a) Neuronal co-existance of putative transmitters in the spinal cord and brainstem of the rat. In: Cuello (ed) Co-transmission. MacMillan, London, pp 51–75

Gilbert RFT, Emson PC, Hunt SP, Bennett GW, Marsden CA, Sandberg BEB, Steinbusch HWM, Verhofstad AAJ (1982b) The effects of monoamine neurotoxins on peptides in the rat spinal cord. Neuroscience 7:69–87

Gilbey MP (1980) Characteristics of bulbo-spinal sympatho-inhibitory pathways descending from the ventromedial reticular formation and raphe nuclei of the medulla. PhD Thesis, University of Birmingham

Gilbey MP, Coote JH, Macleod VH, Peterson DF (1981) Inhibition of sympathetic activity by stimulation of the raphe nuclei and the role of 5-hydroxytrypamine in this effect. Brain Res 226:131–142

Gilbey MP, Coote JH, Fleetwood-Walker SM, Peterson DF (1982a) The influence of the paraventriculo-spinal pathway, and oxytocin and vasopressin on sympathetic preganglionic neurones. Brain Res 251:283–290

Gilbey MP, Peterson DF, Coote JH (1982b) Some characteristics of sympathetic preganglionic neurones in the rat. Brain Res 241:43–48

Gilbey MP, McKenna KE, Schramm LP (1983) Effects of substance P on sympathetic preganglionic neurones. Neurosci Lett 41:157–159

Gilbey MP, Jordan D, Numao Y, Spyer KM, Wood LM (1985) Respiratory modulation of cervical sympathetic preganglionic neurones in the anaesthetised rat. J Physiol (Lond) 369: 145

Gilbey MP, Numao Y, Spyer KM (1986) Discharge patterns of cervical sympathetic preganglionic neurones related to central respiratory drive in the rat. J Physiol (Lond) 378:253–265

Gilliatt RW (1948) Vasoconstriction in the finger after deep inspiration. J Physiol (Lond) 107:76–88

Gilliatt RW, Guttmann L, Whitteridge D (1948) Inspiratory vasoconstriction in patients after spinal injuries. J Physiol (Lond) 107:65–75

Gillis RA, Helke CJ, Hamilton BL, Morgenroth UH (1976) Cardiac arrhythmias produced by electrical stimulation of the mid brain raphe in the cat. Soc Neurosci Abst 2:74
Gilmore JP, Tomomatsu E (1985) Renal mechanoreceptors in non-human primates. Am J. Physiol 248:R202–R207
Glazer EJ, Ross LL (1980) Localisation of noradrenergic terminals on sympathetic preganglionic nuclei of the rat: demonstration by immunocytochemical localisation of dopamine hydroxylase. Brain Res 185:39–49
Glick SM, Brownstein MJ (1980) Vasopressin content of rat brain. Life Sci 27:1103–1110
Glick G, Wechsler AS, Epstein SE (1969) Reflex cardiovascular depression produced by the stimulation of pulmonary stretch receptors in the dog. J Clin Invest 48:467–473
Gootman PM, Cohen MI (1970) Efferent splanchnic activity and systemic arterial pressure. Am J Physiol 219:897–903
Gootman PM, Cohen MI (1971) Evoked splanchnic potentials produced by electrical stimulation of medullary vasomotor regions. Exp Brain Res 13:1–14
Gootman PM, Cohen MI (1973) Periodic modulation (cardiac and respiratory) of spontaneous and evoked sympathetic discharge. Acta Physiol Pol 24:97–109
Gootman PM, Cohen MI (1974) The interrelationships between sympathetic discharge and central respiratory drive. In: Umbach W, Koepchen HP (eds) Central rhythmic and regulation. Hippokrates, Stuttgart, pp 195–209
Gootman PM, Cohen MI (1980) Origin of rhythms common to sympathetic outflows at different spinal levels. In: Sleight P (ed) Arterial baroreceptors and hypertension. Oxfort University Press, Oxford, pp 154–160
Gootman PM, Cohen MI (1981) Sympathetic rhythms in spinal cats. J Auton Nerv Syst 3:379–387
Gootman PM, Cohen MI, Piercey MP, Wolotsky P (1975) A search for medullary neurones with activity patterns similar to those in sympathetic nerves. Brain Res 87:395–406
Gootman PM, Feldman JL, Cohen MI (1980) Pulmonary afferent influences on respiratory modulation of sympathetic discharge. In: Koepchen HP, Hilton SM, Trzebski A (eds) Central interactions between respiratory and cardiovascular control systems. Springer, Berlin Heidelberg New York, pp 172–178
Graham LT, Shank RP, Werman R, Aprison MH (1967) Distribution of some synaptic transmitter suspects in cat spinal cord: glutamic acid, aspartic acid, γ-aminobutyric acid, glycine and glutamine. J Neurochem 14:465–472
Green JH, Heffron PF (1967) Observations on the origin and genesis of a rapid sympathetic rhythm. Arch Int Pharmacodyn Ther 109:403–411
Gregor M, Janig W (1977) Effects of systemic hypoxia and hypercapnia on cutaneous and muscle vasconstrictor neurones in the cats hind limb. Pflügers Arch 368:71–81
Gregor M, Janig W, Riedel W (1976) Response patterns of cutaneous postganglionic neurones to the hind limb on spinal cord heating and cooling in the cat. Pflügers Arch 363:135–140
Greving R (1928) Die zentralen Anteile des vegetativen Nervensystems. In: Möllendorf W, Bargmann W (eds) Handbuch der mikroskopischen Anatomie des Menschen, vol 4. Springer, Berlin Heidelberg New York, pp 917–1060
Grillner S, Lund S (1968) The origin of a descending pathway with monosynaptic action on flexor motoneurones. Acta Physiol Scand 74:274–284
Grillner S, Hongo T, Lund S (1970) The vestibulo-spinal tract. Effects on alpha motoneurones in the lumbosacral spinal cord in cat. Exp Brain Res 10:94–120
Grosse M, Janig W (1976) Vasoconstrictor and pilomotor fibres in skin nerves to the cat's tail. Pflügers Arch 361:221–229
Guertzenstein PG, Silver A (1974) Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. J Physiol (Lond) 242:489–503
Gustafsson B (1974) After-hyperpolarisation and the control of repetitive firing in spinal neurones of the cat. Acta Physiol Scand [Suppl] 416:1–47
Gustafsson B, Lipski J (1980) Effect of membrane polarisation on synaptic activity of the timing of antidromic invasion. Brain Res 181:121–133

Guyenet PG (1980) The coeruleospinal noradrenergic neurones: anatomical and electrophysiological studies in the rat. Brain Res 189:121–133
Guyenet PG, Cabot JB (1981) Inhibition of sympathetic preganglionic neurones by catecholamines and clonidine: mediation by an adrenergic receptor. J Neurosci 1:908–917
Guyenet PG, Stornetta RL (1982) Inhibition of sympathetic preganglionic discharges by epinephrine and methyl epinephrine. Brain Res 235:271–283
Guyton AC, Harris JW (1951) Pressor receptor autonomic oscillation: a possible cause of vasomotor waves. Am J Physiol 165:158–166
Haeusler G (1977) Neuronal mechanisms influencing transmissions in the baroreflex arc. Prog Brain Res 47:95–109
Haigler HJ, Aghajanian GK (1974) Lysergic diethylamide and serotonin. A comparison of effects on serotonin neurones and neurones receiving a serotonin input. J Pharmacol Exp Ther 188:688–699
Hancock MB (1976) Cells of origin of hypothalamo-spinal projections in the rat. Neurosci Lett 3:179–184
Hancock MB (1982) Leu-enkephalin, substance P, somatostatin immunohistochemistry combined with retrograde transport of horseradish peroxidase in sympathetic preganglionic neurones. J Auton Nerv Syst 6:263–272
Hancock MB, Peveto CA (1979) A preganglionic autonomic nucleus in the dorsal gray commissure of the lumbar spinal cord of the rat. J Auton Nerv Syst 183:45–72
Hanna BD, Lioy F, Polosa C (1979) The effect of cold blockade of the medullary chemoreceptors on the CO_2 modulation of vascular tone and heart rate. Can J Physiol Pharmacol 57:461–468
Harada Y, Takahashi T (1983) The calcium component of the action potential in spinal motoneurones of the rat. J Physiol (Lond) 335:89–100
Hare BD, Neumayr RJ, Franz DN (1972) Opposite effects of L-Dopa and 5-HTP on spinal sympathetic reflexes. Nature 239:226–227
Hass HL, Felix D, Celio MR, Ingami T (1980) Angiotensin II in the hippocampus. A histochemical and electrophysiological study. Experientia 36:1394–1395
Hedreen JC, McGrath S (1977) Observations of labelling of neuronal cell bodies, axons, and terminals after injection of horseradish peroxidase into rat brain. J Comp Neurol 176:225–246
Helke CJ, Neil JJ, Massari VJ, Loewy AD (1982) Substance P neurones project from the ventral medulla to the intermediolateral cell column and the ventral horn in the rat. Brain Res 243:147–152
Helke CJ, Sayson SC, Keeler JR, Charlton CG (1985) Evidence for bulbospinal thyrotropin releasing hormone (TRH) projections: co-existence with serotonin. Fed Proc 44:1388
Helke CJ, Sayson SC, Keeler JR, Charlton CG (1986) Thyrotropin-releasing hormone immunoreactive neurones project from the ventral medulla to the intermediolateral cell column: partial co-existence with serotonin. Brain Res 381:1–7
Henry JL, Calaresu FR (1972a) Distribution of cardiovascular sites in the intermediolateral nucleus of the cat. Am J Physiol 222:700–704
Henry JL, Calareus FR (1972b) Topography and numerical distribution of neurones of the thoraco-lumbar intermediolateral nucleus in the cat. J Comp Neurol 144:205–214
Henry JL, Calaresu FR (1974a) Excitatory and inhibitory inputs from medullary nuclei projecting to spinal cardioacceleratory neurones in the cat. Exp Brain Res 20:485–504
Henry JL, Calaresu FR (1974b) Pathways from medullary nuclei to spinal cardioacceleratory neurones in the cat. Exp Brain Res 20:505–514
Henry JL, Calaresu FR (1974c) Origin and course of crossed medullary pathways to spinal sympathetic neurones in the cat. Exp Brain Res 20:515–526
Henry JL, Calaresu FR (1974d) Responses of single units in the intermediolateral nucleus to stimulation of cardioregulatory medullary nuclei in the cat. Brain Res 77:314–319
Heuser JE, Reese TS (1973) Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J Cell Biol 57:315–344

Heymans C, Bouckaert JJ, Farber S, Hsu FY (1936) Spinal vasomotor reflexes associated with variations in blood pressure. Am J Physiol 117:619–625

Hilaire G, Monteau R (1976) Connexions entre les neurone inspiratoires bulbaires et les motoneurones phréniques et intercostaux. J Physiol (Paris) 72:987–1000

Hilton SM, Marshall JM (1982) The pattern of cardiovascular response to the carotid chemoreceptor stimulation in the cat. J Physiol (Lond) 326:495–513

Hilton SM, Marshall JM, Timms RJ (1983) Ventral medullary relay neurones in the pathway from the defence areas of the cat and their effect on blood pressure. J Physiol (Lond) 345:149–166

Hokfelt T, Fuxe K, Goldstein M, Jonsson O (1974) Immunohistochemical evidence for the existence of adrenaline neurones in the rat brain. Brain Res 66:235–251

Hokfelt T, Fuxe K, Johansson O, Jeffcoate S, White N (1975) Thyrotropin releasing hormone (TRH) containing nerve terminals in certain brainstem nuclei and in the spinal cord. Neurosci Lett 1:133–139

Hokfelt T, Ljungdahl A, Steinbusch H, Verhofstad A, Nilsson G, Brodin E, Pernov B, Goldstein M (1978) Immunohistochemical evidence of a substance P like immunoreactivity in some 5-hydroxytryptamine containing neurones in the rat central nervous system. Neuroscience 3:517–538

Hokfelt T, Terenius L, Kuypers HGJM, Dann O (1979) Evidence for enkephalin immunoreactive neurones in the medulla oblongata projecting to the spinal cord. Neurosci Lett 14:55–60

Hokfelt T, Johansson O, Ljungdahl A, Lundberg JM, Schultzberg M (1980) Peptidergic neurones. Nature 284:515–521

Holets V, Elde R (1982) The differential distribution and relationship of serotonin and peptidergic fibers to the sympathoadrenal neurones in the intermediolateral cell column of the rat: a combined retrograde axonal transport and immunofluorescence study. Neuroscience 7:1155–1174

Holets V, Elde R (1983) Sympathoadrenal preganglionic neurons: their distribution and relationship to chemically-coded fibres in the kitten intermediolateral cell column. J Auton Nerv Syst 7:149–163

Holstege G, Kuypers HGJM (1982) The anatomy of brainstem pathways to the spinal cord in cat. A labelled amino acid tracing study. Prog Brain Res 57:145–175

Holstege G, Kuypers HGJM, Boer RC (1979) Anatomical evidence for direct brainstem projections to the somatic motoneurone cell groups and autonomic preganglionic cell groups in cat spinal cord. Brain Res 171:329–323

Hongo T, Ryall RW (1966) Electrophysiological and microelectrophoretic studies on sympathetic preganglionic neurones in the spinal cord. Acta Physiol Scand 68:96–104

Hongo T, Jankowska E, Lundberg A (1968) Postsynaptic excitation and inhibition from primary afferents in neurones in the spinocervical tract. J Physiol (Lond) 199:569–592

Horeyseck G, Janig W (1974a) Reflex activity in postganglionic fibres within skin and muscle nerves elicited by somatic stimuli in chronic spinal cats. Exp Brain Res 21:155–168

Horeyseck G, Janig W (1974b) Reflexes within postganglionic fibres within skin and muscle nerves after mechanical non-noxious stimulation of skin. Exp Brain Res 20:115–123

Horeyseck G, Janig W (1974c) Reflexes in postganglionic fibres within skin and muscle nerves after noxious stimulation of skin. Exp Brain Res 20:125–134

Hosoya Y (1980) The distribution of spinal projecting neurones in the hypothalamus of the rat, studied with the HRP method. Exp Brain Res 40:79–87

Howe PRC, Costa M, Furness JB, Chalmers JP (1980) Simultaneous demonstration of phenylethanolamine *N* methyl transferase immunofluorescent and catecholamine fluorescent nerve cell bodies in the rat medulla oblongata. Neuroscience 5:2229–2238

Howe PRC, Khun DM, Minson JB, Stead BH, Chalmers P (1983) Evidence for a bulbospinal serotonergic pressor pathway in the rat brain. Brain Res 270:29–36

Hubbard JE, Di Carlo V (1974a) Fluorescence histochemistry of monoamine containing cell bodies in the brainstem of the squirrel monkey (*Siamiri sciureus*). II. Catecholamine containing groups. J Comp Neurol 153:369–384

Hubbard JE, Di Carlo V (1974b) Fluorescence histochemistry of monoamine containing cell bodies in the brain stem of the squirrel monkey (*Saimiri sciureus*). III. Serotonin containing groups. J Comp Neurol 153:385–398

Huber JF (1936) Nerve roots and nuclear groups in the spinal cord of the pigeon. J Comp Neurol 65:43–91

Ikeda H, Gotah J (1971) Distribution of monoamine containing cells in the central nervous system of the chicken. Jpn J Pharmacol 21:763–784

Illert M, Gabriel M (1970) Mapping the spinal cord of the cat for sympathetic and blood pressure responses. Brain Res 23:274–276

Illert M, Gabriel M (1972) Descending pathways in the cervical cord of cats affecting blood pressure and sympathetic activity. Pflügers Arch 335:109–124

Illert M, Seller H (1969) A descending sympatho-inhibitory tract in the ventrolateral column of the cat. Pflügers Arch. 313:343–360

Ipp E, Dobbs R, Unger RH (1978) Morphine and β-endorphin influence the secretion of the endocrine pancreas. Nature 276:190–191

Iriki M, Kozawa E (1975) Factors controlling the regional differentiation of sympathetic outflow – influence of the chemoreceptor reflex. Brain Res 87:281–291

Ishikawa M, Shimada S, Tanaka C (1975) Histochemical mapping of catecholamine neurones and fiber pathways in the pontine tegmentum of the dog. Brain Res 86:1–16

Iwamura Y, Uchino Y, Ozawa S, Kudo N (1969) Excitatory and inhibitory components of somato-sympathetic reflex. Brain Res 16:351–358

Jackson IMD (1980) TRH in the rat nervous system: identity with synthetic TRH on high performance liquid chromatography following affinity chromatography. Brain Res 201:245–248

Jackson I, Reichlin S (1974) Thyrotropin releasing hormone (TRH): distribution in hypothalamic and extrahypothalamic brain tissue of mammalian and submammalian chordates. Endocrinology 95:854–862

Jacobsohn L (1908) Über die Kerne des menschlichen Rückenmarks. Anhang zu den Abhandlungen der Königlichen Preußischen Akademie der Wissenschaften. Reimer, Berlin

Janig W (1979) Reciprocal reaction patterns of sympathetic subsystems with respect to various afferent inputs. In: Brooks C McC, Koizumi K, Sato A (eds) Integrative functions of the autonomic nervous system. University of Tokyo Press, Tokyo and Elsevier, Amsterdam, pp 263–274

Janig W (1985) Organisation of the lumbar sympathetic outflow to skeletal muscle and skin of the hind limb and tail. Rev Physiol Biochem Pharmacol 102:119–213

Janig W, Kummel H (1977) Functional discrimination of postganglionic neurones to the cat's hind paw with respect to skin potentials recorded from the hairless skin. Pflügers Arch 371:217–225

Janig W, Kummel H (1981) Organisation of the sympathetic innervation supplying the hairless skin of the cat's paw. J Auton Nerv Syst 3:215–230

Janig W, McLachlan EM (1986) Identification of distinct topographic distributions of lumbar sympathetic preganglionic neurones projecting to end organs with different functions in the cat. J Comp Neurol 246:104–112

Janig W, Rath B (1977) Electrodermal reflexes in the cat's paws elicited by natural stimulation of the skin. Pflügers Arch 369:27–32

Janig W, Schmidt RF (1970) Single unit responses in the cervical sympathetic trunk upon somatic nerve stimulation. Pflügers Arch 314:199–216

Janig W, Spilok N (1978) Functional organisation of the sympathetic innervation supplying the hairless skin of the hind paws in chronic spinal cats. Pflügers Arch 377:25–31

Janig W, Szulczyk P (1979) Conduction velocity in spinal descending pathways of baro- and chemoreceptor reflex. Brain Res 168:404–407

Janig W, Szulczyk P (1980) Functional properties of lumbar preganglionic neurones. Brain Res 186:115–131

Janig W, Sato A, Schmidt RF (1972) Reflexes in the postganglionic cutaneous fibres by stimulation of group I to group IV somatic afferents. Pflügers Arch 331:244–256

Jansen JKS, Nicholls JG (1973) Conductance changes, an electrogenic pump and the hyperpolarisation of leech neurones following inpulses. J Physiol (Lond) 229:635–655
Jenkins JS, Ang VTY, Hawthorn J, Rossor MN, Iverson LL (1984) Vasopressin, oxytocin and neurophysins in the human brain and spinal cord. Brain Res 291:111–117
Johansson B (1962) Circulatory responses to stimulation of somatic afferents. Acta Physiol Scand 57 [Suppl 198]:1–92
Johansson B, Langston JB (1964) Reflex influence of mesenteric afferents on renal, intestinal and muscle blood flow and on intestinal motility. Acta Physiol Scand 61:400–412
Johansson B, Jansson O, Ljung B (1968) Tonic supraspinal mechanisms influencing the intestino-intestinal inhibitory reflex. Acta Physiol Scand 72:200–204
Johansson O, Hokfelt T, Pernow B, Jeffcoate SL, White N, Steinbusch HWM, Verhofstad AAJ, Emson PC, Spindel E (1981) Immunohistochemical support for three putative transmitters in one neurone: co-existence of 5-hydroxytryptamine, substance P and thyrotropin releasing hormone like immunoreactivity in the medullary neurones projecting to the spinal cord. Neuroscience 6:1857–1881
Johnson RH, Crampton-Smith A, Walker JM (1969) Heart rate and blood pressure of spinal cats inspiring CO_2 before and after injection of hexamethonium. Clin Sci 36:257–265
Jones DJ, Kendall DE, Enna SJ (1982) Adrenergic receptors in rat spinal cord. Neuropharmacology 21:367–370
Jones EG (1975) Possible determinants of the degree of retrograde neuronal labelling with horseradish peroxidase. Brain Res 85:249–254
Kadzielawa K (1983a) Inhibition of the activity of sympathetic preganglionic neurones and neurones activated by viceral afferents by alpha-methyl noradrenaline and endogenous catecholamines. Neuropharmacology 22:3–17
Kadzielawa K (1983b) Antagonism of the excitatory effects of 5-hydroxytryptamine on sympathetic preganglionic neurones and neurones activated by visceral afferents. Neuropharmacology 22:19–27
Kaminski RJ, Meyer GA, Winter DL (1970) Sympathetic unit activity associated with Mayer waves in the spinal dog. Am J Physiol 219:1768–1771
Kardon FC, Winokur A, Utiger RD (1977) Thyrotropin releasing hormone (TRH) in rat spinal cord. Brain Res 122:578–581
Katholi RE, Hazeman GR, Whitlow PL, Woods WT (1983). Hemodynamic and afferent nerve responses to intrathecal adenosine in the dog. Hypertension 5 [Suppl 1]:149–154
Kaufman A, Koizumi K (1971) Spontaneous and reflex activity of single units in lumbar white rami. In: Kao FF, Vassalle M, Koizumi K (eds) Research in physiology. A liber memorialis in honour of Professor CM Brooks. Aulo Gaggi, Bologna, pp 469–481
Kaufman A, Sato A, Sato Y, Sugimoto H (1977) Reflex changes in heart rate after mechanical and thermal stimulation of the skin at various levels in cats. Neuroscience 2:103–109
Kaya R, Starling EH (1909) Note on asphyxia in the spinal animal. J Physiol (Lond) 39:346–353
Keeler JR, Helke CJ (1985) Spinal cord substance P mediates bicuculline induced activation of cardiovascular responses from the ventral medulla. J Auton Nerv Syst 13:19–33
Keeler JR, Charlton CG, Helke CJ (1985) Cardiovascular effects of spinal cord substance P: studies with stable receptor agonist. J Pharmacol Exp Ther 233:755–760
Kehrel H, Mutharoglu N, Weidinger H (1962) Über phasische Einflüsse und den Einfluß der Asphyxie auf den Tonus des sympathischen Kreislaufzentrums. Z Kreislaufforsch 51:334–351
Kell JF, Hoff EC (1952) Descending spinal pathways mediating pressor responses of cerebral origin. J Neurophysiol 15:229–311
Kerr FWL (1975) Neuroanatomical substrates of nociception in the spinal cord. Pain 1:325–356
Kerr FWL, Alexander S (1964) Descending autonomic pathways in the spinal cord. Arch Neurol Psychiatr, Chicago 10:249–261
Khayutin VM, Lukoshkova EV (1970) Spinal mediation of vasomotor reflexes in animals with intact brain studied by electrophysiological methods. Pflügers Arch 321:197–222

Khimonidi RK, Baklavadjan OG, Lebedev VP, Saruchanjan RV (1980) Specialisation of the descending sympatho-excitatory pathways of the spinal cord dorsolateral funiculus. Fiziol Zh SSSR 66:1031–1038 (In Russian)

Kirchner F, Sato A, Weidinger H (1970) Central pathways of reflex discharges in the cervical sympathetic trunk. Pflügers Arch 319:1–11

Kirchner F, Sato A, Weidinger H (1971) Bulbar inhibition of spinal and supraspinal sympathetic reflex discharges. Pflügers Arch 326:324–333

Kirchner F, Wyszogrodski I, Polosa C (1975a) Some properties of sympathetic preganglionic neurone inhibition by depressor area and intraspinal stimulation. Pflügers Arch 357:349–360

Kirchner F, Kirchner D, Polosa C (1975b) Spinal organisation of sympathetic inhibition by spinal afferent volleys. Brain Res 87:161–170

Knowles WD, Phillips MI (1980) Angiotensin II cells in the organum vasculosum lamina terminals (OVLT) recorded in the hypothalamic brain slices. Brain Res 197:256–259

Koepchen HP (1962) Die Blutdruckrhythmik. Steinkopff Darmstadt

Koizumi K, Brooks CM (1972) The integration of autonomic systems and their reactions: a discussion of autonomic reflexes, their control and their association with somatic reaction. Ergeb Physiol: 67:1–68

Koizumi K, Brooks C McC (1984) The spinal cord and the autonomic nervous system. In: Davidoff RA (ed) Handbook of the spinal cord, vols 2, 3. Dekker, New York, pp 779–816

Koizumi K, Sato A (1972) Reflex activity of single sympathetic fibres to skeletal muscles produced by electrical stimulation of somatic and vasodepressor afferent nerves. Pflügers Arch 332:283–301

Koizumi K, Suda I (1963) Induced modulations in autonomic efferent neurone activity. Am J Physiol 205:738–744

Koizumi K, Sato A, Kaufman A, Brooks C McC (1968) Studies of sympathetic neuron discharges modified by central and peripheral excitation. Brain Res 11:212–224

Koizumi K, Collin R, Kaufman A, Brooks CM (1970) Contribution of unmyelinated afferent excitation to sympathetic reflexes. Brain Res: 20:99–106

Koizumi K, Seller H, Kaufman A, Brooks C McC (1971) Pattern of sympathetic discharges and their relation to baroreceptor and respiratory activities. Brain Res 27:281–294

Kojima M, Takecuchi Y, Goto M, Sano Y (1982) Immunohistochemical study of the distribution of serotonin fibres in the spinal cord of the dog. Cell Tissue Res 226:477–491

Kollai M, Koizumi K (1977) Differential responses in sympathetic outflow evoked by chemoreceptor activation. Brain Res 138:159–165

Konishi M (1968) Fluorescence microscopy of the spinal cord of the dog with special reference to the autonomie lateral horn cells. Arch Histol Jpn 30:33–44

Konturek SJ, Tasler J, Cieszkowski M, Jaworek J, Coy DH, Schally AV (1978) Inhibition of the pancreas secretion by enkephalin and morphine in dogs. Gastroenterology 74:851–855

Kopp UC, Olson LA, DiBona GF (1984) Reno-renal reflex responses to mechano- and chemoreceptor stimulation in the dog and rat. Am J Physiol 246:F67–F77

Kopp UC, Smith LA, DiBona GF (1985) Renorenal reflexes: neuronal components of ipsilateral and contralateral renal responses. Am J Physiol 249:F507–F517

Kostreva DR, Seaguard JL, Castaner A, Kampine JP (1981) Reflex effects of renal afferents on the heart and kidney. Am J Physiol 241:R286–R292

Krasyukov AV, Lebedev VP, Nikitin SA (1981) White rami responses to stimulation of the ventral surface of the medulla. Fiziol Zh SSSR 6:1057–1066

Krieder MS, Winokur A, Utiger RD (1979) TRH immunoreactivity in rat hypothalamus and brain, assessment by gel-filtration and thin layer chromatography. Brain Res 171:161–165

Krnjevic K, Lisiewicz A (1972) Injection of calcium ions into spinal motoneurones. J Physiol (Lond) 225:363–390

Krnjevic K, Randic M, Siesjo BK (1965) Cortical CO_2 tension and neuronal excitability. J Physiol (Lond) 176:105–122

Krukoff TL, Ciriello J, Calaresu FR (1985a) Segmental distribution of peptide and 5HT like immunoreactivity in nerve terminals and fibres of thoracolumbar sympathetic nuclei of the cat. J Comp Neurol 240:103–116

Krukoff TL, Ciriello J, Calaresu FR (1985b) Segmental distribution of peptide like immunoreactivity in cell bodies of the lumbar sympathetic nuclei of the cat. J Comp Neurol 240:90–102

Kubeck MJ, Lorincz MA, Wilber JF (1977) The identification of thyrotropin releasing hormone (TRH) in hypothalamic and extrahypothalamic loci of the human nervous system. Brain Res 126:196–200

Kuhn DM, Wolf WA, Lovenberg W (1980a) Pressor effects of electrical stimulation of the dorsal and median raphe nuclei in anaethestised rats. J Pharmacol Exp Ther 214:403–409

Kuhn DM, Wolf WA, Lovenberg W (1980b) Review of the role of central serotonergic neuronal system in blood pressure regulation. Hypertension 2:243–255

Kumazawa T, Perl ER (1976) Differential excitation of dorsal horn and substantia gelatinosa marginal neurones by primary afferent units with fine (A and C) fibres. In: Zotterman Y (ed) Sensory functions of the skin in primates. Pergamon, Oxford, pp 67–87

Kuo DC, Yamasaki OS, Krauthamer GM 1980) Segmental organisation of sympathetic preganglionic neurones of the splanchnic nerve as revealed by retrograde transport of horseradish peroxidase. Neurosci Lett 17:11–16

Kuo DC, Nadelhaft I, Hisamitsu T, De Groat WC (1983) Segmental distribution and central projections of renal afferent fibres in the cat studied by transganglionic transport of horseradish peroxidase. J Comp Nuerol 216:162–174

Kuypers HGJM, Maisky VA (1975) Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups. Neurosci Lett 11:9–14

Kuypers HGJM, Martin GF (1982) Anatomy of descending pathways to the spinal cord. Prog Brain Res 57:1–411

Lackner KJ (1980) Mapping of monoamine neurones and fibres in the lower brainstem and spinal cord. Anat Embryol (Berl) 161:169–195

Lane JD, Smith JE, Hall PV, Campbell RL (1978) Distribution of taurine and putative amino acid neurotransmitters in eight areas of the canine lumbar spinal cord. Brain Res 152:386–390

Lang RE, Heil J, Ganten D, Herman K, Rascher W, Unger T (1983) Effect of lesions in the paraventricular nucleus on vasopressin and oxytocin contents in brainstem and spinal cord. Brain Res 260:326–329

Laruelle L (1937) La structure de la moelle épinière en coupes longitudinales. Rev Neurol (Paris) 44:695–725

Laskey W, Schondorf R, Polosa C (1979) Intersegmental connections and interactions of myelinated somatic and visceral afferents with sympathetic preganglionic neurones in the unanaesthetised spinal cat. J Auton Nerv Syst 1:69–76

Lebedev VP, Skobelev VA, Bushmarina TA (1974) Study of the lateral horn sympathetic preganglionic neurones in the cat lumbar spinal cord. Neirofiziologiia 6:295–303

Lebedev VP, Petrov VI, Skobelev VA (1976a) Antidromic discharges of sympathetic preganglionic neurones located outside the spinal cord lateral horns. Neurosci Lett 2:325–329

Lebedev VP, Rosanov NN, Skobelev VA, Smirnov KA (1976b) Study of the early somato-sympathetic reflex response. Neurosci Lett 2:319–323

Lebedev VP, Baklavadjan OG, Khimonidi RK (1979) The level of realization of the baroreceptor sympatho-inhibitory influences. Fiziol Zh SSSR 66:1015–1023

Lebedev VP, Petrov VI, Skobelev VA (1980) Do sympathetic preganglionic neurones have a recurrent inhibitory mechanism? Pflügers Arch 383:91–97

Lebedev VP, Krasyukov AV, Nikitin SA (1986) Electrophysiological study of sympathoexcitatory structures of the bulbar ventrolateral surface as related to vasomotor regulation. Neuroscience 17:189–203

Lechan RM, Molich ME, Jackson IMD (1983) Distribution of immunoreactive growth hormone-like material and thyrotropin-releasing hormone in the rat central nervous system: evidence for their co-existence in the same neurones. Endocrinology 112:877–884

Lechan RM, Snapper SB, Jacobson S, Jackson IMD (1984) The distribution of thyrotropin-releasing hormone (TRH) in the Rhesus monkey spinal cord. Peptides Suppl 1, 5:185–194

Legendre P, Simmonet G, Vincent JD (1984) Electrophysiological effects of angiotensin II on cultured mouse spinal cord neurones. Brain Res 279:287–296

Leong SK, Tay SS, Wong WC (1983) Preganglionic neurones projecting to the first thoracic sympathetic ganglion in the terrapin (*Trionyx sinensis*) J Auton Nerv Syst 9:585–593

Levi-Montalcini R, Angeletti PU (1964) Hormonal control of the NGF content of the submaxillary glands of mice. In: Steenby LM, Meyer J (ed) Salivary glands and their secretions. Pergamon, Oxford, pp 129–141

Light AR, Perl ER (1979) Re-examination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibres. J Comp Neurol 186:117–132

Lind RW, Swanson LW, Ganten D (1985) Organisation of angiotensin II immunoreactive cells and fibres in the rat central nervous system. Neuroendocrinology 40:2–24

Linden RJ (1979) Atrial receptors and heart rate. In: Hainsworth R, Kidd C, Linden RJ (eds) Cardiac receptors. Cambridge University Press, Cambridge, pp 165–191

Lioy F, Hanna BD, Polosa C (1978) CO_2 dependent component of the neurogenic vascular tone in the cat. Pflügers Arch 374:187–191

Lipski J (1981) Antidromic activation of neurones as an analytic tool in the study of the central nervous system. J Neurosci Methods 4:1–32

Lipski J, Trzebski A (1975) Bulbospinal neurones activated by baroreceptor afferents and their possible role in inhibition of preganglionic sympathetic neurones. Pflügers Arch 356:181–192

Lipski J, Coote JH, Trzebski A (1977) Temporal patterns of antidromic neurones related to the central inspiratory activity and pulmonary stretch receptor reflex. Brain Res 135:162–166

Ljungdahl A, Hokfelt T, Nilsson G (1978) Distribution of substance P like immunoreactivity in the central nervous system of the rat: cell bodies and nerve terminals. Neuroscience 3:861–943

Llinas R, Hess R (1976) Tetrodotoxin-resistant dendritic spikes in avian purkinje cells. Proc Natl Acad Sci USA 73:2520–2523

LoPachin RM, Rudy TA (1983) Sites and mechanisms of action for the effects of intrathecal noradrenaline on thermoregulation in the rat. J Physiol (Lond) 341:527–544

Loewy AD (1981) Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. Brain Res 222:129–133

Loewy AD, McKellar S (1980) The neuroanatomical basis of central cardiovascular control. Fed Proc 39:2495–2503

Loewy AD, McKellar S (1981) Serotonergic projections from the ventral medulla to the intermediolateral cell column in the rat. Brain Res 211:146–152

Loewy AD, Neil JJ (1981) The role of descending monoaminergic systems in the central control of blood pressure. Fed Proc 40:2778–2785

Loewy AD, Sawyer WB (1982) Substance P antagonist inhibits vasomotor responses elicited from the ventral medulla in rat. Brain Res 245:379–383

Loewy AD, Gregorie EM, McKellar S, Baker RP (1979a) Electrophysiological evidence that the A_5 catecholamine cell group is a vasomotor centre. Brain Res 178:196–200

Loewy AD, McKellar S, Saper CB (1979b) Direct projections from the A_5 catecholamine cell group to the intermediolateral cell column. Brain Res 174:309–314

Loewy AD, Wallach JH, McKellar S (1981) Efferent connections of the ventral medulla oblongata in the rat. Brain Res Rev 3:63–80

Loewy AD, Marson L, Parkinson D, Perry MA, Sawyer WB (1986) Descending noradrenergic pathways involved in the A_5 depressor response. Brain Res 386:H44–H51

Loh HH, Li CH (1977) Biologic activities of β-endorphin and its related peptides. Ann NY Acad Sci 297:115–128

Loizou LA (1972) The postnatal ontogeny of monoamine containing neurones in the central nervous system of the albino rat. Brain Res 40:395–418

Lorenz RG, Saper CB, Wong DC, Ciaranello RD, Loewy AD (1985) Co-localisation of substance P and phenylethanolamine *N*-methyl transferase-like immunoreactivity in neurons of

ventrolateral medulla that project to the spinal cord: potential role in central vasomotor tone. Neurosci Lett 55:255–260
Lovick TA (1987a) Cardiovascular control from neurones in the ventrolateral medulla. In: Taylor EW (ed) Studies in neuroscience. Manchester University Press, Manchester (In press)
Lovick TA (1987b) Differential control of cardiac and vasomotor activity by neurones in nucleus paragigantocellularis lateralis in the cat. J Physiol (Lond) 389:23–35
Lovick TA, Hilton SM (1985) Vasodilator and vasoconstrictor neurones of the ventrolateral medulla in the cat. Brain Res 331:353–357
Lovick TA, Hilton SM (1986) Cardiovascular neurones in the ventral medulla. J Auton Nerv Syst (Suppl) 121–124
Lovick TA, Hunt SP (1983) Substance P immunoreactive and serotonin containing neurones in the ventral medulla of the cat. Neurosci Lett 36:223–228
Low PA, Okazaki H, Dyck PJ (1977) Splanchnic preganglionic neurones in man. Acta Neuropathol (Berl) 40:55–61
Luiten PGM, ter Horst GJ, Karst H, Steffens AB (1985) The course of paraventricular hypothalamic efferents to autonomic structures in medulla and spinal cord. Brain Res 329:374–378
Lund S, Pompeiano O (1968) Monosynaptic excitation of alpha motoneurones from supraspinal structures in the cat. Acta Physiol Scand 73:1–21
Lundberg JM, Hockfelt T, Anggard A, Kimmel J, Goldstein M, Markey K (1980) Co-existance of an avian pancreatic polypeptide (APP) immunoreactive substance with catecholamines in some peripheral and central neurones. Acta Physiol Scand 110:107–109
Ma RC, Dun NJ (1985a) Norepinephrine depolarises lateral horn cells of neonatal rat spinal cord in vitro. Neurosci Lett 60:163–168
Ma RC, Dun NJ (1985b) Vasopressin depolarises lateral horn cells of the neonatal rat spinal cord in vitro. Brain Res 348:36–43
Ma RC, Dun NJ (1986) Excitation of lateral horn neurones in neonatal rat spinal cord by 5-hydroxytryptamine. Brain Res 24:89–98
MacDonald RL, Cohen DH (1970) Cells of origin of sympathetic pre- and post-ganglionic cardioacceleratory fibres in the pigeon. J Comp Neurol 40:343–358
Magnusson T (1973) Effect of chronic transection on DA, NA and 5-HT in the rat spinal cord. Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol 278:13–22
Malliani A (1979) Afferent cardiovascular sympathetic nerve fibres and their function in the neural regulation of the circulation. In: Hainsworth R, Kidd C, Linden RJ (eds) Cardiac receptors. Cambridge University Press, Cambridge, pp 319–338
Malliani A, Lombardi F, Pagani M (1981) Functions of afferents in cardiovascular sympathetic nerves. J Auton Nerv Syst 3:231–236
Malliani A, Pagani M, Pizzinella P, Furlan R, Guzzetti S (1983) Cardiovascular reflexes mediated by sympathetic afferent fibres. J Auton Nerv Syst 7:295–301
Mannard A, Polosa C (1973) Analysis of background firing of single sympathetic preganglionic neurones of cat cervical nerve. J Neurophysiol: 36:398–408
Mannard A, Rajchgot P, Polosa C (1977) Effect of post-impulse depression on background firing of sympathetic preganglionic neurones. Brain Res 126:243–261
Marino PL, Lamb TW (1975) Effect of CO_2 and the extra-cellular H^+ iontophoresis on single cell activity in the cat brainstem. J Appl Physiol 38:688–695
Marson L, Loewy AD (1985) Topographical organisation of substance P and monoamine cells in the ventral medulla of the cat. J Auton Nerv Syst 14:271–285
Martin GF, Humbertson AO, Laxson C, Panneton WM (1979) Evidence for direct bulbospinal projections to lamina, IX, X and the intermediolateral cell column. Studies using axonal transport techniques in the North American opposum. Brain Res 170:165–171
Martin GF, Cabana T, DiTirro FJ, Ho RH, Humberston AO (1982) Reticular and raphe projections to the spinal cord of the North American opossum. Evidence for connectional heterogeneity. Prog Brain Res 57:109–129
Massazza A (1923a) La citoarchitettonica del midollo spinale umano. Riv Pathol Nerv Ment 28:22–43

Massazza A (1922) La citoarchitettonica del midollo spinale umano. Nota I. Arch Anat Histol Embryol (Strasb) 1:323–410

Massazza A (1923b) La citoarchitettonica del midollo spinale umano. Nota II. Arch Anat Histol Embryol (Strasb) 2:1–56

Massazza A (1924) La citoarchitettonica del midolla spinale umano. Nota III. Arch Anat Histol Embryol (Strasb) 3:115–189

Mathison GC (1910) The action of asphyxia upon the spinal animal. J Physiol (Lond) 41:416–449

Maurin Y, Buck SH, Wamsley JK, Burke TF, Yamamura HI (1984) Light microscopic autoradiographic localisation of (^{3}H) substance P binding sites in the rat thoracic spinal cord. Life Sci 34:1713–1716

Mayer S (1876) Studien zur Physiologie des Herzens und der Blutgefäße. V. Über spontane Blutdruckschwankungen. Akad Wiss Wien Math Naturwiss 74:281–307

McAllen RM (1985) Bulbospinal neurones of the "glycine sensitive" area in the cat. J Physiol (Lond) 361:48

McAllen RM (1986a) Location of neurones with cardiovascular and respiratory function at the ventral surface of the cats medulla. Neuroscience 18:43–49

McAllen RM (1986b) Action and specificity of ventral medullary vasopressor neurones in the cat. Neuroscience 18:51–59

McAllen RM, Neil JJ, Loewy AD (1982) Effects of kainic acid applied to the ventral surface of the medulla oblongata on vasomotor tone, the baroreceptor reflex and hypothalamic autonomic responses. Brain Res: 238:65–76

McCall RB (1983) Serotonergic excitation of sympathetic preganglionic neurones: microiontophoretic study. Brain Res 289:121–127

McCall RB (1984) Evidence for serotonergically mediated sympatho-excitatory response to stimulation of medullary raphe nuclei. Brain Res 311:131–139

McCall RB, Gebber GL (1975) Brain stem and spinal synchronisation of sympathetic neurone discharge. Brain Res 89:139–143

McCall RB, Gebber GL, Barman SM (1977) Spinal interneurones in the baroreceptor reflex arc. Am J Physiol 236:H657–H665

McKellar S, Loewy AD (1982) Efferent projections of the A_1 catecholamine cell groups in the rat: an autoradiographic study. Brain Res 241:11–29

McKenna KE, Schramm LP (1985) Mechanisms mediating the sympathetic silent period: studies in the isolated spinal cord of the neonatal rat. Brain Res 329:233–240

McLachlan EM, Hirst GDS (1980) Some properties of preganglionic neurones in the upper thoracic spinal cord of the cat. J Neurophysiol 43:1251–1265

McLachlan EM, Oldfield BJ, Sittiracha T (1985) Localisation of hind limb vasomotor neurones in the lumbar spinal cord of the guinea pig. Neurosci Lett 54:269–275

Meckler RL, Weaver LC (1985) Splenic, renal and cardiac nerves have unequal dependence upon tonic supraspinal inputs. Brain Res 338:123–135

Meites J, Branei JF, Van Vugt DA, Smith AF (1978) Relation of endogenous opioid peptides and morphine to neuroendocrine function. Life Sci 24:1325–1336

Mendelsohn FAO, Quirion R, Saavedra JM, Aquilera G, Catt KJ (1984) Autoradiographic localisation of angiotensin II receptors in rat brain Proc Natl Acad Sci USA 81:1575–1579

Millan MJ, Millan MH, Czlonkowski A, Herz A (1984a) Vasopressin and oxytocin in the rat spinal cord: distribution and origins in comparison to [metenkephalin], dynorphin and related opioids and their irresponsiveness to stimuli modulating neuro-hypophyseal secretion. Neuroscience 13:179–187

Millan MJ, Millan MH, Czlonkowski A, Herz A (1984b) Contrasting interactions of the locus coeruleus as comparison to ventral noradrenergic bundle with CNS and pituitary pools of vasopressin, dynorphin and related opioid peptides in the rat. Brain Res 298:243–252

Miller RJ, Cuatrecasas P (1978) Enkephalins and endorphins. In: Munson PL, Glover J, Diczfusy E, Olsen RE (eds) Vitamins and hormones, vol 36. Academic, New York, pp 297–382

Millhorn DE, Eldridge FL (1986) Role of ventrolateral medulla in regulation of respiratory and cardiovascular systems (a review). J Appl Physiol 61:1249–1263

Mitchell RA, Herbert DA (1974) The effect of carbon dioxide on the membrane potential of medullary respiratory neurones. Brain Res 75:345–349

Mizukawa K (1980) The segmental detailed topographical distribution of monoaminergic terminals and their pathways in the spinal cord of the cat. Anat Anz 147:125–144

Mo N, Dun NJ (1987) Is glycine an inhibitory transmitter in rat lateral horn cells? Brain Res 400:139–144

Morrison SF, Gebber GL (1984) Raphe neurones with sympathetic related activity: baroreceptor responses and spinal connections. Am J Physiol 243:R49–R59

Murata Y, Shibata H, Chiba T (1982) A correlative quantitative study comparing the nerve fibres in the cervical sympathetic trunk and the locus of the somata from which they originate in the rat. J Auton Nerv Syst 6:323–333

Nakayama S, von Baumgarten R (1964) Lokalisierung absteigender Atmungsbahnen im Rückenmark der Katze mittels antidromer Reizung. Pflügers Arch 281:231–244

Nauta HJW, Pritz MB, Lusek RJ (1974) Afferents to the rat caudoputamen studied with horseradish peroxidase. An evaluation of retrograde neuroanatomical research method. Brain Res 67:219–238

Navaratnam V, Lewis PR (1970) Cholinesterase containing neurones in the spinal cord of the rat. Brain Res 18:411–425

Neil JJ, Loewy AD (1982) Decreases in blood pressure in response to *L*-glutamate microinjections in the A_5 catecholamine cell group. Brain Res 241:271–278

Neuhuber W (1982) The central projections of visceral primary afferent neurones of the inferior mesenteric plexus and hypogastric nerve and the location of the related sensory and preganglionic cell bodies in the rat. Anat Embryol (Berl) 164:413–425

Neumayr RJ, Hare BD, Franz DN (1974) Evidence for bulbospinal control of sympathetic neurones by monoaminergic pathways. Life Sci. 14:793–806

Nicholaidis S, Ishibashi S, Gueguen B, Thornton SN, de Beaurepaire R (1984). Iontophoretic investigation of identified SFO angiotensin-responsive neurones firing in relation to blood pressure changes. Brain Res Bull 10:357–363

Nicholl RA, Barker JL (1971) Excitation of supraoptic neurosecretory cells by angiotensin II. Nature 233:172–174

Nijima A (1971) Afferent discharges from arterial mechanoreceptors in the kidney of the rabbit. J Physiol (Lond) 219:477–485

Nilaver G, Zimmerman EA, Wilkins J, Michaels J, Hoffman D, Silverman AJ (1980) Magnocellular hypothalamic projections to the lower brain stem and spinal cord of the rat. Neuroendocrinology 30:150–158

Ninomiya I, Nisimaru N, Irisawa H (1971) Sympathetic nerve activity to the spleen, kidney and heart in response to baroreceptor input. Am J Physiol 221:1346–1351

Nisimaru N (1971) Comparison of gastric and renal nerve activity. Am J Physiol 220:1303–1308

Niwa M, Shigematsu K, Saavedra JM (1986) Changes in substance P receptors of the intermediolateral cell column of the thoracic spinal cord of young spontaneously hypertensive rats. In: Nakamura K (ed) Brain and blood pressure control. Elsevier, Amsterdam, pp 225–230

Nobin A, Björklund A (1973) Topography of monoamine systems in the human brain as revealed in fetuses. Acta Physiol Scand 88 Suppl 388 1–4

Nygren OG, Olsen L (1977) Intracisternal neurotoxins and monoamine neurones innervating the spinal cord: acute and chronic effects on cell and axon counts and nerve terminal densities. Histochemistry 52:281–306

Ogawa N, Yamawaki Y, Kuroda H, Ofuji T, Itoga E, Kito S (1981) Discrete regional distributions of thyrotropin-releasing hormone (TRH) receptor binding in monkey central nervous system. Brain Res 205:169–174

Ohye C, Bouchard R, Poirier LJ (1970) Spontaneous activity of the putamen after chronic interruption of the dopaminergic pathway: effect of *L*-dopa. J Pharmacol Exp Ther 175:700–708

Okada H, Fox IJ (1967) Respiratory grouping of abdominal sympathetic activity in the dog. Am J Physiol 213:48–56

Oldfield BJ, McLachlan EM (1981) An analysis of the sympathetic preganglionic neurones projecting from the upper thoracic spinal roots of the cat. J Comp Neurol 196:239–345

Oliver C, Eskay RL, Ben-Jonathan N, Porter JC (1974) Distribution and concentration of TRH in rat brain. Endocrinology 95:540

Ono T, Nishimo H, Sasaka K, Yano I, Simpson A (1978) Paraventricular nucleus connections to the spinal cord and pituitary. Neurosci Lett 10:141–146

Onuf B (1900) On the arrangement and function of the cell groups of the sacral region of the spinal cord in man. Arch Neurol Psychopathol 3:387–412

Onuf B, Collins J (1898) Experimental researches on the localisation of sympathetic nerve in the spinal cord and brain, and contributions to its physiology. J Nerv Ment Dis 25:661–678

Otsuka M, Takahashi T (1977) Putative peptide neurotransmitters. Annu Rev Pharmacol Toxicol 17:425–439

Pagani M, Schwartz P, Banks R, Lombadi F, Malliani A (1974) Reflex responses of sympathetic preganglionic neurones initiated by different cardiovascular receptors in spinal animals. Brain Res 68:215–225

Palkovits M, Jacobowitz DM (1974) Topographical atlas of catecholamine and acetylcholinesterase-containing neurones in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). J Comp Neurol 157:29–42

Papajewski W, Klee MR, Wagner A (1969) The action of raised CO_2 pressure on the excitability of spinal motoneurones. Electroencephalogr Clin Neurophysiol 17:618 (Abstr)

Pardini BJ, Wurster RD (1984) Identification of the sympathetic preganglionic pathway to the cat stellate ganglion. J Auton Nerv Syst 11:13–25

Parent A (1973) Demonstration of a catecholamine pathway from the midbrain to the striomygdaloid complex in the turtle (*Chrysemys picta*). J Anat 114:379–387

Parent A (1975) The monoaminergic innervation of the telencephalon of the frog, *Rana pipiens*. Brain Res 99:35–47

Parent A (1978) Monoaminergic systems of the brain. In: Gans C, Ulinski P, Northcutt RG (eds) Biology of the reptilia. Academic London, pp 247–285

Pearson J, Pytel BA (1978) Quantitative studies of sympathetic ganglion and spinal cord intermediolateral grey columns in familial dysautonomia. J Neurol Sci 39:47–59

Perri V, Sachi O, Casella C (1970) Electrical properties and synaptic connections of the sympathetic neurones in the rat and guinea pig superior cervical ganglion. Pflügers Arch 314:40–54

Peterson BW, Maunz RA, Pitts NG, Macken RG (1975) Patterns of projecting and branching of reticulospinal neurones. Exp Brain Res 23:333–351

Peterson BW, Pitts NG, Fukishima K (1979) Reticulospinal connections with limb and axial motoneurones. Exp Brain Res 36:1–20

Petras JM, Cummings JF (1972) Autonomic neurones in the spinal cord of the rhesus monkey: correlation of the findings of cytoarchitectonics and sympathectomy with fibre degeneration following dorsal rhizotomy. J Comp Neurol 146:189–218

Petras JM, Faden AI (1978) The origin of sympathetic preganglionic neurones in the dog. Brain Res 144:353–357

Phillips JW, Linacher JJ (1974) Excitation of cerebral cortical neurones by various polypeptides. Exp Neurol 43:414–423

Phillips MI, Nelson PG, Neal E, Quinlan J (1980) Angiotensin-induced phasic firing of spinal cord neurones in culture. Soc Neurosci Abstr 6:619

Pilowsky P, West M, Chalmers J (1985) Renal sympathetic nerve responses to stimulation, inhibition and destruction of the ventrolateral medulla in the rabbit. Neurosci Lett 60:51–55

Pilowsky PM, Kapoor V, Minson JB, West MJ, Chalmers JP (1986) Spinal cord serotonin release and raised blood pressure after brainstem kainic acid injection. Brain Res 366:354–357

Pin C, Jones B, Jouvet M (1968) Topographie des neurones monoaminergiques du cérébral du chat: étude par histofluorescence. CR Soc Biol (Paris) 162:21–36

Pitts RF, Bronk DW (1942) Excitability cycle of the hypothalamus sympathetic nervous system. Am J Physiol 135:504–522

Poitras D, Parent A (1978) Atlas of the distribution of monoamine-containing nerve cell bodies in the brainstem of the cat. J Comp Neurol 179:699–718

Poljak S (1924) Die Struktureigentümlichkeiten des Rückenmarkes bei den Chiroptern. Zugleich ein Beitrag zu der Frage über die spinalen Zentren des Sympatheticus. Z Anat Entwicklungsgesch 74:509–576

Polosa C (1967) Silent period of sympathetic preganglionic neurones. Can J Physiol Pharmacol 45:1033–1045

Polosa C (1968) Spontaneous activity of sympathetic preganglionic neurones. Can J Physiol Pharmacol 46:887–896

Polosa C, Rosenberg P, Mannard A, Wolkove N, Wyszogrodski I (1969) Oscillatory behaviour of the sympathetic system induced by picrotoxin. Can J Physiol Pharmacol 47:815–826

Polosa C, Mannard A, Laskey W (1979) Tonic activity in the autonomic nervous system; functions, properties, origins. In: Brooks CM, Koizumi K, Sato A (eds) Integrative functions of the autonomic nervous system. University of Tokyo Press, Tokyo, pp 342–354

Polosa C, Gerber U, Schondorf R (1980) Central mechanisms of interaction between sympathetic preganglionic neurones and the respiratory oscillator. In: Koepchen HP, Hilton SM, Trzebski A (eds) Central interactions between respiratory and cardiovascular control systems. Springer, Berlin Heidelberg New York, pp 137–143

Polosa C, Schondorf R, Laskey W (1982) Stabilisation of the discharge rate of sympathetic preganglionic neurones. J Auton Nerv Syst 5:45–54

Pomeranz B, Wall PD, Weber WV (1968) Cord cells responding to fine myelinated afferents from viscera, muscle and skin. J Physiol (Lond) 199:511–532

Porter JP, Brody MJ (1985) Role of spinal vasopressinergic mechanisms in the cardiovascular effects produced by stimulation of the paraventricular nucleus. Soc Neurosci Abstr 11:P36

Preiss G, Polosa C (1974) Patterns of sympathetic activity associated with Mayer waves. Am J Physiol 226:724–730

Preiss G, Polosa C (1977) The relation between end-tidal CO_2 and discharge patterns of sympathetic preganglionic neurones. Brain Res 122:255–267

Preiss G, Kirchner F, Polosa C (1975) Patterning of sympathetic preganglionic neurone firing by the central respiratory drive. Brain Res 87:363–374

Prout BJ, Coote JH, Downman CBB (1964) Supraspinal inhibition of a cutaneous vascular reflex in the cat. Am J Physiol 202:303–307

Prout BJ, Coote JH, Downman CBB (1965) Independence of central controls of vascular and sweat gland responses in the paw of the cat. J Neurol Neurosurg Psychiatry 28:223–227

Przybyla AC, Wang SC (1967) Neurophysiological characteristics of cardiovascular neurones in the medulla oblongata of the cat. J Neurophysiol 30:645–660

Ramage AG (1985) The effects of ketanserin, methysergide and LY53857 on sympathetic nerve activity. Eur J Pharmacol 113:295–303

Randic M, Miletic V (1978) Depressent actions of methionine-enkephalin and somatostatin on cat dorsal horn neurones activated by noxious stimuli. Brain Res 152:196–202

Rando TA, Bowers CW, Zigmond RE (1981) Localisation of neurones in the rat spinal cord which project to the superior cervical ganglion. J Comp Neurol 196:73–83

Rang HP, Ritchie JM (1968) On the electrogenic sodium pump in mammalian non-myelinated nerve fibres and its activation by various external cations. J Physiol (Lond) 196:183–221

Ranson SW (1913) The course within the spinal cord of the non-medullated fibres of the spinal dorsal roots: a study of Lissauer's tract in the cat. J Comp Neurol 23:259–274

Ranson SW, Billingsley PR (1916) Afferent spinal paths and the vasomotor reflexes. Studies on vasomotor reflex arcs VI. Am J Physiol 42:16–35

Rao UC, Bijlani V (1980) The intermediolateral gray column in the spinal cord of *Macaca mulatta*). J Auton Nerv Syst 2:259–267

Recordati GM, Moss NG, Waselkov L (1978) Renal chemoreceptors in the rat. Circ Res 43:534–543

Recordati GM, Moss NG, Genovesi S, Rogenes P (1980) Renal receptors in the rat sensitive to chemical alterations of their environment. Circ Res 46:395–405

Recordati G, Genovesi S, Cerati D (1982) Renorenal reflexes in the rat elicited upon stimulation of renal chemoreceptors. J Auton Nerv Syst 6:127–142
Reid JL, Zivin JA, Foppen F, Kopin IJ (1975) Catecholamine neurotransmitters and synthetic enzymes in the rat spinal cord. Life Sci 16:975–984
Renaud LP, Martin JB, Brazeau P (1978) Depressant action of TRH, LH-RH and somatostatin on activity of central neurones. Nature 255:233–235
Renshaw B (1941) Influence of discharge of motoneurones upon excitation of neighbouring motoneurones. J Neurophysiol 4:167–183
Rethelyi M (1972) Cell and neuropil architecture of the intermediolateral (sympathetic) nucleus of the cat spinal cord. Brain Res 46:203–213
Rethelyi M (1974) Spinal transmission of autonomic processes. J Neural Transm (Suppl) 11:195–212
Rexed B (1954) A cytoarchitectonic atlas of the spinal cord in the cat. J Comp Neurol 100:297–379
Riedel W, Iriki M, Simon E (1972) Regional differentiation of sympathetic activity during peripheral heating and cooling in anaesthetised rabbits. Pflügers Arch 322:239–247
Riley HA (1960) An atlas of the basal ganglia, brain stem and spinal cord. Hafner, New York
Riphagen CL, Pittman QT (1985) Cardiovascular responses to intrathecal administration of arginine vasopressin in rats. Peptides 10:293–298
Riphagen CL, Bauce L, Veale WL, Pittman QJ (1986) The effects of intrathecal administration of arginine-vasopressin and substance P on blood pressure and adrenal secretion of epinephrine in rats. J Auton Nerv Syst 16:91–99
Rizzoli AA (1968) Distribution of glutamic acid, aspartic acid, γ-aminobutyric acid and glycine in six areas of cat spinal cord before and after transection. Brain Res 11:11–18
Robertson DR (1987) Sympathetic preganglionic neurones in frog spinal cord. J Auton Nerv Syst 18:1–11
Rogenes PR (1982) Single unit and multiunit analysis of renorenal reflexes elicited by stimulation of renal chemoreceptors in the rat. J Auton Nerv Syst 6:143–156
Rohlicek CV, Polosa C (1981) Hypoxic responses of sympathetic preganglionic neurones in the acute spinal cat. Am J Physiol 241:H679–H683
Rohlicek CV, Polosa C (1983) Hypoxic responses of sympathetic preganglionic neurones in sinoaortic-denervated cats. Am J Physiol 244:H681–H686
Romanes GJ (1951) The motor cell columns of the lumbo-sacral spinal cord of the cat. J Comp Neurol 94:313–363
Ross CA, Ruggiero DA, Joh TH, Park DH, Reis DJ (1983) Adrenaline synthesising neurones in the rostral ventrolateral medulla: a possible role in tonic vasomotor control. Brain Res 273:356–361
Rubin E, Purves D (1980) Segmental organisation of sympathetic preganglionic neurones in the mammalian spinal cord. J Comp Neurol 192:163–174
Saavedra JM, Palkovits M, Brownstein M, Axelrod J (1974) Serotonin distribution in the nuclei of rat hypothalamus and preoptic region. Brain Res 77:157–165
Salisbury PF, Cross CE, Rieben PA (1960) Reflex effects of the left ventricular distension. Circ Res 8:530–534
Sangdee C, Franz DN (1979) Enhancement of central norepinephrine and 5-hydroxtrypamine transmission by tricyclic antidepressants: a comparison. Psychopharmacology (Berlin) 62:9–16
Sangdee C, Franz DN (1983) Evidence for inhibition of sympathetic preganglionic neurones by bulbospinal epinephrine pathways. Neurosci Lett 37:167–173
Saper CB, Loewy AD (1980) Efferent connections of the parabrachial nucleus in the rat. Brain Res 197:291–317
Saper CB, Loewy AD, Swanson LW, Cowans WM (1976) Direct hypothalamic-autonomic connections. Brain Res 117:305–312
Sarnoff SJ, Yamada SI (1959) Evidence for reflex control of arterial blood pressure from abdominal receptors with special reference to the pancreas. Circ Res 7:325–335

Sato A (1972a) Somato-sympathetic reflex discharges evoked through supramedullary pathways. Pflügers Arch 332:117–126
Sato A (1972b) The relative involvement of different reflex pathways in somato-sympathetic reflexes, analysed in spontaneously active single preganglionic sympathetic units. Pflügers Arch 333:70–81
Sato A (1972c) Spinal and supraspinal inhibition of somato-sympathetic reflexes by conditioning afferent volleys. Pflügers Arch 336:121–133
Sato A (1973) Spinal and medullary components of the somato-sympathetic reflex discharges evoked by stimulation of the group IV somatic afferents. Brain Res 51:307–318
Sato A, Schmidt RF (1966) Muscle and cutaneous afferents evoking sympathetic reflexes. Brain Res 2:399–401
Sato A, Schmidt RF (1971) Spinal and supraspinal components of the reflex discharges into lumbar and thoracic white rami. J Physiol (Lond) 212:839–850
Sato A, Schmidt RF (1973) Somato-sympathetic reflexes: afferent fibres, central pathways, discharge characteristics. Physiol Rev 53:916–947
Sato A, Tsushima N, Fujimori B (1965) Reflex potentials of lumbar sympathetic trunk with sciatic nerve stimulation in cats. Jpn J Physiol 15:532–539
Sato A, Sato I, Ozawa I, Fujimori B (1967) Further observation of the reflex potential in the lumbar sympathetic trunk. Jpn J Physiol 17:294–307
Sato A, Kaufman A, Koizumi K, Brooks CM (1969) Afferent nerve groups and sympathetic reflex pathways. Brain Res 14:575–587
Sato A, Sato Y, Shimada F, Torigata Y (1975) Changes in gastric motility produced by nociceptive stimulation of the skin in rats. Brain Res 94:465–474
Sato Y, Terui N (1976) Changes in the duodenal motility produced by noxious mechanical stimulation of the skin in rats. Neurosci Lett 2:189–193
Satoh K, Tohiyama M, Yamamoto K, Sukumoto T, Shimizu N (1977) Noradrenaline innervation of the spinal cord: studies by the horseradish peroxidase method combined with monoamine oxidase staining. Exp Brain Res 30:175–186
Schiebel ME, Schiebel AB (1968) Terminal axonal patterns in cat spinal cord. II. The dorsal horn. Brain Res 9:32–58
Schimert J (1939) Das Verhalten der Hinter-würzelkollateralen im Rückenmark. Z Anat Entwicklungsgesch 109:665–687
Schläfke ME, Loeschke HH (1967) Lokalisation eines an der Regulation von Atmung und Kreislauf beteiligten Gebietes an der ventralen Oberfläche der Medulla Oblongata durch Kälteblockade. Pflügers Arch 297:201–220
Schläfke ME, See WR (1980) Ventral medullary surface stimulus response in relation to ventilatory and cardiovascular effects. In: Koepchen HP, Hilton SM, Trzebski A (eds) Central interaction between respiratory and cardiovascular control systems. Springer, Berlin Heidelberg New York, pp 56–63
Schmidt RF (1974) Pre- and postganglionic neurones as final common path of somato-sympathetic reflexes. In: Umbach W, Koepchen HP (eds) Central rhythmic and regulation. Hippokrates, Stuttgart, pp 178–190
Schmidt RF, Schönfuss K (1970) An analysis of the reflex activity in the cervical sympathetic trunk induced by myelinated somatic afferents. Pflügers Arch 314:175–198
Schmidt RF, Weller E (1970) Reflex activity in the cervical and lumbar sympathetic trunk induced by unmyelinated afferents. Brain Res 24:207–218
Schondorf R, Laskey W, Polosa C (1983) Upper thoracic sympathetic neurone responses to input from urinary afferents. Am J Physiol 245:R311–R320
Schramm LP, Adair JR, Stribling JM, Gray LP (1975) Preganglionic innervation of the adrenal gland of the rat: a study using horseradish peroxidase. Exp Neurol 49:540–553
Schramm LP, Stribling JM, Adair JR (1976) Developmental reorientation of sympathetic preganglionic neurones in the rat. Brain Res 106:166–171
Schramm LP, Howland EW, McKenna KE, Barton GN (1979) Spinal pathways mediating splanchnic sympathetic excitation and sympathetic silent periods in the rat. Brain Res 167:396–401

Schroder HD (1985) Anatomical and pathoanatomical studies on the spinal efferent systems innervating pelvic structures. J Auton Nerv Syst 14:23–48
Sears TA (1964) The slow potentials of thoracic respiratory motoneurones and their relation to breathing. J Physiol (Lond) 175:404–424
Sell R, Erdelyi A, Schaefer H (1958) Untersuchungen über den Einfluß peripherer Nervenreizung auf die sympathische Aktivität. Pflügers Arch 267:566–581
Seller H (1973) The discharge pattern of single units in thoracic and lumbar white rami in relation to cardiovascular events. Pflügers Arch 343:317–330
Selzer M, Spencer WA (1969) Interactions between visceral and cutaneous afferents in the spinal cord: reciprocal primary afferent fibre depolarisation. Brain Res 14:349–366
Silver A, Wolstencroft JH (1971) The distribution of cholinesterase in relation to the structure of the spinal cord in the cat. Brain Res 34:205–227
Simon OR, Schramm LP (1983) Spinal superfusion of dopamine excites renal sympathetic nerve activity. Neuropharmacology 18:697–700
Simon OR, Schramm LP (1984) The spinal cord and medullary termination of myelinated renal afferents in the cat. Brain Res 290:239–247
Sinha JN, Atkinson JM, Schmidt H (1973) Effects of clonidine and *L*-dopa on spontaneous and evoked splanchnic nerve discharges. Eur J Pharmacol 24:113–119
Skok V, Ivanov AY (1983) What is the ongoing activity of sympathetic neurones? J Auton Nerv Syst 7:263–270
Skok VI (1980) Ganglionic transmission: morphology and physiology. In: Kharkevich DA (ed) Pharmacology of ganglionic transmission. Springer, Berlin Heidelberg New York, pp 9–39
Smith OA (1965) Anatomy of central neural pathways mediating cardiovascular functions. In: Randall, WC (ed) Nervous control of the heart. Williams and Wilkins, Baltimore, pp 34–53
Smits JFM, Brody MJ (1984) Activation of afferent renal nerves by intrarenal bradykinin in conscious rats. Am J Physiol 247:R1003–R1008
Smits JFM, van Essen H, Struyker Boudier HAJ (1978) Serotonin-mediated cardiovascular responses to electrical stimulation of the raphe nuclei in the rat. Life Sci 23:173–178
Smolen AJ, Glazer EJ, Ross LL (1979) Horseradish peroxidase histochemistry combined with glyoxylic acid induced fluorescence used to identify brainstem catecholaminergic neurones which project to the chick thoracic spinal cord. Brain Res 160:353–357
Sofroniew MV (1980) Projections from vasopressin, oxytocin and neurophysin neurones to neural targets in the rat and human. J Histochem Cytochem 28:475–478
Sofroniew MV, Weindl A (1981) Central nervous distribution of vasopressin, oxytocin and neurophysin. In: Martinez JL, Jenson RA, Messing RB, Rigter H, McGaugh JL (eds) Endogenous peptides, learning and memory process. Academic, New York, pp 377–389
Spindel E, Wurtman RJ (1980) TRH immunoreactivity in rat brain regions, spinal cord and pancreas: validation by high pressure liquid chromatography and thin layer chromatography. Brain Res 201:279–288
Spyer KM (1981) Neural organisation and control of the baroreceptor reflex. Rev Physiol Biochem Pharmacol 88:24–124
Stanek KA, Neil JJ, Sawyer WB, Loewy AD (1984) Changes in regional blood flow and cardiac output after *L*-glutamate stimulation of A_5 cell group. Am J Physiol 246:H44–H51
Stein RD, Genovesis S, Demarest K, Weaver LC (1986) Capsaicin treatment attenuates the reflex excitation of sympathetic activity caused by chemical stimulation of intestinal afferent nerves. Brain Res 397:145–151
Steinbusch HWM (1981) Distribution of serotonin immunoreactivity in the central nervous system of the rat: cell bodies and terminals. Neuroscience 6:557–618
Stella A, Colin R, Busnardo I, Zanchetti A (1984) Effects of afferent renal nerve stimulation on renal hemodynamic and excretory functions. Am J Physiol 247:H576–H583
Stock G, Schmelz M, Knuepfer M, Forssman WG (1983) Functional and anatomic aspects of central nervous cardiovascular regulation. In: Ganten D, Pfaff D (eds) Central cardiovascular control. Springer, Berlin Heidelberg New York, pp 1–30 (Current topics in neuroendocrinology, vol 3)

Sudakov KV, Sherstnev VV, Osipovskii SD (1976) Direct action of angiotensin II on central neurones. Bull Exp Biol Med (Rus) 82:899–902

Sun MK, Guyenet PG (1985) GABA mediated baroreceptor inhibition of reticulo-spinal neurones. Am J Physiol 249:R672–R680

Suter C, Coote JH (1987) Intrathecally administrated angiotensin II increases sympathetic activity in the rat. J Auton Nerv Syst 19:31–37

Swanson LW, Kuypers HGJM (1980) The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organisation of projections to the pituitory, dorsal vegal complex and spinal cord as demonstrated by retrograde double-labelled methods. J Comp Neurol 194:555–570

Swanson LW, McKellar S (1979) The distribution of oxytocin and neurophysin stained fibres in the spinal cord of the rat and the monkey. J Comp Neurol 188:87–106

Swanson LW, Sawchenko PE (1980) Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. Endocrinology 31:410–417

Szentagothai J (1948) Anatomical considerations of monosynaptic reflex arcs. J Neurophysiol 11:445–454

Szentagothai J (1964) Neuronal and synaptic arrangments in the substantia gelatinosa Rolandi. J Comp Neurol 122:219–240

Szentagothai J, Rethelyi M (1973) Cyto- and neuropil architecture of the spinal cord. In: Desmedt JE (ed) New developments in electromyography and clinical neurophysiology, vol 3. Karger, Basel, pp 20–27

Szulczyk P, Trzebski A (1976) The local effects of pH changes in the cerebrospinal fluid on the ventrolateral areas of the medulla oblongata and the spinal cord surface on the activity of cardiac and vertebral sympathetic nerves. Acta Physiol Pol 27:9–17

Szulczyk P, Trzebski A (1979) Effect of ischaemic decerebration on escape of sympathetic activity during carotid baroreceptor sympathetic reflex. Brain Res 168:404–407

Szulczyk P, Wilk M (1985) Neural organisation of the viscero-cardiac reflexes. Brain Res 326:261–271

Takahashi D (1913) Zur vergleichenden Anatomie des Seitenhorns im Rückenmark der Vertebralen. Arb Neurol Inst Wien Univ 20:62–83

Takano Y, Loewy AD (1985) [^{3}H]substance P binding in the intermediolateral cell column and striatum of the rat. Brain Res 311:144–147

Takano Y, Martin JE, Leeman SE, Loewy AD (1984) Substance P immunoreactivity released from rat spinal cord after kainic acid excitation of the ventral medulla oblongata: a correlation with increases in blood pressure. Brain Res 291:168–172

Takano Y, Sawyer WB, Loewy AD (1985) Substance P mechanisms of the spinal cord related to vasomotor tone in the spontaneously hypertensive rat. Brain Res 334:105–116

Takano Y, Sawyer WB, Kamiya H, Loewy AD (1986) Substance P mechanisms of the spinal cord related to vasomotor tone in rats. In: Nakamura K (ed) Brain and blood pressure control. Elsevier, Amsterdam, pp 205–210

Tan CK, Wong WC (1975) An ultrastructural study of the synaptic glomeruli in the intermediolateral neurones of the rat. Experientia 31:201–203

Tappaz ML, Zivin JA, Kopin IJ (1976) Intraspinal glutamic decarboxylase distribution after transection of the cord at the thoracic level. Brain Res 111:220–223

Tauber ES (1974) Physiology of sleep. Adv Sleep Res 1:133–172

Taylor DG, Brody MJ (1976) Spinal adrenergic mechanisms regulating sympathetic outflow to blood vessels. Cir Res 38 [Suppl 11]:10

Taylor DG, Gebber GL (1973) Sympathetic unit responses to stimulation of the cat medulla. Am J Physiol 225:1138–1146

Taylor DG, Gebber GL (1975) Baroreceptor mechanisms controlling sympathetic neurone rhythms of central origin. Am J Physiol 228:1002–1013

Terni T (1923) Ricerche anatomiche sul sistema autonomi degli uccelli. 1. Il sistema prengangliare spinale. Arch Ital Anat Embriol 24:407–531

Terui N, Saeki Y, Kuwaki T, Kumada M (1986) Role of neurons of the ventrolateral medulla oblongata in the arterial baroreceptor reflex in rabbits. In: Nakamura K (ed) Brain and blood pressure control. Elsevier, Amsterdam, pp 49–54

Torigoe Y, Cernucan RD, Nishimoto JAS, Blanks RHI (1985) Sympathetic preganglionic efferent and afferent neurones mediated by the greater splanchnic nerve in rabbit. Exp Neurol 87:334–348

Trzebski A, Lipski J, Majcherczyk S, Szulczyk P, Chruscielewski L (1975) Central organisation and interaction of the carotid baroreceptor and chemoreceptor sympathetic reflex. Brain Res 87:227–237

Tucker DC, Saper CB (1985) Specificity of spinal projections from hypothalamic and brainstem areas which innervate sympathetic preganglionic neurones. Brain Res 360:159–164

Uchida Y, Kamisaka K, Ueda H (1971) Two types of renal mechanoreceptors. Jpn Heart J 12:233–241

Ueda H, Uchida Y, Kamisaka K (1967) Mechanism of the reflex depressor effect by kidney in dog. Jpn Heart J 8:597–606

Undesser EK, Shinnick-Gallagher O, Gallagher JP (1981) Spinal sympathetic reflex and random activity in the isolated amphibian spinal cord. Am J Physiol 240:R295–R300

Unnerstall JR, Kopajtic TA, Kuhar MJ (1984) Distribution of α_2-agonist binding sites in the rat and human central nervous system: analysis of some functional anatomic correlates of the pharmacological effects of clonidine and related adrenergic agents. Brain Res Rev 7:69–101

Van der Guyten J, Palkovits M, Wijnen HLJM, Versteeg DHG (1976) The regional distribution of adrenaline in the rat brain. Brain Res 107:171–175

Wallis DI, North RA (1978) Synaptic input to cells of the rabbit superior cervical ganglion. Pflügers Arch 374:145–152

Wang GH (1964) The neural control of sweating. University of Wisconsin Press, Madison

Wang GH, Brown VW (1965a) Changes in galvanic skin reflex after acute spinal transection in normal and decerebrate cats. J Neurophysiol 19:446–451

Wang GH, Brown VW (1965b) Supraspinal inhibition of an autonomic reflex. J Neurophysiol 19:564–572

Wang GH, Stein P, Brown VW (1956) Effects of transections of central neuraxis on galvanic skin reflex in anaesthetised cats. J Neurophysiol 19:340–349

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol 21:165–204

Wayner MJ, Ono T, Nolley D (1973) Effects of angiotensin II on central neurones. Pharmacol Biochem Behav 1:679–691

Weaver LC (1977) Cardiopulmonary sympathetic afferent influences on renal nerve activity. Am J Physiol 233:H592–H599

Weaver LC, Mecklem LJ, Reimann KA, Meckler RL, Oehl RS (1979) Organisation of thoracic sympathetic afferent influences on renal nerve activity. Am J Physiol 237:H44–H50

Weaver LC, Fry HK, Meckler RL, Oehl RS (1983) Multisegmental spinal sympathetic reflexes originating from the heart. Am J Physiol 245:R345–R352

Weidinger H, Huber U (1964) Beziehung zwischen sympathischen Aktionspotentialen, zentraler Atmung und Blutdruck bei chronisch decerebrierten Katzen. Pflügers Arch 281:259–270

Weidinger H, Leschhorn V (1964) Sympathische Tonisierung und rhythmische Blutdruckschwankungen. Z Kreislaufforsch 53:985–1002

Wessendorf MV, Anderson EG (1983) Single unit studies of identified bulbospinal serotonergic units. Brain Res 279:93–103

Wessendorf MV, Proudfit HK, Anderson EG (1981) The identification of serotonergic neurones in the nucleus raphe magnus by conduction velocity. Brain Res 214:168–173

Westlund KN, Bowker RM, Ziegler MG, Coulter JD (1982) Descending noradrenergic projections and their spinal terminations. In: Kuypers HGJM, Martin GF (eds) Descending pathways to the spinal cord. Prog Brain Res 57. Elsevier, Amsterdam, pp 219–238

Westlund KN, Bowker RM, Ziegler MG, Coulter JD (1983) Noradrenergic projections to the spinal cord of the rat. Brain Res 203:15–32

Whitwam JG, Kidd C, Fussey IV (1979) Responses in sympathetic nerves of the dog evoked by stimulation of somatic nerves. Brain Res 165:219–233

Wilson VJ, Peterson BW (1981) Vestibulospinal and reticulospinal systems. In: Handbook of physiology, sect 1: The nervous system, vol 11. American Physiological Society, Bethesda, pp 667–702

Wolstencroft JH (1964) Reticulospinal neurones. J Physiol (Lond) 174:91–108

Wong RK, Prince DA (1978) Participation of calcium spikes during intrinsic burst firing in hippocampal neurones. Brain Res 159:385–390

Wong WC, Tan CK (1974) Degeneration in the adult rat spinal cord following systemic treatment with 6-hydroxydopamine. Electron microscopy study. Experientia 30:1455–1458

Wong WC, Tan CK (1980) The fine structure of the intermediolateral nucleus of the spinal cord of the monkey (*Macaca fascicularis*). J Anat (Lond) 130:263–277

Wood JD (1975) Neurophysiology of Auerbach's plexus and control of intestinal motility. Physiol Rev 55:307

Wood JD, Mayer CJ (1979) Serotonergic activation of tonic type enteric neurones in guinea pig small intestine. J Neurophysiol 42:582–593

Wurster RD (1977) Spinal sympathetic control of the heart. In: Randall EC (ed) Neural regulation of the heart. Oxford University Press, Oxford, pp 211–246

Wyszogrodski I (1972) Central inhibition in the sympathetic nervous system. PhD thesis, McGill University, Canada

Wyszogrodski I, Polosa C (1973) The inhibition of preganglionic neurones by somatic afferents. Can J Physiol Pharmacol 51:29–38

Yaksh TL, Rudy TA (1976) Chronic catheterisation of the spinal sub-arachnoid space. Physiol Behav 17:1031–1037

Yamada KA, Norman WP, Hamosh P, Gillis RA (1982) Medullary ventral surface GABA receptors affect respiratory and cardiovascular function. Brain Res 248:71–78

Yamada KA, McAllen RM, Loewy AD (1984) GABA antagonists applied to the ventral surface of the medulla oblongata block the baroreceptor reflex. Brain Res 297:175–180

Yamaji K, Fujita M, Otsuki Y, Tamiyawa O (1981) Development of radioimmunoassay for oxytocin in the spinal cord and brain of rats. Psychoneuroendocrinology 6:347–351

Yamashita H, Inenaga K, Koizumi K (1984) Possible projections from regions of paraventricular and supraspinal nuclei to the spinal cord: electrophysiological studies. Brain Res 296:373–378

Yashpal K, Gauthier SG, Henry JL (1985) Substance P given intrathecally at the spinal T_9 level increases adrenal output of adrenaline and noradrenaline in the rat. Neuroscience 15:529–536

Yen CT, Blum PS, Spath JA (1983) Control of cardiovascular function by electrical stimulation within the medullary-raphe region of the cat. Exp Neurol 79:666–679

Yoshimura M, Nishi S (1982) Intracellular recordings from lateral horn cells of the spinal cord in vitro. J Auton Nerv Syst 6:5–11

Yoshimura M, Polosa C, Nishi S (1986a) Noradrenaline modifies sympathetic preganglionic neurone spike and after potential. Brain Res 362:370–374

Yoshimura M, Polosa C, Nishi S (1986b) Electrophysiological properties of sympathetic preganglionic neurones in the cat spinal cord in vitro. Pflügers Arch 406:91–98

Yoshimura M, Polosa C, Nishi S (1986c) After-hypopolarisation mechanisms in cat sympathetic preganglionic neurones in vitro. J Neurophysiol 55:1234–1246

Yusof AMP (1986) Factors concerned in the regulation of sympathetic activity during paradoxical sleep. PhD thesis, University of Birmingham

Yusof AMP, Coote JH (1986a) Excitatory and inhibitory action of 5-hydroxytryptamine (5-HT) on sympathetic neurones in rat spinal cord. Neurosci Lett [Suppl] 24:S38

Yusof AMP, Coote JH (1986b) Is vasomotor tone dependent on a substance P sympatho-excitatory pathway in the spinal cord? Neurosci Lett [Suppl] 24:S15

Yusof AMP, Coote JH (1987) The action of substance P antagonist on sympathetic nerve activity in the rat. Neurosci Lett 75:329–333
Zhang T, Rohlicek CV, Polosa C (1982) Responses of sympathetic preganglionic neurones to systemic hypercapnia in the acute spinal cat. J Auton Nerv Syst 6:381–389
Zieglgänsberger W, Tulloch IF (1979) Effects of substance P on neurones in the dorsal horn of the spinal cord of the cat. Brain Res 166:273–282
Zivin JA, Reid JL, Saavedra JM, Kopin IJ (1975) Quantitative localisation of biogenic amines in the spinal cord. Brain Res 99:293–301

Subject Index