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ROLE OF NEUROPEPTIDES

IN CONTROL OF SPINAL AUTONOMIC OUTPUT

REGULATING HEART, BLOOD VESSELS AND ADRENAL MEDULLA

by

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the Faculty of Graduate Studies and Research
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ABSTRACT

This thesis focuses on the possible roles of a number of neuroactive peptides in the regulation of sympathetic output at the spinal level. The five peptides selected, namely substance P, angiotensin II, thyrotropin-releasing hormone, somatostatin and oxytocin, were chosen because each peptide has been found in discrete descending anatomical pathways to sympathetic preganglionic neurones. However, there was no physiological evidence indicating the type of functional pathway in which they are involved. Therefore, these peptides were administered intrathecally at the second and ninth thoracic spinal levels to study their possible involvement in the regulation of arterial pressure, heart rate and adrenal medullary output of catecholamines in the rat. The results reveal that each peptide is unique in terms of the type of effect observed. Thus, in some cases a peptide had a greater effect at one spinal level versus the other. One peptide, oxytocin, had a positive chronotropic effect on heart rate without altering arterial pressure. This physiological evidence suggests a selective control of sympathetic output and indicates that the precision in the regulation of this output can be accounted for by central pathways which can be identified by their presumed chemical mediators of synaptic transmission.

RESUME

Cette thèse rapporte l'étude des rôles possibles de plusieurs peptides agissant dans le Système Nerveux Central sur l'output sympathique au niveau spinal. Cinq peptides, soit la Substance P, l'Angiotensine II, l'Hormone de Libération Thyréotrope, la Somatostatine et l'Oxytocine, ont été choisis parce que chacun de ces peptides a été trouvé dans des faisceaux anatomiques descendant vers les neurones sympathiques pré-ganglionnaires. Cependant, il n'y avait pas de preuves physiologiques quant à la nature fonctionelle de ces faisceaux. En conséquence, ces peptides furent administrés par voie intrathécale au second et au neuvième niveaux thoraciques pour déterminer leur rôle possible dans le contrôle de la pression artérielle, du rythme cardiaque et de la production de catécholamines par la médullo-surrénale, chez le rat. Les résultats démontrent que chaque peptide est unique quant à la nature des effets observés. Ainsi, dans certains cas, le peptide a eu un effet plus marqué à un niveau spinal plutôt qu'à l'autre. Un peptide, l'Oxytocine, avait un effet stimulant sur le rythme cardiaque sans changer la pression artérielle. Ces données physiologiques suggèrent un contrôle sélectif de l'output sympathique et indiquent que la précision dans ce contrôle peut être expliquée par des systèmes centraux identifiables par leurs médiateurs chimiques présumés de transmission synaptique.

PREFACE

In accordance with the Guidelines Concerning Thesis Preparation, the Candidate has chosen the option of including as part of her thesis the text of original papers already published by learned journals, or original papers submitted or suitable for submission to learned journals for publication. The thesis conforms to other requirements explained in the Guidelines Concerning Thesis Preparation, McGill University, Faculty of Graduate Studies and Research, revised April, 1984.

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Yashpal, K., Gauthier, S. and Henry, J.L. Oxytocin administered intrathecally selectively increases heart rate not arterial pressure in the rat. (manuscript in preparation;

Chapter IX of this thesis)

Miss Nancy Lang participated in the experiments involving measurement of catecholamines that are described in Chapters II, III, IV and V.

Miss Ruth Cridland and Mr. Vito Romita, both graduate students using the intrathecal technique in their studies, participated in the project described in Chapter I.

Drs. Serge Gauthier and James Henry were co-holders of the operating grant from the Quebec Heart Foundation which funded the present work. They guided me throughout this project and contributed to the discussions that preceded the experimental work, participated in the analysis of the results and provided advice in preparation of the manuscripts.

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Many beautiful events have happened to befall me. This thesis is one of them. Writing these few words of acknowledgement stirs up many emotions within me which mere words cannot express.

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Siddharth, my son, I love you for putting up with the lousy dinners.

To my two very special friends, Mr. Vito V. Romita and Miss Ruth Cridland, who shared my tears and laughter throughout this period, it is a pleasure to express my gratitude for their emotional support.

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I INTRODUCTION

The autonomic nervous system controls the life-sustaining functions of the body, maintaining the stability of the "milieu interieur". Walter Cannon, in 1929, regarded the sympathetic nervous system as a system for uniform mass action to help cope with emergencies and physiological imbalances facing the organism. He said "... the neurone relations in the sympathetic division of the autonomic system seem devised for widespread diffusion of nervous impulses." Thus, Cannon viewed the sympathetic nervous system as a functional syncitium, activated in a massive, non-specific fashion in response to a stressful situation. We know now that the autonomic nervous system acts in a much more sophisticated and selective way than was thought in Cannon's time. Stimuli of the internal and external environments provoke diverse yet specific autonomic responses increasing output at some levels and decreasing it at others, so that the overall response provides the appropriate adjustment for any given stimulus.

Even the concept of a vasomotor centre in the medulla as the sole site for cardiovascular regulation has been abandoned. This concept reached its peak of definition in 1946, with the publication of detailed topographical maps of the vasomotor centres by Alexander. The tenacity of his diagrams and the associated concepts of the central control of the cardiovascular system is apparent even today, in the many

general textbooks of physiology in which this control is portrayed in such terms (see for example textbooks by Ganong,1981; Guyton, 1981; Mountcastle, 1980; Schmidt and Thews, 1983).

Today, instead, a new concept of a hierarchical neuraxis, modelled after the organization proposed in the early 20th century by Sherrington, is more popularly accepted. The concept has emerged in which the spinal cord, the pons and medulla, the hypothalamus and limbic and telencephalic structures each mediates its own characteristic type of reflex and each does so via specific reflex pathways arching through specific nuclei. This concept has initiated a search to identify the specific pathways and the specific nuclei involved in the central nervous control of identifiable autonomic functions, as well as the particular mechanisms by which specific autonomic responses are brought about.

Recently, new anatomical and biochemical methods have demonstrated that a number of cell groups originating from the hypothalamus and brain stem project to the sympathetic preganglionic neurones in the spinal cord. The descending autonomic pathways contain a variety of neurotransmitters, including catecholamines, serotonin and a variety of neuropeptides. Now the challenge is to elucidate the particular roles of each of these putative neurotransmitters in normal regulation of autonomic function and, further, to identify the particular dysfunction in neurotransmitter systems associated with specific clinical disorders.

This thesis examines the roles of a number of peptides in spinal autonomic pathways. The experimental paradigm involves the intrathecal administration of peptide agonists or antagonists onto the spinal cord and an examination of the effects of this administration specifically on heart rate, arterial blood pressure and adrenal medullary output of catecholamines. Thus, it provides a unique insight into the efferent mechanisms regulating the heart, the vessels and the adrenal medullae.

The following review of the relevant literature is designed to present the reader with some of the experimental and historical context within which these experiments were done. Briefly, the present survey of the literature concerns itself with the description of the anatomical, morphological and physiological properties of sympathetic preganglionic neurones, particularly those involved in the control of autonomic cardiovascular and adrenomedullary functions, the descending anatomical pathways from supraspinal structures to these neurones and the possible chemical mediators of synaptic transmission between these pathways and the sympathetic preganglionic neurones.

II LITERATURE REVIEW

1. Sympathetic Preganglionic Neurones - Anatomical Organization

The intermediolateral nucleus is a remarkable region of the spinal grey matter in terms of its structure and function. The nucleus corresponds to a small lateral expansion - the classical lateral horn - slightly dorsal from the horizontal plane passing through the dorsal edge of the central canal. Gaskel in 1886 proposed that the lateral horn of the thoracic spinal cord is associated with the sympathetic nervous system. Now, it is generally accepted that cell bodies of sympathetic preganglionic neurones are located in the intermediolateral nucleus of the thoraco-lumbar spinal cord. However, as will be presented later in this section, sympathetic preganglionic neurones are also found in the other regions of the spinal cord.

As early as 1898, Onuf and Collins observed chromatolytic changes in neurones in the lateral horn, in the intermediate grey region extending medially from the lateral horn towards the central canal and in the region dorsal to the central canal after excision of the stellate ganglion, or of the thoracic sympathetic chain in the cat. Since that time, various investigators have confirmed these results using the same technique of retrograde degeneration and have extended the area to include the lateral funiculus as a site of origin

of sympathetic preganglionic axons (Cummings, 1969; Henry & Calaresu, 1972a; Petras and Cummings, 1972). More recently, with the advent of the new technique of retrograde transport of markers (typically horseradish peroxidase; HRP), the organization and distribution of sympathetic preganglionic neurones have been determined more precisely (Chung et al., 1975; 1979; Dalsgaard & Elfvin, 1979,1981; Deuschl & Illert, 1981; Faden and Petras, 1978; Hancock, 1982; Hancock & Peveto, 1979; Murata et al., 1982; Oldfield & McLachlan, 1981; Petras & Cummings,1978; Petras & Faden, 1978; Rando et al., 1981; Schramm et al., 1975). These recent studies have confirmed and expanded upon the earlier studies regarding the location of sympathetic preganglionic neurones, and have identified yet another region, the ventral horn, where a small number of these neurones can also be found. An additional advantage of these experiments is that they have provided extensive information on the morphology and cytoarchitectonics of these cells in various spinal segments.

Table 1 summarizes the results of the relevant studies. We shall now turn to a detailed survey of the localization, morphology and cytoarchitecture of sympathetic preganglionic neurones in each of the individual regions.

(i) Lateral horn

The lateral horn was the earliest part of the spinal cord to be implicated in sympathetic pathways. The initial observations were based on the indirect evidence that when

Table I. Summary of anatomical experiments showing spinal localization of sympathetic preganglionic neurones

INVESTIGATORS	SPECIES	TECHNIQUE USED	STRUCTURES IMPLICATED
Anderson, 1902	cat	cervical sympathectomy	lateral horn
Chung et al., 1975	cat	HRP into stellate ganglion	lateral horn, lateral funiculus, intermediate grey, central canal ventral horn
Cummings, 1969	dog	adrenal medullectomy	lateral horn, lateral funiculus, intermediate grey, central canal
Dalsgaard & Elfvin, 1979	guinea-pig	HRP into superior cervical ganglion	lateral horn, intermediate grey central horn
Dalsgaard & Elfvin, 1981	guinea-pig	HRP into stellate ganglion	lateral horn, lateral funiculus, intermediate grey, central canal
Deuschl & Illert, 1981	cat	HRP into lumbar paravertebral ganglion	lateral horn, lateral funiculus, central canal, intermediate grey, ventral horn
Faden & Petras, 1978	dog	HRP into lumbar or thoracic paravertebral ganglion	lateral horn, intermediate grey, central ganglion
Hancock, 1982	hamster	HRP to intermesenteric or lumbar sympathetic trunk	lateral horn, intermediate grey, central canal

Hancock & Peveto, 1979	rat	HRP to hypogastric nerve	lateral horn, intermediate grey, central canal
Herring, 1903	cat	cervical sympathectomy	lateral horn
Murata et al., 1982	rat	HRP to cervical sympathetic trunk	lateral horn, lateral funiculus, intermediate grey, central canal
Oldfield & McLachlan 1981	cat	HRP into stellate ganglion or superior cervical trunk	lateral horn, lateral funiculus, intermediate grey, central canal, ventral horn
Onuf & Collins, 1898	cat	thoracic and lumbar sympathectomy	lateral horn, intermediate grey, central canal
Petras & Cummings, 1972	monkey	thoracic and lumbar sympathectomy	lateral horn, lateral funiculus, intermediate grey, central canal
Petras & Cummings, 1978	dog	HRP into bladder	lateral horn, intermediate grey
Petras & Faden, 1978	dog	HRP into lumbar or thoracic paravertebral ganglion	lateral horn, intermediate grey, central canal
Rando et al., 1981	rat	HRP into superior cervical ganglion	lateral horn, lateral funiculus, intermediate grey, central canal
Schramm et al., 1975	rat	HRP into adrenal medulla	lateral horn, lateral funiculus, intermediate grey, central canal

viewed longitudinally the distribution of neurones in the lateral horn appeared to correspond to the spinal segments from which the white rami communicantes emerge. Subsequent studies by Onuf & Collins (1898), Andersen (1902) and Herring (1903) provided more direct evidence that sympathetic preganglionic neurones are located in this region. These studies involved sectioning of lumbar and thoracic white rami communicantes and exploring the spinal cord for neurones displaying chromatolytic changes. These results were later confirmed by subsequent degeneration studies using the then more refined Nauta technique (Cummings, 1969; Petras & Cummings, 1972). Retrograde transport of intracellular marker proteins such as HRP has yielded the most unequivocal anatomical approach to identifying neural pathways. Thus, retrogradely labelled neurones have been observed in the thoraco-lumbar lateral horn following injection of HRP into paravertebral ganglia (Chung et al., 1975, 1979; Dalsgaard & Elfvin, 1979, 1981; Deuschl & Illert, 1981; Faden & Petras, 1978; Oldfield & McLachlan, 1981; Petras & Faden, 1978; Rando et al., 1981), prevertebral ganglia (Dalsgaard & Elfvin, 1979), the lumbar sympathetic or the intermesenteric trunk (Hancock, 1982), the cervical sympathetic nerve (Murata et al., 1982), the hypogastric nerve (Hancock & Peveto, 1979), the adrenal medulla (Schramm et al., 1975) and the urinary bladder (Petras & Cummings, 1978).

The topography of the lateral horn, and the quantitative distribution of the preganglionic neurones found there were studied by Henry and Calaresu (1972a) in serial

transverse sections of the cat spinal cord. The lateral horn extends from the C8-T1 border to L4 and contains between 32,790 and 53,340 neurones. The number of neurones in the lateral horn varies from segment to segment with the greatest number occurring at the levels of the rostral thoracic (T1-T2) and middle lumbar (L3-L4) segments. In addition, the number of neurones varies from one section to another: 15 μm thin sections contain from 0 to 22 neurones. More recently, Oldfield and McLachlan (1981) have made a detailed quantitative and topographical analysis of the preganglionic neurone population in the upper thoracic spinal cord of the cat after injecting horseradish peroxidase into the stellate ganglion. From reconstruction of different segmental levels, they found most of these cells to lie in a column 200 μm in diameter, composed of cell aggregations of 20-150 neurones at intervals of approximately 300 um.

The morphology of the neurones in the intermediolateral nucleus varies from segment to segment. Sympathetic preganglionic neurones caudal to T2 are generally fusiform or spindle shaped, with proximal dendrites aligned rostrocaudally. However, the T1 and T2 segments have predominantly multipolar rather than fusiform cells, and their dendrites are aligned mediolaterally. In the ventral parts of the nucleus in the more rostral segments, both fusiform and triangular cells are aligned with their long axes in the dorsoventral direction. In addition, stellate cells also occur in small numbers in conjunction with the fusiform cell bodies throughout the

lateral horn at all segmental levels between T1 and T9, with the greatest abundance in T1 and T2. Recently, a report has appeared based on the very difficult technique of the intracellular injection of HRP into the somata of single sympathetic preganglionic neurones in vivo (Dembowsky et al., 1985). From a total of 28 neurones injected intracellularly in the lateral horn of the third thoracic segment, seven could be reconstructed in serial sections of the spinal cord. Three neurone types were identified: spindle-shaped with the long axis oriented rostro-caudally, large multipolar and small oval cells. Five to eight primary dendrites arise from the cell body and dendritic branches could be traced up to 1330 µm from the cell body. The dendritic fields are oriented rostro-caudally with total lengths of 1500 to 2540 µm.

In terms of the ultrastructure of sympathetic preganglionic neurones, electron microscopic techniques have provided much useful information. For example, Chung et al (1980) found two prominent bouton populations terminating on sympathetic preganglionic neurones, one with round vesicles and another with pleomorphic vesicles. On the somata pleomorphic terminals outnumber those containing round vesicles, while the two types are represented almost equally on the dendrites. Furthermore, the total coverage by boutons is found to be greater on proximal dendrites than on perikarya. Chung et al. (1980) suggested that inputs to the sympathetic preganglionic somata are predominantly inhibitory while the dendrites receive a more equal balance of inhibitory

and excitatory inputs. They based their suggestion on the following: in attempts to correlate the morphology of neurones with physiological function, round synaptic vesicles have been found to be associated with excitation, while inhibitory systems posses axon terminals with pleomorphic vesicles (Uchizono, 1967). Furthermore autoradiographic studies have shown that axon terminals with round vesicles concentrate acetylcholine, a putative excitatory neurotransmitter (Marchbanks '69), whereas terminals with flattened vesicles concentrate glycine (Matus and Demisen, '71) or gamma amino butyric acid (Hökfelt and Ljungdahl, '72), both of which are putative inhibitory transmitters.

Axons of sympathetic preganglionic neurones in the lateral horn closely follow the lateral border of the grey matter down through the ventral horn, to exit from the spinal cord through the ventral roots (Dalsgaard & Elfvin, 1981; Deuschl & Illert, 1981; Rethelyi, 1972). These axons do not appear to give rise to collaterals (Dembowsky et al., 1985).

(ii) Lateral funiculus

Henry and Calaresu (1972a) suggested that the lateral funiculus contains sympathetic preganglionic neurones on the basis of the similar morphological appearance of neurones found there to those of the lateral horn. Experimental support for this was provided by Cummings (1969) in the cat and Petras & Cummings (1972) in the rhesus monkey, where they observed chromatolytic neurones in the lateral funiculus following

thoracic sympathectomy. Since then, many studies have reported retrograde labelling of cells in lateral funiculus following injection of HRP into paravertebral ganglia (Chung et al., 1975, 1979; Dalsgaard & Elfvin, 1981; Deuschl & Illert, 1981; Oldfield & McLachlan, 1981; Rando et al., 1981), cervical sympathetic nerves (Murata et al., 1982) and adrenal medulla (Schramm et al., 1975). However, there are some papers which did not report these cells in the lateral funiculus following injection of HRP into sympathetic axons innervating prevertebral ganglia (Dalsgaard & Elfin, 1979; Hancock, 1982; Hancock & Peveto, 1979).

The shape of the cell bodies in the lateral funiculus is either multipolar (triangular or oval; 19 x 20 μm to 42 x 25 μm) or round (20-32 μm diameter). These neurones tend to be oriented obliquely or mediolaterally, with their dendrites extending laterally into the white matter and medially into the lateral horn (Deuschl & Illert, 1981). The course of their axons resembles that described above for the axons of neurones located in the lateral horn (Dalsgaard & Elfvin, 1981).

(iii) Intermediate grey and central canal regions.

Even in one of the earliest studies, that in 1898 by Onuf and Collins, chromatolytic changes were observed in neurones in the intermediate grey region extending medially from the lateral horn towards the central canal and in the region dorsal to the central canal following excision of the stellate ganglion or the lumbar or thoracic sympathetic chain

in the cat. These results were confirmed later by Cummings (1969) in experiments performed on neonatal dogs, where, following adrenal medullectomy and splanchnic nerve transection, degenerative changes in neurones were observed in the middle region of the intermediate gray as well as in areas dorsolateral and dorsal to the central canal. Following unilateral thoracic or abdominal sympathectomy in the macaque, Petras and Cummings (1972) found an abundance of chromatolytic neurones in the intermediate grey region but not near the central canal region. More recently, retrogradely labelled neurones have been observed in these regions following injection of HRP into paravertebral (Chung et al., 1975, 1979; Dalsgaard & Elfvin, 1979, 1981; Deuschl & Illert, 1981; Faden and Petras, 1978; Oldfield & McLachlan, 1981; Petras and Faden, 1978; Rando et al., 1981) and prevertebral ganglia(Dalsgaard & Elfvin, 1979), the lumbar sympathetic or the intermesenteric trunk (Hancock, 1982), the cervical sympathetic nerve (Murata et al., 1982), the hypogastric nerve (Hancock & Peveto, 1979), the adrenal medulla (Schramm et al., 1975) and the bladder (Petras & Cummings, 1978).

Although cell bodies in these regions are mostly fusiform (16 x 12 μm to 43 x 13 μm) or multipolar (26 x24 μm to 42 x 21 μm) in shape, the orientation of the neurones in the two regions is slightly different.

In the intermediate grey region where HRP was injected into cervical or thoracic paravertebral (Oldfield & McLachlan, 1981) or prevertebral (Dalsgaard & Elfvin, 1979) ganglia the

labelled cell bodies and dendrites were oriented mediolaterally with dendrites extending laterally into the lateral horn and medially as far as the contralateral side of the central canal. Following injection of HRP into lumbar paravertebral ganglia (Deuschl & Illert, 1981) a similar orientation of cells was observed with the addition that some neurones projected into the dorsal horn.

In the central canal region sympathetic preganglionic neurones are oriented mediolaterally, with dendrites occasionally extending into the contralateral side of the spinal cord. In lumbar segments, some neurones are oriented rostrocaudally (Deuschl & Illert, 1979; Hancock & Peveto, 1979; Oldfield & McLachlan, 1981).

The course of the axons of cell bodies in the intermediate grey region differs somewhat from that of the axons arising from cell bodies near the central canal. Three different efferent pathways have been described for axons of preganglionic neurones in the intermediate grey region. Following injection of HRP into stellate ganglion, Dalsgaard & Elfvin (1981) observed some labelled axon coursing obliquely through the dorsal part of the ventral horn to the lateral border of the ventral horn, following at this point the efferent pathway of axons of neurones in the lateral horn. Other axons appeared to follow the medial border of the ventral horn. The third group of fibres is described by Deuschl and Illert (1981) where the labelled axons course straight down through the ventral horn, following injection of HRP into

lumbar paravertebral ganglia. The axons of the cell bodies in the central region, on the other hand follow an efferent pathway only along the medial border of the ventral horn (Dalsgaard & Elfvin, 1981).

(iv) Ventral horn

Several groups of investigators have observed labelled cell bodies in the ventral horn region following injection of HRP into paravertebral ganglia (Chung et al., 1975; Deuschl & Illert, 1981; Oldfield & McLachlan, 1981). These neurones, which are usually fusiform or stellate in shape, are similar in size to neurones in the lateral horn and are located on or near the medial, ventral or lateral border of the grey matter. Their dendrites are aligned dorsoventrally. To my knowledge the functional significance of these neurones has not been studied. Their identification as sympathetic preganglionic neurones is based only on their anatomical projection to paravertebral ganglia. Whether they act as somatic motoneurones with axons coursing through these ganglia or whether they are aberrant sympathetic preganglionic neurones whose cell bodies lie outside their normal distribution remains to be determined.

(v) Longitudinal distribution

When HRP is injected into any given structure, the distribution of labelled neurones in each spinal region tends to vary longitudinally. Oldfield and McLachlan (1981) reported that while more than 70% of the neurones projecting to the

stellate ganglion lie in the lateral horn region, there is a progressive decrease rostrally to caudally in the number of these cells. On the other hand, labelled cells in the intermediate region and around the central canal show a progressive increase caudal to T5. Furthermore, in experiments where HRP was injected at different sites (Oldfield & McLachlan, 1981), depending on where the HRP is injected the relative number of labelled neurones within each spinal segment may vary. For example, a relatively greater percentage of the labelled neurones was observed in more medial spinal regions in the rostral thoracic segments, following injection of HRP into the cervical sympathetic trunk, in contrast to the distribution of labelled neurones observed following injection of HRP into the stellate ganglia.

On the basis of the observation that lateral horn neurones appear to innervate thoracic and lumbar paravertebral ganglia more massively, it was suggested by Petras and Faden (1978) that axons of sympathetic preganglionic neurones in the medial regions of the spinal cord project to the "more distally located sympathetic ganglia". Support for this suggestion comes from various studies. It was shown, for example, that when HRP was injected into the inferior mesenteric ganglion, which is a prevertebral ganglion, as many as one-third of the total number of labelled neurones were located in the intermediate grey region (Dalsgaard & Elfvin, 1979). On the other hand, injection of HRP on the proximal portion of the cut end of the hypogastric nerve showed 80% of the labelled neurones in the

region around the central canal (Hancock & Peveto, 1979). Further support for the suggestion of Petras and Faden (1978) comes from a study where different neurone markers were applied simultaneously to axons in the sympathetic trunk innervating lumbar paravertebral ganglia (Hancock, 1982). Although the segmental distribution of neurones innervating both nerve trunks was similar, the spinal sites of origin were different. Neurones projecting to the lumbar paravertebral ganglia were located primarily in lateral horn regions while neurones projecting to the intermesenteric trunk were both in the lateral horn region and in the region around the central canal. It is noteworthy that no double labelled neurones were observed.

(vi) Intraspinal sympathetic preganglionic pathways

Any classical description of the sympathetic nervous system would state that the axon of a sympathetic preganglionic neurone projects to a paravertebral ganglion through the ipsilateral ventral root of the same spinal segment. The axon then travels through a communicating ramus to reach the sympathetic chain, where it may synapse with postganglionic neurones located in sympathetic ganglia at the same or more distant segmental level. Faden's group has suggested the existence of a longitudinally projecting intraspinal sympathetic preganglionic pathway on the basis of their physiological and anatomical experiments in the spinal cord. These studies in cats and dogs showed that the axons of

sympathetic preganglionic neurones may travel through several segments within the spinal cord before exiting through a distant ventral root to reach the sympathetic trunk (Faden and Petras 1978; Faden et al., 1979). The existence of intraspinal pathway explains the results of many studies described in earlier sections where retrogradely labelled neurones were found in several spinal segments.

These results, however, do not conform to the findings of Oldfield and McLachlan (1980) and Rubin and Purves (1980). In these studies, when the HRP was applied to a single ramus communicantes, labelled neurones were confined to a length of spinal cord approximately equal to the length of a single segment. Similarly, when HRP was applied to the splanchnic nerve following transection of selected thoracic spinal nerves, labelled neurones were confined to the segments with intact spinal nerves (Kuo et al., 1980). This discrepancy in results may be largely due to the methodology used in some of the experiments. In those of Faden and Petras (1978) it is not clear exactly how the ganglion injected with HRP was isolated. First of all the authors state that the sympathetic chain was cut ~"cranial and caudal to the ganglion while preserving [which ?] communicantes". Secondly, the ganglion was isolated after the injection of HRP, leaving the possibility that some of the HRP came into contact with fibres besides those in the nerve which finally remained intact. Thirdly, it is not clear if adequate care was taken to safeguard against leakage of HRP from the site of injection; it is possible that after the

experiment HRP diffused extensively to be taken up by damaged axons at the cut ends of the sympathetic chain. In fact, when Rubin and Purves (1980) used the method described by Faden and Petras (1978) and Chung et al. (1979), labelled neurones were not restricted to a single segment but were found in several segments. It seems, therefore, that in spite of some data for intraspinal projection of the axons of sympathetic preganglionic neurones, this proposal has not withstood subsequent experimental examination.

(vii) Ipsilateral vs contralateral axonal projection

When HRP is injected into the cervical sympathetic nerve or into the stellate ganglion, the vast majority of labelled neurones observed are ipsilateral to the site of injection (Oldfield and McLachlan, 1981). However, this is not the case for sympathetic preganglionic neurones projecting elsewhere. Following unilateral lumbar sympathectomy, chromatolytic cell bodies are observed bilaterally in the lateral horn and in the intermediate grey region in lumbar and thoracic segments. Similar bilateral distribution of neurones is observed following unilateral injection of HRP into lumbar paravertebral ganglia (Petras & Faden, 1978). Bilateral labelling of neurones has also been reported following injection of HRP into the inferior mesenteric ganglion (Dalsgaard & Elfvin, 1979), into the hypogastric nerve (Hancock & Peveto, 1979) and into the intermesenteric trunk (Hancock, 1982).

(viii) Summary.

The anatomical organization and distribution of sympathetic preganglionic neurones in the spinal cord have been described. The neurones are localized in the lateral horn, in the lateral funiculus, in the intermediate grey region in a thin band extending medially from the lateral horn towards the central canal and in the region dorsal to the central canal. A few cells are also located in the ventral horn. In each of these regions, neurones are characterized with specific morphological properties including the orientation of their dendrites and the route of projection of their axons. The spinal distribution of neurones projecting to a given ganglion is stereotyped. It appears that while paravertebral ganglia are preferentially innervated by neurones in the lateral horn, the prevertebral ganglia are preferentially innervated by neurones in more medial regions of the spinal cord. The projections of neurones to thoracic paravertebral ganglia is predominantly ipsilateral, while that to lumbar paravertebral or prevertebral ganglia is bilateral.

2. Sympathetic Preganglionic Neurones - Functional Organization

(i) Sympathetic preganglionic neurones innervating heart and blood vessels

The synaptic connection between preganglionic and postganglionic sympathetic neurones in the sympathetic ganglia prevents the accurate anatomical localization of neurones in the intermediolateral nucleus which project to the heart. Thus, the only information available comes from physiological studies in which the spinal cord was stimulated using microelectrodes and positive sites were marked from which electrical stimulation elicited changes in heart rate (Henry & Calaresu, 1972b). These studies demonstrated that sites from which cardioacceleration could be elicited lay between the first and fifth thoracic segments, with minor changes occurring with stimulation of the sixth thoracic segment as well. Interestingly, increases in arterial pressure could be elicited from the first thoracic segment to the ninth, the most caudal segment tested. It was concluded from this study that neurones in cardioacceleratory pathways lie in the intermediolateral nucleus between the first and fifth thoracic segments and that neurones in pressor pathways are found more extensively throughout the longitudinal extent of the nucleus.

(ii) Sympathetic preganglionic neurones innervating the adrenal medullae

Sympathetic inputs to the adrenals are by preganglionic neurones and therefore the cell bodies of these neurones can be easily identified using anatomical tracing techniques. Elliott in 1913 observed that following transection of the splanchnic nerves in the cat, myelinated nerve fibers degenerated to their endings in the adrenal medulla. He thought it probable that the primary preganglionic nerves directly innervated chromaffin cells of the adrenal medulla without the normal innervation via postganglionic cells which characterizes sympathetic innervation. Elliott's observation was confirmed by later studies which in addition started to describe the spinal rami communicantes of the peripheral autonomic nerves innervating the adrenal medulla. Hollinshead in 1936 emphasized the importance of the lesser splanchnic nerve and the branches from the lumbar symphathetic chain in adrenal innervation. Swinyard in 1937, observed that the transection of the last eight thoracic and first two lumbar ventral roots resulted in loss of all myelinated nerves to the adrenal gland and a 50-70% reduction in nerve bundles entering the gland. Maycock and Heslop (1939) indicated that the bulk of secretory-motor fibres to the adrenal medulla were carried in the greater splanchnic nerve, rather than in the lesser splanchnic nerve, thus contradicting the earlier observation of Hollinshead. Using the nerve fibre degeneration technique in cats, Young (1939) showed that the medulla was innervated ipsilaterally by

spinal roots T6 to L3. Later, using a similar technique in dogs and cats, it was concluded that the majority of preganglionic fibres to the adrenal gland left the spinal cord via ventral roots T7-T9 (Kiss 1951).

More conclusive and more detailed studies regarding the innervation of the adrenal medulla had yet to come. Cumming's (1969) experiments on dogs investigated the origin of preganglionic innervation by studying the distribution of chromatolytic neurones in the spinal cord after either transection of sphlanchnic nerves or adrenal medullectomy. He found that the preganglionic neurones were mainly localized in the thoracic segments of the cord and, in particular, in the intermediolateral cell column. In a more systematic study, similar results were obtained in rats (Schramm et al. 1975) using the retrograde horseradish peroxidase tracing technique. In this study, labelled neurones were seen almost exclusively in the intermediolateral cell columns of the T1 to L1 segments of the spinal cord. Recently, Haase et al. (1982) confirmed the above findings of Schramm et al. (1975). According to these investigators, labelled neurones were found in the ipsilateral intermediolateral cell column of the T2 through T13 segments of the spinal cord, with most of the labelling in the T6 to T10 segments, following HRP injection in the adrenal medulla. In addition, they combined the HRP method with the acetylcholinesterase (AchE) technique. The rationale behind this was that since neurones in the intermediolateral cell column possess a strong

acetylcholinesterase activity (Navaratnam et al., 1970) and the ultrastructural localization of AchE in the adrenal medulla is associated with preganglionic fibers (Lewis & Shute, 1969; Somogyi et al., 1975), preganglionic neurones innervating the adrenal medulla should be AchE-positive. In these experiments, the neurones labelled with horseradish peroxidase indeed demonstrated a strong acetylcholinesterase activity.

Holets and Elde (1983) also applied a sophisticated method to elucidate some of the subtleties of the innervation of the adrenal glands. They retrogradely labelled the preganglionic neurones with the fluorescent dye "Fast-Blue" and looked for the distribution of chemically-coded fibres and their relation to sympathetic preganglionic neurones that project to the adrenal medulla in kittens. They found that 89.9 % of all labelled neurones were located in the ipsilateral intermediolateral nucleus. Enkephalin, serotonin and substance P immunoreactive fibers appeared to surround both retrogradely labelled and unlabelled lateral horn neurones. Somatostatin immunoreactive fibres were observed only in proximity to labelled neurones while only a sparse population of neurophysin and oxytocin immunoreactive fibers were observed around sympathoadrenal neurones.

3. Anatomy of Descending Sympathetic Regulatory Pathways

(i) Inputs from primary afferents

Unlike the sacral parasympathetic preganglionic neurones which receive a sparse yet distinct input directly from primary afferent sensory fibres (Kawatani et al.,1983; Morgan et al., 1981; Nadelhaft & Booth, 1984; Nadelhaft et al., 1983; Roppolo et al., 1985) direct connections from primary afferent fibres onto sympathetic preganglionic neurones are almost nonexistent (Kuo et al., 1983). Thus, inputs to these neurones are essentially all from within the central nervous system, and most of those identified to date arise from specific supraspinal structures.

(ii) Pons and medulla

This region, as mentioned earlier in the Introduction, was originally thought of as containing a "vasomotor centre", including both vasodepressor and vasoconstrictor centres. The pons and medulla are now thought of as containing a number of nuclei, each of which plays its own unique role in cardiovascular regulation. Together, they are the most caudal link in a longitudinal series of reflex systems in the neuraxis. This series extends from as high as the cortex, which can control and integrate any number of afferent and efferent inputs for appropriate cardiovscular regulation. The nuclei in the medulla and pons not only organize patterns of

somatic motor and autonomic systems but are also responsible for the maintenance of normal levels of blood pressure and heart rate. Certain nuclear groups contain complex networks of interneurones which interact by inhibiting and exciting each other, thus causing any variety of mixed responses in the systemic circulation (Reis, 1972). This section, however, will focus mainly on the pontine and medullary structures which give rise to fibers projecting to sympathetic preganglionic neurones.

Investigations designed to identify these structures have adopted two principal experimental approaches. One is electrophysiological, the other anatomical. The first electrophysiological studies investigating the descending inputs from the pons and medulla to sympathetic preganglionic neurones were reported by Henry and Calaresu (1974a-d). They plotted various sites of antidromic responses in the lower brain stem to electrical stimulation of axon terminals in the lateral horn of the cat. A number of structures were identified that send axons to the intermediolateral nucleus. Of these structures, a number were attributed specifically a cardio- and pressure-regulatory function on the basis of effects of electrical stimulation of brain stem sites on heart rate and arterial pressure; these structures included the caudal raphe nuclei, the nucleus paramedium reticularis, the nuclei medullae oblongatae centralis, the nucleus lateralis reticularis and the nucleus parvocellularis.

Support for the results of these studies is

overwhelming from both subsequent electrophysiological studies and from anatomical studies (see Tables II and III for details). More recently, however, the electrophysiological studies are making an attempt to understand precisely the role of a specific structure or a pathway involved in mechanisms regulating cardiovascular function (Bernthal & Koss, 1984; Brown and Guyenet, 1984, 1985; Cabot et al., 1979, 1981; Caverson et al., 1983; Dampney et al., 1980, 1982; Hilton, 1982; Hilton et al., 1983; Morrison & Gebber, 1982; Mraovitch et al., 1982).

For example, Brown and Guyenet (1984, 1985) have demonstrated reticulospinal neurones in a discrete region of the rostral ventrolateral medulla oblongata with activity highly correlated to arterial pressure levels and the cardiac cycle in rats. These neurones appear also to support tonic sympathetic vasomotor tone, mediate baroreceptor reflexes and participate in the vascular components of the defence reaction.

Raphespinal neurones in the cat have been reported to exhibit restricted axonal branching in some cases and widespread patterns in other cases, suggesting that the medullary raphe complex is capable of regional as well as global control over spinal sympathetic outflow (Morrison and Gebber, 1985).

Another example of identifying the complex mechanisms involved can be seen in the report of Doba and Reis (1974), in which the nucleus paramedium reticularis appears to mediate a

Table II. Summary of literature on supraspinal sites giving rise to descending pathways to the lateral horn - electrophysiological studies

INVESTIGATORS	SPECIES	STIMULATION SITE	STRUCTURES IMPLICATED AND CARDIOVASCULAR EFFECT
Adair et al.,	cat	medulla oblongata	caudal raphe nuclei
Brown & Guyenet, 1984	rat	spinal cord	rostral ventrolateral medulla, intermediate nucleus paragigantocellularis lateralis
Brown & Guyenet, 1985	rat	medulla oblongata	rostral ventrolateral medulla mediates baroreceptor reflex & exerts tonic vasomotor tone
Cabot et al., 1979	pigeon	medulla	caudal raphe nuclei exert tonic sympathetic inhibition
Caverson et al., 1983	cat	central canal region	bilateral projection from ventrolateral medulla mediating carotid inputs to spinal cord
Dampney et al., 1982	rabbit	medulla oblongata	ventrolateral medulla is vasomotor in function and receives inputs from NTS and parabrachial n.
Henry & Calaresu, 1974a	cat	axon terminals in intermediolateral nucleus	caudal raphe nn., n. paramedian reticularis, n. lateralis reticularis, n. gigantocellularis, n. parvocellularis, n. medullae oblongatae centralis, n. pontis centralis caudalis

Henry & Calaresu, 1974b	cat	medulla & pons	caudal raphe nn. & n. paramedium reticularis elicit cardiac slowing and decrease pressure; n.lateralis reticularis & n. medullae ob. centr. elicit cardioaccleration & increased pressure
Hilton et al., 1983	cat	brain stem (to elicit defence response)	pathway from defence areas relays in ventral medulla
Loeschcke et al, 1970	cat	medulla	ventrolateral medulla is vasopressor
Lovick, 1985	cat	lower thoracic cord	n. paragigantocellularis
Morrison & Gebber, 1985	cat	intermediolateral n.	raphe-spinal neurones are of two types
Mraovitch et al.,	cat	medulla oblongata	n. parabrachialis involved in pressor response
Neumayr et al., 1974	cat	medulla oblongata	region of n. lateralis reticularis
Ward & Gunn, 1976	rabbit	medulla oblongata	caudal raphe nuclei

powerful pressor response elicited by electrical stimulation of the fastigial nucleus. The fastigial nucleus appears to receive inputs from the vestibular apparatus which can occur upon assumption of an upright posture. Thus, impulses for the orthostatic circulatory reflex are conveyed from the vestibular organs along the vestibular nerve to the fastigial nucleus in the cerebellum. The fastigial nucleus in turn activates the paramedium reticular nucleus and this pathway mediates the increased sympathetic outflow which maintains blood pressure and increases heart rate when going from a supine to a standing position.

Although much of the knowledge of the functional neuroanatomy of pontine and medullary structures involved in projections to sympathetic preganglionic neurones is coming from electrophysiological experiments, a more complex circuitry of pathways in regulating cardiovascular function is also emerging from the application of a number of relatively new neuroanatomical techniques for tracing neural pathways. These techniques include the autoradiographic technique, which is based on the anterograde axonal transport of radio-labelled proteins from the cell body to the axon terminals, and the retrograde axonal transport of horseradish peroxidase or other proteins from axon terminals or damaged axons toward the cell bodies. They also include immunohistochemical techniques for the identification of transmitter-specific pathways and the 2-deoxyglucose technique as a marker of metabolic activity in functionally specific pathways. Because of the very large

Table III. Summary of literature on supraspinal sites giving rise to descending pathways to the lateral horn - anatomical studies

INVESTIGATORS	SPECIES	TECHNIQUE	STRUCTURES IMPLICATED
Amendt et al., 1978	cat	HRP into lateral horn	n. tractus solitarius, raphe nn., ventrolateral reticular formation
Amendt et al., 1979	cat	HRP into lateral horn	n. tractus solitarius,, n. gracilis, lateral reticular n., postpyramidal & inferior central nn. of the raphe
Basbaum et al., 1978	cat	[^{3}H] amino acids into brain stem	n. raphe magnus, n. gigantocellularis
Blessing et al., 1981	rabbit	HRP into lateral horn	n. raphe magnus, n. gigantocellularis
Farlow et al., 1984	rabbit	HRP into spinal cord	ventrolateral medulla
Holstege et al., 1979	cat	[^{3}H] amino acids into brain stem	n. subcoeruleus, reticular formation n. raphe obscurus
Kuypers & Maisky, 1975	cat	HRP into spinal cord	reticular formation, vestibular complex, sorsal hypothalamus, locus coeruleus, n. subcoeruleus

Loewy & Burton, 1978	cat	[^{3}H] amino acids into brain stem	n. tractus solitarius
Martin et al., 1979, 1982	opossum	[^{3}H] leucine into raphe pallidus & obscurus and n. gigantocellularis	projections from these nn to intermediolateral n., laminae IX & X
Miura et al, 1983	cat	HRP into lateral horn	origins: 72.1% medulla, 10.2% pons, 8.5% midbrain, 9.2% hypothalamus
Ono et al., 1978	rat	HRP into lateral horn	paraventricular n.
Zemlan & Pfaff,1979 Zemlan et al., 1984	rat	HRP into spinal cord	n. reticularis ventralis, n. gigantocellularis, n. reticularis magnocellularis

amount of information now available, for brevity the results from those studies on descending inputs from the lower brain stem are summarized in Table III.

Experiments using the technique of injecting horseradish peroxidase (HRP) into the spinal cord and looking for the retrogradely labelled cells in the brain stem have been done in a variety of species, including the rat (Martin et al., 1985; Ono et al., 1978; Zemlan & Pfaff, 1979), the cat (Amendt et al., 1978, 1979; Kuypers & Maisky, 1975; Hays & Rustioni, 1981; Miura et al., 1983; Tohyama et al., 1979), the rabbit (Blessing et al., 1981; Farlow et al., 1984) the opossum (Crutcher et al., 1978; Martin et al., 1979, 1981) and the monkey (Castiglioni et al., 1978; Kneisley et al., 1978). Most of these studies are important in that they demonstrate a widespread distribution of supraspinal regions which give rise to descending inputs to the spinal cord; many of these studies, however, are of little significance in terms of the specificity of the structures innervating sympathetic preganglionic neurones because HRP injections are often diffuse and regionally non-specific. Only a few investigations have used localized injections of HRP.

Studies involving injections of tritiated amino acids into specific nuclear groups in the brain stem and then examining the spinal cord for anterogradely labelled terminals in the vicinity of the lateral horn have provided more specific information. As a result, a number of nuclear groups have been identified in the cat (Basbaum et al., 1978;

Holstege et al., 1979; Loewy & Burton, 1978; Loewy & McKellar, 1981) and in the opossum (Martin et al., 1979, 1982, 1985). Various identified areas are summarized in Table III.

The identification of various regions in the pons and medulla projecting to spinal preganglionic neurones have led to the question as to their functional roles in regulation of the cardiovascular ssystem. Pressor and/or cardioacceleratory responses have been elicited by electrical stimulation of the parahypoglossal area (Calaresu & Henry, 1970), the nucleus parabrachialis (Mraovitch et al., 1982), the nucleus lateralis reticularis (Henry & Calaresu, 1974a; Neumayr et al., 1974), the nucleus parvocellularis (Henry & Calaresu, 1974a) and the caudal raphe nuclei (Adair et al., 1977). Other areas of the medulla have caused inhibition of spinal preganglionic neurones. Depressor and/or cardiac slowing responses have been evoked by electrical stimulation of the caudal raphe nuclei (Adair et al., 1977; Coote & MacLeod, 1974a; Henry & Calaresu, 1974a; Neumayr et al., 1974; Ward and Gunn, 1976), the nucleus paramedium reticularis and the nuclei medullae oblongatae centralis, subnucleus ventralis (Henry & Calaresu, 1974a). The ventrolateral surface of the medulla, also known as the "chemosensitive area", has been shown to elicit pressor responses by electrical stimulation (Hilton, 1982; Hilton et al., 1983; Loeschcke et al., 1970;) or by perfusing it with kainic acid (McAllen et al., 1982). Depressor responses have been produced by lesioning or by perfusing the nucleus with glycine (Guertzenstein & Silver, 1974) or with pentobarbitone

(Feldberg & Guertzenstein, 1972). In addition, activation of central chemoreceptors by CO_2 has been shown to increase the rate of discharge of sympathetic preganglionic neurones (Hanna et al., 1981).

The studies reported above show quite convincingly that pontine and medullary structures do project to sympathetic preganglionic neurones and are involved in regulating the various cardiovascular parameters. The next logical question, then, is to ask if any of these brain stem structures receives afferents from cardiovascular-related nerves. The carotid sinus and aortic depressor nerves, known collectively as buffer nerves, have been the subject of considerable attention over the past several decades (Calaresu et al., 1975; Spyer, 1981, 1982), primarily because these nerves convey exclusively cardiovascular information concerning arterial pressure, from baroreceptors, and arterial gas partial pressures, from chemoreceptors, to the central nervous system. Furthermore, electrical stimulation of these nerves has been shown to alter the resting level of arterial pressure (Ciriello and Calaresu, 1979; Fink et al., 1980; Kirchheim, 1976; Krieger, 1964; Patel et al., 1981).

Buffer nerve afferent fibres in the cat have been shown to terminate throughout the rostrocaudal extent of the nucleus of the tractus solitarius with densest labelling in the caudal half of the nucleus (Berger, 1979; Ciriello et al., 1981). Several additional subnuclei of the solitary nucleus complex also receive direct projections from the carotid sinus

nerve, specifically the parvocellular nucleus, the interstitial nucleus and the ventrolateral nucleus (Berger, 1979; Ciriello & Calaresu, 1981; Ciriello et al., 1981; Davies & Kalia, 1981; Panneton & Loewy, 1980). The carotid sinus nerve has also been shown to project to other brain stem areas, including the area postrema (Davies & Kalia, 1981; Panneton & Loewy, 1980), the dorsal motor nucleus of the vagus, the nucleus ambiguus (Davies & Kalia, 1981), the reticular formation just ventral to the solitary nuclear complex and the external cuneate nucleus (Ciriello et al., 1981).

A similar distribution for afferent fibres of the aortic depressor nerve in the solitary nuclear complex has been described (Ciriello & Calaresu, 1981; Ciriello et al., 1981; Kalia & Welles, 1980). It is interesting to note that most studies are done in the rat and rabbit because in these species only the baroreceptor fibres are carried in the aortic depressor nerve (Chalmers et al., 1967; Sapru et al., 1981). The termination of these afferents has been shown to occur exclusively in the nuclei of the solitary tract (Cirello, 1983; Wallach & Loewy, 1980), with the densest projections in the interstitial nucleus and the dorsolateral aspect of the solitary complex.

It is worth noting that the area receiving the greatest projection from the aortic baroreceptor afferent fibres in the rat (Ciriello, 1983) corresponds to the area of the solitary nuclear complex in which bilateral lesions in the

cat result in acute fulminating hypertension (Doba & Reis, 1973). Since bilateral transection of the aortic depressor nerves in the rat has been shown to produce a mild chronic elevation in arterial pressure (Ciriello et al., 1981; Fink et al., 1980; Krieger, 1964; Patel et al., 1981) it is likely that the hypertension that follows lesions of the nuclei of the tractus solitarius is due primarily to the destruction of aortic baroreceptor afferent fibres and their reflex relay neurones.

(iii) Hypothalamus

One of the most important areas of the brain, the hypothalamus, has often been thought of as an integrative centre for cardiovascular responses because it can integrate inputs which involve somatic, endocrine and autonomic functions as well as emotional states. Stimulation of various areas of the hypothalamus can cause either vasopressor or vasodepressor responses, increases or decreases in heart rate, and inhibition of baroreceptor inputs to the lower brain stem. Much knowledge about the hypothalamus has come through studies on the defense reaction. Stimulation of the posterior hypothalamus produces very similar changes to those occurring during "fight or flight". From the circulatory standpoint, blood pressure, heart rate, muscular blood flow and cardiac output are increased in this case, whereas the baroreceptor reflexes and blood flow to the mesentary are decreased.

Again the emphasis is upon the hypothalamus not as a

centre unto itself but rather as a relay station which integrates various longitudinally oriented patterns of responses. For example, stimulation of the anterior hypothalamus produces sympathetic cholinergic vasodilatation, a physiological response which occurs in anticipation of exercise (Uvnas, 1954; Hilton, 1966). However, the sympathetic vasodilator pathways have their origin in the motor cortex, pass caudally to the hypothalamus, turn dorsally to the collicular area, make an abrupt turn ventrally, and pass through the ventrolateral portion of the medulla to the lateral horn of the spinal cord (Uvnas, 1954). Thus, while stimulation of one area may cause a particular response, the response itself should be considered in the context of the function of the whole brain.

Although the role of the hypothalamus in regulation of the cardiovascular system is well established, the search for the inputs to and outputs from the hypothalamus has withstood a long controversy. This section, however, will focus mainly on the descending control from the hypothalamus upon autonomic regulation of cardiovascular parameters.

Skimming through the literature on descending projections from the hypothalamus, one senses a kind of frustration among the earlier investigators for not identifying the direct pathway from the hypothalamus to the sympathetic preganglionic neurones. The first suggestion of the existence of such a pathway came in 1930 from Beattie and his co-workers who observed pressor responses upon electrical

stimulation of various sites in the hypothalamus. Following surgical lesions of these areas they also found degenerating fibres in the region of the lateral horn.

Later attempts with lesions made to interrupt specific descending pathways indicated that the fibres mediating the hypothalamic pressor response were diffuse in the midbrain, in the pontine tegmentum (Magoun et al, 1938) and in the lateral medullary reticular formation (McQueen et al., 1954; Wang & Ranson, 1939). Electrophysiological information on these pathways demonstrates that hypothalamic pressor responses are mediated by two separate pathways in the cat. One activates sympathetic fibres with a long latency and this activation is inhibited reflexly by increases in arterial pressure. The other activates sympathetic preganglionic neurones with a shorter latency but this response is not inhibited by increases in arterial pressure (Gebber et al, 1973). The precise anatomical location of these two pathways could not be identified. Ciriello and Calaresu (1977) also failed to observe degenerating axons or terminals within the lateral horn following electrolytic lesioning of sites within the posterior and ventrolateral hypothalamic regions in the cat.

Thus, a concept of polysynaptic pathways prevailed until the late seventies. According to this concept the hypothalamus influenced the autonomic centres of the brain stem and spinal cord through a system of poorly defined connections through the brain stem reticular formation. This issue was raised again more recently by increasing evidence

showing the existence of long descending projections from the hypothalamus directly to the lower brain stem and spinal cord in various mammals such as the rat (Hancock, 1976; Hosoya & Matsushita, 1979; Hosoya, 1980; Luiten et al., 1985; Ono et al., 1978; Saper et al., 1976), the cat (Caverson et al., 1984; Kuypers & Maisky, 1975; Saper et al., 1976; Yamashita et al., 1984), the rabbit (Blessing & Chalmers, 1979), the opossum (Crutchner et al., 1978) and the monkey (Castiglioni et al., 1978; Kneisley et al., 1978; Saper et al., 1976).

Kuypers and Maisky (1975) and Hopkins (1975) found that after large quantities of HRP are injected into the spinal cord or medulla an appreciable number of neurones in the hypothalamus can be retrogradely labelled, and Conrad and Pfaff (1976) traced a pathway from the vicinity of the paraventricular nucleus to the dorsomedial part of the medulla using the autoradiographic method. Both types of observation have been confirmed by Saper et al. (1976) who found that a continuous population of neurones extending from the paraventricular nucleus through the lateral and posterior hypothalamic areas into the mesencephalic central grey, projects directly to the Edinger-Westphal nucleus, to the vagal nuclear complex and to the intermediolateral column in the spinal cord of the rat, cat and monkey. By immunohistochemical technique, using antibodies either to oxytocin or to its carrier protein, neurophysin I (also called "estrogen-stimulated neurophysin"), Swanson (1977) and Swanson & McKeller (1979) demonstrated descending fibres in the rat

passing through the lateral tegmentum of the brain stem and the dorsolateral funiculus of the spinal cord to the intermediolateral nucleus. More recent studies have not only supported these earlier results but have described in much more detail the location of precise nuclear groups in the hypothalamus which project directly to the spinal cord, the course taken by these fibers and the precise sites of their termination. Thus, Hosoya's study (1980) with the HRP method has revealed that spinally projecting neurones originate in the paraventricular nucleus, the lateral hypothalamic area, the posterior hypothalamic area, the dorsal hypothalamic area and the retrochiasmatic area, and that the paraventricular nucleus and the lateral hypothalamic area contained 70-80% of the total neurones projecting directly to spinal levels. Luiten et al. (1985), using the anterograde transport technique of Phaseolus Vulgaris leucoagglutinin, a lectin which is carried in an orthograde direction by axonal transport mechanisms, have described the precise course of the entire descending paraventricular fibre system in rats. According to their study, two major bundles of descending fibres can be distinguished. At spinal cord levels the fibres course through the dorsolateral funiculus and can be followed in this position over the entire length of the cord to the lower lumbar segments. In the thoraco-lumbar cord a continuous range of collaterals leaves the main bundle in the lateral funiculus and branches in a complex but organized pattern in the intermediolateral nucleus and in area X of Rexed just dorsal to the central canal.

Recently two electrophysiological studies have verified the presence of a direct hypothalamo-spinal pathway in cats. Action potentials evoked antidromically by electrical stimulation of the intermediolateral nucleus, were recorded in the paraventricular nucleus of the hypothalamus by Caverson et al. (1984). In the second study Yamashita et al. (1984) recorded orthodromic sympathetic preganglionic discharges evoked by microstimulation of certain regions in or near the paraventricular nucleus and also recorded antidromic responses in the paraventricular and supraoptic nuclei to stimulation of the intermediolateral nucleus of the thoracic cord.

Thus, the experiments in the last decade have ended a long time controversy and have confirmed the existence of a direct hypothalamo-spinal pathway.

(iv) Summary

It is well established that many neuronal groups from the pons and medulla do project to the sympathetic preganglionic neurones. While anatomical studies have traced the actual descending pathways, electrophysiological studies have helped to some extent in deciphering the functional correlates to these regulating pathways. It is further shown that at least some of the regions of the pons and medulla, which give rise to pathways regulating sympathetic output are involved in effecting the various cardiovascular responses and that these regions also receive information from the buffer nerves.

Going rostrally in the neuraxis, from the pons and medulla to the hypothalamus, again anatomical studies with new tracer techniques have ended a long controversy in demonstrating a direct descending pathway from the hypothalamus to intermediolateral nucleus. Recent electrophysiological data also support the existence of this pathway.

Although integration of cardiovascular control from higher centers is not discussed here, it must be emphasized that any autonomic adjustment is controlled by multiple integrative sites within the central nervous system. Suprapontine, bulbar, cerebellar and spinal mechanisms all play important and complementary roles in normal cardiovascular regulation.

4. Descending Peptidergic Inputs to Sympathetic Preganglionic Neurones

In the past decade, a large number of peptides has been discovered within the vertebrate central nervous system. This discovery has opened up a new era in studying the biological organism and has given it a fresh look. It has opened up new possibilities in deciphering many as yet unknown mechanisms of central nervous regulation, including regulation of the cardiovascular system.

The detection of these peptides within the central nervous system has raised many questions regarding their source, mechanism of action and function. The first studies on

peptides naturally focussed on peptide distribution and peptide synthesis within the central nervous system. Our understanding of their physiological roles and mechanisms of action is still in its infancy.

Until recently, the monoamines (noradrenaline, adrenaline, dopamine and serotonin), acetylcholine and the amino acids glycine, glutamate and gamma-aminobutyric acid were thought to be the only neurotransmitters. Based on the tentative estimates of the number of synapses occupied by each of the types of neurotransmitter present in the central nervous system Snyder (1980) found that approximately 40 percent of known synapses could be accounted for as being occupied by these neurotransmitters. It seems reasonable, therefore, to anticipate that many neuroactive peptides, which are thought to have neurotransmitter or neuromodulatory roles, will account for the occupancy of the majority of thus far unclassified synaptic sites.

Among an increasing list of neuroactive peptides, five are the focus of this thesis. They are substance P, angiotensin II, thyrotropin releasing hormone (TRH), somatostatin and oxytocin. These peptides are carefully chosen on the basis that all five are present in the intermediolateral nucleus and seem to be present in identifiable pathways descending from specific supraspinal structures to the intermediolateral nucleus. They are usually concentrated in nerve terminals and in many cases are released by electrical or chemical stimulation in a calcium dependent manner, which

suggests that these peptides may serve as chemical mediators of synaptic transmission. This section will attempt to describe each peptide in relation to its involvement in control of sympathetic output regulating cardiovascular parameters.

Of all these peptides, substance P has the longest history and is probably the best characterized as far as its distribution, release and biological properties are concerned.

SUBSTANCE P

THE DEAN OF PEPTIDES

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

The story of substance P began in 1931 in London when Professors von Euler and Gaddum reported the discovery of a pharmacologically active substance in extracts of horse brain and intestine. The material was noted to have hypotensive (in vivo) and gut contracting (in vitro) properties in rabbits. As these investigators stored their new-found material in the form of a powder they attributed to it the name Substanze P, where P stood for powder (von Euler and Gaddum, 1931).

In the early 1950's, the distribution of substance P was studied with the bioassay technique by Pernow (1953), Lembeck (1953), and Amin et al. (1954). They made the remarkable observation that there is more substance P in the dorsal roots than in the ventral roots, and, based on this finding Lembeck (1953) proposed the important hypothesis that substance P might be the transmitter of primary sensory neurones. This hypothesis was supported by the studies made in the early 1960's by Lembeck & Holasek (1960), von Euler and Lishajko (1961), Gaddum (1961), Inouye & Kataoka (1962), and Ryall (1962) that substance P is concentrated in synaptosomal fractions. In spite of these encouraging results, investigators were unable to show any direct action of substance P on individual neurones. This represented a major obstacle to the acceptance of Lembeck's hypothesis. In addition, iontophoretic studies by Galindo et al. (1967) showed no effect of substance P, either excitatory or inhibitory on central neurones. Just when the situation appeared to be in a

deadlock, a breakthrough was made by Leeman & her co-workers (Chang et al., 1971), who determined the chemical structure of substance P as an undecapeptide and thus opened up new horizons. The isolation and chemical characterization of substance P, followed by its availabiltiy in synthetic form (Tregear et al., 1971) and the generation of the first anti-substance P antibody (Powell et al., 1973) were important milestones in the scientific saga of substance P. These discoveries opened possibilities to develop highly specific immunohistochemical and radioimmunological techniques for detailed mapping of the distribution and release of substance P and stimulated much research which has exploded during the last few years.

Substance P has attracted considerable attention because of its possible role in the functioning of a large number of biological systems. It is widely but selectively distributed in both the peripheral and central nervous systems. It has been shown to satisfy the majority of the criteria necessary for it to be considered as a neurotransmitter. Infusion of substance P in the central nervous system excites many neurones and evokes a variety of behavioural responses. Peripherally, it contracts a large number of smooth muscles and is a potent secretagogue and vasodilator (Pernow, 1953). Of particular interest is the possibilityy that substance P is the transmitter for the sensory fibres relaying nociceptive stimuli into the spinal cord and brain stem (Cuello, 1978; Henry, 1976; Hokfelt et al., 1975b, 1977a,b; Jessell &

Iversen, 1977; Ljungdahl et al., 1978; Mudge et al., 1979; Nicoll et al., 1980; Randic & Miletic, 1977; Takahashi & Otsuka, 1975.), and that it may also play a role in neurogenic inflammation as the mediator of the axon reflex. (Lembeck & Gamse, 1982).

The ability of substance P to alter blood pressure was first reported by von Euler and Gaddum over 50 years ago. They found that the intravenous administration of a crude extract of substance P decreased the blood pressure of an anesthetized rabbit. Since then it has been established that substance P is one of the most potent peripheral vasodilators known. It dilates arteries in several vascular beds (Bury & Mashford, 1977; Couture et al., 1980) probably via a direct action on specific substance P receptors (Chahl & Walker, 1981; Couture et al., 1980; Eklund et al., 1977). Conversely, substance P appears to have no direct effects on cardiac function (Burcher et al., 1977; Bury & Mashford, 1977). Administered centrally (either intracerebroventricularly or intracisternally) substance P evokes a pressor response (Fuxe et al., 1981; Haeusler & Osterwalder, 1980; Unger et al., 1981) which is mediated by the sympathetic nervous system (Petty & Reid, 1981; Unger et al., 1981). This involvement of substance P in neural pathways regulating the cardiovascular control is the topic of this section.

Substance P is widely distributed in the spinal cord. The highest levels are localized in the dorsal horn, in the ventral horn, in the intermediolateral nucleus and in the

lamina X area (Ditirro et al., 1981;Hökfelt et al., 1975, 1976, 1977; Takahashi & Otsuka, 1975). In the dorsal horn substance P is mainly contained in the terminals of small diameter dorsal root sensory neurones, whereas in the ventral horn and the lateral horn, the majority of substance P fibres seem to be of supraspinal origin (DeLanerolle & La Motte, 1982; Helke et al., 1982; Hökfelt et al., 1977; Kanazawa et al., 1979).

Substance P has been implicated as a neurotransmitter in several spinal cord neuronal systems. The peptide is released from spinal tissue following potassium depolarization (Gamse et al., 1979; Jessel & Iversen, 1977). It is also released from the spinal cord during electric stimulation of the dorsal roots (Otsuka & Konishi, 1976) and following activation of nociceptive afferents (Yaksh et al., 1980). Substance P excites dorsal horn neurones that are themselves excited by noxious stimuli (Henry, 1976; Piercey et al., 1980; Wright & Roberts, 1980). Studies with putative substance P antagonists have provided further evidence that spinal cord substance P is involved in regulation of transmission of noxious stimuli (Åkerman et al., 1982; Couture et al., 1985; Yashpal & Henry, 1984). These antagonists also alter motor and autonomic functions (Couture et al., 1985; Loewy & Sawyer, 1982), thus supporting observations that substance P excites ventral horn motor neurones (Henry et al., 1975;Otsuka & Yanagisawa, 1980; Yanagisawa et al., 1982) as well as sympathetic preganglionic neurones (Backman & Henry, 1984). Therefore, as a neurotransmitter in the spinal cord, substance

P is probably involved in the regulation of nociception (Henry, 1976; Wright & Roberts, 1980), motor function (Otsuka & Konishi, 1977; Yanagisawa et al., 1982) and autonomic output (Åkerman et al., 1982; Keeler & Helke, 1984).

It is suprising to find that inspite of the attention paid to the physiological roles of substance P in the spinal cord, the substance P receptors in the spinal cord have not been well characterized. There exist a number of studies demonstrating substance P receptors in homogenates of brain (Hanley et al., 1980; Perrone et al., 1983; Torrens et al., 1983), salivary glands (Liang & Cascieri, 1980), and small intestine (Buck et al., 1984) as well as autoradiographic localization of these receptors in slices of forebrain (Shults et al., 1982; Quirion et al., 1983), hind brain (Helke et al., 1984) and the whole brain (Wolf et al., 1985).

In the spinal cord, on the other hand, only two major studies have appeared (Maurin et al., 1984; Takano & Loewy, 1984). In the first study Maurin et al. (1984) describe the distribution of substance P receptors in the thoracic spinal cord by using autoradiography. The results indicate the presense of dense receptor sites in the substantia gelatinosa, the intermediolateral cell column and around the central canal. In the second study, Takano and Loewy (1984) report that the intermediolateral region and the striatum contain a single high affinity (^{3}H)-substance P binding component. In 1985, Helke's group have shown qualitative and quantitative differences in substance P binding sites in membrane

homogenates, (Charlton & Helke, 1985a) and their segmental localization, quantification and characterization in the rat spinal cord (Charlton & Helke, 1985b). These results show a marked presence of substance P receptors in the dorsal horn, in the intermediolateral nucleus, around lamina X and in the phrenic motor nucleus as well as a lower distribution in other regions of the ventral horn.

One common finding of all these studies in the spinal cord is the concentration of substance P receptors in the intermediolateral nucleus. This implies a potential role for substance P in the regulation of sympathetic output. Support for this possibility can be seen in a recent report by Takano and co-workers (Takano et al., 1985) which deals with substance P mechanisms involved in regulation of vasomotor tone at the spinal cord level in normotensive Wistar-Kyoto and spontaneously hypertensive rats. The results indicate that the intermediolateral region of 16 week old hypertensive rats contains more (^{3}H)-substance P binding sites than normotensive rats. Thus, one abnormality exists within the lateral cell column. The second abnormality consists of the increased content of substance P in the lateral cell column which could be due either to the existence of more substance P neurones from supraspinal structures projecting to the intermediolateral column or due to some indirect neural mechanism affecting the regulation of the descending substance P neurones. "Thus, factors regulating both the amount of substance P in this system as well as the receptor number on

the sympathetic neurons may in fact account for the higher levels of sympathetic excitation of the vasomotor outflow in the SHR" (Takano et al., 1985).

Evidence from various studies supports the proposal that the binding in the intermediolateral nucleus is probably associated with the substance P nerve terminal system that originates in the ventral medulla (Helke et al., 1982). This system has been implicated in the regulation of information to the sympathetic preganglionic neuronal cell bodies in the lateral horn (Keeler & Helke, 1984; Loewy & Sawyer, 1982; Oldfield et al., 1985). Oldfield et al. (1985) examined the distribution of substance P-positive fibres within the intermediate zone of the thoracolumbar spinal cords of rabbits, cats and monkeys. In all three species substance P fibres were concentrated in areas known to contain sympathetic preganglionic neurones. Nerve terminals containing substance P-like immunoreactive material are found in the intermediolateral nucleus (Gilbert et al., 1982; Ho, 1983; Holets & Elde, 1982; Ljungdahl et al., 1978). According to Oldfield et al. (1985) the greatest accumulation of substance P fibres was found in the T_3-T_5 and L_2-L_4 regions, again in the intermediolateral nucleus.

Several studies have reported the origin of these nerve terminals from nuclear groups in the brain stem. The mammalian brain stem contains several substance P-positive cell groups (de Lanerolle & La Motte, 1982; Dittirro et al., 1981; Ljungdahl et al.,1978). Of these, the caudal pontine medullary

raphe complex is proposed as an origin of substance P containing fibres which project to sympathetic preganglionic neurones (Gilbert et al., 1982; Johansson et al., 1981). The anatomical studies using anterograde and retrograde transport of tracer substances have provided support for this midline caudal brain stem region as the source of serotonin input to sympathetic preganglionic neurones (Cabot et al., 1982; Loewy & Neil, 1981; Loewy & McKeller, 1981). In addition, Chan-Palay and co-workers (1978, 1979) provided further evidence establishing the co-existence of serotonin and substance P within some raphe neurones in the rat; and Bowker et al. (1981) showed the projections from this region to the spinal cord. A further support for this came when Gilbert et al. (1982) presented the evidence that the destruction of serotonin containing raphe neurones results in the partial loss of substance P terminals within the rat sympathetic neuropil. These data were sufficient evidence to support the hypothesis that neurones within the caudal raphe complex co-transport both substance P and serotonin to terminals within the intermediolateral nucleus.

In spite of this evidence, Helke's group (Helke et al., 1982) questioned these results on the basis of their experiments. They found that electrolytic lesions of the raphe were ineffective in reducing substance P levels in the intermediolateral nucleus, as measured by radioimmunoassay. Furthermore, injections of 5,7-dihydroxytryptamine, which destroys medullary serotonin cells, had no effect on the

substance P content in the lateral horn. Support for Helke's results came from Loewy and his co-workers. They observed that kainic acid stimulation of the ventral medulla causes a release of substance P from the spinal cord which is not blocked by intracerebroventricular injections of the serotonin neurotoxin 5,7-dihydroxytryptamine (Takano et al., 1984).

Helke's group, however, has shown that substance-P immunoreactive innervation of the intermediolateral cell column is reduced after lesions of ventral medulla. As intrathecal injections of substance P antagonists reduce blood pressure and block the pressor response that follows kainic acid excitation of the ventral medulla, Loewy and Sawyer (1982) have suggested that substance P neurones of the ventral medulla may be the neurones critical for the maintenance of vasomotor tone.

In spite of these controversies on the exact origin of substance P fibres descending to sympathetic preganglionic neurones, the fact remains that a descending substance P-pathway, originating in supraspinal structures and projecting to the sympathetic preganglionic cell column is one of the major central pathways regulating vasomotor tone. What is lacking is the evidence on physiological role played by this undecapeptide in autonomic regulation. Two iontophoretic studies (Backman & Henry, 1984a; Gilbey et al., 1983) have concluded that substance P may be a transmitter or modulator mediating excitatory inputs to sympathetic preganglionic neurones. In addition, Keeler et al (1985) have recently reported that intrathecal administration of a synthetic analog

of substance P increases arterial pressure and heart rate as well as plasma levels of adrenaline and noradrenaline.

In view of the fact that no study exists by which substance P has been shown to be involved in the regulation of sympathetic output, specifically to the cardiovascular system or to the adrenal medullae, part of my study was done with substance P.

ANGIOTENSIN II

A COMPLEX PEPTIDE

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

The literature on the octapeptide angiotensin II, is not only exhaustive but also complex. This complexity has arisen from the fact that until the mid-seventies almost all research on the renin-angiotensin system was focused on the peripheral components of the enzyme-peptide-steroid regulation of blood pressure and volume homeostasis. But recent years have accumulated enough evidence to show that angiotensin II exerts a myriad of effects which are evoked via central mechanisms. The effects include the increased secretion of vasopressin, stimulation of drinking and a marked pressor response (Reid, 1977). It is believed that peripheral angiotensin is unable to enter the brain in significant amounts. Naturally the question comes to mind: how can angiotensin evoke the reponses which are obviously mediated centrally?

The anatomists searched for angiotensin receptors in the central nervous system, biochemists for the enzymes necessary for the renin-angiotensin system, and physiologists for a central site and mechanism of action of angiotensin II. Although a vast number of experiments have helped in furthering the knowledge about this peptide, the existence of a dual system of angiotensin II has made the story very complex. As a result, many questions remain unanswered as we will see in the following text which will focus only on the aspects which involve this peptide's role in the regulation of the cardiovascular system.

Since peripheral angiotensin II is unable to enter the brain in significant amounts, several areas where the blood brain barrier is permeable (eg. the circumventricular organs) are believed to be the site of action mediating the peptide's central effects. These areas include the subfornical organ, the AV3V region and the area postrema (Broadwell & Brightman, 1976). Binding sites for blood-borne angiotensin II have been localized in these regions, particularly the subfornical regions (Van Houten et al., 1980), and, after angiotensin II injection into the cerebral ventricles, in the organum vasculosum of the lamina terminalis (Landas et al., 1980).

In addition to the role established for this circulating hormone, it has been now shown that an endogenous angiotensin system exists in the central nervous system. All components of the renin-angiotensin system (Lang et al., 1983) including renin (Ganten & Speck, 1978; Hirose et al., 1978), angiotensin converting enzyme (Saavedra & Chevillard, 1982; Yang & Neff, 1972) and the polypeptide precursor angiotensinogen (Campbell et al., 1984; Printz & Lewicki, 1977) have been identified in the brain.

A large number of studies has also identified receptors for angiotensin II in the membrane fraction of brain (Baxter et al., 1980; Bennett & Snyder, 1980; Harding et al., 1981; Sirett et al., 1977, 1979). Mendelsohn (1984) has shown a very high density of angiotensin II receptors in the subfornical organ, the paraventricular nucleus of the hypothalamus, the nucleus of the tractus solitarius and the

area postrema; moderate receptor concentrations are found in the organum vasculosum of the lamina terminalis and low concentrations in the grey matter of the spinal cord of the rat (for a complete list of structures see reference Mendelsohn, 1984). Similarly, Speth and co-workers (1985) have shown the existence of angiotensin II receptors in the canine central nervous system.

Angiotensin II-containing nerve terminals and neurones have been localized in the brain by immunohistochemistry (Changaris et al., 1978; Fuxe et al., 1976; Ganten et al., 1978; Kilcoyne et al., 1980, Quinlan & Phillip, 1981; Weyhenmeyer & Phillips, 1982). High densities were found in the substantia gelatinosa of the spinal cord and spinal trigeminal nucleus, the intermediolateral cell column, the medial external layer of the median eminence and the hypothalamus.

Angiotensin II has been implicated in central control of the cardiovascular system (for recent references see Crowin et al., 1985; Guo & Abboud, 1984; Lappe & Brody, 1984; Scholkens et al., 1982). When angiotensin II is injected directly into the cerebral ventricles, particularly into the third ventricle, pressor and drinking responses are elicited. The effects do not seem to be due to leakage of the peptides into the periphery (Hoffman & Phillips, 1976) and the site of action appears to be in the ventral anterior third ventricle (Phillips & Hoffman, 1977). When angiotensin II is injected systemically, the same responses can be seen, but the site of

action seems to be the areas where the blood brain barrier is thin and incomplete, such as the area postrema (Joy & Lowe, 1970) and the subfornical organ (Simpson & Routtenberg, 1973). It appears then, that there are two kinds of receptors for angiotensin in the brain: those accessible in brain tissue and those accessible on the ventricular surface. It is thus possible that some of the effects of angiotensin II on the central nervous system may be attributed to a peptide derived from a central nervous system renin-angiotensin system, while others may be due to circulating angiotensin II that has diffused across the more permeable parts of the blood-brain barrier.

In dogs and rabbits, the major receptor site for the pressor activity has been shown to reside in the area postrema (Ferrario et al.,1972). This area lies in close proximity to the nucleus of the tractus solitarius and exerts a tonic influence on arterial pressure (Ferrario & Barnes, 1981). Small but significant lowering of the blood pressure is shown by lesion of the area postrema (Ferrario et al., 1979) while electrical stimulation of this area produces a pressor response (Barnes & Ferrario, 1981).

In the rat, on the other hand, angiotensin seems to exert its pressor effects mainly by activating receptors in the periventricular region of the preoptic area of the anterior hypothalamus, the so-called AV3V region. By using tritiated amino acid tracing techniques, two primary pathways from the AV3V region to the lower brain stem have been described (Conrad

& Pfaff, 1976; Swanson et al., 1978). One of the pathways passes through the ventromedial hypothalamic-median eminence region; integrity of this pathway seems to be necessary for the pressor response evoked by this peptide (Johnson et al., 1981). Bealer (1982) has further shown that this neural pathway mediates the pressor response to intraventricular angiotensin II and is distinct from the neural pathway mediating the drinking response in rats. Thus, the participation of angiotensin in the central mechanism of blood pressure regulation is generally accepted.

Looking into the mechanism of the pressor effect, earlier investigators attributed it to two components: mediation by sympathetic nerve activation (Severs et al., 1966) and by the release of vasopressin (Hoffman et al., 1977; Keil et al., 1975; Uhlick et al., 1975). At present, despite a wealth of experimental data, the hemodynamic consequences of central angiotensin receptor stimulation are still not fully explained. The answer is not as simple as was proposed by earlier studies. The mutual interaction of sympathetic activation, release of vasopressin, contribution of the baroreflex, etc., has still to be understood. Investigations into the role of the sympathetic nervous system have yielded contradictory results. There are major discrepancies in the literature (Aars & Akre, 1968; Ferrario et al., 1972; Mann et al.,1982; Morrison & Pickford, 1969; Severs & Daniels-Severs, 1973; Stein et al., 1984; Tobey et al., 1983; Unger et al., 1985).

One noticeable point that has emerged from the above studies on angiotensin II is that most investigators seem to restrict their studies to supraspinal structures, whether they are looking for the receptors or for mechanisms of action. It is all the more noticeable in physiological studies where sympathetic mechanisms have been implicated (Lappe & Brody, 1984; Scholkens et al.,1982; Unger et al., 1981). The spinal cord has often been overlooked as a possible site for sympathoexcitatory effects of angiotensin II (Buckley & Jandhyala, 1977; Ganten et al., 1978; Scholkens et al., 1982).

A closer look at the literature would suggest the spinal cord to be a potential candidate for one of the sites of action for this peptide. This is based on the following facts. Fuxe et al. (1976) observed a high density of nerve terminals containing immunofluorescent angiotensin II located in the intermediolateral nucleus in the rat. Ganten et al. (1978) subsequently confirmed this observation and added that this immunoreactivity was not observed in nerve terminals in the intermediolateral nucleus one week after transection of the spinal cord. Accumulation of the immunoreactive material was observed in the descending fibres in the lateral funiculus, although the cell bodies giving rise to these descending fibres were not identified. In colchicine-treated rats, Brownfield et al. (1982) found that the cell bodies containing immunoreactive material were restricted to the magnocellular cells in the paraventricular, supraoptic and accessory magnocellular nuclei of the hypothalamus in the rat, suggesting

that the angiotensin-containing nerve terminals in the intermediolateral nucleus are associated with the fibres descending from the hypothalamus directly to the spinal sympathetic neurones. Radioautographic studies on the central binding sites of angiotensin also implicate angiotensin in synaptic transmission in the spinal cord. While the existence of high affinity binding sites in central nervous tissue has been known for some time, Mendelsohn et al. (1984) have recently demonstrated low concentrations of binding sites specifically in the spinal grey matter.

The physiological significance of angiotensin in the spinal cord, at present is only a matter of speculation. The marked pressor response induced by intraventricular administration, the activated renin-angiotensin system in hypertensive animals and the involvement of sympathetic mechanisms raises the possibility that the spinal cord is one of the sites of action in producing the elevated blood pressure by this peptide. The experiments described in this thesis with angiotensin II are done in this context.

THYROTROPIN-RELEASING HORMONE

THE FIRST ISOLATED RELEASING FACTOR

pGlu-His-Pro

The field of neuroendocrinology was ushered in just over a decade ago with the first isolated releasing factor, which was designated thyrotropin-releasing hormone (TRH) by virtue of its capacity to stimulate the release of thyroid-stimulating hormone from the mammalian anterior pituitary. TRH was isolated from ovine (Burgus et al., 1969) and porcine (Nair et al., 1970) hypothalamic tissue and was characterized as a tripeptide, consisting of pGlu-His-Pro-NH_2. This discovery was soon to be followed by a group of polypeptides consisting of either hypothalamic release or release inhibiting factors or hormones regulating the release of anterior pituitary hormones.

Further research on this tripeptide, originally found to subserve a neuroendocrine function in regulation of the pituitary-thyroid axis, has revealed that it has other potent pharmacological and behavioral actions which are unrelated to its established endocrine effects. TRH injected intravenously or intracerebroventricularly gives rise to a variety of biological actions which include release of prolactin, tremor, respiratory stimulation, increased visceral movements, a pressor response and depending on the species, hyperthermia or hypothermia and miosis or mydriasis. Interestingly, behavioral changes, psychostimulant effects, euphoric and analeptic properties of TRH have also been extensively documented. Although, TRH testing has become an invaluable research tool, as well as an established diagnostic procedure for the

evaluation of patients with disorders of the hypothalamo-pituitary system and thyroid gland function, other unique and novel functions have also generated a considerable interest in this hormone.

Thus, when radioimmunoassay and immunohistochemical techniques presented the evidence of its ubiquitous distribution throughout the central nervous system of several mammalian and sub-mammalian species it was not a surprise. In fact, such observations have often led to the hypothesis that certain hypothalamic hormones may subserve non-endocrine functions in the central nervous system, such as synaptic modulation and/or neurotransmission.

Much as I would enjoy describing the more interesting psychological and euphoric actions of TRH this part nevertheless focuses upon its role in eliciting certain cardiovascular responses which often are an important component of the psychological and physiological states of the organism. For example, circulatory shock following endotoxemia, hemorrhage (Holaday et al., 1981 a, b.) or spinal cord trauma (Faden et al., 1981) has been shown to be reversed by TRH. These protective effects involve changes in cardiovascular and respiratory functions and appear to be mediated by central autonomic actions, independent of its endocrine actions.

As yet, few studies have specifically focussed their attention to the involvement of TRH in the mediation of the pressor response. Beale et al. (1977) reported that

intracisternal injections of nanogram amounts of thyrotropin-releasing hormone produced the pressor response in rabbits without any effect on heart rate. However, other investigators found increases in both heart rate and arterial pressure by injecting TRH intraventricularly in cats (Delbarre et al., 1977), goats (Eriksson & Gordin, 1981) and rats (Holaday & Faden, 1983; Koivusalo et al., 1979). In all these experiments TRH was injected into the ventricular system of the brain.

Other investigators have administered microinjections of thyrotropin-releasing hormone into specific nuclei in the hypothalamus. Feuerstein et al. (1983) found increases in heart rate and arterial pressure following injections into the medial preoptic nucleus. These authors have suggested on the basis of their results that the medial preoptic nucleus is an important forebrain site for autonomic regulation by thyrotropin-releasing hormone, mediated through the sympathetic nervous system. Diz and Jacobowitz (1984) have also provided evidence that the medial preoptic nucleus as the site of action for thyrotropin-releasing hormone in mediating the tachycardia and pressor responses.

These areas have been found to contain a large number of TRH binding sites. TRH has been immunochemically localized in various hypothalamic nuclei (Brownstein et al., 1974; Hokfelt et al., 1975; Leppaluoto et al., 1978; Okon & Cock, 1976; Ogawa et al., 1981; Taylor & Burt, 1982; Winkour & Utiger, 1974) as well as within brain stem nuclei such as the

nucleus of the tractus solitarius, the nucleus ambiguus and the dorsal motor nucleus of the vagus (Eskay et al., 1983; Hokfelt et al., 1975a). The localization of TRH and the presence of its receptors in some of the nuclei involved in cardiovascular regulatory mechanisms is predictive of a role for TRH in modulating cardiovascular function. Burt and Taylor (1980) have reported the presence of (^{3}H)-TRH binding sites in many of the autonomic areas which are adjacent to the ventricular spaces. Eskay et al., (1983), have proposed that the presence of high concentrations of immunoreactive-TRH in the nucleus of the tractus solitarius suggests a possible role for this hormone "in the so called vegetative function such as respiration, heart rate, blood pressure and taste".

In additon to the presence of TRH in supraspinal structures, it has also been shown to be localized in the spinal cord. Hokfelt et al., (1975b) have reported TRH-positive nerve terminals around motoneurones in the spinal cord of the rat. Also, while the results of Kardon et al. (1977) show that the spinal cord contains substantial quantities of TRH with high concentrations in the ventral horn and the central canal region, Prasad and Edwards (1984) show a uniform binding throughout the rat spinal cord. These latter results are closer to Sharif and Burt's (1983) findings where binding sites in different segments of the spinal cord were observed to be generally similar in the rabbit, although density in the thoracic segments seemed to be somewhat higher.

The widespread occurence of TRH outside the

median eminence suggests a physiological role besides that of a releasing hormone. As a matter of fact, about two-thirds of the total brain TRH is localized outside the hypothalamus (Winkour & Utiger, 1974). TRH is different in this respect from luteinizing hormone-releasing hormone which is found exclusively in the arcuate nucleus and the median eminence. These facts suggest that TRH may act both as a hormone, released into the portal vessels, and as a neurotransmitter or modulator, released at synapses in discrete regions of the brain and spinal cord.

From the literature described so far on TRH, two points can be made. One is that TRH is distributed widely in the central nervous system; sites include certain regions which are involved in regulating cardiovascular regulation. The other is that central administration of TRH evokes pressor responses and tachycardia. These two points can help shed some light into the mechanisms by which TRH acts.

In studies designed to address the potential mechanism of TRH in cardiovascular interactions, Horita et al. (1977) reported that the pressor effect of intracerebroventricular administration of this peptide was abolished by cervical spinal transection and that this central action of TRH was unaltered by adrenergic or cholinergic antagonists (Horita et al., 1979). By contrast, others have shown that this peptide does evoke changes in sympathetic tone. Specifically, following intraventricular injection of TRH increases in splanchnic nerve firing rates were observed

along with a pressor response and tachycardia (Delbarre et al., 1977). More recently, Brown (1981) observed that TRH acts within the brain to increase sympatho-medullary outflow as demonstrated by elevations of plasma catecholamines, glucose and glucagon; similar findings have recently been reported by Feuerstein et al.(1983). Another recent paper by Diz and Jacobowitz (1984) also shows the involvement of sympathetic and parasympathetic nervous system in the cardiovascular responses to TRH.

The role played by TRH and its mechanism of action in the central regulation of the cardiovascular system are not known. The evidence regarding the specific preoptic and hypothalamic sites of action for TRH suggests a physiological role for it in these brain regions (Diz & Jacobowitz, 1984; Feuerstein et al., 1983). Further evidence for physiological and/or pharmacological actions of thyrotropin-releasing hormone in these areas include reports that iontophoretic applications of thyrotropin-releasing hormone onto hypothalamic and septal area neurones results in inhibition of firing rates (Dyer & Dyball, 1974; Renaud et al., 1975). While inhibition is seen in hypothalamic sites, excitation is seen in the spinal cord. Backman & Henry (1984a) have shown that TRH, when applied iontophoretically onto sympathetic preganglionic neurones produces an excitatory effect.

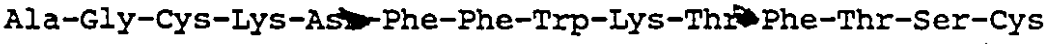
The anatomical support for the presence of this peptide in various areas has been described above. While the anatomical pathways that are responsible for the cardiovascular

actions of TRH are not known, there is ample evidence (anatomical and physiological) for interconnection among the various preoptic and hypothalamic nuclei in addition to direct and polysynaptic connections between these nuclei, brain stem cardiovascular areas and the preganglionic origins of the sympathetic and parasympathetic nervous systems.

To my knowledge, no study exists at present where heart rate and arterial blood pressure were monitored while applying TRH at the spinal cord level. It is known that sympathetic preganglionic neurones do regulate the heart, the blood vessels and the adrenal medullae. It is also known that TRH is present in the spinal cord, in the brain stem and in the hypothalamus. Therefore, an initial approach to study the mechanisms of action of TRH, to my mind, would be to apply it at the lowest level of neuraxis i.e. the spinal cord, before going higher up in the neuraxis with complex neural circuits. This is precisely what has been done in this thesis with TRH

SOMATOSTATIN

THE INHIBITOR ?



The story of the discovery of growth hormone (GH) - inhibiting factor (GIF), also known as GH release-inhibiting hormone (GIH), somatotropin-release inhibiting factor (SRIF) and somatostatin, is a short one but very interesting. In 1967, Krulich was screening fractions from sephadex fractions of sheep and rat hypothalami to locate the fractions which release growth hormone. To his surprise he found that certain fractions consistently inhibited the release of growth hormone, whereas others stimulated it. The results were interpreted to mean that hypothalamic extracts of rat and sheep contain a growth hormone inhibiting factor in addition to the growth release factor previously found in these extracts (Krulich et al., 1968).

Encouraged by these results, Krulich and his collaborators undertook the further purification of somatostatin from sheep hypothalami. These results indicated that somatostatin could be obtained in highly purified form by application of procedures that had been successfully used in purification of other hypothalamic releasing factors. This group later showed that somatostatin had no effect on release of ACTH, FSH, or LH from pituitaries incubated in vitro (Crighten et al., 1969; Dhariwal et al., 1969). It therefore appeared to have a specific inhibitory effect of hypophyseal somatotrophs. The excitement at the discovery of somatostatin was clearly stated in their paper (Krulich et al., 1968).

Unfortunately, in spite of the many reproducible observations reported by Krulich et al., the reports of somatostatin were met by scepticism. In fact, Guillemin constantly cast doubts on the existence of such a factor (Burgus & Guillemin, 1970). This was ironically followed by rediscovery of somatostatin by Brazeau et al. (1973) in Guillemin's laboratory. They too were attempting to purify growth releasing factors in hypothalamic extracts and noted as the Krulich group did that certain factors inhibited the release of growth hormone. By chance they used a sensitive assay for the inhibitor. They isolated it, determined its structure and synthesized the molecule, which turned out to be a tetradecapeptide. Only 500,000 sheep hypothalami were required for the characterization of this factor, the subject of this section.

As was expected, high amounts of somatostatin immunoreactivity could immediately be shown in the median eminence, mainly in its external layer (Dubois et al., 1974; Hokfelt et al., 1974). With ultrastructural immunocytochemistry, it could also be demonstrated that the peptide was present in nerve ending vesicles (Pelletier et al., 1974). Soon it was revealed that like TRH, somatostatin was not confined to median eminence only, but was present in very high concentrations in other areas of the central nervous system also. And therefore, like TRH, it probably had other physiological roles besides inhibition of growth hormone.

The extrahypothalamic presence of somatostatin instigated a search throughout the regions of the brain where it could be found and indeed, an impressive amount of data is presently available which shows a wide and uneven distribution of somatostatin in the central nervous system.

This section opens with the description of the distribution of cells containing somatostatin in the central nervous system. Subsequently, its physiological functions, with special focus on its role as a hypotensive agent, will be described. We shall conclude with evidence that somatostatin may be involved as a chemical mediator of synaptic transmission.

Distribution studies present strong evidence for the occurrence of extensive somatostatin-containing neurone systems in the rat brain (for a comprehensive study refer to Johansson et al., 1984). Although these neurones can be found in various nuclei, it is very common that "aggregates" of somatostatin-positive cell bodies and fibres do not conform to the well defined anatomical nuclei, but are located in areas between well-established nuclei or occupy only parts of such nuclei.

These somatostatin-containing neurones are found in all parts of cerebral cortex. Large number of cells are present in the hippocampus, the amygdala and the pyriform cortex. Also, subcortical areas such as the caudate nucleus and nucleus accumbens are rich in somatostatin-positive cells. The hypothalamus shows several nuclei which contain very high

numbers of somatostatin-positive cell bodies; these include the anterior periventricular area and the ventromedial and arcuate nuclei. In fact, the periventricular area of the hypothalamus contains the largest number of such cells.
Somatostatin-containing cell bodies are also seen in high densities close to the lateral lemniscus, adjacent to and partly within the pontine reticular nuclei, in the dorsal cochlear nucleus and immediately dorsal to the nucleus of the tractus solitarii.

Somatostatin-containing axons and nerve terminals have been detected in several extrahypothalamic brain areas. Dense or very dense fibre networks are seen in the nucleus accumbens, the caudate nucleus, the central amygdaloid nucleus, the parabrachial nucleus and the nucleus ambiguus. The nucleus of the tractus solitarius is rich in somatostatin-positive fibres as has been described in detail by Kalia and collaborators (1984).

It is known that the spinal cord content of somatostatin is the second highest in the nervous system, being lower only than that of the hypothalamus (Patel and Reichlin, 1978). Somatostatin in the spinal cord is mainly concentrated in the dorsal horn (Hokfelt et al., 1976; Johansson et al., 1984; Stine et al., 1984). Since these cell bodies are observed in spinal ganglia (Hokfelt et al., 1976) it appears that at least part of the somatostatin fibres in the dorsal horn are of primary sensory origin. This is supported by the fact that dorsal root ganglionectomy decreases somatostatin levels in the

dorsal cord (Stine et al., 1982). The large number of small immunoreactive cells in lamina II suggests that a large proportion of fibres in the superficial laminae have a spinal origin (Burnweit & Forssmann, 1979; Dalsgaard et al., 1981; Forssmann, 1978). This agrees well with rather small immunohistochemical changes observed in this region after dorsal rhizotomy and capsaicin application to sensory nerves (Jancso et al., 1981; Nagy et al., 1981). Somatostatin positive neurones can also be found in more ventral areas of the spinal cord as shown by many investigators (Burnweit & Forssmann, 1979; Dalsgaard et al., 1981; Forssmann, 1978) including neurones in the sympathetic intermediolateral nucleus (Dalsgaard et al., 1981). Furthermore, there is also evidence that somatostatin is present in descending pathways (Holets & Elde, 1982) particularly innervating sympathetic preganglionic neurones (Hancock, 1982; Holets & Elde, 1982). In fact, it has been shown that somatostatin content decreases both rostral and caudal to spinal transection, indicating the presence of ascending and descending somatostatin pathways within the spinal cord (Stine et al., 1982). The presence of a dense fibre network containing this peptide in the spinal cord has led Forssmann (1978) to propose the existence of a whole somatostatinergic system in the spinal cord itself.

Somatostatin is increasingly being shown in nerve tracts, in the classical sense of a compact bundle of fibres. This was shown earlier in the median eminence where fibres run in the external layer (Hokfelt et al., 1978a, b). Later, a long

descending projection from the amygdaloid complex to nucleus reticularis in the medulla oblongata was described by Kawai et al. (1982). Takatsuki et al, (1982) demonstrated a pathway from the cochlear nuclei to the contralateral inferior colliculi. Holets and Elde (1982) described a supraspinal pathway descending to the intermediolateral cell column in the spinal cord.

In other brain areas, immunocytochemical techniques have revealed individual axons rather than tracts (Krisch, 1978). This finding and the wide distribution of somatostatin-containing neurones in several brain regions suggests that at least some of the extrahypothalamic somatostatin-containing cells are interneurones with short processes. However, this assumption contradicts some of the evidence presented by Epelbaum (1982) and Palkovits et al. (1980). These studies isolated periventricular somatostatin cells from other brain areas by knife cuts and resulted in decreased amounts of somatostatin in several regions of the brain. The reduction of somatostatin may of course be due to either degeneration of somatostatin-containing axons or to a trans-synaptic trophic effect resulting in the failure of cells to synthesize somatostatin.

Somatostatin is implicated in neuroendocrine, motor, and higher cortical brain functions (Reichlin, 1983). Cortical somatostatin levels are selectively reduced in Alzheimer's dementia and Parkinson's disease, while striatal concentrations are increased in Huntington's disease (Epelbaum et al., 1983;

Reichlin, 1983). Other physiological effects include an increase in small intestine motility (Bueno & Ferre, 1982; Helke, 1984) and hypotension (Weitzman et al., 1979).

The possibility of some influence of somatostatin in cardiovascular regulation is not surprising. The anatomical distribution of this peptide as described above shows that somatostatin immunoreactivity is present in neurones of the central nucleus of amygdala which was retrogradely labelled by injection of fluorescent dyes into the nucleus of the tractus solitarius and dorsal motor nucleus of the vagus nerve. This suggests the presence of a long descending somatostatin-containing neurone system from the amygdaloid complex (Higgins & Schwaber, 1983; Kawai et al., 1982). In addition, the presence of this peptide in the hypothalamus, in specific brain stem structures such as dorsal motor nucleus of the vagus and nucleus tractus solitarius (Koda et al., 1985) and the presence of supraspinal pathway descending to the intermediolateral nucleus containing sympathetic preganglionic neurones (Holets & Elde, 1982) provides further evidence of a possible role in cardiovascular regulatory pathways. With these facts in mind it seems reasonable to raise the possibility that somatostatin may be participating in the control of the cardiovascular system.

Several investigators have implicated the area postrema as an important site for regulation of pressor responses (Szilagyi & Ferrario, 1981). Somatostatin-like immunoreactivity within the area postrema of the rat and cat has been demonstrated. In addition, possible origins of area postrema

somatostatin like immunoreactivity include autonomic nuclei that contain somatostatin cell bodies (e.g. dorsal motor nucleus and nucleus of the tractus solitarius) or projections from other pontine, medullary and hypothalamic nuclei (Helke, 1984; Krisch, 1981). The increased amounts of somatostatin-like immunoreactivity along the ventrolateral area postrema of the rat and cat with the nucleus tractus solitarius and dorsal motor nucleus may represent the point of entry of somatostatin fibres into the area postrema (Newton & Maley, 1985).

Somatostatin has been shown to cause hypotension when administered intracerebroventricularly in rats (Weitzman et al., 1979), and in cats (Delbarre et al., 1977). Recently, Koda et al. (1985) have directly probed into the possible role of medullary somatostatin as a neurotransmitter involved in blood pressure regulation in the rat. Their data indicate that intramedullary injection of somatostatin into the nucleus of the tractus solitarius evokes an immediate but transient hypotensive and bradycardic effect which is mediated through the parasympathetic nervous system. In addition,these responses are blocked by adrenergic blocking agents suggesting that central adrenergic mechanisms are involved. To our knowledge this is the first study examining the mechanisms by which somatostatin may alter the autonomic regulation of heart and blood pressure. It would be interesting to see how somatostatin behaves when given into the spinal cord directly. We find in the spinal cord not only an extensive somatostatinergic network but also fibres containing somatostatin descending to the

intermediolateral nucleus. Elde et al. (1984) have demonstrated that somatostatin immunoreactive fibres preferentially occupy the neuropil surrounding sympathoadrenal neurones. On the basis of this observation, they suggested a specific role for these fibres in regulating the adrenal medulla as opposed to oxytocin immunoreactive fibres which are preferentially distributed to other neurones besides sympathoadrenal neurones. This suggests that oxytocin is not involved in regulation of adrenal function.

On the basis of this information, the present experiments with somatostatin have attempted to specify some of its roles in regulation of heart rate, blood pressure and adrenal output of catecholamines. At the present time there is enough evidence for somatostatin to be considered as a putative transmitter. Its presence in synaptosomal fractions from several regions of the central nervous system, its calcium-dependent release from central tissues (Lee & Iversen, 1981) and its binding to specific receptors in the central nervous system (Reubi et al., 1981; Srikant & Patel, 1981) provide evidence for a putative role as a chemical mediator of synaptic transmission.

In view of the evidence implicating somatostatin in regulation of sympathetic preganglionic neurones and the additional evidence implicating it as a chemical mediator of synaptic transmission in other regions of the central nervous system, it was imperative that this be among the peptides surveyed in this study.

OXYTOCIN

THE MILKY WAY !

Cys-Tyr-Ile -Gln-Asn-Cys-Pro-Leu-Gly-NH_2

It is not easy to write a small review on the neurohypophyseal peptide oxytocin for two reasons: first, it is a difficult task to bring together the recent explosion of research on neurohypophyseal hormones; and second it is difficult to comment on oxytocin alone without mentioning vasopressin since most papers describe both of these hormones together. Nevertheless, an attempt will be made to keep the spotlight on oxytocin - the topic of this section.

The hypothalamo-neurohypophysial system has been studied extensively as a classical neuroendocrine unit. Neurones in the supraoptic and paraventricular nuclei synthesize the nonapeptide hormones arginine-vasopressin and oxytocin and their associated neurophysins. These peptides are transported then to terminal fields in the neural lobe of the hypophysis, from which they are released into the general circulation for delivery to distant target tissues such as the kidney, uterus and mammary gland (Scharrer & Scharrer, 19554; Sachs, 1969).

The major source of these peptides has been confirmed by immunohistochemical studies. In addition, they have revealed their presence in a large number of other neurones located within as well as outside of the hypothalamus. Additional magnocellular vasopressin or oxytocin neurones have been identified in a number of so called accessory nuclei scattered in various regions of the hypothalamus. These accessory nuclei contain intermingled populations of magnocellular vasopressin

and oxytocin neurones which for the most part project to the posterior pituitary. On the other hand, parvocellular vasopressin and oxytocin neurones have now been identified both inside and outside the hypothalamus which do not appear to project to the posterior pituitary.

Recently, neuroanatomical studies utilizing retrograde tracer and immunohistochemical techniques have demonstrated that immunoreactivity corresponding to oxytocin, vasopressin and neurophysins can be detected in neurones projecting from the paraventricular nucleus to the neuropil of the medulla especially the nucleus of the tractus solitarius and the spinal cord, including the autonomic preganglionic cell column (Swanson & Kuypers, 1980; Sawchenko & Swanson, 1982). This observation is of particular interest here because it indicates a possible role of these projections in integrating autonomic function.

Anatomical evidence for the existence of direct pathway(s) originating from paraventricular nucleus of the hypothalamus and projecting to the other brain areas including intermediolateral nucleus of the spinal cord is well established (Hancock, 1976; Hosoya & Matsushita, 1979; Kuypers & Maisky, 1975; Nance, 1981; Ono et al., 1978; Saper et al., 1976; Swanson & Kuypers, 1980). In the brain stem, these fibres appear to terminate mainly in the caudal brain regions, including the nucleus of the tractus solitarius, the nucleus ambiguus (Buijs, 1980), the locus coeruleus and the dorsal vagal complex (Swanson & Hartman, 1980).

In the spinal cord a long projection from the paraventricular nucleus to intermediolateral nucleus has been shown. For example, Kuypers and Maisky (1975) demonstrated that neurones in the paraventricular nucleus were retrogradely labelled following horseradish peroxidase injection into the spinal cord. Their results were confirmed by Saper et al. (1976) who studied the anterograde transport of tritiated amino acids from the paraventricular nucleus to the spinal cord and found labelled fibre terminals in the intermediolateral nucleus. The existence of this pathway has also been supported by electrophysiological studies (Caverson et al., 1984; Yamashita et al., 1984; for details see section on hypothalamus, 3. iii).

Some of the fibres in these projections display immunoreactivity for arginine-vasopressin, oxytocin or their respective neurophysins (Buijs, 1978; Nilaver et al., 1980; Sawchenko & Swanson, 1982; Sofroniew, 1980; Sofroniew & Weindl, 1978; Swanson, 1977; Swanson & McKellar, 1979; Swanson & Sawchenko,1980). For example, some fibres have been stained immunohistochemically with an antiserum to neurophysin I in rat and bovine brains (Swanson, 1977), and with antisera to oxytocin (Buijs, 1978; Nilaver et al., 1978; Swanson, 1978) and vasopressin (Buijs, 1978) in the rat.

A noticeable point in these pathways is that oxytocin-containing fibres appear to be more predominant than the vasopressin-containing fibres. In fact, the ratio of oxytocin to vasopressin fibres seems to be approximately 4 to 1

at the level of the caudal medulla oblongata (Sofroniew & Schrell, 1981).

In the spinal cord, it was reported that the fibres stained with a cross-absorbed antiserum to bovine neurophysin I (Swanson, 1977). Since it is known that neurophysin I is specifically associated with oxytocin in bovine brain (Vandesande et al., 1975), it was concluded that these fibres were oxytocinergic, originating in the paraventricular nucleus and perhaps in the adjacent cells of the lateral hypothalamic area and zona incerta. This conclusion is supported by the observation that a neurophysin-stained paraventriculo-spinal tract can be traced from the hypothalamus, through ventrolateral parts of the reticular formation, to the lateral funiculus (Swanson 1977), and by the fact that neurophysin-stained neuronal cell bodies have not been found in the brain stem or spinal cord.

The oxytocin-stained fibres have been reported in the dorsal horn, central gray (Buijs, 1978; Nilaver et al., 1978; Swanson, 1978) and intermediolateral nucleus (Nilaver et al., 1978; Swanson, 1978) of the rat. These fibres are also present in lateral horn of the monkey spinal cord (Swanson, 1978). Interestingly, the homozygous Brattleboro rat, which does not synthesize vasopressin, shows normal terminal fields of the oxytocin projections (Buijs, 1978; Nilaver et al., 1978; Swanson & McKellar, 1979).

It is noteworthy that oxytocin-containing fibres innervate the marginal zone of the dorsal horn equally densely at all levels of the cord, whereas the innervation of the lateral horn is more dense at upper thoracic, lower thoracic and lumbar cord segments (Jenkins et al., 1984). This suggests the possibility that the specific visceral organs are influenced preferentially by descending information from the paraventricular nucleus. For instance, Swanson & McKellar (1979) have suggested a preferential oxytocinergic innervation of the preganglionic neurones between T_9-T_{11} regulating the adrenal gland (Schramm et al., 1975). These results, however, are in direct conflict with the recent study by Elde et al. (1984) who have demonstrated that somatostatin immunoreactive fibres preferentially occupy the neuropil surrounding sympathoadrenal neurones while oxytocin immunoreactive fibres are preferentially distributed to other neurones besides sympathoadrenal neurones. On the basis of these observations, they suggested that somatostatin is involved in regulating the adrenal medulla and that oxytocin is not involved in regulation of adrenal function.

The presence of oxytocin nerve fibres and terminals in specific brain stem nuclei and spinal cord regions which are thought to be involved in cardiovascular control, and the possible function of this peptide as a neuro-regulator (Versteeg et al., 1979) suggest a possible role of oxytocin in central cardiovascular regulation. Versteeg et al. (1983) have reported that oxytocin administered intracisternally in

anaesthetized rats attenuates the pressor response and produces bradycardia evoked by electrical stimulation of the mesencephalic reticular formation, the active sequence within the oxytocin molecule being the C-terminal tripeptide propyl-leucyl-glycinamide. In other such studies, while Zerbe et al. (1983) have shown that oxytocin given into the ventricles of the awake rats produces tachycardia but has no effect on blood pressure, Feuerstein et al. (1984) show evidence that with the same route and with the same dose oxytocin produces no change in either heart rate or blood pressure in the awake rat.

Intracisternal oxytocin has been reported to produce an increase in arterial pressure in the dog (Montastruc & Tran, 1984; Tran et al., 1982), but these results are in conflict with a recent paper in which intracisternal administration of oxytocin had no effect on mean arterial pressure or heart rate in the rat (Petty et al. 1985). In this latter study intravenous injection, however, induced a dose related biphasic change in mean arterial pressure accompanied by bradycardia.

Focussing specifically on the spinal cord, iontophoretic application of oxytocin has been reported to produce a delayed, slow and prolonged excitation of sympathetic preganglionic neurones in the intermediolateral nucleus (Backman & Henry, 1984b). In addition, electrical stimulation of the paraventricular nucleus causes release of oxytocin along with vasopressin from the spinal cord (Pittman et al., 1984).

Mechanisms of action by which oxytocin produces cardiovascular changes remain unclear. Two studies have claimed that oxytocin induces an increase in blood pressure independent of variation in sympathetic tone since the increase in the pressor response persists in demedullated dogs or the dogs with diabetes insipidus (Montastruc & Tran, 1984; Tran et al., 1982). Versteeg & coworkers (1983) implicate medullary raphe areas as a possible site of action.

As in any other integrative action of a peptide, control by oxytocin may involve a number of variables at various levels of the neuraxis. Neuroanatomical data showing its localization in various nuclei and pathways already exists in abundance; what is needed now is more systematic physiological data to understand the functional significance of this peptide. This need establishes the rational for the respective experiments described in this thesis.

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THE CHALLENGE !

"Solutions to important basic and clinical problems, once exclusively within the domain of cardiovascular physiology and cardiology, will ultimately require detailed integrative and cellular studies of the brain. This increasing appreciation of the role of the nervous system in cardiovascular disease is stimulating the development of a "cardiovascular neurobiology".

Cohen and Cabot (1979)

The Scenario

The involvement of the central nervous system in the regulation and modulation of cardiovascular functions is beyond doubt. There is also enough anecdotal history to suggest that certain external environmental effects on the nervous system, particularly of a stressful nature, can have profound and lasting cardiovascular consequences. This has led to a proliferation of epidemiological studies emphasizing the role of environmental, psychological and social factors in the etiology of cardiovascular diseases such as hypertension, myocardial infarction, stroke and sudden cardiac death (Cohen & Obrist, 1975; Heart Facts, 1979). Consequently, there has been the development of behavioral models investigating normal and abnormal cardiovascular responses to environmental events, including models emphasizing the importance of previous experiences in life (Cohen & Obrist, 1975; Smith, 1974). There has also been the development of physiological models which have studied the effects of stimulation, lesions or drugs on various cardiovascular parameters including pathological conditions such as orthostatic hypotension, myocardial lesions, cardiac arrhythmias, etc. (de Jong et al., 1977).

Recently, the recognition of the role of peptides in the control of arterial blood pressure has been one of the most dramatic steps toward this research. Although still in its infancy, the challenge is there to try to integrate the existing data and to further this understanding by systematic research.

This challenge has an immediacy which is refelcted in the following statistics: of the nearly one million deaths from cardiovascular disease in the United States in 1976, approximately 65% resulted from heart attack (Heart Facts, 1979). More than 100,000 of these individuals died suddenly and unexpectedly with no previous history of heart disease (Lown, 1979). These sudden deaths generally are caused by ventricular fibrillation and there is evidence to show the involvement of brain mechanisms in the initiation of this malignant arrhythmia (Lown, 1979; Schwartz et al., 1978). As another example, Sudden Infant Death Syndrome claims 10,000 lives annualy in United States. The leading hypothesis maintains that it results from a lethal arrhythmia that may be related to an abnormality in the sympathetic cardiac innervation or in its development (Schwartz, 1976).

How do we meet this challenge systematically? The first step toward this goal is the identification and characterization of the neural circuitary influencing cardiovascular function. With the advent of new methods, there is now an adequate anatomical foundation to begin studying the physiological aspects regarding the roles of peptides in how the brain influences cardiovascular activity.

The results from the application of anatomical methods to studying the circuitry of central pathways regulating the cardiovascular system have tended to emphasize the concept of descending functional pathways that mediate patterns of cardiovascular adjustments specific to given behavioural

demands (Cohen & Obrist, 1975; Hilton, 1970). For instance, exercise requires increased cardiac output and increased blood flow in the active muscle beds, and a descending pathway originating in the motor cortex has been hypothesized to mediate this cardiovascular response (Uvnas, 1954; refer to section 3. (iii) of this thesis for details). On the other hand, assumption of an upright posture (orthostasis) requires cardiovascular adjustments to maintain a constant mean arterial blood pressure and especially adequate cerebral circulation which is mediated by a different pathway arising in a restricted region of the cerebellum (Doba & Reis, 1974; refer to section 3. (ii) for details). The concept that multiple descending pathways are involved in cardiovascular control is a significant departure from the traditional 'centre' concept and lays a solid foundation for the contemporary 'connectionistic' approach.

With this conceptual shift comes an appreciation for the high degree of specificity in the sympathetic outflow. While this kind of specificity can more readily be seen in the parasympathetic system, Cannon's concept of a regionally and functionally non-specific sympathetic output was prevalent until recently. With our growing awareness of the specificity of descending pathways comes the concept that certain pathways may be more involved in mediating phasic changes (for example during orthostasis and exercise) while other pathways would be involved primarily in mediating more tonic or homeostatic functions.

The objective

The experimental framework for the present thesis was designed in the light of this background. Five peptides namely, substance P, angiotensin II, thyrotropin-releasing hormone, somatostatin and oxytocin were chosen for the reasons discussed earlier, in each respective section. The experiments were designed to study the physiological roles of these peptides in spinal autonomic pathways in the rat. Previous evidence cited is mainly anatomical evidence, that these peptides exist in the lateral horn and are located specifically in pathways descending from supraspinal structures involved in cardiovascular control. What is lacking, however, is direct evidence that these peptides have an action in autonomic pathways, an action specifically on neurones involved in control of cardiovascular parameters.

The peptides used in the present study were administered into the spinal intrathecal space of the rat and measurements were made of arterial pressure, of heart rate and of adrenal medullary output of catecholamines. Thus the unique contributions of this approach were to establish whether there is any physiologically relevant action and whether the actions of a particular peptide have any functional significance specifically in the regulation of cardiovascular parameters.

There are some important specific questions which this experimental approach can elucidate, which other available techniques can not. The intrathecal catheter can be placed at

the desired level of the spinal cord. Thus, by comparison of the effects on heart rate after administration of peptides into the upper thoracic spinal cord, on the one hand, and of the effects on adrenal medullary output of administration into the lower thoracic spinal cord, on the other, differences in the neurochemical properties of descending pathways controlling the two types of output can be identified. For example, it might be found that although peptide A and peptide B are both localized in terminals in the lateral horn, peptide A induces changes in the heart rate but not in adrenal medullary output, while peptide B induces changes in adrenal medullary output but not in heart rate. In this case, the results would provide physiological evidence that control mechanisms regulating different autonomic functions can be differentiated on a neurochemical basis.

Electrophysiological studies can identify cellular actions of peptides on single sympathetic preganglionic neurones and thereby provide precise information on the specific sites of action. However, if a peptide acts on interneurones connected to sympathetic preganglionic neurones regulating cardiovascular parameters, such an action would go undetected in the electrophysiological experiments and the conclusion could be made that the peptide plays no role in control of sympathetic output at the spinal level. In the present intrathecal experiments, however, such an action would manifest itself as a change in these parameters. The present study, therefore, in being non-selective in the cellular site

of action, could provide information on which peptides have a physiological function in spinal autonomic pathways regardless of site.

Various studies on the physiological roles of peptides in central control of the cardiovascular system have applied different peptides at the level of the medulla, the hypothalamus or in forebrain structures. To our thinking this involves a complicated circuitry which makes it difficult to interpret the results obtained from such experiments. This by no means reduces the importance of such studies. It only seems more appropriate to start the study at the level of the spinal cord, with a relatively simple circuitry. This one step, taken in the present thesis, toward a global understanding of the central regulation of the cardiovascular system will perhaps shed some light on the role of peptidergic, descending, functional pathways.

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CHAPTER I

PEPTIDE DISTRIBUTION AFTER INTRATHECAL ADMINISTRATION

INTRODUCTION

Despite the widespread use of the intrathecal technique in neurobiology, little is known about the normal diffusion or penetration of the injected material along the axis or into the grey matter of the spinal cord. Attempts have been made to trace the diffusion of radiolabelled morphine in the intrathecal space as well as the progression of analgesia (Yaksh & Rudy, 1977) but, as those agents pass readily across the blood brain barrier they can hardly be considered reliable indicators of the fate of all substances delivered into the intrathecal space. This led us to undertake a project which would evaluate the intrathecal technique in a multifaceted way, applying various methodologies to study the movement of injected substances both into the grey matter and along the axis of the spinal cord. Since we are interested in peptides, we have chosen a radiolabelled peptide for this study, ^{125}I-substance P.

METHODS

Animal preparation

Male Sprague-Dawley rats (approx. 350g) were anaesthetized with urethane (2.5g/kg i.p.). An intrathecal catheter (Intramedic PE-10) was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the desired vertebral level. Spinous processes were used as landmarks. In our preliminary experiments the validity of this method for correct positioning of the inner tip of the catheter was confirmed in X-rays of rats implanted with wire filled catheters. (Yashpal et al., 1985). Labelled peptides were administered intrathecally via this implanted catheter.

In some experiments a second catheter was inserted into the right femoral vein for the withdrawal of blood samples.

Diffusion of Fast Green Dye along the rostro-caudal axis of the spinal cord

For this particular study, the rat was prepared as described above with an intrathecal catheter, but the spinal cord was exposed to the full length by laminectomy, so that the spread of the dye after injection could be recorded on videotape.

Ten µl of Fast Green dye solution were injected, followed by 10 µl of artificial cerebrospinal fluid to flush the intrathecal catheter. At this point, the timer was started

simultaneously with the video camera. The movement of the dye was videotaped throughout a 30 min period, the duration of the experiment.

Diffusion of ^{125}I-substance P along the rostro-caudal axis of the spinal cord

In this series of experiments, the animals were anaesthetized with urethane and an intrathecal catheter was implanted as described above. Substance P, ^{125}I-labelled with Bolton Hunter reagent (68 TBq q/mmol; Amersham laboratories) was mixed in artificial CSF to yield a solution containing 29×10^3 cpm/µl. A 10µl solution of the radiolabelled substance P (7400Bq; 0.2 µCi) was administered via the intrathecal catheter followed by 10 µl of CSF to flush the catheter. At one or 10 min post injection, the catheter was removed and the animal was sacrificed by decapitation. The spinal cord was ejected rapidly using a modified technique of Yaksh and Harty (1981). The time required for decapitation and removal of spinal cord was approximately 2 to 3 min. The cord was then cut into cervical, thoracic, lumbar and sacral regions on a paraffin-covered glass plate. Each piece of spinal cord was rinsed with cold saline, weighed and stored at -4 ^{o}C. The amount of radioactivity (cpm) present in different spinal cord regions was determined using a Searle gamma counter. In addition, the spinal catheter itself was counted to determine how much radiolabelled substance P was adhering to the plastic catheter.

Penetration of ^{125}I-substance P into the spinal cord

The animal preparation for this part of the study was the same as described above. The same solution of radiolabelled substance P was injected intrathecally. However, in this series of experiments, instead of separating all the regions of the spinal cord, only the appropriate region, where the catheter was implanted, e.g. thoracic or lumbar, was cut from the whole spinal cord. This piece of spinal cord was then immersed immediately for two minutes in CanLab Quick Freeze (Dichlorodifluoromethane: $C\text{-}Cl_2F_2$) maintained at -50°C ± 1°C. The sections were stored at -80°C until further processing.

Transverse and longitudinal 20 micron sections of frozen spinal cord were cut in a cryostat at -20°C under a safety light. The sections were mounted on slides and dried immediately with a hair blower for 20 to 30 sec. The autoradiographs were prepared by exposing the spinal sections to (^{3}H)-ultrofilm LKB Instrument Sweden for 24 days. The films were subsequently developed using Kodak D19 developer. The transverse tissue sections were counter-stained with a 0.05% solution of Cresyl Violet.

Passage of ^{125}I-substance P into the blood

To determine if any intrathecally administered labelled substance P crossed into the blood, 1 ml samples of blood were taken from a catheter implanted in the femoral vein. After a baseline sample of blood was withdrawn, radiolabelled substance

P was injected via the intrathecal route. The blood samples were then withdrawn at one, 6 and 11 min following injection. After each sample, the blood was replaced with an equal volume of warm, heparinized saline. The samples were placed in a Searle gamma counter for 2 min to determine the amount of radioactivity present.

RESULTS

Diffusion of Fast Green dye along the rostro-caudal axis of the spinal cord

Experiments with the green dye injection showed that at one min after the administration, the dye was present at one segment above and 2 segments below the tip of the catheter. At ten min post injection it had spread to 2 segments above and 2 segments below the tip of the catheter. At 30 min the dye could be seen 2 segments rostral and 3 segments caudal to the tip of the intrathecal catheter. Thus, the total spread remains in the vicinity of the injected site and does not bathe the whole length of the spinal cord.

Diffusion of ^{125}I-substance P along the rostro-caudal axis of the spinal cord.

The results showing the diffusion of radiolabelled substance P along the rostro-caudal axis of the spinal cord are presented in Figs 1 and 2. It can be seen that when the peptide was injected intrathecally at the thoracic level, the

radioactivity counts were significantly higher in that region at 1 min period post injection. These counts remained high even at 10 min after injection.

Penetration of ^{125}I-substance P into the spinal cord.

Representative autoradiographs showing the penetration of ^{125}I-substance P into the spinal cord grey matter are presented in Figs. 3 and 4. It was consistently observed that penetration to the lateral horn region occurred within 1 min of administration and that penetration at 10 min was similar.

Passage of ^{125}I-substance P into the blood.

Fig. 5 shows blood levels of label at 1, 6 and 11 min after radiolabelled substance P was given intrathecally. Data in Fig. 5 are expressed as counts per ml of blood; given that the average body weight of the rats used was 380 g, and that the blood volume of rats of this weight range is approximately 60 ml/kg, these data translate into whole blood contents of 0.69% at 1 min, and 2.3% at 6 and 11 min.

Discussion

One question often asked regarding the injection of drugs into the intrathecal, or spinal subarachnoid space is whether the injected compound remains in the vicinity of the catheter tip or whether it spreads to distant sites. One may also ask about the extent of penetration of the peptide into

the spinal cord. As the experiments described in this thesis have been based on the intrathecal technique, some preliminary experiments were done to answer these questions.

We first examined the possibility that the injected material might move longitudinally along the cord in the subarachnoid space, perhaps even as far as vegetative structures in the lower brain stem. Our experiments with Fast Green dye demonstrated that the injected material remained within the vicinity of the tip of the catheter during the 30 min time period of the experiment.

In a similar study with bromophenol blue dye, Yaksh and Rudy (1976b) reported that a 10 µl solution produced an even staining around the cord and failed to diffuse more than 2.5 cm from the tip of the catheter. Their study, however, examined only one time frame, 10 min after injection. With a 20 µl volume, the extent of spread was in some cases between 3-5 cm while 40 µl solution virtually bathed the entire cord (Fig. 6).

In our second series of experiments radio-labelled substance P was injected intrathecally to assess the spread of the peptide into spinal tissue rostral and caudal to the tip of the catheter. The highest counts were in the thoracic region, where the tip of the catheter lay. Relatively small amounts of radioactivity were observed in the cervical or sacral regions (Figs. 1 and 2). These results are supported by those of Candeletti et al. (1984) who have shown that the highest concentration of iodinated peptides (calcitonin and dynorphin) given intrathecally are localized in that particular region of

the spinal cord where the tip of the catheter is placed (Fig. 7). Moreover, these investigators have also shown that there is no significant diffusion of the injected substances into supraspinal structures. In a study of Wolf and Mohrland (1984), radiolabelled ^{3}H-leucine enkephalin or ^{3}H-leucine enkephalin plus (^{125}I-Tyr)-substance P diluted with unlabelled substance P was injected intrathecally at the lumbar region. The highest levels of radioactivity at 30 min after injection were present at the level of the tip of the catheter.

These results of Wolf and Mohrland agree with our results in terms of localization of the injected material; however, they differ from those of Candeletti et al. (1984) in terms of the diffusion to supraspinal structures. In this study of Wolf and Mohrland, while the presence of radioactivity in the lumbar enlargement was 139 to 680 times higher than the background levels found in skeletal muscle (0.1-2.3 cpm/mg tissue), detectable levels of radioactivity were also found in the medulla (14 times background) and the periaqueductal grey (2 times background) suggesting the drug distribution was not confined exclusively to the spinal cord.

Wolf and Mohrland's results not only disagree with Candeletti's group regarding the diffusion above the level of spinal cord but also are in conflict with that of Piercey et al. (1981) who presented evidence that following intraspinal injection of radiolabelled Tyr^{8}-substance P (3 mCi ^{125}I/mol) negligible amounts were found in supraspinal structures. In fact, when the distribution data are applied to

ED_{50} doses, it was found that intrathecal administration of substance P would result in a maximum of 0.02 pmoles in brain structures, 0.33 pmol in the cervicothoracic cord region, and 0.59 pmol in the lumbosacral region of the spinal cord (Fig. 8).

The assumption that the spinally injected material does not move into more rostral brain regions is further substantiated by studies in which ^{14}C-labelled morphine sulphate was injected via a spinal catheter and animals were sacrificed at intervals up to 60 minutes after the injection (Yaksh & Rudy, 1976a). At even the longest intervals neither forebrain nor brain stem radioactivity ever exceeded 0.15 percent of that recovered from the spinal cord.

The results from our own experiments and the data presented above show that the spread of the peptide after intrathecal administration is restricted to the vicinity of the tip of the catheter. This suggests that the diffusion to the supraspinal structures must be negligible.

With regard to penetration of substance P into spinal cord matter, autoradiographs of longitudinal sections show that this diffusion is restricted to less than 1 cm rostro-caudally at 1 and 10 min. This information is more relevant to physiological studies than the information described above because the presumed sites of action in these physiological studies lie in the spinal grey matter. It can be concluded, then, that the diffusion into spinal tissue is considerably more localized longitudinally than one would assume from the

diffusion of dye or other markers in the subarachnoid space.

Another important conclusion from this series of experiments is that beyond the initial distribution at one min following administration, 10 min distribution is not different in the rat. In fact, a parallel study by Yaksh and Rudy (1977) in which radiolabelled morphine was given intrathecally and transverse spinal slices were sampled for levels of radioactivity, it was found that the longitudinal distribution of percent of recovered activity was almost identical at two and at 30 min after administration (Fig. 9).

The autoradiographs of transverse sections in our study provided even further information on the penetration of the label because the internal structure of the spinal cord could be identified more clearly in these sections. Given that the probable site of action of substance P in provoking the sympathetic responses is in the lateral horn, the autoradiographs demonstrated that some label penetrated to this region of the spinal cord. These radiographs also demonstrated the phenomenon described above, that little difference exists in the distribution of the label at one, and 10 min after administration.

The fourth series of experiments was done to determine if the injected peptide, might pass from the perispinal space into the circulation. Thus a central or peripheral action of drug can be assessed. As can be seen in Fig. 5, negligible amounts (less than 1%) of radiolabelled peptide was found in the blood. Similar results were obtained upon intrathecal

administration of radiolabelled oxytocin.

Whenever a drug administered to any part of the central nervous system has a physiological effect, it implies some form of binding interaction between the drug and the nervous tissue. The binding could be specific or nonspecific. However, a more fundamental significance is suggested when a drug is found to bind, react with, or accumulate in, a specific anatomical structure of the brain. One sole purpose of studying the spread or penetration of the intrathecally injected drug in most studies described above was to find two types of information: whether the observed effects were localized in the spinal cord and whether higher brain structures were involved in eliciting those effects and to what extent. In other words, is the spinally injected material travelling up to the higher centres and, if it is, in what quantities. Most investigators have satisfactorily explained the functional significance of their results on the basis of the anatomical spread of the drug in the spinal cord. For instance, Yaksh and Rudy (1977) conclude in their study that "the effects of morphine and naloxone administered into the spinal subarachnoid space seem to be mediated by their effect on receptors limited to spinal cord". In our previous studies, physiological effects have been observed at one min after administration in the tail-flick paradigm (Yashpal et al., 1982; Yashpal & Henry, 1983, 1984) and the abruptness of these effects has been interpreted as supporting a local rather than a remote site of action.

However, the administration of drugs into the spinal

subarachnoid space must be interpreted with caution. Care must be taken in the experiments that administration does not cause irreversible damage to the cord either through hemorrhage or by physical pressure on the cord and nerve roots. In addition, changes in osmolarity, in ionic composition and in pH could alter spinal function.

Regarding the site of action, it is possible that the effect is mediated via an action on the nerve roots, or on the cell bodies and synapses which lie within the dorsal horn rather than those in the lateral horn and dorsolateral funiculus (Henry & Calaresu, 1972). If it is to be assumed that the ability of the drug to produce the effect is contingent upon the ability to reach the grey matter then the lipid solubility of the drug is an important factor. In the case of substance P, it is highly lipid soluble (Banks & Kastin, 1985). Thus, the time course of the effect in this case, not surprisingly, is rapid. In addition, Yaksh and Rudy (1977) observed that the onset of the antinociceptive action of fentanyl was much more rapid than the time required for an equianalgesic dose of morphine to produce its effect. On the basis of these results they suggested that the time difference may be due to the difference in penetration time resulting from the differential lipid partition coefficients of two drugs, fentanyl being highly lipid soluble while morphine is only slightly so. Since the spinal cord may be visualized as a myelin-covered column, the possibility that some drugs may penetrate only poorly, must be considered in evaluating the

time course of the drug effects.

This study indicates that the penetration of labelled peptide into the spinal cord is much more limited than one could have surmised from earlier studies. The rostro-caudal distance of this penetration was approximately one cm. In terms of depth of penetration, the label reached the lateral horn, even though the quantity reaching this depth is small. Furthermore, it was interesting to note that the diffusion of the label was approximately the same at one and ten min after administration. Finally, passage of the label into the blood was negligible.

On the basis of these studies it was concluded that the technical basis of the experiments justified the use of the intrathecal technique in the physiological experiments reported in this thesis.

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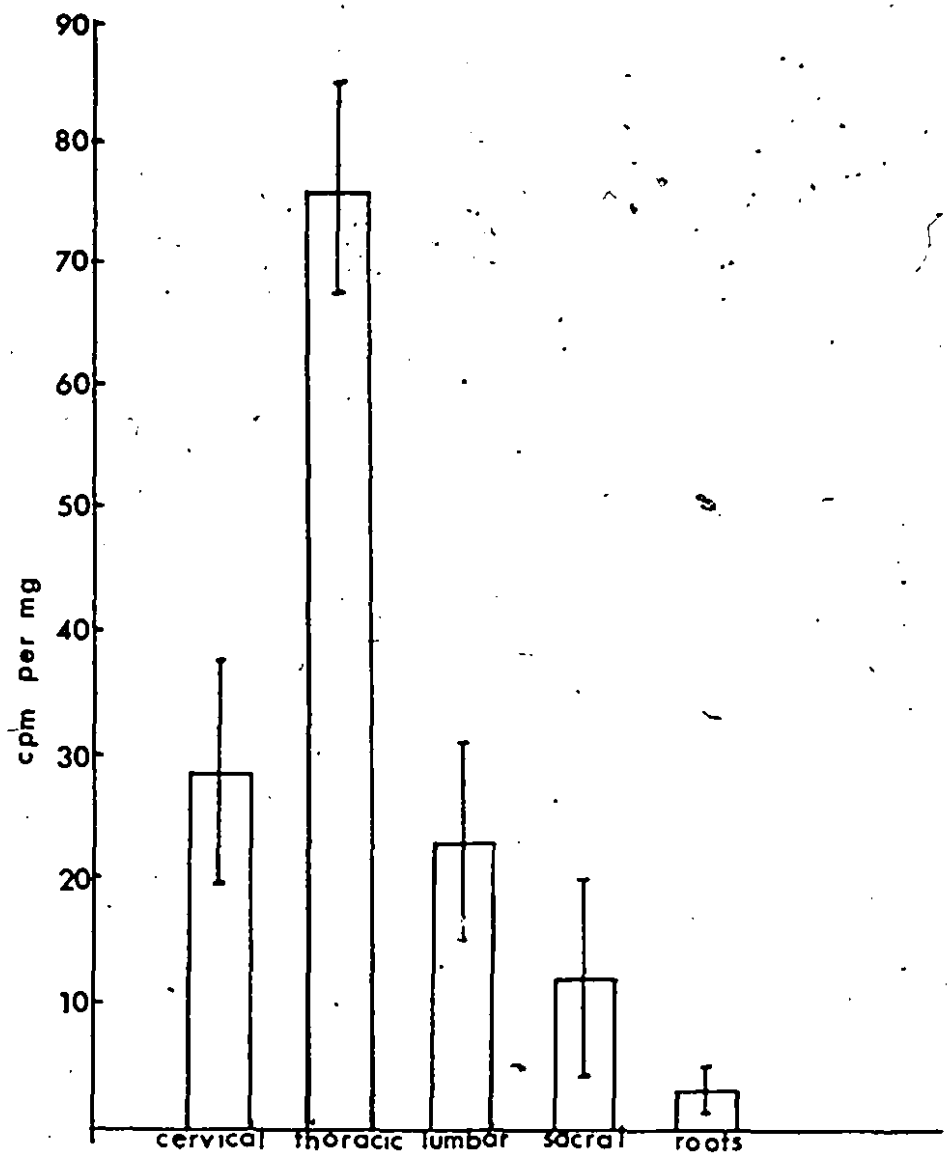


Figure 1. Histograms showing mean quantities of radioactive label counted in various spinal regions after injection of [^{125}I]-substance P at the ninth thoracic vertebral level. Spinal cords were removed at 1 min after injection. (n = 11)

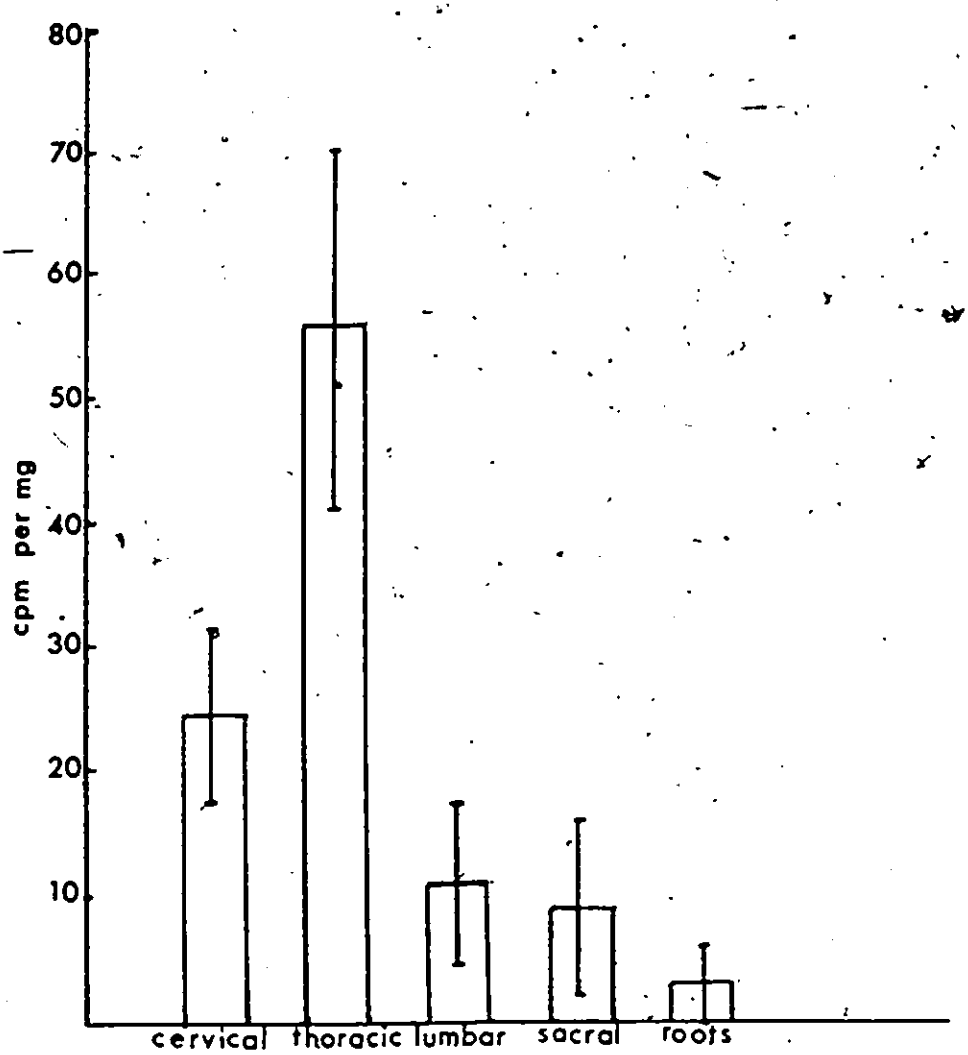
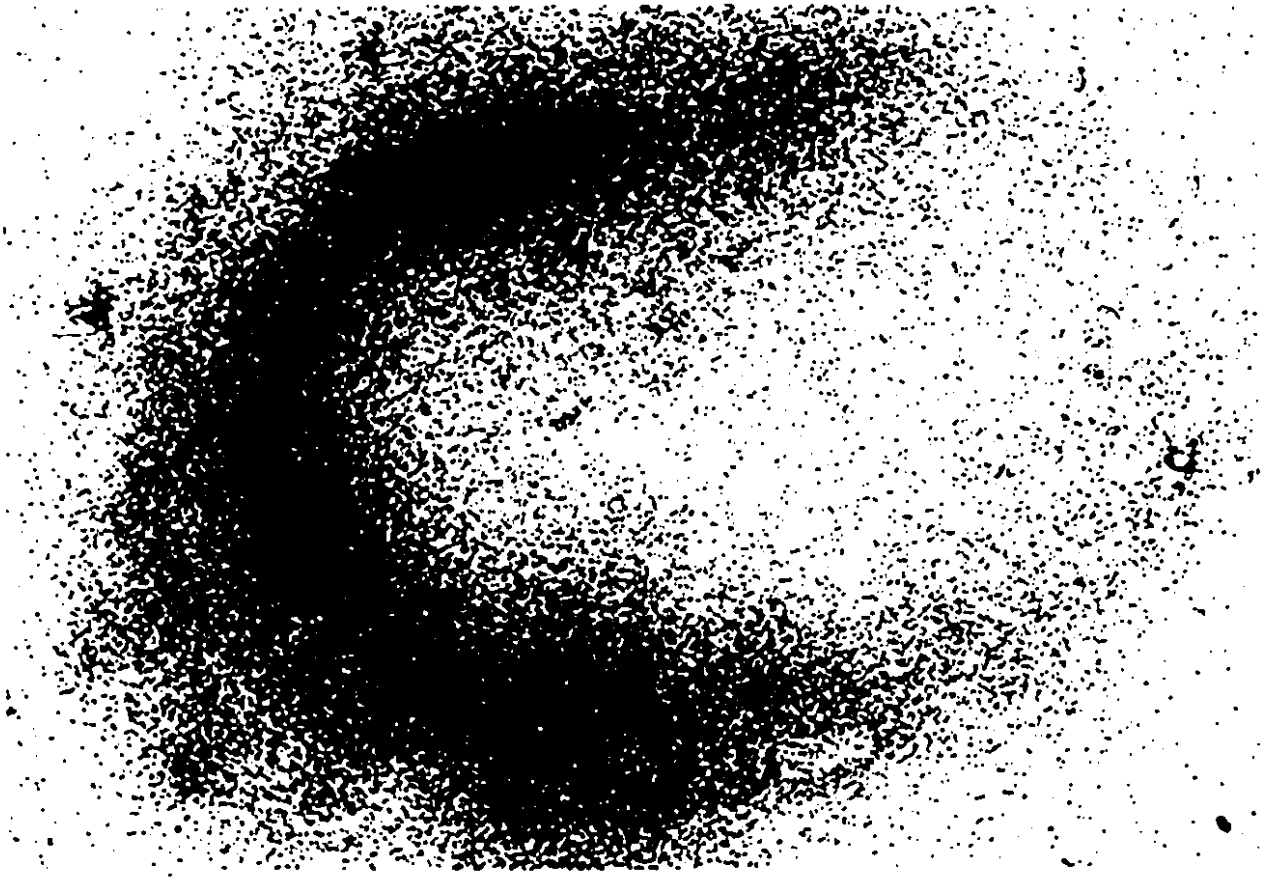


Figure 2. Histograms similar to those in Figure 1, but in rats (n = 7) from which spinal cords were removed 10 min after [^{125}I]-substance P was given intrathecally.

Figure 3. Penetration of [^{125}I]-substance P into transverse section of the ninth thoracic segment of the spinal cord of the rat. Upper photomicrograph (A) is one transverse section, 20 μm thick, stained with cresyl violet to show cell bodies. Lower photograph (B) is autoradiograph from same section, showing degree of penetration of label into the spinal cord. The spinal cord represented was removed 10 min after the labelled substance P was given.

A

B



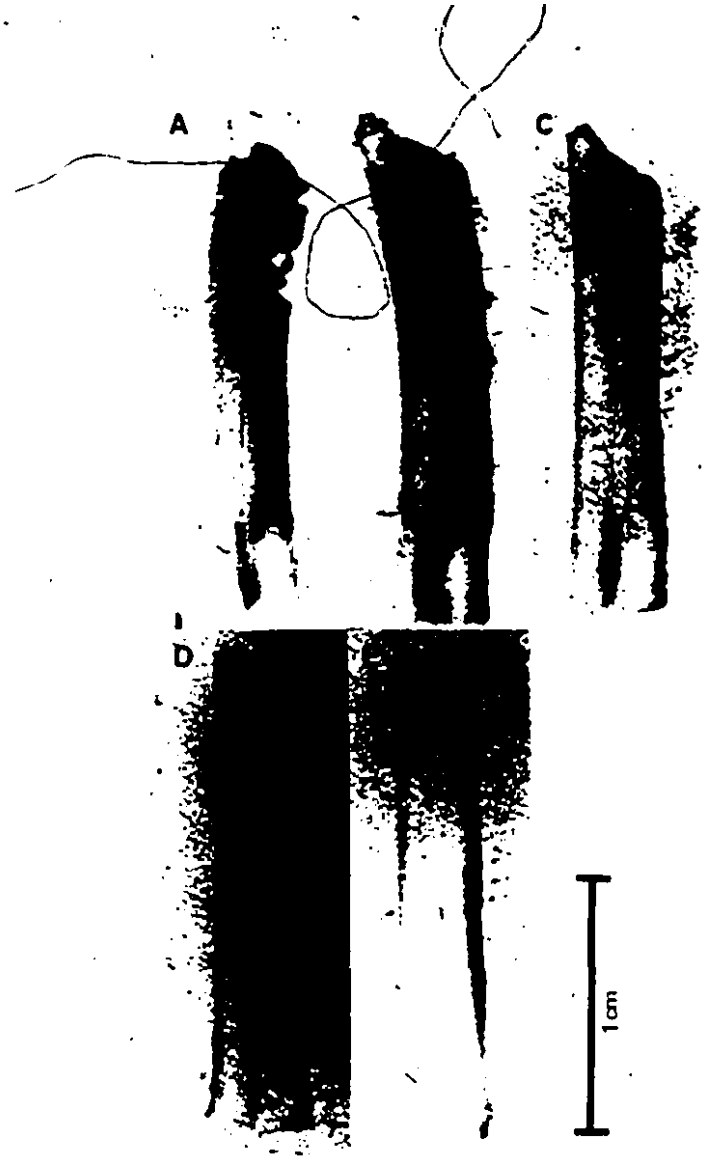


Figure 4. Autoradiographs of longitudinal sections of spinal cord around the ninth thoracic segment of one animal, showing distance of penetration of the label into the spinal cord. In this case the spinal cord was removed 1 min after injection of the labelled substance P. Each section shown is 20 μm thick. Sections A to E were 20, 80, 200, 400 and 560 μm, respectively, from the surface of the cord. It can be seen that the penetration into spinal tissue was concentrated within less than 1 cm rostrally and caudally from the site of injection.

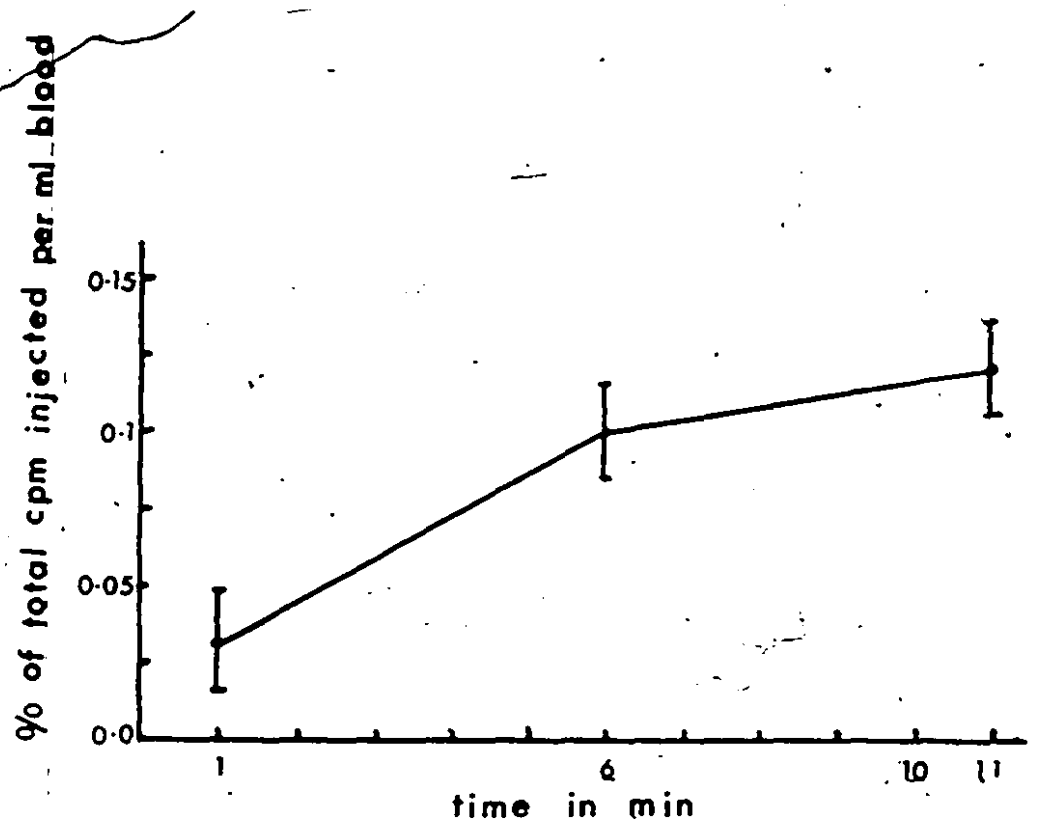


Figure 5. Passage of label into the circulation after intrathecal administration of [^{125}I]-substance P. Data shown are expressed as % of the total counts per min injected per ml of blood.

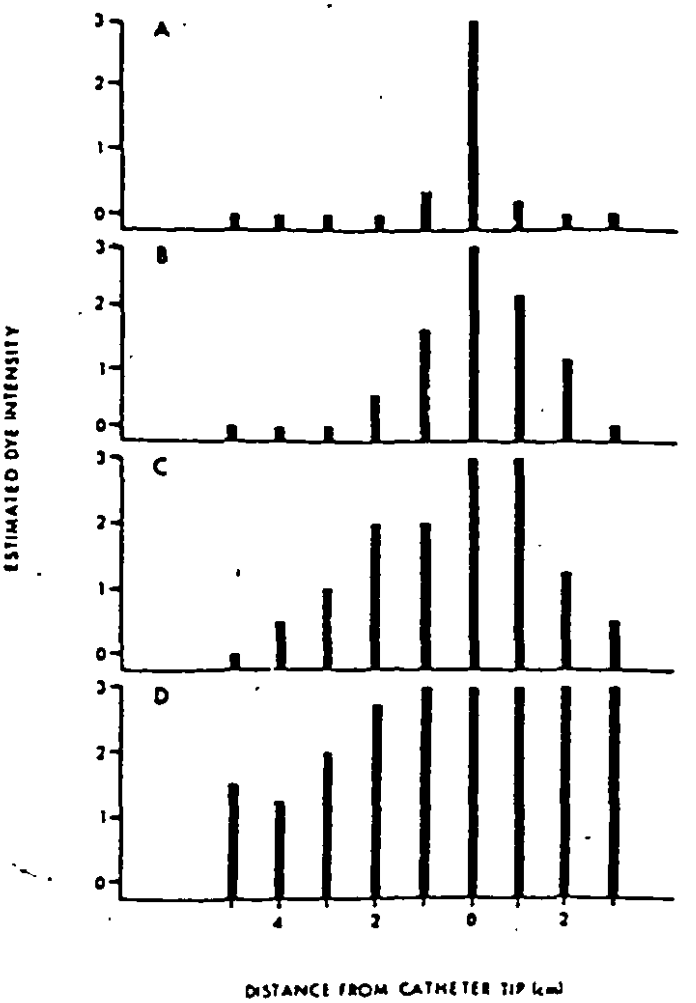


Figure 6. Figure from Yaksh and Rudy (1976b) showing extent of longitudinal diffusion of bromophenol blue dye along the spinal cord (the experimental animal is not stipulated, but it is assumed that it was from the rat). Animals were sacrificed 10 min after administration of the dye.

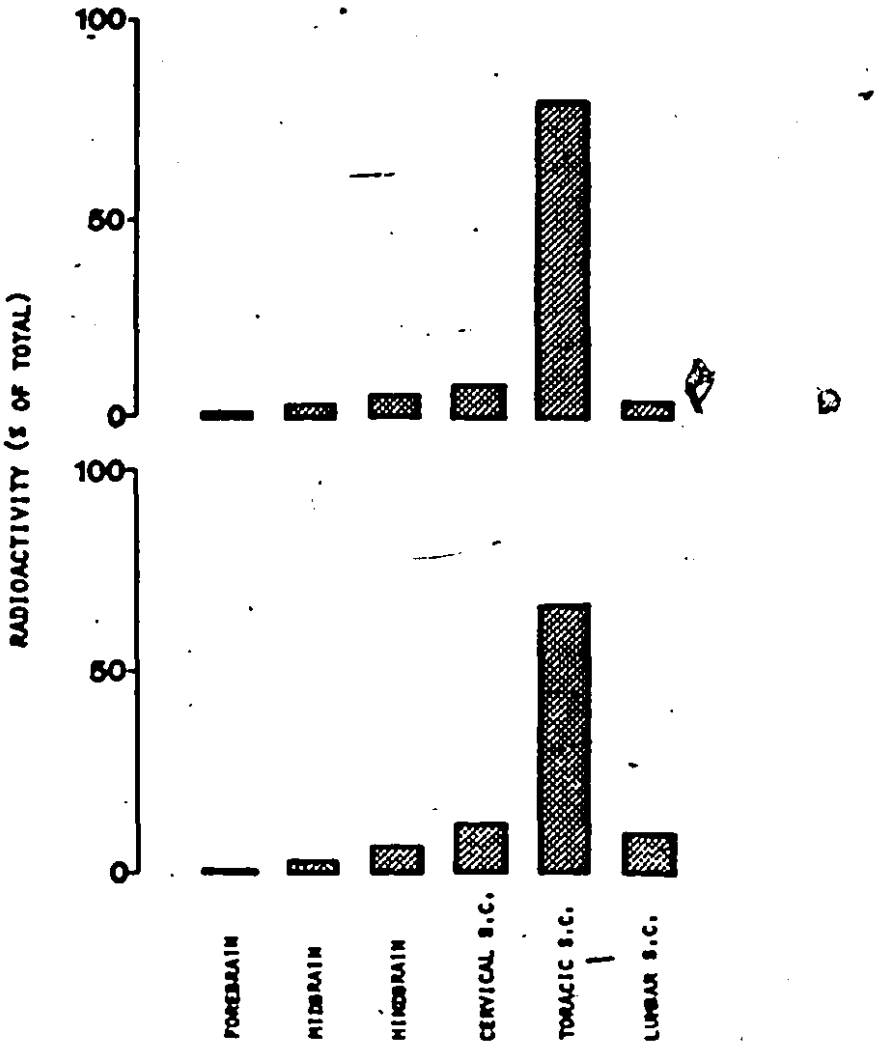


Figure 7. Histogram from Candeletti et al. (1984) showing regional localization of label after intrathecal administration of [^{125}I]-dynorphin (top panel) or [^{125}I]-calcitonin in the rat. Animals were sacrificed 60 min after administration of the peptide.

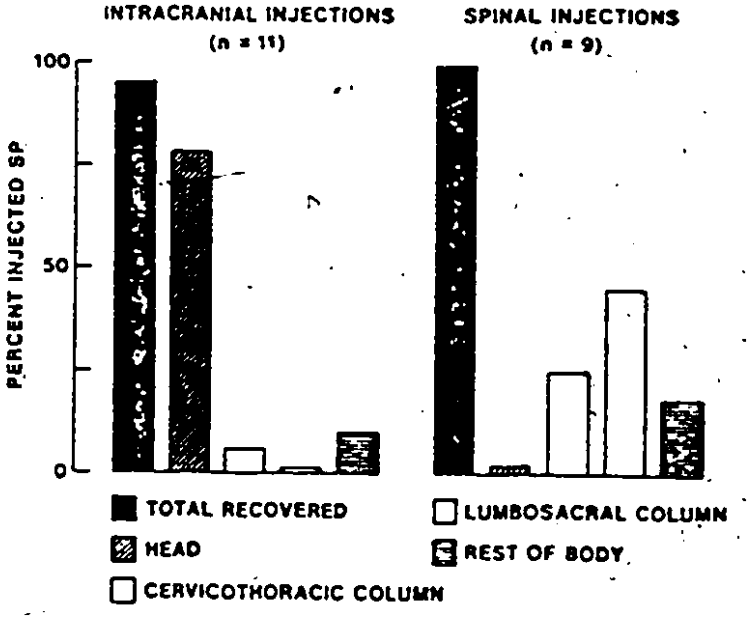


Figure 8. Histogram from Piercey et al. (1981) indicating regional distribution of label after intrathecal administration of labelled substance P to the lumbosacral region of the spinal cord in the mouse. Samples were taken at approximately 1 min after intrathecal administration.

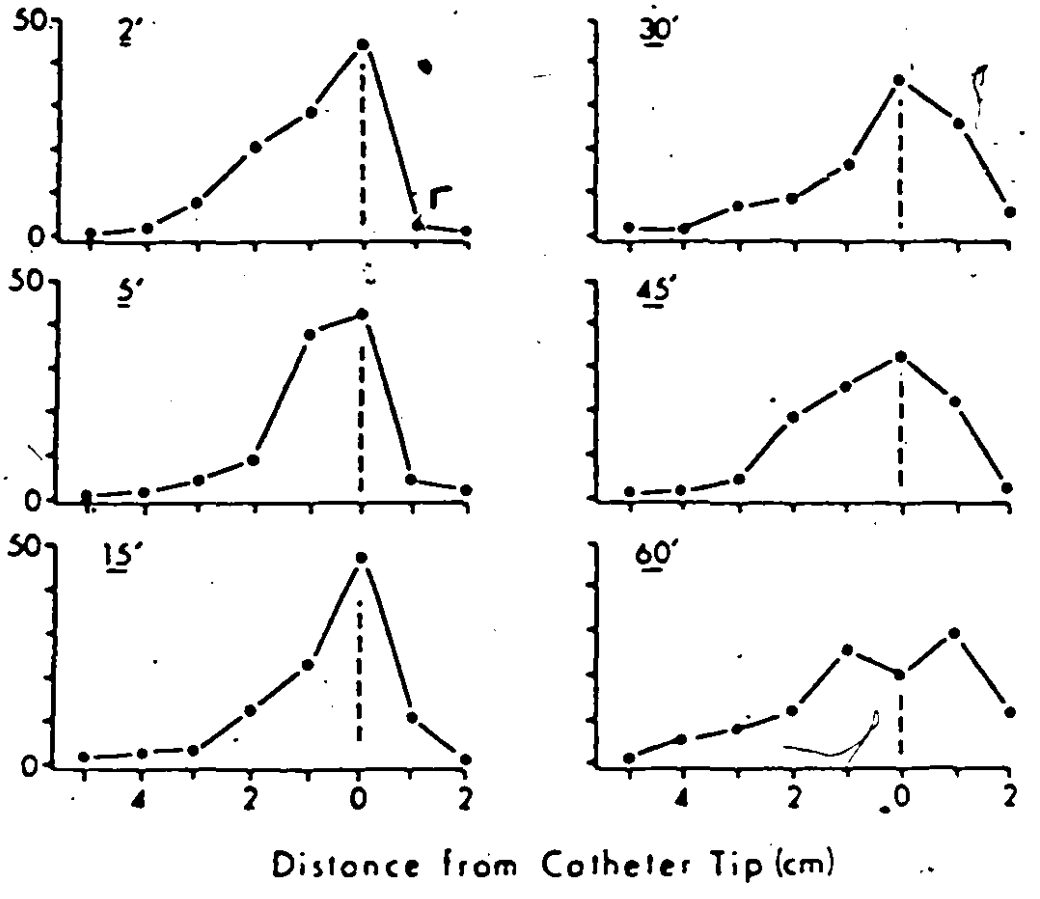


Figure 9. Figure from Yaksh and Rudy (1977) showing comparable distances of distribution of radiolabelled morphine at 2 to 60 min after intrathecal administration in the rat.

CHAPTER II

SUBSTANCE P GIVEN INTRATHECALLY AT THE SPINAL T9 LEVEL INCREASES ADRENAL OUTPUT OF ADRENALINE AND NORADRENALINE IN THE RAT

ABSTRACT

Administration of 10 µg of substance P intrathecally to the spinal T_9 level of the adult rat, anaesthetized with urethane, provoked an increase in free catecholamines in plasma taken from the inferior vena cava. Adrenaline levels at 1 min after administration were 154.8 ± 10.8% (mean ± SE; n = 11) of preadministration levels and noradrenaline levels were 153.5 ± 11.8% of preadministration levels. Differences between the values of free catecholamines in animals given substance P vs those given vehicle only were statistically significant at 1 and 10 min postinjection, but not at 30 min. Administration of a substance P analogue with central antagonistic properties 15 min before substance P was given prevented expression of the effects of substance P.

These results suggest that substance P may be an excitatory chemical mediator of synaptic transmission in spinal pathways controlling adrenal medullary output. Thus dysfunction of substance P mechanisms may underlie some animal models of hypertension and may be involved in some cases of essential hypertension in man as well as in autonomic dysfunction associated with some neurological entities.

INTRODUCTION

Essential hypertension in man has been attributed to dysfunction in the central nervous system and alterations in central nervous function exacerbate the increased arterial pressure in some animal models of hypertension. These facts provide the rationale for our on-going interest in central nervous regulation of the cardiovascular system and for our experimental approach. In the present study the focus has been on the role of substance P in spinal autonomic pathways, specifically those regulating adrenal function. Injection of Fast Blue or True Blue into the adrenal medulla of the rat has demonstrated that the majority of retrogradely labelled cells is located in the intermediolateral nucleus of segments T_7-T_{12}, with T_9 containing the largest number within any single segment (Holets & Elde, 1982). Nerve terminals containing substance P-like immunoreactive material are observed in the intermediolateral nucleus (Ljungdahl et al., 1978) surrounding sympathetic preganglionic neurons (Ditirro et al., 1981; Holets & Elde, 1982), including those neurons projecting directly to the adrenal medullae (Holets & Elde, 1982). The probable source of most, if not all, of these terminals is from cells in the brain stem because substance P is found in cell bodies of a number of brain stem nuclei, including the caudal raphe nuclei (Hokfelt et al., 1978; Chan-Palay, 1979), which have been implicated in cardiovascular control (Henry & Calaresu, 1974), as well as in a region near

the ventral surface of the medulla (Helke et al., 1982), where neuronal excitation by microinjection of kainic acid elicits an increase in arterial pressure (Loewy & Sawyer, 1983). This evidence suggests that substance P is a chemical mediator in some of the autonomic pathways descending directly from specific brain stem structures to neurons in the intermediolateral nucleus, including the sympathetic preganglionic neurons which project to the adrenal medullae. The recently demonstrated excitation of identified single sympathetic preganglionic neurons by the iontophoretic application of substance P (Gilbey et al., 1983; Backman & Henry, 1984) leads to the further possibility that this peptide is a mediator of excitatory input to these neurons.

In the present study on the physiological role of substance P in spinal pathways controlling specifically adrenal medullary output, the experiments were designed to determine the effects of the intraspinal administration of substance P on the levels of catecholamines in venous plasma. Some of the findings have been briefly reported in abstract form (Yashpal et al., 1983).

EXPERIMENTAL PROCEDURES

Animal Preparation

Male Sprague-Dawley rats (approx. 500g) were anesthetized with urethane (2.5 g/kg, i.p.). Three catheters were implanted under microscopic control. An intrathecal

catheter (Intramedic PE-10) was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the T_9 vertebral level, using spinal processes as landmarks. In our preliminary experiments the validity of this method for correct positioning of the inner tip of the catheter was confirmed in X-rays of rats implanted with wire-filled catheters (Fig. 1). It was via this catheter that substance P was administered intrathecally.

A second catheter (Intramedic PE-60) was passed via the femoral vein into the inferior vena cava so that the inner tip lay between the renal veins and the heart (see Fig.1). This catheter was used for the collection of blood.

The third catheter (Intramedic PE-60) was placed in the left common carotid artery facing the heart for continuous monitoring throughout the experiment of pressure, monitored via a Statham transducer (P23 DC) connected to a Grass P5 polygraph.

Rectal temperature was maintained at approximately 37°C by a heating pad.

Experiments with substance P

After surgical preparation a 30 min period was allowed for stabilization. Then a basal sample of 1.5 ml of blood was withdrawn and placed immediately into cooled heparinized Eppendorf tubes; this blood was immediately replaced with 1.5 ml of warm (37°C) physiological saline, heparinized to keep the catheter patent. After another 5 min, substance P

(Peninsula Laboratories) was administered in a single dose of 10 μg (6.5 nmol) delivered over a period of 15-20 sec in 10 μl of artificial cerebrospinal fluid (CSF; aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$). Following delivery of the peptide the intrathecal catheter was flushed with 10 μl of CSF (catheter volume was 6-8 μl). Blood samples were then taken, as before at 1, 10 and 30 min after substance P administration. The four samples were centrifuged at 2,000 rev/min at 4°C and the plasma was stored at -70°C until assayed for free catecholamines.

A dose of 6.5 nmol was selected because in earlier studies on intrathecal administration of substance P at the lumbar level, this dose was found to be effective in eliciting nociceptive responses (Piercey et al., 1981; Yashpal et al., 1982; Yashpal & Henry, 1985).

Experiments with [D-Pro^2,D-Phe^7,D-Trp^9]substance P

An additional series of experiments was done to test the effects of the substance P analogue [D-Pro^2, D-Phe^7, D-Trp^9]substance P, which has antagonist properties to substance P in the central nervous system (Piercey et al., 1981; Fuxe et al., 1982; Salt et al., 1982; Hanley, 1983; Stoppini et al., 1983). In this case, 5 min after the base sample of blood was taken, the analogue was given intrathecally in a dose of 10 μg in 10 μl of CSF, and the catheter was flushed as above. In a previous study (Yashpal & Henry, 1985)

this dose was found to block totally the effects of intrathecal administration of substance P on tail flick latency. Ten minutes later a sample of blood was taken as before and 15 min after the analogue was given substance P was administered in the manner described above. Three more blood samples were taken, at 1, 10 and 30 min after substance P; thus the effects of substance P were monitored as in the earlier experiments, except that the analogue had been given 15 min previously.

As a control for experiments with [D-Pro^2, D-Phe^7, D-Trp^9]substance P, vehicle replaced the analogue solution.

Assay techniques

Free adrenaline and free noradrenaline in each blood sample were quantitated by high pressure liquid chromatography (Waters M45 solvent delivery system, U6K manual injector, Bioanalytic Systems LC-4 electrochemical detector) using a method modified from Hallman et al., 1978. The mobile phase consisted of 92 parts of 0.1 M Na phosphate monobasic, 1 mM NaOH octylsulphate and 0.1 mM disodium ethylene-diaminetetra-acetate (pH adjusted to 5.5 with 2 N NaOH) and of 8 parts of methanol. The column was a Waters micro-Bondapak C_{18} maintained at 30°C with a water jacket to reduce the retention times. The electrochemical detector employed a glassy carbon electrode, with voltage set at +0.7 V and a sensitivity of 2.0 nA/V (filter C). The reliable detection limit was 0.5 ng/ml.

Statistical analysis

The levels of adrenaline and of noradrenaline were each calculated as a percent of their basal level taken 5 min prior to peptide administration. The rats were divided into two groups, those receiving substance P and those receiving vehicle, and the percent values were averaged within each group for each post administration sample time, i.e. 1, 10 and 30 min. The mean percent values thus obtained are plotted in Figs. 2 and 3. To determine statistical significance Student's t-test was applied to compare the two values (substance P vs vehicle groups) at each sample time for each of adrenaline and noradrenaline.

In experiments on the substance P analogue the same general procedure was followed, except that the values compared included also those taken 10 min after the analogue (i.e. 5 min before substance P) was given.

RESULTS

Our preliminary studies demonstrated that the diffusion of Fast Green dye over 30 min was limited to 2 segments rostrally and 3 segments caudally. Furthermore the onset of the response at 1 min suggests a local, rather than a remote site of action, and this latency is similar to that of intraspinal administration of substance P on tail flick latency (Yashpal et al., 1982).

In early experiments the validity of the technique of

intrathecal injection in studying chemicals for their effects on adrenal medullary output was tested by administering artificial CSF in a manner similar to that used for injection of substance P. This was done to determine whether injection per se altered this output; that this injection failed to alter vena caval plasma levels of either adrenaline or noradrenaline during the next 30 min is illustrated in Figs. 2 and 3.

Effects of substance P on plasma levels of adrenaline and noradrenaline

Intrathecal administration of substance P increased plasma levels of adrenaline. This effect was observed at 1 min after injection, when the first sample was taken, and was still present at 10 min after administration. The results obtained are summarized in the graph of Fig. 2.

Prior to administration the base values were the same for the rats given CSF and those given substance P, with means of 9.75 ± 1.81 (SEM) ng/ml plasma for the former (n = 12) and 8.29 ± 1.33 ng/ml for the latter (n = 17). However, at 1 min after administration, while the levels of adrenaline in plasma from control rats had a mean of 94.1 ± 4.5% of base value, those from substance P-treated rats were 154.8 ± 10.8%. This difference was statistically significant ($P < 0.005$). At 10 min after administration these values were 101.7 ± 5.5 and 141.9 ± 12.1, respectively, and at 30 min they were 101.3 ± 4.8 and 147.3 ± 19.4, respectively. The difference at 10 min was statistically significant ($P < 0.05$) but that at 30 min was not.

Noradrenaline levels were also elevated by intrathecal administration of substance P. The results obtained are summarized in Fig. 3. Mean base values were 3.96 ± 0.97 (n = 12) and 4.80 ± 1.01 (n = 18) for CSF- and substance P-treated rats, respectively. These values originated from the same plasma samples which gave rise to adrenaline values. One assay of adrenaline from a CSF-treated rat and one from a substance P-treated rat were unreliable and hence the numbers of samples giving rise to the data were different for adrenaline and noradrenaline. In substance P-treated rats the mean values at 1, 10 and 30 min after administration were 153.2 ± 11.8, 142.8 ± 10.7 and 128.6 ± 13.8 percent of base value, respectively. In contrast, the mean values in rats given vehicle only were 91.9 ± 7.5, 100.8 ± 7.8 and 102.3 ± 5.4 at 1, 10 and 30 min, respectively. The values at 1 and 10 min were significantly different between the two groups of animals ($P < 0.001$ and $P < 0.05$, respectively).

Effects of [D-Pro^2, D-Phe^7, D-Trp^9]substance P on responses to substance P injection

In experiments on the effects on plasma adrenaline of the intrathecal administration of substance P after the prior intrathecal administration of CSF or the analogue antagonist [D-Pro^2, D-Phe^7, D-Trp^9]substance P, base values were 9.06 ± 1.89 ng/ml plasma for rats given CSF (n = 12) and 8.73 ± 3.56 ng/ml for those given the analogue (n = 10). The results

are summarized in Fig. 4. One min after substance P was injected adrenaline levels in rats given CSF had become 196.4 ± 17.2% of the respective base values, while in those given the antagonist they were 104.3 ± 9.6%. These values for the two groups, at 1 min after substance P administration, were statistically significantly different ($P < 0.005$). The values were 126.9 ± 10.0 and 133.6 ± 16.8% at 10 and 30 min, respectively, after substance P administration in CSF-pretreated rats, and 94.4 ± 10.4 and 93.7 ± 8.3 for the same respective values in rats pretreated with the analogue: these differences were not statistically significant.

Similar results were obtained when plasma levels of noradrenaline were determined (see Fig. 5). Base values were 1.94 ± 0.34 ng/ml plasma for CSF-treated rats and 1.82 ± 0.22 ng/ml plasma for rats given [D-Pro^2, D-Phe^7, D-Trp^9]substance P. Respective values at 1 min after substance P administration were 184.9 ± 19.4% of base value for the former group of rats and 113.4 ± 12.6% for the latter. These differences were statistically significant ($P < 0.025$). At 10 and 30 min after substance P administration these values were 137.3 ± 20.0 and 134.5 ± 13.4%, respectively, for CSF-treated rats and 104.6 ± 23.9 and 114.8 ± 20.4%, respectively, for rats given the analogue; these differences were not statistically significant.

Effects of [D-Pro^2, D-Phe^7, D-Trp^9]substance P on plasma levels of adrenaline and noradrenaline

Ten minutes after the substance P analogue was given plasma levels of free adrenaline were 99.6 ± 8.7% of base value and those of free noradrenaline were 98.1 ± 15.2%. In comparison, the respective levels 10 min after CSF administration were 110.8 ± 20.0 and 113.8 ± 11.5%. Neither the values for adrenaline nor those for noradrenaline were statistically significantly different between the two groups of rats.

Other effects of [D-Pro^2, D-Phe^7,D-Trp^9] substance P

The analogue alone did induce changes in arterial pressure. Initially, there was an increase which was maximal at 5-10 min. This was followed by a decrease which persisted to the end of the longest measurement, at 1h. A similar effect on arterial pressure has been reported for a higher molar dose of another analogue antagonist of substance P (Loewy & Sawyer, 1983). However, in a number of our experiments the hypotension was enough to cause respiratory arrest within 20-30 min of administration; these animals were excluded from the results. In the animals which were included, however, the mean arterial pressure after 20 min began to fall below 80 mmHg.

DISCUSSION

We have thus demonstrated that administration of substance P to the ninth thoracic spinal segment increases levels of free adrenaline and noradrenaline in plasma collected from the inferior vena cava. This effect occurs when substance P is given in animals pretreated with intrathecal administration of CSF but not after pretreatment with [D-Pro^2, D-Phe^7, D-Trp^9]substance P, which blocks central effects of substance P in other paradigms as well (Piercey et al., 1981; Fuxe et al., 1982; Salt et al., 1982; Hanley, 1983; Stoppini et al., 1983; Yashpal & Henry, 1985). As administration was at the spinal level of origin of sympathetic fibers to the adrenal medullae (Almazon et al., 1982; Holets & Elde, 1982) the probable source of most of the increase in catecholamines is the adrenal medulla (Micalizzi & Pals, 1979), although some noradrenaline may be of neural origin. The most likely explanation for the observed effects then is that substance P excited sympathetic preganglionic neurons in the spinal cord, including those projecting directly to the adrenal medullae and this excitation in turn provoked an increase in the release of free catecholamines from adrenal medullary chromaffin cells. It is important to note that this indirect effect of substance P on adrenal chromaffin cells, an effect mediated via sympathetic preganglionic neurons, is opposite to the direct inhibitory effect of substance P on the release of catecholamines from adrenal chromaffin cells in vitro (Livett

et al., 1979; Role et al., 1981) because the two sites of action are different.

Our evidence supports the possibility that substance P is a chemical mediator of synaptic transmission in descending inputs to lower thoracic sympathetic preganglionic neurons (Ljungdahl et al., 1978; Chan-Palay, 1979; Ditirro et al., 1981; La Motte & De Lanerolle, 1981; Helke et al., 1982; Holets & Elde, 1982; Loewy & Sawyer, 1983). If this is the case then our data suggest that these inputs are distributed at least to sympathetic preganglionic neurons projecting to the adrenal medullae. Furthermore our results suggest that substance P mediates specifically an excitatory input to these neurons, acting to excite the preganglionic neurons which stimulate the release of catecholamines from the adrenals.

Possible clinical implications

This possibility in turn has important clinical implications. For example, in analogy to the potential role of overabundant levels of substance P in contributing to the pain of arachnoiditis (Hosobuchi et al., 1980), it is possible that an overabundant production and/or release of substance P at the lower thoracic spinal level may underlie some animal models of hypertension and perhaps be involved also in some cases of essential hypertension in man in which there is an excessive release of catecholamines from the adrenal glands.

Altered activity in substance P-containing pathways may also be involved in the autonomic dysfunction associated with

neurological entities such as Multiple System Atrophy with autonomic failure (Shy-Drager syndrome). For example, it has been found that patients with this syndrome have significantly reduced levels of substance P in lumbar cerebrospinal fluid compared to patients with other neurological deficits but lacking the autonomic failure (Nutt et al., 1980). Furthermore, Shy-Drager patients are characterized by a lack of the orthostatic reflex, a compensatory shunting of blood when rising from the supine to the standing position, and recent evidence (Ziegler et al., 1977) has been presented that while recumbent these patients have normal resting levels of noradrenaline, but they fail to show the normal increase in these levels upon standing or upon exertion. Therefore considering this evidence in the light of our present results, it is suggested that substance P-containing pathways, descending to the sympathetic preganglionic neurons which regulate adrenal medullary function, may participate not so much in the regulation of baseline release of catecholamines, but under conditions for which an appropriate response is a sudden and perhaps prolonged increase in this release. Thus in the absence of long-term pathology, substance P may participate in the activation of sympathetic preganglionic neurons during increased catecholamine release associated with such conditions as stress, loss of blood, hypothermia, hypoglycemia, hypoxia and extreme physical work.

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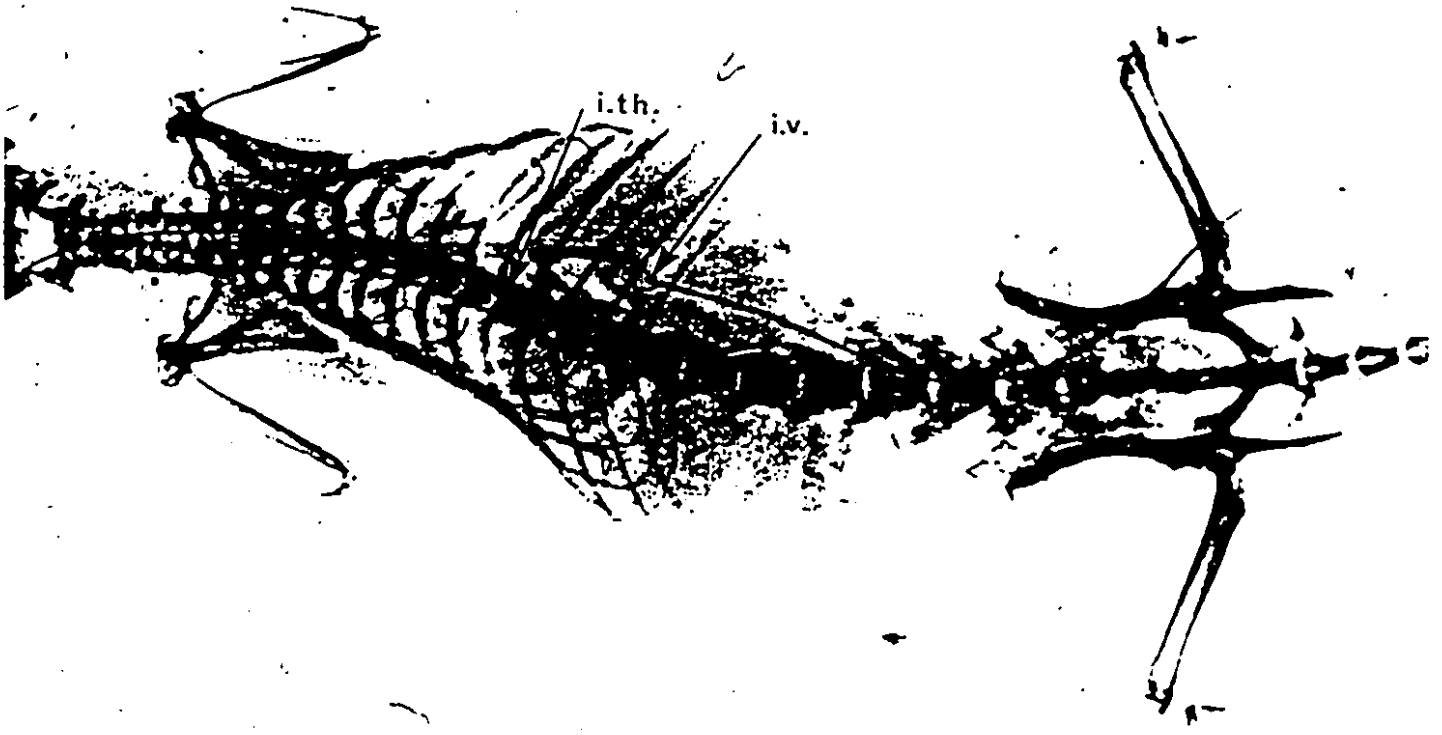

Figure 1. X-ray of rat illustrating positions of intrathecal (i.th.) and intravenous (i.v.) catheters in anesthetized rat. Once the catheters were implanted, fine stainless steel wires were inserted so that the inner ends were flush with the tips of the respective catheters. The tip of the intrathecal catheter was at the level of the ninth thoracic vertebra; that of the intravenous catheter was in the inferior vena cava at a level between the adrenal veins and the heart.

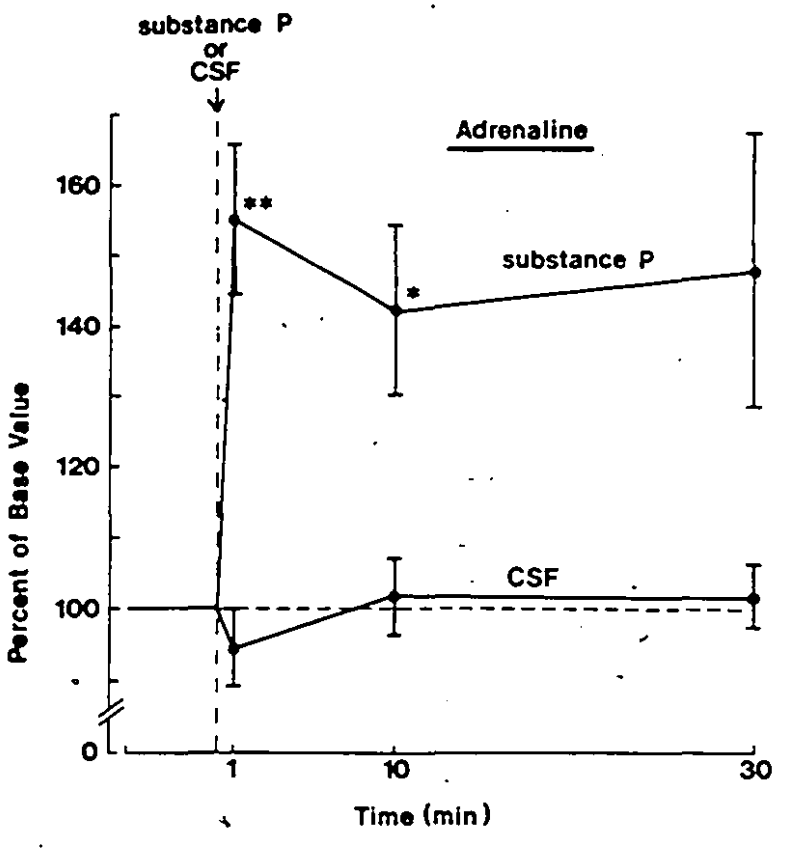

Figure 2. Time-effect curve for the effect on venous plasma free adrenaline of substance P (10 µg; n = 12) and of artificial cerebrospinal fluid (CSF; n = 11) injected intrathecally at the T_9 spinal level of the rat. The ordinate shows adrenaline level expressed as a mean percentage of the base value in blood which was taken prior to intrathecal injection. Vertical bars each represent the SEM. The abscissa shows the time after intrathecal injection; the vertical arrow indicating the time of injection taken as 0 min. *, $P < 0.05$; **, $P < 0.005$.

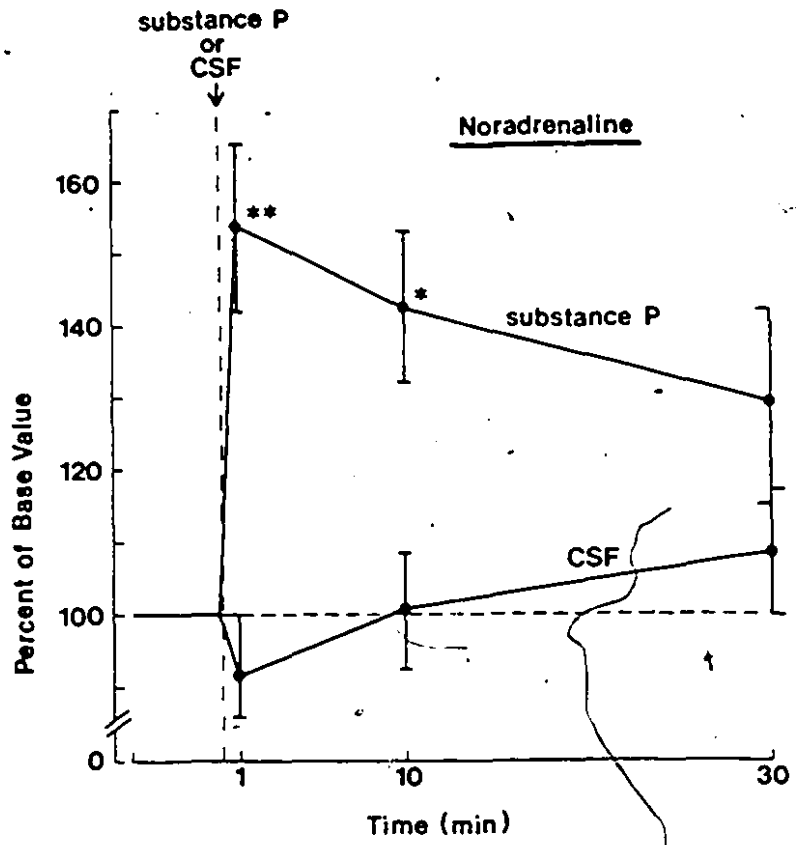

Figure 3. Time-effect curve for the effect on venous plasma free noradrenaline of substance P (10 µg; n = 18) and of artificial cerebrospinal fluid (CSF; n = 12). The curve is otherwise similar to that in Fig. 2. *, $P < 0.005$; **, $P < 0.001$.

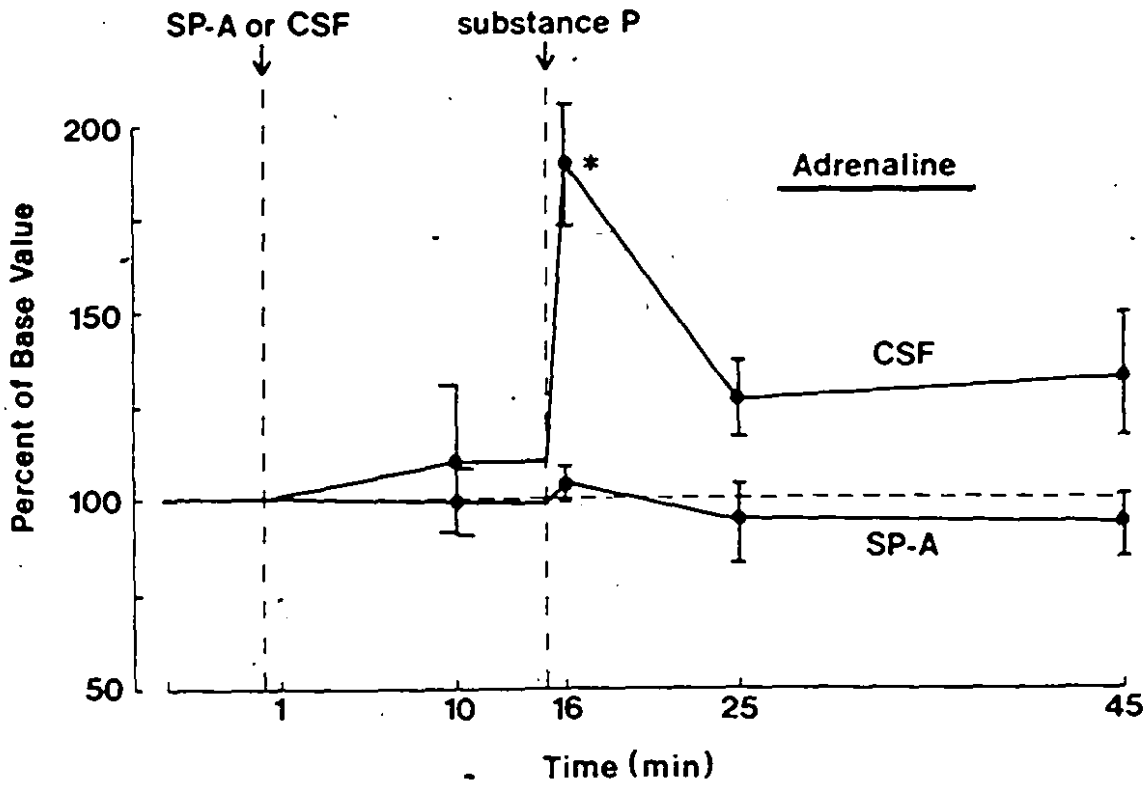

Figure 4. Effects of intrathecal administration of substance P (10 μg; at arrow in middle of abscissa) on venous plasma free adrenaline in rats pretreated with artificial cerebrospinal fluid (CSF; at arrow near left of abscissa; n = 12) or with [D-Pro^2, D-Phe^7, D-Trp^9]substance P (SP-A; at arrow near left of abscissa; n = 10). Refer to legend of Fig. 2 for other details. *, $P < 0.005$.

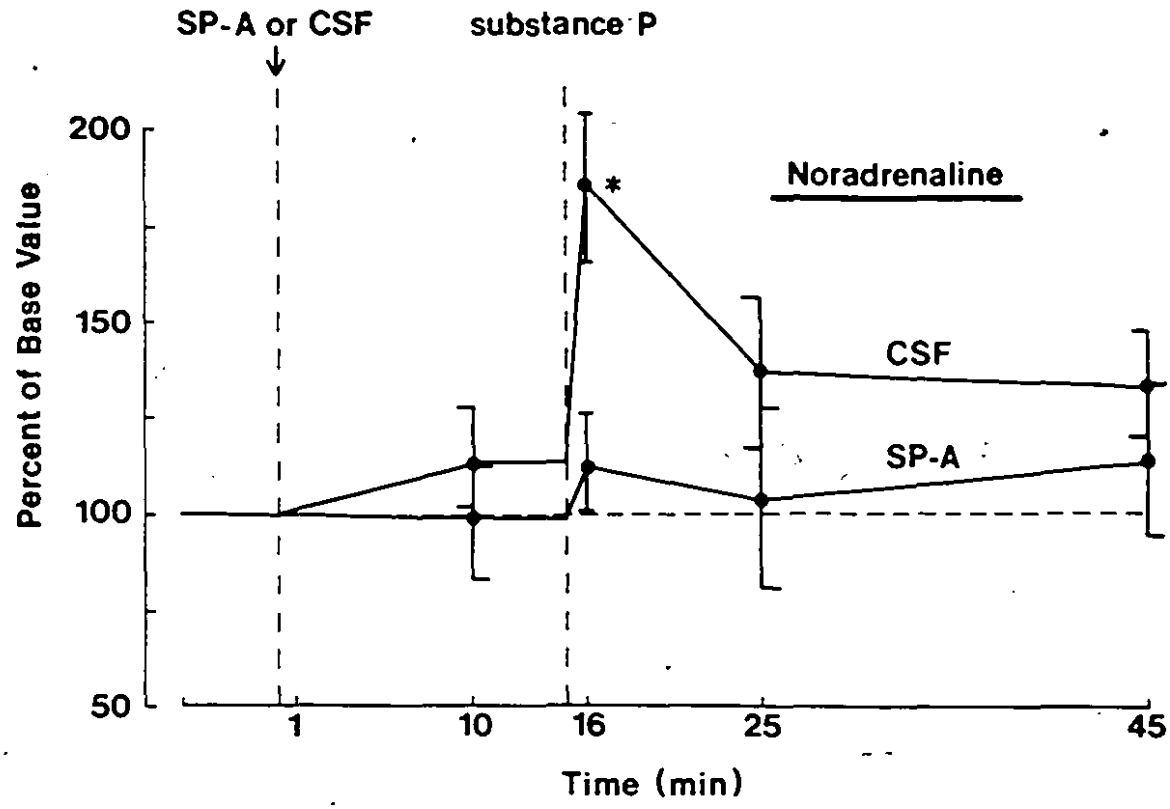

Figure 5. Effects of intrathecal administration of substance P on venous plasma free noradrenaline after prior administration of CSF or of [D-Pro^2, D-Phe^7, D-Trp^9]substance P. Refer to legends of Figs. 2 and 4 for other details. *, $P < 0.025$.

CHAPTER III

SUBSTANCE P GIVEN INTRATHECALLY AT THE SPINAL T9 LEVEL

INCREASES ARTERIAL PRESSURE AND HEART RATE

IN THE RAT

ABSTRACT

Administration of 10 µg of substance P intrathecally at the spinal T9 level of the unanaesthetized and of the anaesthetized rat provoked an increase in arterial pressure and an increase in heart rate. Both cardiovascular responses began within one to two min of administration, and the peak of each occurred at 4-10 min. In the anaesthetized rat, which gave rise to the bulk of the responses reported, peak arterial pressure was approx. 20 mmHg greater than pre-administration levels, and peak heart rate was approx. 50 beats per min greater. Similar administration of vehicle failed to alter either parameter. Arterial pressure and heart rate in substance P-treated rats were significantly different from those in vehicle-treated rats up to 15-20 min after administration. Pretreatment with the sympathetic ganglion blocker, hexamethonium (10 mg/kg, i.v.), prevented the responses to intrathecal administration of substance P. Pretreatment with [D-Pro^2,D-Phe^7,D-Trp^9]-substance P, and analogue with antagonist properties in the central nervous system, blocked both responses to substance P but failed to alter similar responses provoked by intrathecal administration of angiotensin II. Pretreatment with vehicle had no effect on responses to substance P or to angiotensin II. The antagonist also had partial agonistic effects. Both arterial pressure and heart rate were transiently increased, but this effect was reversed within 6 min; in the case of heart rate, values returned to the pre-application level but arterial

pressure fell to approx. 15 mmHg below this level. These results demonstrate a pharmacologically specific excitatory effect of substance P on spinal mechanisms controlling sympathetic output to the vessels and the heart; this output can be either via the adrenal medullae or via nerve pathways to the vessels and the heart. Our results also support the possibility that dysfunction of substance P systems at the spinal level may underly some models of hypertension and may be involved in some cases of essential hypertension in man, as well as in autonomic dysfunction associated with some neurological disorders.

INTRODUCTION

Substance P has been implicated in neural mechanisms regulating sympathetic output at the spinal level. The intermediolateral nucleus has a single high affinity and saturable binding component (Maurin et al., 1984; Takano and Loewy, 1984). Substance P-like immunoreactive material is found in the intermediolateral nucleus (Ljungdahl et al., 1978) in presumed axon terminals surrounding sympathetic preganglionic neurones (Ditirro et al., 1981; Holets & Elde, 1982). These neurones are excited by the iontophoretic application of substance P (Backman & Henry, 1984; Gilbey et al., 1983) and the vasomotor response elicited by injection of kainic acid into the ventral medulla of the rat is blocked by the intrathecal administration of a substance P antagonist (Loewy & Sawyer, 1982).

We have recently found that the intrathecal administration of substance P at the spinal T9 level increases plasma levels of epinephrine and norepinephrine in the rat (Yashpal et al., 1985), and the present experiments were done to determine the effects of this administration at the T9 level on arterial pressure and heart rate. Some of these data have been presented in abstract form (Yashpal et al., 1984).

METHODS

Experiments on Awake Animals.

Male Sprague-Dawley rats (approx. 350 g) were used. In experiments on awake animals, each rat had been implanted previously under Na-pentobarbital anaesthesia (65 mg/kg i.p.) with a chronic intrathecal catheter (Intramedic PE-10) which had been passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the T9 vertebral level. Spinous processes were used as landmarks. In our preliminary experiments the validity of this method for correct positioning of the inner tip of the catheter was confirmed in X-rays of rats implanted with wire-filled catheters. It was via this catheter that substance P was administered intrathecally. Only those rats which showed no neurological deficit throughout a recovery period of 7 days or more were used experimentally.

The indirect tail cuff method was used to measure systolic arterial pressure and heart rate. Each animal was placed in a darkened perspex container with a warming plate and, after an equilibration period of 20 min, baseline readings were taken over a 5 min period or until such readings had become stable. Substance P (Peninsula Laboratories) was then administered via the intrathecal catheter. It was delivered over a period of 15-20 sec in a dose of 10 µg (6.5 nmoles) dissolved in 10 µl of artificial cerebrospinal fluid (CSF; aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$).

Following delivery of the peptide the intrathecal catheter was flushed with 10 µl of CSF (catheter volume was 6-8 µl).

Experiments on Anaesthetized Animals

Each rat was anaesthetized with urethane (2.5 g/kg i.p.) and an intrathecal catheter was inserted as described above.

A second catheter (Intramedic PE-60) was inserted into the left common carotid artery facing the heart for direct monitoring of arterial pressure via a Statham transducer (Gould P23 ID) connected to a Grass P5 polygraph. Heart rate was calculated from this record. Systolic and diastolic pressures were measured from the ratemeter records and mean arterial pressure was calculated from these measurements. The number of heart beats in a 10 sec period was counted, and that number was multiplied by six to obtain heart rate in beats per min.

In some experiments a third catheter was inserted into the right femoral vein for the intravenous infusion of drugs.

Rectal temperature was maintained at approx. 37^{0}C by a heating pad.

After surgical preparation, a 30 min period was allowed for stabilization. Baseline readings of arterial pressure and heart rate were then taken over a five min period and substance P was administered intrathecally as described above. Readings of arterial pressure and heart rate were taken each minute for the next 15 min, and then at 20 and 30 min.

An additional series of experiments was done on anaesthetized animals to determine the pharmacological

specificity of the effects of substance P: [D-Pro^2, D-Phe^7, D-Trp^9]-substance P, which has antagonistic properties in the central nervous system (Fuxe et al., 1982 Hanley, 1982; Piercey et al., 1981; Stoppini et al, 1983; Yashpal & Henry, 1984) was administered prior to substance P to determine whether the response to substance P was altered. Thus, in this series of experiments, after baseline readings of arterial pressure and heart rate were taken, the antagonist was given intrathecally in a dose of 10 µg in 10 µl and the catheter was flushed as above. In a previous study (Yashpal et al., 1982), this dose was found to block totally the effects of substance P on tail flick latency, when the two peptides were given intrathecally. Measurements of arterial pressure and heart rate were taken at one min intervals as before. Then, 15 min after administration of the analogue, substance P was given and measurements were taken each min for the next 15 min, and then at 20 and 30 min. As a control for [D-Pro^2, D-Phe^7, D-Trp^9]-substance P, vehicle replaced the analogue solution. As a control for substance P in this series, a small number of experiments was done with angiotensin II (Peninsula Laboratories; 10 µg in 10 µl of CSF).

Statistical Analysis

Results from each rat were tabulated as changes in arterial pressure and in heart rate from the baseline values determined before intrathecal injection. Data for the figures were summarised by taking the mean ± S.E. of the values from

each group of rats at each min following administration. To determine statistical significance Student's t-test was applied to compare the two values (substance P vs CSF groups) at each sample time after administration of substance P or of the analogue.

RESULTS

Our preliminary experiments demonstrated that the diffusion of Fast Green dye over 30 min was limited to 2 segments rostrally and 3 segments caudally from the site of injection. Furthermore, the rapid onset of the response at 1 min, as seen in earlier experiments (Yashpal et al, 1985; Yashpal et al, 1982), suggests a local rather than a remote site of action.

Effects of Intrathecal Administration of Substance P in the Awake Rat

Intrathecal administration of substance P to the awake, restrained rat increased arterial pressure and heart rate. The mean baseline value for systolic arterial pressure was 129.8 ± 6.1 (S.E.M.) mmHg and for heart rate it was 362.5 ± 15.2 bpm (n = 7). At one min after administration these mean values were 143.7 ± 6.0 mmHg and 400.5 ± 24.5 bpm, respectively. At five min they were 151.9 ± 3.8 mmHg and 379.5 ± 21.8 bpm, respectively. The results are summarised in Figure 1. In some cases, some of the dramatic behavioural responses described in a previous paper

(Yashpal et al, 1982) were observed. It was concluded that the cardiovascular responses observed may be secondary to these behavioural responses and therefore that the unanaesthetized animal is an unreliable model in which to study the cardiovascular effects of intrathecal administration of substance P.

Accordingly, the remaining results were obtained from anaesthetized rats.

Effects of Intrathecal Administration of Substance P on Arterial Pressure in the Anaesthetized Rat

Intrathecal administration of substance P to the anaesthetized rat provoked an increase in mean arterial pressure. In pilot experiments the magnitude of the response was found to be related to the dose administered; 5 µg produced a smaller response than did 10 µg and 1 µg barely altered cardiovascular parameters. This effective dose range resembles that found in other experiments at the spinal level (Cridland & Henry, 1986; Yashpal et al., 1982). This effect was observed one min after injection and progressed to about 4 min, when it peaked. From this time there was a gradual decrease in arterial pressure toward pre-administration levels. A characteristic response from one animal is shown in Figure 2. In control experiments, when CSF was given instead of the substance P solution there was no change in either arterial pressure or heart rate. The data from these experiments are displayed graphically in Figure 3A.

Before administration the baseline systolic and diastolic pressures were 117 ± 3.6 and 65 ± 3.1 mmHg, respectively, in substance P-treated rats and 113 ± 4.9 and 65 ± 4.3 mmHg, respectively, in CSF-treated rats. At 4 min post-administration, systolic and diastolic pressures were 136 ± 4.7 and 83 ± 6.4 mmHg, respectively, in CSF-treated rats. The changes from baseline values were significantly different between the two groups of rats at 2-20 min inclusive after administration (2 min, $p < 0.05$; 3 min, $p < 0.005$; 4-15 min, $p < 0.001$; 20 min, $p < 0.025$; 1 and 30 min, not significant). Pulse pressure remained constant throughout the sample period.

To pursue the possibility that substance P delivered into the intrathecal space might pass into the circulation and express its effects via a peripheral action, these experiments were repeated, except that the same dose of substance P was administered intravenously in a volume of 500 µl of physiological saline. Prior to administration the mean systolic and diastolic pressures of the four animals tested were 124.5 ± 2.3 and 60.3 ± 3.9 mmHg, respectively. Changes from these baseline values were 0.5 ± 7.4, -4.0 ± 3.9, -8.0 ± 5.3 and -7.0 ± 4.8 mmHg at 1, 5, 10 and 15 min, respectively, after administration.

Effects of Intrathecal Adminstration of Substance P on Heart Rate in the Anaesthetized Rat

Heart rate showed a roughly similar effect of substance P. There was an increase which could be observed at one min

after injection. This increase progressed to peak at about 9 min, after which there was a gradual decrease toward pre-administration levels. Injection of vehicle alone had no effect on heart rate. The data from these experiments are summarized in Figure 3B.

Baseline heart rate was 323 ± 12.1 bpm in substance P-treated rats and 332 ± 15.4 bpm in CSF-treated rats. At 9 min after administration these values were 379 ± 18.5 and 328 ± 16.6 bpm in substance P- and CSF-treated rats, respectively. The values were significantly different between the two groups of rats at 1-15 min, inclusive ($p < 0.001$).

Effects of Ganglion Block on Responses to Substance P

To puruse the possibility that substance P delivered into the space might be passing into the circulation and producing its cardiovascular responses via a peripheral mechanism the experiments were repeated in the same manner as above except that prior to administration of substance P, hexamethonium was given systemically to block synaptic transmission in sympathetic ganglia. The rationale was that this would eliminate responses due to activation of sympathetic preganglionic neurones, leaving responses provoked via other mechanisms.

Mean baseline systolic and diastolic pressures in the four animals tested were 116.3 ± 12.1 and 45.0 ± 5.3 mmHg, respectively. Administration of 10 mg/kg of hexamethonium i.v. decreased pressure by approximately 20 mmHg. Subsequent

admministration of substance P failed to alter systolic or diastolic pressure. These results are illustrated in Figure 4A, along with results obtained from six control animals in which CSF replaced the substance P solution.

Mean baseline heart rate was 378.3 ± 34 bpm in the substance P-treated rats. Administration of hexamethonium increased heart rate by about 25 bpm but subsequent administration of substance P failed to alter heart rate further. These results are illustrated in Figure 5B.

Effects of [D-Pro^2, D-Phe^7, D-Trp^9]-substance P on responses to substance P

[D-Pro^2, D-Phe^7, D-Trp^9]-substance P was given 15 min prior to substance P in a further series of experiments to determine whether the responses to substance P could be blocked by an analogue with central antagonist properties in other experimental paradigms. Mean baseline values of systolic and diastolic pressures were 114 ± 3.8 and 65 ± 3.1 mmHg respectively, in rats given the analogue (n = 7). In control experiments, in which vehicle solution replaced the analogue solution, these same respective values were 109 ± 5.3 and 63 ± 4.6 mmHg.

When substance P was given in rats pre-treated with CSF, arterial pressure showed roughly the same response as in the earlier experiments: an increase over the first 4 min followed by a slow decline toward pre-administration levels. However, when substance P was given after the antagonist this response

was completely absent. The results are illustrated in Figure 6A. The differences between the two groups of animals were statistically significant at minutes 1-15 inclusive after substance P administration ($p < 0.01$).

Heart rate measurements revealed a similar antagonism of the substance P response by [D-Pro^2, D-Phe^7, D-Trp^9]-substance P, yet the typical response to substance P was observed in rats pre-treated with CSF (Fig. 6B). The results are illustrated in Figure 4B. Baseline values for analogue-treated and CSF-treated rats were 333 ± 10.5 and 328 ± 15.3 bpm, respectively. Post-substance P values were significantly different at minutes 1-15 inclusive after administration ($p < 0.01$).

The pharmacological specificity of the substance P analogue in blocking the responses to substance P was investigated by administering it prior to the administration of angiotensin II, which had effects on heart rate and arterial pressure similar to those of substance P (Yashpal et al., submitted). The same protocol was followed as that described above; 10 ug of angiotensin II given to CSF-pretreated rats increased both parameters. When the [D-Pro^2, D-Phe^7, D-Trp^9]-substance P solution replaced the CSF, the effects of angiotensin II were roughly the same.

Other Effects of [D-Pro^2, D-Phe^7, D-Trp^9]-substance P

[D-Pro^2, D-Phe^7, D-Trp^9]-substance P had two observable effects besides the antagonism described above.

One of those effects was a transient increase in both arterial pressure and heart rate. These responses may be seen in Figure 5. Arterial pressure at one min after injection had already reached a mean of 22 mmHg greater than pre-administration levels and it remained distinctly greater than the pressure in CSF-treated animals up to 4 min. At 9 min, this situation was reversed and the mean of the arterial pressure in animals given the analogue fell below that in animals given CSF, remaining below to the end of the 15 min period. An increase in heart rate also occurred, although it was somewhat slower, reaching a peak at about 5 min, while a decrease to below control levels did not occur.

[D-Pro^2, D-Phe^7, D-Trp^9]-substance P also led to death in about 60% of the animals given the analogue; animals which died were excluded from the results listed above. Furthermore, of the animals which were included in the results, when substance P was given the mean systolic and diastolic arterial pressures were 100.0 $\pm$ 5.9 and 55.0 $\pm$ 3.5 mmHg, respectively, in those pretreated with the substance P analogue while these pressures were 113.6 $\pm$ 6.2 and 60.7 $\pm$ 5.1 mmHg, respectively, in animals pretreated with CSF. It should be noted from Figure 6 that recovery from the depressant effect of the substance P analogue had occurred by 35 min after its administration.

DISCUSSION

These results have demonstrated that the intrathecal administration of substance P to the ninth segment of the thoracic spinal cord increases arterial pressure and heart rate. These responses are absent after prior administration of a substance P analogue with central antagonistic properties (Fuxe et al., 1982; Hanley, 1982; Piercey et al., 1981; Stoppini et al., 1983; Yashpal & Henry, 1984). This antagonism appears to be pharmacologically specific to substance P because the increases in arterial pressure and heart rate in response to similar administration of angiotensin II (which fail to occur after administration of hexamethonium; Yashpal et al., 1986) are not blocked by the substance P analogue. In addition to demonstrating that cardiovascular responses occur upon spinal administration of substance P, the present study also provides physiological evidence that the increase in the output of catecholamines from the adrenals, which is induced by similar administration of substance P (Yashpal et al., 1985), may be great enough to alter cardiovascular parameters.

These responses to substance P are probably due to an action in the spinal cord because they were absent in animals in which the sympathetic ganglia had been blocked and because intravenous administration of substance P fails to produce similar effects. Furthermore, substance P is known as a potent vasodilator peptide peripherally (Tenner et al., 1980). Although the possibility exists that substance P activated or mimicked

primary afferents (cf. Henry, 1976) and may have thus brought about the cardiovascular changes, it seems more likely that the action was on sympathetic preganglionic neurones. The basis for this conjecture is twofold: substance P-containing terminals are particularly dense in the intermediolateral nucleus of the thoracic spinal cord (Ditirro et al., 1981; Holets & Elde, 1982; Ljungdahl et al., 1978) and substance P applied by microiontophoresis onto these neurones causes excitation (Backman & Henry, 1984; Gilbey et al., 1983).

These pressure responses were observed in unanaesthetized as well as in anaesthetized rats. The qualitative similarity of the responses in the two types of preparation was not surprising. In the first place, as the action is in the spinal cord, relatively few central synapses are involved in mediating the effects compared to studies in which agents are administered intracerebrally. Secondly, of the various possible anaesthetics, urethane has less of a disruptive effect on baseline parameters and on cardiovascular reflexes as well as on respiratory parameters than do other anaesthetics in the rat (Armstrong. 1981; DeWildt et al., 1983; Sapru & Krieger, 1979).

The anaesthetized rat was considered to be better suited to these experiments because in unanaesthetized rats the cardiovascular changes might be secondary to behavioural responses elicited as the primary effect of peptide administration (Hylden & Wilcox, 1981; Yashpal et al., 1982). Furthermore, heart rate fluctuated widely after administration of substance P in the awake rat. This is indicated by the

varying mean values as well as by the large error bars. Our close attention to proving that qualitatively similar results are observed in both the awake and the anaesthetized animal, and the choice of the unanaesthetized animal for these experiments prompts us to point out that most investigators who study intracranial injection of peptides to determine their effects on cardiovascular parameters do not repeat their experiments in anaesthetized animals and these investigators thereby fail to ensure that the effects observed are not secondary to behavioural responses (Berecek et al., 1983; Falcon et al., 1978; Feuerstein et al., 1984; Fisher et al., 1985; Unger et al., 1981).

The time course of the changes in arterial pressure and heart rate in the anaesthetized rat is slower than that of the responses observed upon electrical stimulation of lateral horn neurones in the cat (cf. Henry & Calaresu, 1972). The onset of the responses we have reported here may be delayed by the time taken to diffuse from the surface of the spinal cord to the site of action; in earlier experiments on the effects of substance P on tail-flick latency (Yashpal et al., 1982) it was found that the peak effect occurred at about one min after administration. The very prolonged nature of the responses in the present experiments, lasting 15-20 min, suggests a prolonged action of the peptide on the spinal neurones, and is consistent with the prolonged effects on adrenal medullary output observed in our earlier experiments (Yashpal et al., 1985).

This very slow time course of the action of substance P may be especially appropriate for control of autonomic function, where regulation is of events spanning seconds or minutes, rather than tens of milliseconds as in the case of control of somatic function. Thus, substance P may be viewed as a slow regulator of excitability in spinal pathways controlling sympathetic output.

The mechanisms by which the changes in arterial pressure and heart rate are elicited probably include direct neural connections (cf. Henry & Calaresu, 1972). In addition, an adrenal component probably also exists because our earlier studies demonstrated that intrathecal administration of substance P at the ninth thoracic level also increases adrenal medullary output of free epinephrine and free norepinephrine (Yashpal et al. 1985).

Increases in arterial pressure and heart rate upon administration of [D-Pro^2, D-Phe^7, D-Trp^9]-substance P suggest transient agonistic effects. Agonistic effects were not observed in our earlier experiments in the tail-flick test in the male Sprague-Dawley rat (Yashpal & Henry, 1984).

The later, prolonged decrease in arterial pressure produced by the substance P antagonist suggests that substance P pathways impose a tonic excitation on the sympathetic preganglionic neurones, at least in our experimental protocol. This possibility is consistent with the earlier report (Henry & Calaresu, 1974) that transection of descending excitatory autonomic fibres in the dorsolateral funiculi leads to a

sustained reduction in arterial pressure and with the hypotensive effects of intrathecal administration of another substance P antagonist (Loewy & Sawyer, 1982).

The premature deaths of some rats after administration of [D-Pro^2, D-Phe^7, D-Trp^9]-substance P may be related to the motor deficit reported previously when this analogue is given intrathecally (Piercey et al., 1981; Yashpal & Henry, 1984) and recent evidence has been presented which suggests that these effects of the antagonist may be due to local anaesthetic actions (Post et al., 1985). Given the possibility that substance P antagonists might be useful clinically for the alleviation of essential hypertension it is important to select an analogue which lacks central "neurotoxic" effects (Couture et al., 1985).

As this paper was about to be submitted for publication a closely related paper appeared by Keeler, Charlton and Helke (1985). They used a synthetic analogue of substance P which is more stable than the endogenous peptide. Their report confirmed our earlier paper (Yashpal et al., 1985) that intrathecal injection of substance P to the lower thoracic spinal cord increases adrenal output of norepinephrine and epinephrine. Keeler et al. (1985) reported qualitatively similar cardiovascular effects to those we report here, except that the effects of the synthetic analogue last longer than those of the endogenous peptide. Keeler et al. also found that pharmacological blockade of sympathetic ganglionic transmission prevents the expression of these cardiovascular changes.

In summary, our results support cumulative evidence implicating substance P as a chemical mediator of synaptic transmission in descending inputs to lower thoracic sympathetic preganglionic neurones (Chan-Palay, 1979; Ditirro et al.,1981; Helke et al., 1982; Hokfelt et al., 1978; Holets & Elde, 1982; Ljungdahl et al., 1978). If this is the case, then our data suggest that these inputs are distributed at least to sympathetic preganglionic neurones in pathways regulating arterial pressure and heart rate. Furthermore, our results suggest that substance P mediates specifically an excitatory synaptic input to these neurones, to bring about an increase in arterial pressure and an increase in heart rate.

ACKNOWLEDGEMENTS

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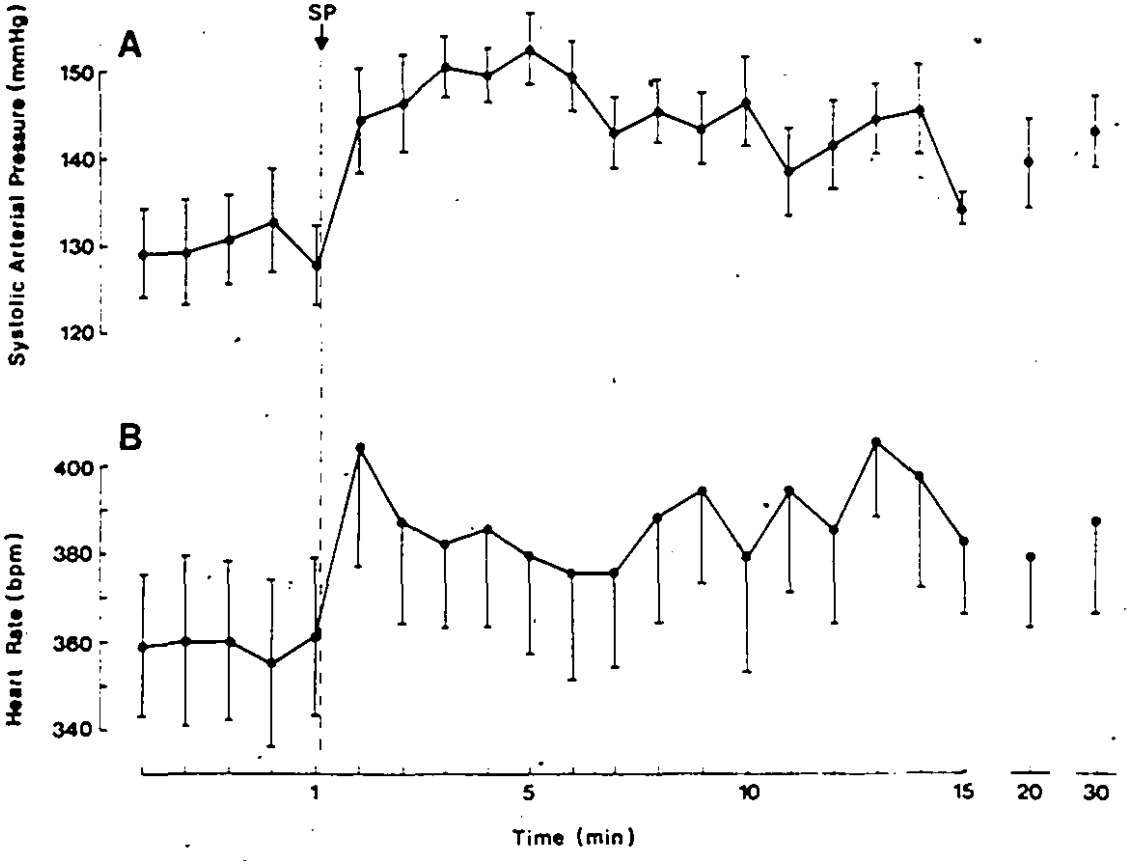

Figure 1. Mean systolic arterial pressure (A) and mean heart rate (B) in the awake, restrained rat (n = 8) induced by substance P (SP) injected intrathecally to the ninth thoracic spinal segment. Substance P was given in a dose of 10 µg dissolved in artificial cerebrospinal fluid. The abscissa indicates time after administration. Vertical bars each represent one S.E.M.

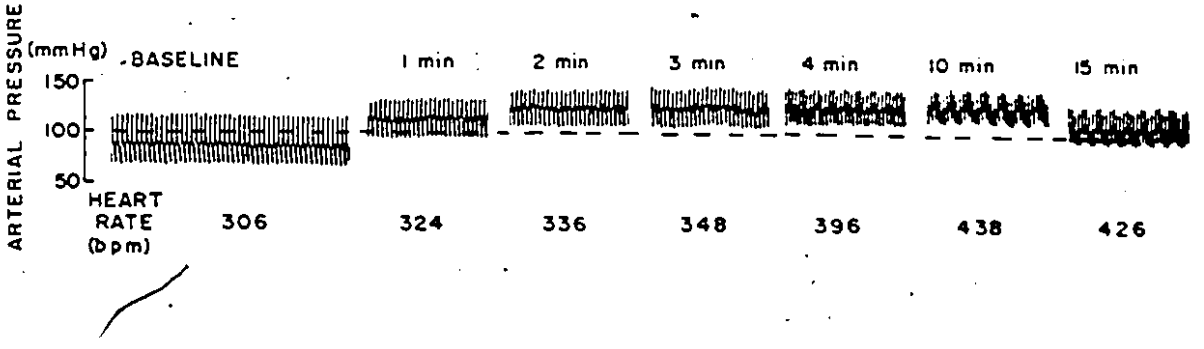

Figure 2. Polygraph records showing arterial pressure response to intrathecal administration of substance P in one rat. Baseline record was taken just prior to administration. This record spans 10 sec of the record. The dashed horizontal line indicates 100 mm Hg. Heart rate at each respective time is given below each record.

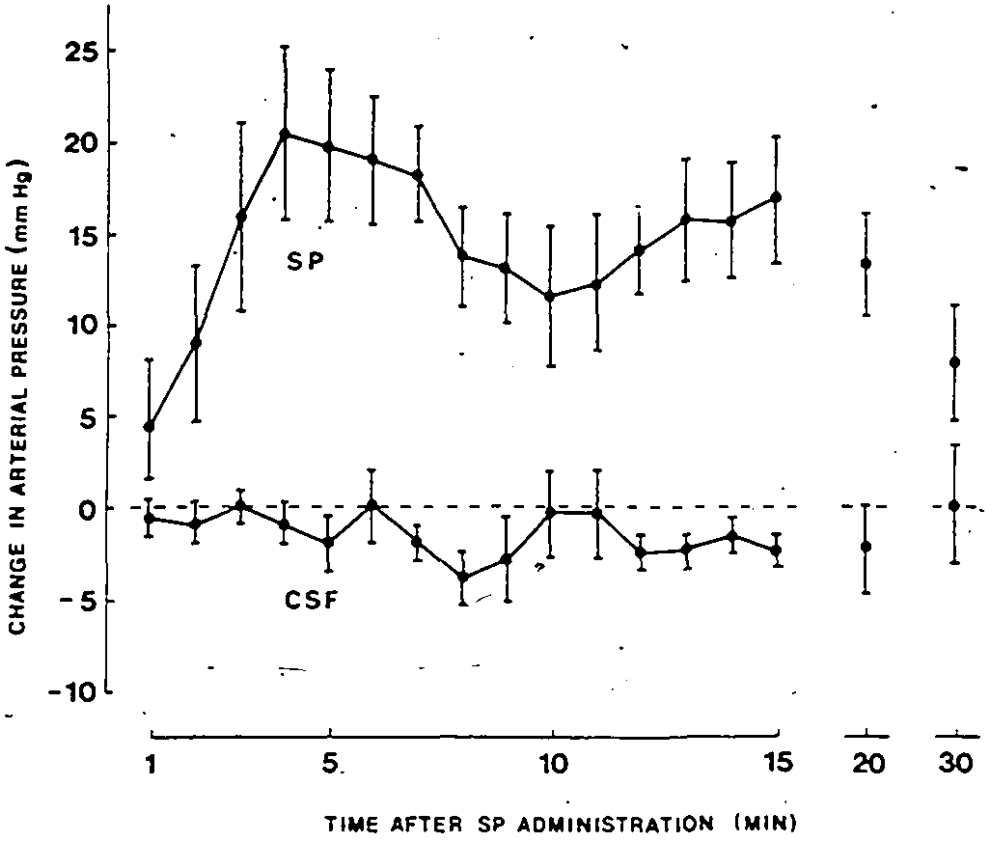

Figure 3. Change in mean arterial pressure induced by artificial cerebrospinal fluid (CSF; n = 11) or substance P (SP; n = 10) injected intrathecally to the ninth thoracic spinal segment of the anaesthetized rat. The ordinate shows the mean change in mean arterial pressure as compared to the pre-administration values. Details are otherwise as in the legend to Fig. 1.

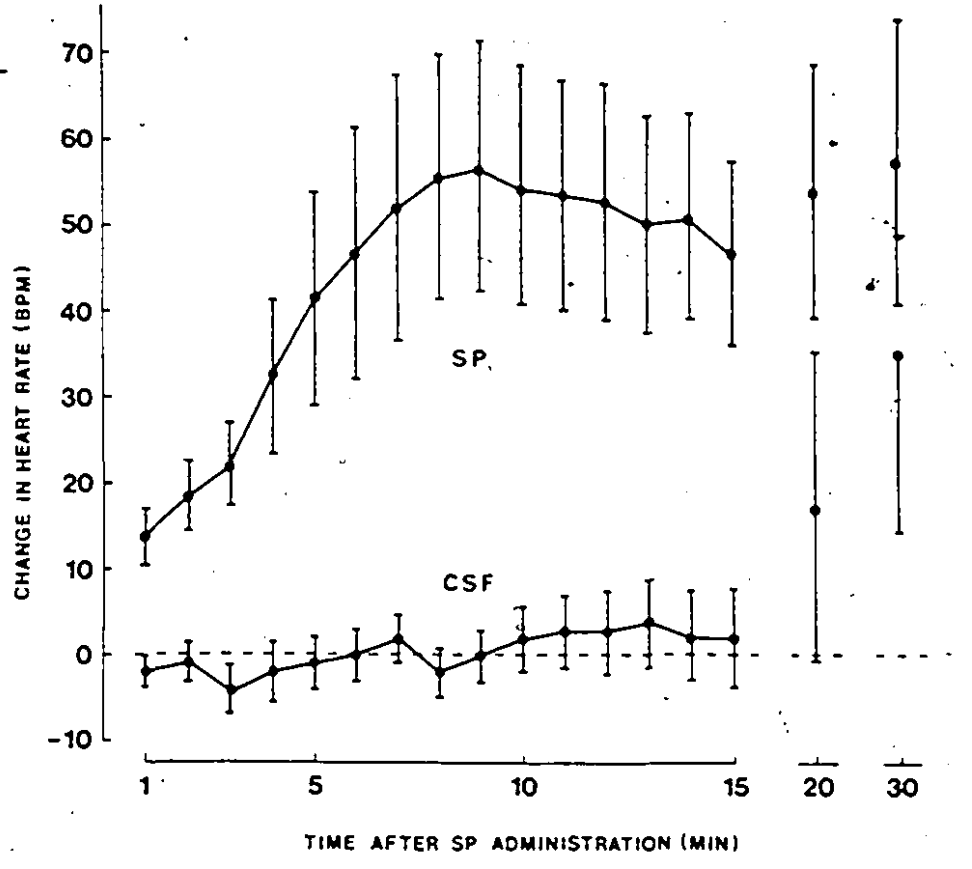

Figure 4. Change in heart rate induced by CSF or substance P injected intrathecally to the ninth thoracic spinal segment of the anaesthetized rat. The ordinate shows the mean change in heart rate as compared to the pre-administration values. Details are otherwise as in the legend to Fig. 1.

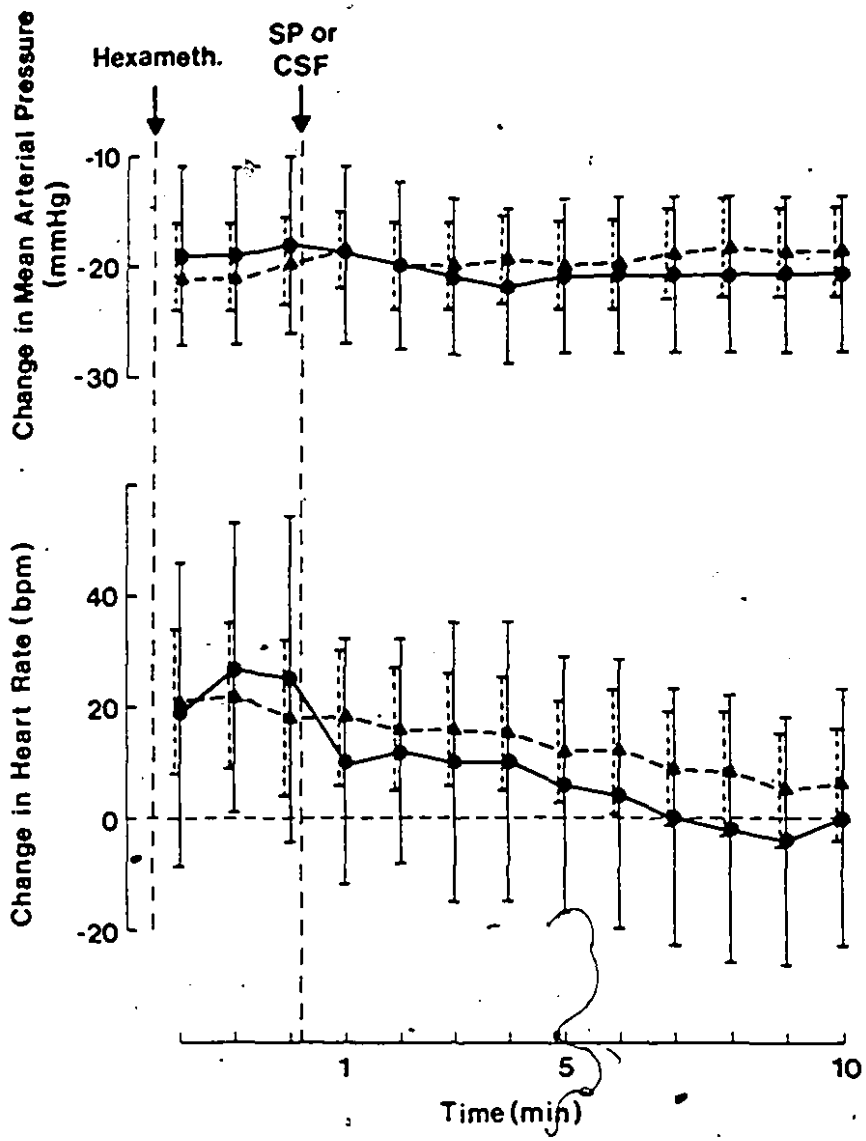

Figure 5. Effects of ganglion block on responses to intrathecal administration of substance P. Hexamethonium (10 mg/kg, i.v.) was given at the arrow at the left. CSF (▲- -▲ n =6) or substance P (●—● n = 4) was given 3 min later. Values are expressed as changes from baseline arterial pressure (A) and from baseline heart rate (B) determined over the 5 min prior to administration of hexamethonium. Details are otherwise as in the legend to Fig. 1.

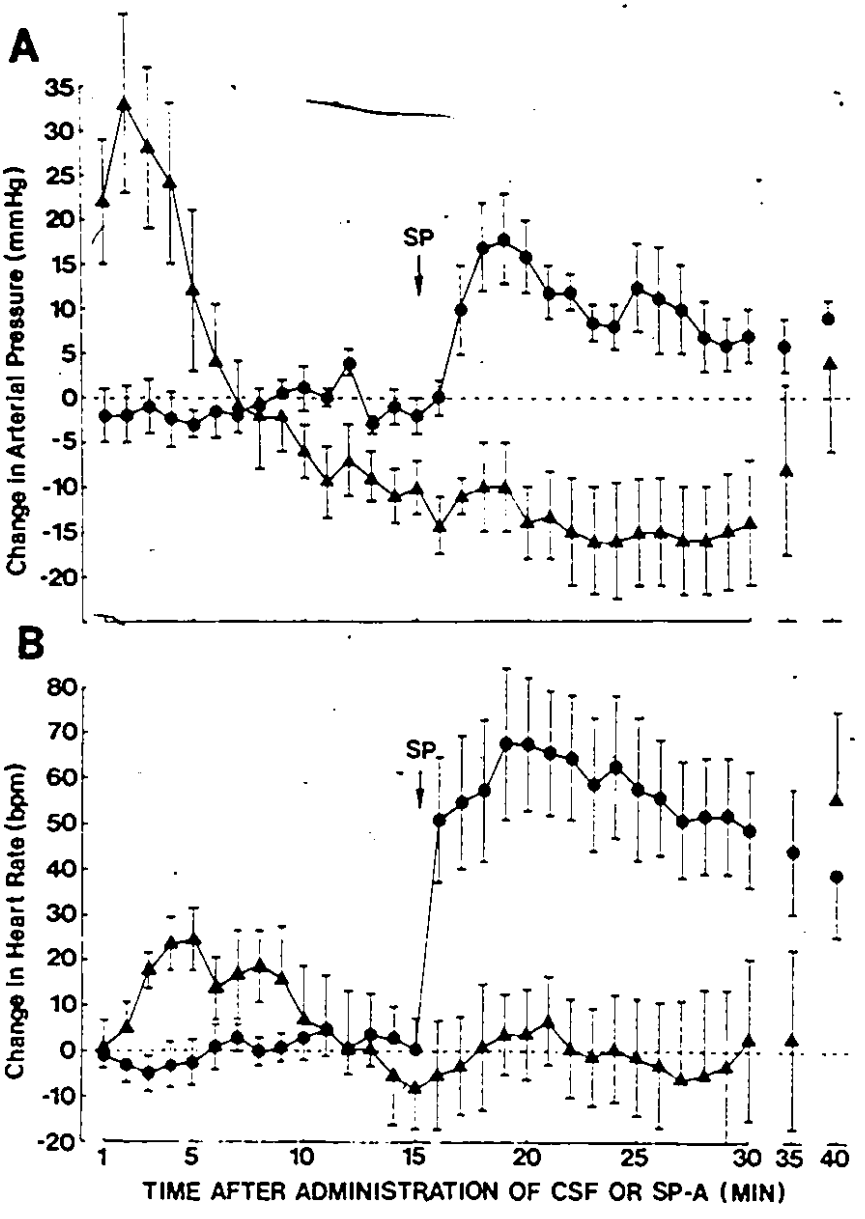

Figure 6. Changes in arterial pressure (A) and in heart rate (B) induced by intrathecal administration of SP (at arrow), 15 min after prior intrathecal administration of CSF (●—● n = 8) or of [D-Pro^2, D-Phe^7, D-Trp^9]-substance P (▲—▲ n = 7). Details are otherwise as in the legend to Fig. 1.

CHAPTER IV

SUBSTANCE P ADMINISTRATION TO THE SECOND THORACIC SPINAL SEGMENT INCREASES ARTERIAL PRESSURE, HEART RATE AND ADRENAL MEDULLARY OUTPUT OF CATECHOLAMINES IN THE RAT

ABSTRACT

Administration of substance P intrathecally to the second thoracic spinal segment of the anaesthetized rat produced an increase in mean arterial pressure and heart rate. This administration also led to an increase in circulating level of adrenaline and a non-significant increase in the levels of noradrenaline. The increase in arterial pressure peaked at about three min after administration, reaching a value of 15 mmHg above pre-administration levels; recovery occurred slowly over the next 10 min. The heart rate response was more rapid in onset, with an increase of 35 bpm at one min after administration and reaching a peak of 50 bpm at 5-10 min; recovery also occurred slowly, with pre-application levels being reached only after 20 min following administration. At one min after administration adrenaline and noradrenaline levels were approximately 150% of baseline levels. Comparison of these results with similar results obtained at the ninth thoracic segment in an earlier study revealed a number of interesting features. The level of significance attained by the changes in catecholamine levels in the present study was considerably less than the level of significance obtained when substance P was given at the ninth thoracic segment; this is not surprising in view of the predominance of neurones projecting to the adrenals from the lower segment. The arterial pressure responses were roughly similar at the two levels of the spinal cord. The heart rate response was

considerably greater and more abrupt at the second thoracic level; again this is not surprising in view of the predominance of cardioacceleratory sympathetic neurones in the upper rather than in the lower thoracic spinal cord. Taken together, our results suggest that substance P has a general excitatory effect on sympathetic neurones throughout the length of the thoracic spinal cord.

INTRODUCTION

Substance P has been implicated as a chemical mediator of synaptic transmission onto sympathetic preganglionic neurones in the spinal cord. Substance P binding sites are found in the intermediolateral nucleus (Charlton & Helke, 1985; Maurin et al., 1984; Takano and Loewy, 1984). The substance P-like immunoreactive material which is also found there (Ljungdahl et al., 1978) is concentrated in nerve terminals surrounding sympathetic preganglionic neurones (Ditirro et al., 1981; Holets & Elde, 1982). In electrophysiological studies, substance P excites single sympathetic preganglionic neurones in vivo (Backman & Henry, 1984; Gilbey et al., 1983).

We have recently reported that intrathecal administration of substance P to the ninth thoracic segment of the anaesthetized rat causes an increase in the adrenal medullary output of catecholamines (Yashpal et al., 1985) as well as an increase in arterial pressure and heart rate (Yashpal et al., submitted). Similar results have been reported by Keeler et al. (1985) using a synthetic analogue of substance P. In view of evidence that different functional types of sympathetic preganglionic neurones are found at different levels of the spinal cord (Henry & Calaresu, 1972 and Holets & Elde, 1982), our earlier experiments were repeated, but in this case substance P was injected at the second thoracic segment rather than at the ninth. The objective was to determine whether different responses to substance P would be elicited from the

two levels. Our experiments were also directed by the possibility that comparison of results from injection at the second with those from the ninth thoracic segment might yield information on similarities or differences in cardiovascular control by humoral (ie. via the ninth thoracic segment and adrenal output) and by neural (ie. via the second thoracic segment and the sympathetic pathways to the heart) mechanisms.

METHODS

Details of the experimental procedures are outlined in previous chapters. The major points are outlined below.

Male Sprague-Dawley rats (approximately 350 g) were anaesthetized with urethane (2.5 g/kg, i.p.). An Intramedic PE-10 catheter was passed intrathecally via a slit in the dura between the occipital bone and the atlas so that the inner tip lay at the level of the second thoracic vertebra. This catheter was used for intrathecal injection of substance P.

In experiments on plasma catecholamines, a second catheter (PE-60) was passed via the left femoral vein into the inferior vena cava so that the inner tip lay between the renal veins and the heart. This catheter was used for the collection of blood.

In all experiments a catheter (PE-60) was placed in the left common carotid artery facing toward the heart for continuous monitoring of arterial pressure via a Statham P23 ID transducer connected to a Grass polygraph.

Rectal temperature was maintained at approximately 37°C with a heating pad.

EXPERIMENTS ON CATECHOLAMINES

Once surgical preparation was complete, 30 min were allowed for stabilization. Then a 1.5 ml sample of blood was taken to establish the baseline, 10 µl of substance P was administered intrathecally 5 min later and three more samples of blood were taken at one, 10 and 30 min after substance P administration. Substance P, from Peninsula Laboratories, was given over a period of 15-20 sec in 10 µl of artificial cerebrospinal fluid (CSF; for composition see Yashpal et al., 1985). Following delivery of the peptide the intrathecal catheter was flushed with 10 µl of CSF. In control rats, CSF alone replaced the substance P solution. Each sample of blood was replaced with an equal volume of warm (37°C), heparinized saline.

Upon withdrawal, each sample of blood was placed into a cooled heparinized Eppendorf tube and centrifuged at 2,000 r.p.m. at 4°C. The plasma was taken and stored at -70°C until assayed for free catecholamines. These were quantified using high pressure liquid chromatography (Waters M45 solvent delivery system, Bioanalytical Systems LC-4 electrochemical detector) using a method modified from Hallman et al. (1978). The reliable detection limit was 0.5 ng/ml.

Levels of adrenaline and noradrenaline in samples taken after substance P or CSF administration were calculated as a

percent of the respective baseline value for each rat. These percent values were then averaged for each group of rats (ie. substance P-treated vs CSF-treated) for each post-administration time. These mean percent values are plotted in Fig. 1. Student's t-test was applied to compare the two values at each sample time, for adrenaline and for noradrenaline.

EXPERIMENTS ON ARTERIAL PRESSURE AND HEART RATE

After surgical preparation at least 30 min were allowed for stabilization. Then baseline readings of arterial pressure and heart rate (calculated from the arterial pressure record) were taken over a 5 min period, and once these readings were stable, substance P was administered via the intrathecal catheter as described above; either 5 or 10 μg were given. Control rats were given CSF instead of the substance P solution. Readings of arterial pressure and heart rate were taken each min for the next 15 min, and then at 20 and 30 min.

Results from each rat were tabulated as changes from the baseline values of arterial pressure or heart rate. Data for the figures were summarized by taking the mean ± S.E. of the values from each group of rats, at each min following administration. Student's t-test was used to compare the two values at each sample time.

RESULTS

In our earliest experiments we found that over a period of 30 min, diffusion of Fast Green dye in the cerebrospinal fluid was limited to two segments rostral and three segments caudal to the level of injection. We have also consistently found that injection of CSF is without effect on plasma catecholamine levels and on heart rate and arterial pressure; similar control experiments were done in the present series, and the results are illustrated in Figs. 1,2 and 4. Furthermore, the rapid onset of the responses at one min, as seen in these and in earlier experiments (Yashpal et al., 1982,1985, submitted), favours a local rather than a remote site of action.

EFFECTS OF SUBSTANCE P ON PLASMA LEVELS OF ADRENALINE

Intrathecal administration of 10 µg of substance P increased plasma levels of adrenaline. This effect, illustrated in Fig. 1, was observed in the first post-injection reading at one min, and persisted until 10 min. Mean baseline values of adrenaline were 5.98 ± 1.42 (S.E.) ng/ml plasma in rats given substance P (n = 9) and 7.72 ± 2.20 in rats given CSF (n = 8). At one min after injection, adrenaline levels in substance P-treated rats had risen to 153.2 ± 22.9 % of the baseline value, while those in CSF-treated rats had risen to 107.1 ± 6.5 % of the baseline value; this difference was not statistically significant ($p > 0.05$). At 10 min, levels were

142.2 ± 15.1 and 104.5 ± 7.4 % for substance P- and CSF-treated rats, respectively; this difference was statistically significant ($p<0.05$). Thirty min after injection, the same respective levels were 137.0 ± 11.9 and 100.3 ± 11.1% of baseline; in this case these values were also significantly different ($p< 0.05$).

EFFECTS OF SUBSTANCE P ON PLASMA LEVELS OF NORADRENALINE

Substance P also increased plasma levels of noradrenaline but in this case the change was not significantly different. The results obtained are summarized in Fig. 1. Mean baseline values were 2.04 ± 0.63 ng/ml plasma from substance P-treated rats (n = 8) and 2.76 ± 0.59 ng/ml from CSF-treated rats (n = 8). Values of noradrenaline were calculated from the same samples of plasma as were those of adrenaline, but in one case, assays of noradrenaline in substance P-treated rats were unreliable, and hence the number of samples giving rise to the data is different for adrenaline and for noradrenaline. Mean levels of noradrenaline after administration of substance P were 164.0 ± 25.4, 159.2 ± 27.1 and 147.8 ± 30.3 % of base values at 1,10 and 30 min, respectively, and in rats given CSF they were 114.8 ± 9.7, 111.4 ± 11.2 and 116.4 ± 11.0%, respectively. Differences between the two groups were not statistically significant at any of the three post-administration sample times.

EFFECTS OF SUBSTANCE P ON ARTERIAL PRESSURE

These experiments were initiated using 10 µg of substance P because this was the dose used in similar experiments at the ninth thoracic segment (Yashpal et al., 1985). From the initial experiments at the second thoracic segment it was concluded that more reliable results would be obtained if the dose were lowered to 5 µg. This conclusion was reached for a number of reasons. One was that with a dose of 10 µg the increase in heart rate was much greater than that observed with the same dose in our earlier experiments at the ninth thoracic level, where the increase with this dose in heart rate peaked at about 60 bpm. In contrast, the mean peak response at the second thoracic level was 120 bpm. A second reason was that the changes in arterial pressure with 10 µg were inconsistent, with some animals showing an increase and others showing a decrease. Finally, in a number of animals there was a considerable decrease in pulse pressure within the first few minutes of substance P administration. This decrease ranged from 20 to 30 % within two minutes. In fact, in one animal pulse pressure decreased from 50 to 30 mm Hg at 3 min post-administration and to 15 mm Hg at 4 min; from this point respiratory depression occurred and the arterial pressure had fallen almost to zero by the time the reading was to be taken at the fifth min. Figure 3 illustrates this dramatic cardiovascular response.

The results obtained with 5 μg of substance P are illustrated in Figure 4. Mean arterial pressure showed a clear increase, starting within two min of administration of substance P and peaking at 3 min. From this time there was a gradual decrease over the next 10 min toward pre-administration levels. When CSF was given instead of the substance P solution there was no change in arterial pressure.

Mean baseline systolic and diastolic pressures before administration were 122.1 ± 4.5 and 70.1 ± 5.3 mmHg, respectively, in CSF-treated rats (n = 14) and were 120.0 ± 4.9 and 64.4 ± 4.1 mmHg, respectively, in substance P-treated rats (n = 13). After administration, mean systolic and diastolic pressures at 3 min were 121.4 ± 5.2 and 68.9 ± 5.0 mmHg, respectively, in CSF-treated rats and 133.8 ± 5.9 and 81.2 ± 7.0 mmHg, respectively, in substance P-treated rats. Baseline values were not significantly different between the two groups. However the mean changes from baseline arterial pressure for the two groups were different from two to 13 min after administration (2 min, $p < 0.25$; 3-11 min, $p < 0.005$; 12 min, $p < 0.25$; 13 min, $p < 0.05$).

EFFECTS OF SUBSTANCE P ON HEART RATE

Heart rate was also increased by administration of 5 μg of substance P. These effects are illustrated in Figure 5. Upon administration of substance P there was an immediate increase in heart rate to about 35 bpm above baseline values. Heart

rate continued to increase until it reached a peak at about 10 min, at which time it was about 55 bpm above baseline values. From this time there was a slow decrease until, at 30 min, the response had ended. In the group of animals given CSF instead of the substance P solution there was a gradual tendency for the heart rate to increase, but there was not the abrupt change seen in the substance P-treated rats.

Mean heart rate before administration was 333.9 ± 11.9 bpm in substance P-treated rats and 361.4 ± 13.2 bpm in CSF-treated rats. At one min after administration mean heart rate was 372.5 ± 17.6 bpm in substance P-treated rats and 364.3 ± 4.3 in CSF-treated rats. At 10 min heart rate was 385.4 ± 11.9 bpm in substance P-treated rats and 368.1 ± 12.7 bpm in CSF-treated rats. When changes from baseline values were compared (these changes are illustrated in Figure 5) the two groups were significantly different between one and 20 min after administration (one min, $p < 0.01$; 2-15 min, $p < 0.005$; 20 min, $p < 0.025$).

DISCUSSION

The results demonstrate that the intrathecal administration of substance P at the second thoracic level of the spinal cord increases arterial pressure and heart rate, and also induces moderate increases in plasma levels of adrenaline and perhaps also of noradrenaline. These responses began within one or two minutes of administration. The rate of onset of these responses is similar to that observed in experiments in which the intrathecal administration of substance P decreased nociceptive reaction time in the tail flick test in the rat (Yashpal et al., 1982). The effects of substance P in the present experiments are probably mediated via an action on substance P receptors because in earlier studies we have found that the effects in the two experimental paradigms (i.e. sympathetic activation and facilitation of tail flick) were blocked by prior administration of substance P antagonists (Yashpal & Henry, 1984; Yashpal et al., 1985, submitted; Couture et al., 1985). In addition, the cardiovascular responses are blocked by administration of hexamethonium (Yashpal et al., submitted; Keeler et al., 1985) suggesting that they were due to sympathetic activation. Finally, similar sympathetic activation occurs with the intrathecal administration of substance P to the awake rat (Yashpal et al., submitted). It is suggested from the present study, then, that substance P may play a role in the regulation of sympathetic output in the upper thoracic spinal cord, and that its action

there is to increase this output.

Similar, but not identical effects to those reported here were observed when this peptide was administered intrathecally at the ninth thoracic segment. These latter results have been presented in two earlier papers (Yashpal et al., 1985, submitted). I shall attempt a comparison of the effects of substance P at the two spinal levels and then present an interpretation of how substance P might be involved in the control of sympathetic output at the spinal level.

Let me first focus on the effects of substance P on heart rate and arterial pressure. In the present experiments there was a clear increase in both parameters, beginning within one to two min of administration, and lasting for 10 to 20 min. While these results might be explained by an action on substance P receptors in somatosensory pathways in the dorsal horn (Henry, 1976), it seems more plausible that the changes in heart rate and arterial pressure are due to an action on sympathetic preganglionic neurones. This latter possibility is supported by the earlier reports that substance P-containing terminals and receptors are found in the intermediolateral nucleus (Charlton & Helke, 1985; Ditirro et al., 1981; Holets & Elde, 1982; Ljungdahl et al., 1978; Maurin et al., 1984; Takano et al., 1984) and that substance P applied by microiontophoresis onto these neurones causes excitation (Backman & Henry, 1984; Gilbey et al., 1983).

Comparison of the results of the present study with those obtained earlier at the ninth thoracic level demonstrates that

at the second thoracic segment the increase in heart rate was much more abrupt than that observed at the ninth thoracic level. This difference might be attributable to the greater preponderance of neurones involved in direct (ie. neural rather than humoral) pathways to the heart in the upper rather than in the middle thoracic segments (Henry & Calaresu, 1972).

Perhaps a more important difference between the results at the two spinal levels is that substance P was considerably more potent in eliciting the heart rate response at the second than at the ninth thoracic level. This may be attributable to the greater preponderance of neurones involved in direct pathways to the heart in the second thoracic segment. In the experiments where 10 µg were given in the present study the cardiovascular changes were not necessarily only of greater magnitude than those observed with 5 µg; rather, the dramatic increase in heart rate accompanied by the decrease in pulse pressure while the mean arterial pressure remained roughly the same, tempts the speculation that despite the marked increase in heart rate, cardiac output decreased profoundly. This could have been in part due to increased peripheral resistance as a result of a marked increase in peripheral vasoconstriction and in part due to a reduced venous return which could also have been due to the marked increase in peripheral vasoconstriction.

With regard to the results with plasma catecholamines, these experiments have shown that there is a delayed increase in adrenaline levels but that noradrenaline shows a non-significant increase after substance P is administered into

the intrathecal space of the second thoracic segment. These results are in contrast to those obtained when substance P was injected at the ninth thoracic level (Yashpal et al, 1985); in this case substance P administration clearly led to abrupt and prolonged increases in plasma levels of noradrenaline and of adrenaline. The difference between the effects at the two levels of the spinal cord is not surprising in view of the observation that of the sympathetic preganglionic neurones projecting directly to the adrenals in the rat 72% lie in the seventh to twelfth thoracic segments of the spinal cord (20% in the ninth thoracic segment alone) while only 9% lie in the first four thoracic segments (Holets & Elde, 1982). Thus, on the basis of numbers alone one could expect this difference to occur.

Comparison of the present results with those obtained at the ninth thoracic segment indicates that substance P activates sympathetic output at the two levels. This lack of segmental selectivity as well as the lack of functional selectivity between neurones in pathways to the heart vs those in pathways to the adrenals, suggests that substance P is a general excitatory agent in spinal sympathetic pathways, stimulating cardiovascular parameters both by neural (via direct neural pathways) and by humoral (via activation of the adrenals) mechanisms. Finally, the roughly equal effects on arterial pressure at the two levels is consistent with the earlier observation that electrical stimulation of the intermediolateral nucleus at levels below those at which

changes in heart rate were observed was still capable of eliciting an increase in arterial pressure (Henry & Calaresu, 1972).

In terms of the functional neuroanatomy of the substance P-containing neurones, although some neurones in the intermediolateral nucleus of the rat may synthesize substance P (Davis et al., 1984), most substance P-containing terminals are probably associated with neurones projecting caudally from a region in the ventrolateral medulla. The evidence supporting this conjecture is that this medullary region has been implicated in cardiovascular control (Dampney & Moon, 1980; Henry & Calaresu, 1974) and neurones in this region project directly to the intermediolateral horn (Henry & Calaresu, 1974; Caverson et al., 1983; Ross et al., 1984). Substance P-containing cell bodies are found in the ventrolateral medulla and these neurones project to the intermediolateral nucleus (Helke et al., 1982; Lovick and Hunt, 1983). Kainic acid injection into this region induces the release of substance P into a perfusate of the thoracic spinal cord (Takano et al., 1984). Injection of kainic acid into this region also produces cardiovascular responses which can be blocked by the intrathecal administration of a substance P antagonist (Loewy & Sawyer, 1982).

The present results complement a growing body of evidence implicating substance P as a chemical mediator of excitatory synaptic transmission between fibres descending from brain stem structures to sympathetic preganglionic neurones in the spinal cord, especially to those neurones specifically involved in regulation of arterial pressure, heart rate and adrenal output of catecholamines. This evidence raises the interesting possibility that excessive activity in descending substance P pathways to sympathetic neurones may underly some animal models of hypertension and may be involved in some cases of essential hypertension in man.

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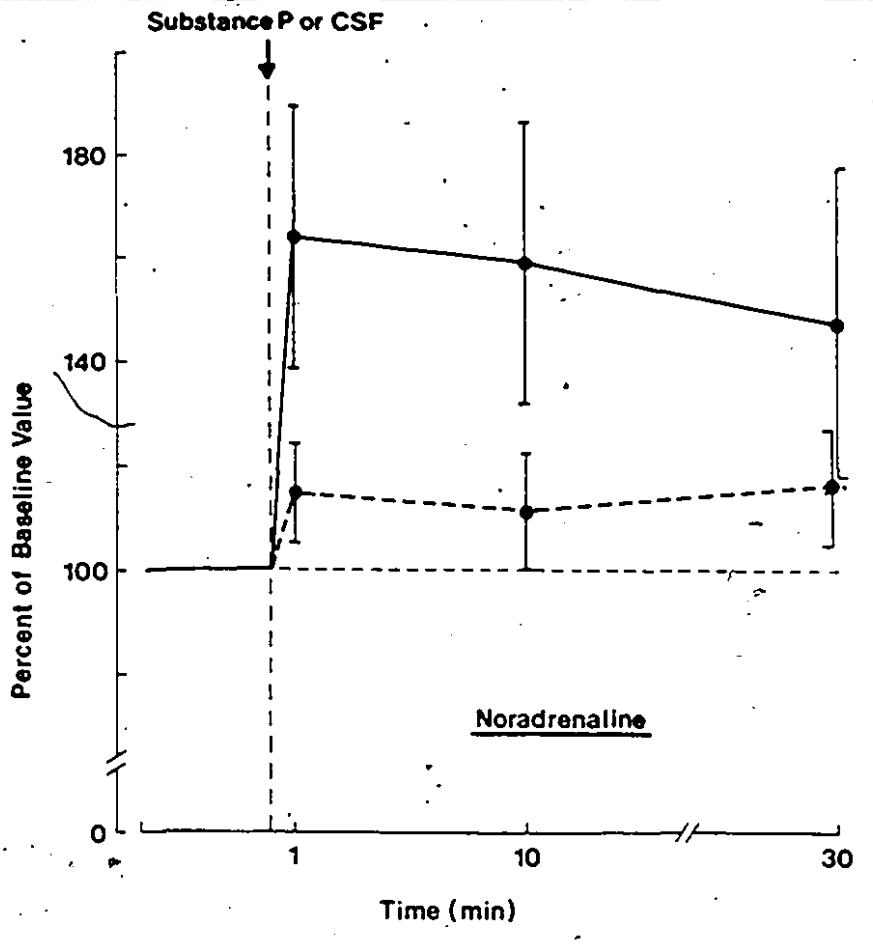

Figure 1. Time effect curve for the effects of the intrathecal administration of substance P (10 µg; n=8; ●——●) or of artificial cerebrospinal fluid (CSF; n = 8; ● - - ●) on plasma levels of noradrenaline in the rat. Administration was at the second thoracic spinal level. The ordinate shows noradrenaline level expressed as a mean percentage of the baseline value in plasma extracted from blood which was taken prior to the intrathecal injection. Vertical bars each represent ± one S.E.M. The abscissa shows the time after intrathecal injection; the vertical arrow indicates the time of injection, taken as 0 min. There was no statistical difference between the two groups at any of the three sample times.

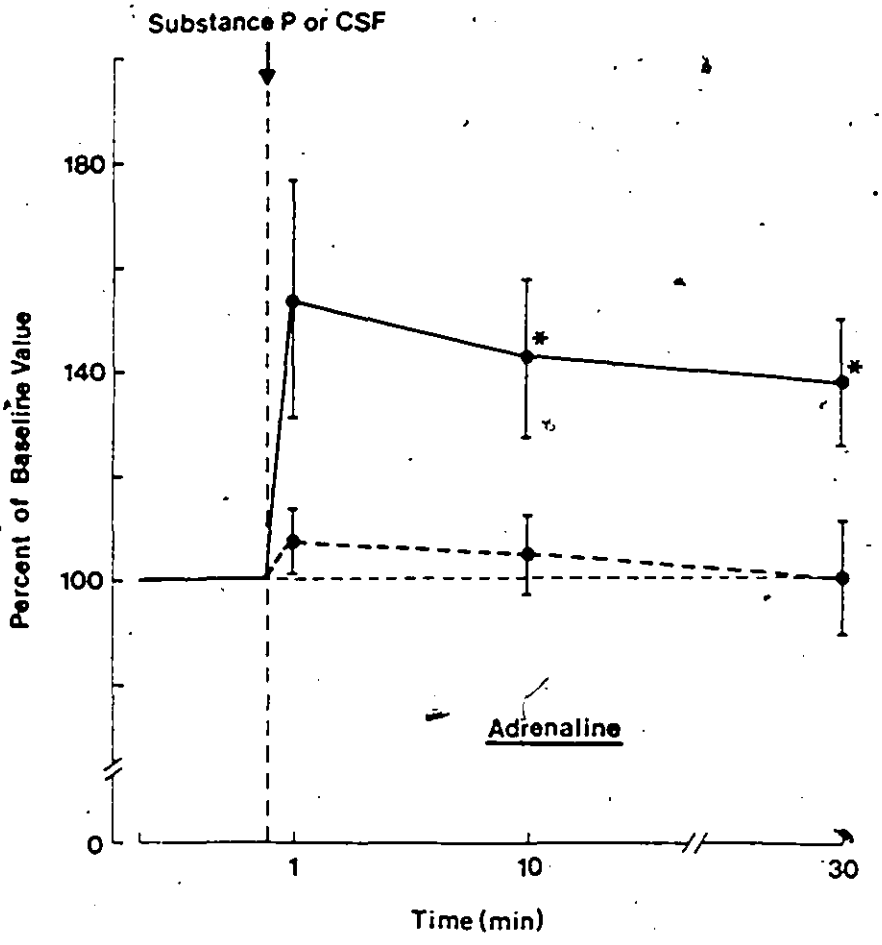

Figure 2. Time-effect curve for the effects of the intrathecal administration of substance P (10 µg; n = 9; ●——●) or of artificial cerebrospinal fluid (CSF; n = 8; ● - - ●) on plasma levels of adrenaline in the rat. Details are otherwise as in the legend to Figure 1. The differences between the two groups were statistically significant at 10 and 30 min, but not at one min post-administration (*, $p<0.05$).

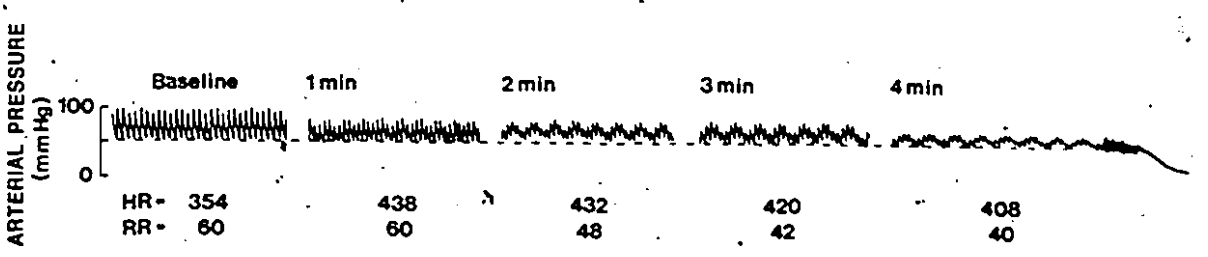

Figure 3. Records from one rat illustrating the rapid increase in heart rate and the decrease in pulse pressure induced by intrathecal injection of 10 µg of substance P at the second thoracic spinal level. Each of the first four records spans a 10 sec period. The last record on the right begins at the same time scale and then is reduced so that the total period covered is about three min. Times indicated above the records are min after injection. The calibration at the left indicates arterial pressure in mmHg. Values below records are heart rate (HR) in beats per min and respiration rate (RR) in breaths per min.

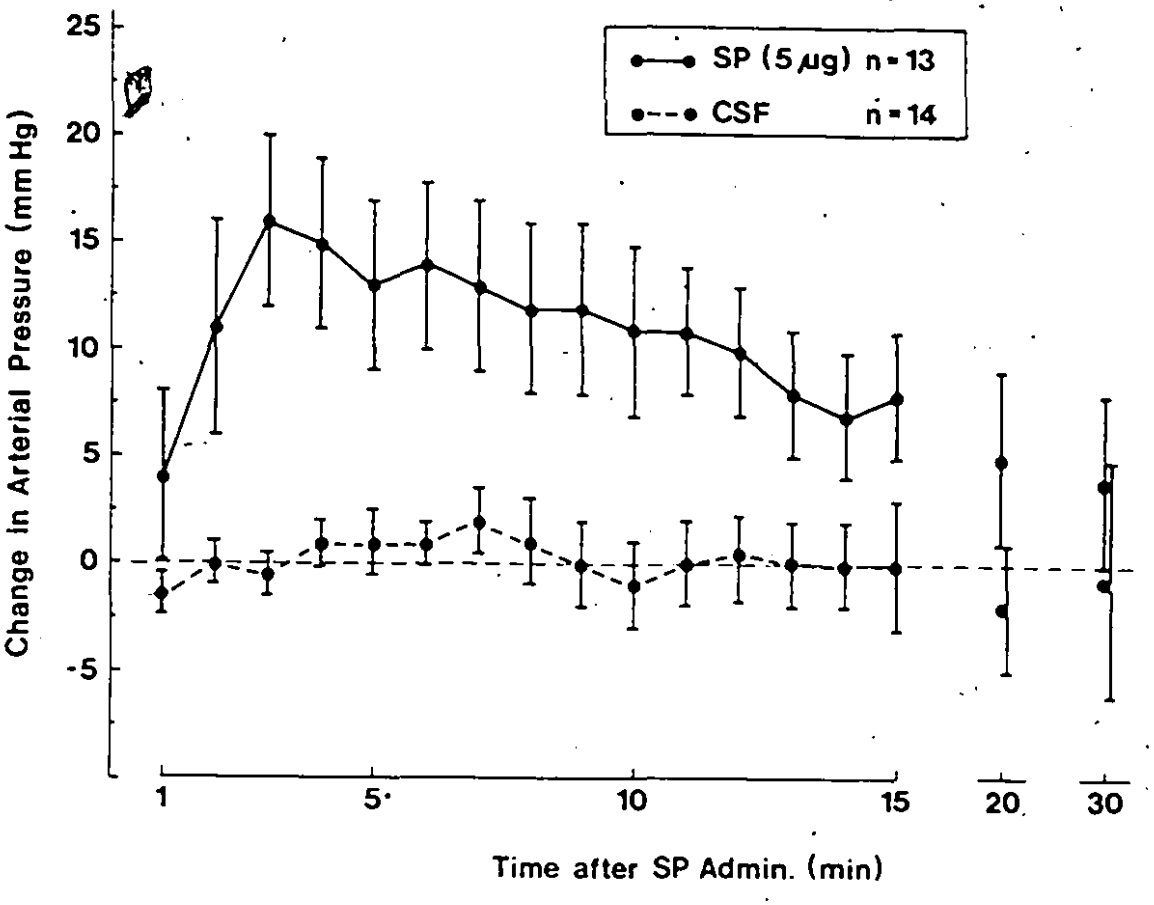

Figure 4. Change in arterial pressure induced by intrathecal injection of substance P (SP) or of artificial cerebrospinal fluid (CSF) to the second thoracic spinal segment of the rat. The ordinate shows the mean change in mean arterial pressure from pre-administration levels. Vertical bars each represent ± one S.E.M. Readings at 2 to 13 min were significantly different between the two groups of animals (p values are given in the text).

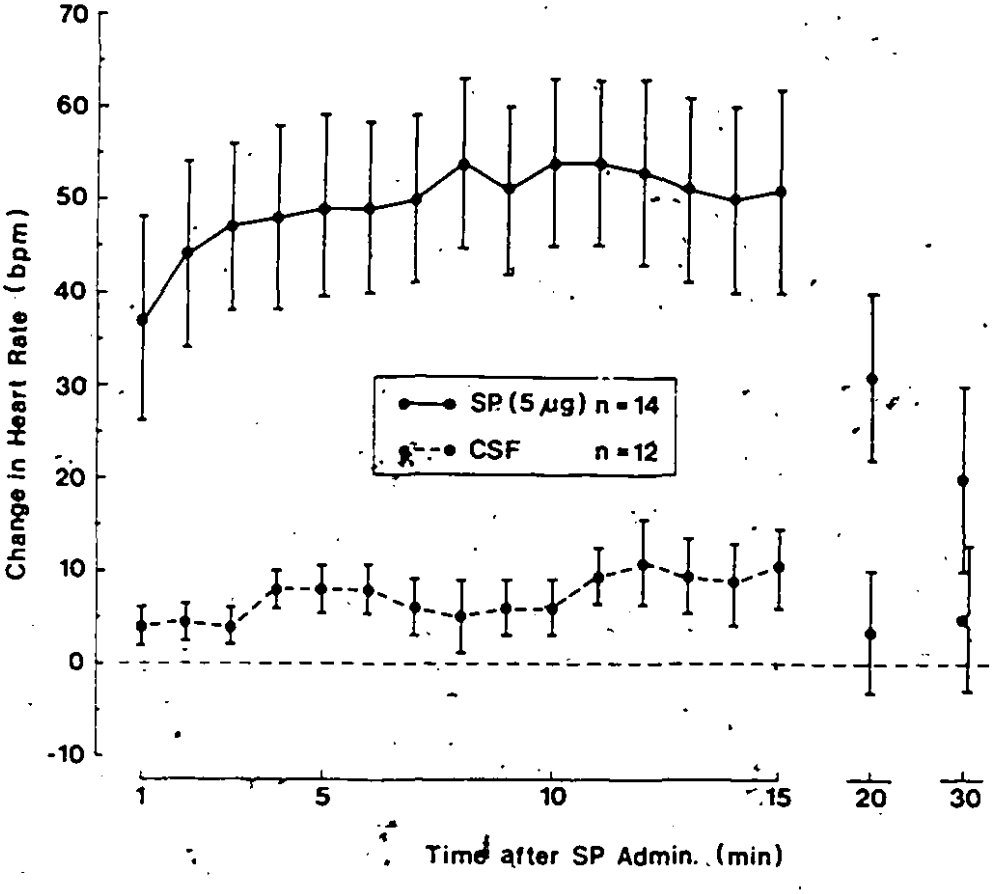

Figure 5. Change in heart rate induced by intrathecal injection of substance P (SP) or of artificial cerebrospinal fluid (CSF). The ordinate shows mean changes in heart rate from pre-administration levels. Readings at one to 20 min were significantly different (p values are given in the text). Details are otherwise as in the legend to Figure 4.

CHAPTER V

EFFECTS OF ADRENALECTOMY ON SYMPATHETIC ACTIVATION INDUCED BY THE INTRATHECAL ADMINISTRATION OF SUBSTANCE P IN THE RAT

Abstract

Administration of substance P into the intrathecal space of the ninth thoracic segment in the anaesthetized rat leads to comparable increases in arterial pressure and heart rate in adrenalectomized and in sham operated animals, whereas similar administration of artificial cerebrospinal fluid is without effect on either parameter. These results suggest that cardiovascular responses to spinal administration of substance P may be elicited via purely neural mechanisms. In view of our earlier finding that this administration of substance P increases the adrenal output of catecholamines, this study also suggests that the contribution of the adrenals to these responses is probably of only minor importance.

INTRODUCTION

In earlier experiments we found that the intrathecal administration of nanomole quantities of substance P at the ninth thoracic spinal level produces sympathetic activation, manifested as increases in heart rate and arterial pressure (Yashpal et al., 1986a) as well as increases in the concentrations of adrenaline and noradrenaline in venous plasma (Yashpal et al., 1985). Our earlier studies have also shown that when substance P is administered at the second thoracic level, the increase in heart rate is more immediate , while the increase in catecholamine output is less significant in comparison to the changes in heart rate and arterial pressure evoked by substance P administration at the ninth thoracic level (Yashpal et al., 1986b). These results suggest that while neuronal components at the second thoracic level are responsible for the increases in heart rate and arterial pressure, a humoral component, via the adrenals, may participate in the cardiovascular responses when substance P is given at the ninth thoracic level.

The present study was done to investigate the involvement of humoral mechanisms via the adrenal glands in mediating the cardiovascular responses to intrathecal administration of substance P. The experimental approach was to repeat the earlier experiments at the ninth thoracic segment, but in adrenalectomized rats. The objective was thus to provide results which could be compared to the earlier results from rats with intact adrenal glands.

EXPERIMENTAL PROCEDURES

As the experimental procedures are described in detail elsewhere (Yashpal et al., 1985, 1986a), only a brief description will be provided here.

Animal preparation

Male Sprague Dawley rats (approx. 350 g) were anaesthetized with urethane (2.5 g/kg, i.p.). Each rat was implanted with an intrathecal catheter (Intramedic PE-10), which was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the ninth thoracic vertebral level. The vertebral spinous processes were used as a basis for determining the length of catheter to be inserted. A second catheter (Intramedic PE-60) was placed in the left common carotid artery facing toward the heart for continuous monitoring of arterial pressure via a Statham P23 ID transducer, connected to a Grass polygraph. Heart rate was calculated from the arterial pressure records. Rectal temperature was maintained at approximately 37°C with a heating pad.

Animals were adrenalectomized bilaterally, using a surgical method. A midline incision was made in the abdomen, and each adrenal gland was exposed. It was separated from its surrounding tissue and removed manually. Sham operated control rats were treated in the same manner, except that the

adrenal glands were not removed.

A stabilization period of 30 min was allowed following surgical preparation. Baseline readings were taken over a five min period and substance P (Peninsula Laboratories) was then administered via the intrathecal catheter. The substance P was delivered over a period of 15-20 sec in a dose of 10 μg (6.5 nmoles) dissolved in 10 μl of artificial cerebrospinal fluid (CSF; an aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$). Following delivery of the peptide the catheter was flushed with 10 μl of CSF (catheter volume was 6-8 μl). In control experiments CSF replaced the substance P solution.

Results from each rat were tabulated as changes from the baseline values of arterial pressure or heart rate. Data for the figures were summarized by taking the mean ± S.E. of the values from each group of rats at each sample time following administration. Student's t-test was used to compare the two values at each sample time. The level of significance was taken as $p < 0.05$.

A number of experiments was done at the beginning of this project to determine the success of the adrenalectomy. In these experiments, a third catheter (Intramedic PE-60) was passed via the left femoral vein into the inferior vena cava so that the inner tip lay between the renal veins and the heart. This catheter was used for collection of blood. A baseline sample of 1.5 ml of blood was withdrawn and placed

immediately into cooled heparinized Eppendorf tubes. This blood was immediately replaced with 1.5 ml of warm (37°C) physiological saline, heparinized to keep the catheter patent. After 5 min, substance P was administered as above. Additional blood samples were taken at 1, 10 and 30 min after substance P administration. The four samples were then centrifuged at 2,000 rev/min at 4°C and the plasma was stored at -70°C until assayed for free catecholamines. This was done by high pressure liquid chromatography using a Waters M45 solvent delivery system and a U6K manual injector. The assay itself was done with a Bioanalytic Systems LC-4 electrochemical detector. The detector had a sensitivity of 2.0 nA/V, and the reliable detection limit was 0.5 ng/ml.

RESULTS

Verification of the correct placement of the inner tip of the intrathecal catheter was done in a number of pilot experiments, in which a wire was passed through the catheter until its end reached the inner tip of the catheter. X-rays of the rats implanted this way confirmed the reliability of our method for placement of the catheter (see Fig. 1 in ref. Yashpal et al., 1985).

We have also found that the diffusion of 10 µg of Fast Green dye is limited to two segments rostral and three segments caudal to the level of injection. Furthermore, the rapid onset of the responses we observed suggests that the site of action of substance P in increasing cardiovascular parameters was in the spinal cord rather than at some remote region, reached only after diffusion of the peptide.

Levels of adrenaline and noradrenaline in the adrenalectomized animals are presented in Table I, along with similar values obtained in our previous study on intact animals; the latter results are fully illustrated in our earlier paper (Yashpal et al., 1985) and are presented here in tabular form only for the convenience of comparison.

Intrathecal administration of substance P increased arterial pressure and heart rate both in sham operated controls and in rats in which the adrenal glands had been removed bilaterally. Administration of CSF had no effect on either parameter. To satisfy ourselves that the rats in each group were comparable in terms of their arterial pressure and heart rate before experimental manipulations, these parameters were examined carefully. The mean baseline systolic pressures were 114.4 ± 5.5 mmHg in the 7 sham operated animals, 118.0 ± 6.1 in the 8 adrenalectomized animals given CSF and 125.4 ± 5.0 in the 15 adrenalectomized animals given substance P; the respective diastolic pressures were 65.0 ± 4.5, 70.0 ± 7.5 and

59.4 ± 5.2 mmHg. Similarly, the mean baseline heart rates were 326.3 ± 17.9, 316.1 ± 7.3 and 317.5 ± 8.0 bpm, respectively, in the three groups.

Adrenalectomy altered these parameters. After the end of the 30 min stabilization period, animals given CSF had a systolic pressure of 105.6 ± 3.9 mmHg, a diastolic pressure of 66.3 ± 2.2 mmHg and a heart rate of 325.1 ± 12.4 bpm. In animals given substance P these parameters were 103.3 ± 5.2 mmHg, 56.1 ± 3.7 mmHg and 326.5 ± 12.1 bpm, respectively.

Figure 1 illustrates the changes in mean arterial pressure for the three groups of animals. Administration of substance P in intact animals had similar effects to those reported in our earlier report (Yashpal et al., 1986) in terms of time course and magnitude of change. Administration of substance P to adrenalectomized animals had a similar effect, at least in terms of the magnitude of the change during the first 10 min. After that time, the arterial pressure in the intact animals tended to show a second increase; this later rise in arterial pressure was also observed in our earlier study. Administration of CSF failed to change mean arterial pressure. (It is perhaps also worth noting that arterial pressure in these adrenalectomized animals did not change over the testing period of the experiment, suggesting that changes in arterial pressure induced by the adrenalectomy had stabilized during the recovery period). The difference between the two adrenalectomized groups (i.e.

substance P-treated vs CSF-treated) was statistically significant at one to 14 min (at 1 min, $p<0.05$; 2-11 min, $p<0.005$; 12-14 min, $p<0.05$). The difference between the two groups given substance P (i.e. adrenalectomized vs intact) was statistically significant only at 15 min ($p<0.05$).

Figure 2 illustrates the changes in heart rate in the three groups of animals. Again, in intact animals the change in heart rate was similar in time course and magnitude to that observed in our earlier study. In adrenalectomized animals the increase occurred much more abruptly, even though the magnitude of the increase was similar between the intact and the adrenalectomized animals at 5 min after administration. Heart rate in CSF-treated animals tended to increase gradually over the period of the experiment; this may have been due to the adrenalectomy, because a similar gradual change in heart rate was not seen in our earlier study in which CSF was given to intact animals. The difference between the two adrenalectomized groups, (substance P-treated vs CSF-treated) was statistically significant for 20 min (1-15 min, $p<0.001$; 20 min $p<0.025$). The difference between the two groups given substance P (i.e. adrenalectomized vs intact) was statistically significant at 1 to 4 min (1 and 2 min, $p<0.01$; 3 min, $p<0.025$; 4 min, $p<0.05$).

DISCUSSION

The principal conclusion which can be drawn from this study is that adrenalectomy does not abolish the cardiovascular responses elicited by the intrathecal administration of substance P to the ninth thoracic segment of the spinal cord and, therefore, that the cardiovascular responses observed in the adrenalectomized animals were elicited by neural rather than humoral mechanisms.

Substance P-containing terminals have been observed surrounding sympathetic preganglionic neurones projecting directly to the adrenal medullae, with the greatest concentration at the ninth thoracic level (Holets & Elde, 1983). As changes in arterial pressure in the adrenalectomized animals were presumably elicited exclusively via neural and not humoral mechanisms in the present study, a further conclusion is that substance P-containing nerve terminals may also be found surrounding sympathetic preganglionic neurones in pathways to the vessels and to the heart.

It is not possible to reliably comment on the importance of the adrenal glands in inducing changes in cardiovascular parameters, at least in the experimental protocol used in these experiments. However, the similarity in the magnitude of the responses elicited in intact and in adrenalectomized animals might suggest that the humoral mechanism is relatively unimportant in comparison with the

neural mechanism. It is interesting in this context that Robinson et al. (1983) stimulated extensively throughout the hypothalamus and monitored arterial pressure and adrenal output of catecholamines. They reported that there was no consistent relationship between the secretion of catecholamines and the concomitant change in arterial pressure. In the present study, the second rise in arterial pressure at 15 min in the intact animals raises the possibility that catecholamines released from the adrenal glands may have given rise to the more prolonged effect in the intact animals. In fact, a biphasic increase in arterial pressure has been reported upon electrical stimulation of the hypothalamus in the rat with intact adrenals (Eferakeya & Bunag, 1974). In this case, the second but not the first phase was abolished by bilateral adrenalectomy, suggesting that the early phase of this biphasic response was elicited via neural mechanisms and that the later phase was elicited via humoral mechanisms.

The more rapid onset of the increase in heart rate in the adrenalectomized animals in the present study is a curious observation and may be due to a factor released by the adrenals, which causes cardiac slowing.

In view of the suggestion that cardioacceleratory neurones lie in the first six thoracic segments (Henry & Calaresu, 1972), the increase in heart rate elicited by neural

mechanisms from the ninth thoracic segment in the present experiments suggests that there may have been some diffusion of substance P to at least the caudal part of the first six thoracic segments. This is entirely feasible using the present experimental paradigm, although the experiments described above using Fast Green dye suggest that this diffusion could not have been extensive. The increase in arterial pressure elicited via neural mechanisms from the region of the ninth thoracic segment in this study is consistent with the earlier report that neurones involved in increasing arterial pressure are also present at this level of the spinal cord (Henry & Calaresu, 1972).

Goadsby (1985) has recently reported that the increase in arterial pressure elicited by electrical stimulation of the locus coeruleus is completely eliminated by bilateral adrenalectomy in the cat. These results suggest that some central pressor pathways exist, which express their effects exclusively via the adrenal glands. Our present study suggests, on the other hand, that descending pressor and cardioacceleratory pathways which release substance P at the spinal level do not require the adrenals. Therefore, one may speculate that the descending pathway from the locus coeruleus does not include a substance P-mediated synapse at the spinal level.

Holets and Elde (1982) have demonstrated a differential distribution of neuropeptides in nerve terminals surrounding

sympathetic preganglionic neurones, with somatostatin being found only in terminals surrounding neurones to the adrenals and with oxytocin being found only in terminals other than those surrounding neurones to the adrenals. Substance P, on the other hand, was observed surrounding both types of sympathetic preganglionic neurone. In view of this varying selectivity in the regulation of sympathetic function, the present results agree with the intimation from the study of Holets and Elde (1982) that the functional role of substance P in spinal autonomic pathways is to regulate sympathetic output in a more global and general way, than to selectively act on one particular population of sympathetic preganglionic neurones, subserving one specific function.

In conclusion, our results show that the intrathecal administration of substance P to the ninth thoracic segment increases heart rate and arterial pressure in the adrenalectomized rat and that the magnitudes of the responses are comparable in both adrenalectomized and intact groups, although there may be differences in the time courses of the responses in the two types of animal. In view of our earlier finding that similar administration of substance P increases adrenal output of catecholamines, these results suggest that substance P regulates the cardiovascular system at the spinal level via both neural and humoral mechanisms, although the extent of the contribution by humoral mechanisms may not be significant, at least in terms of effecting changes in heart rate and arterial pressure.

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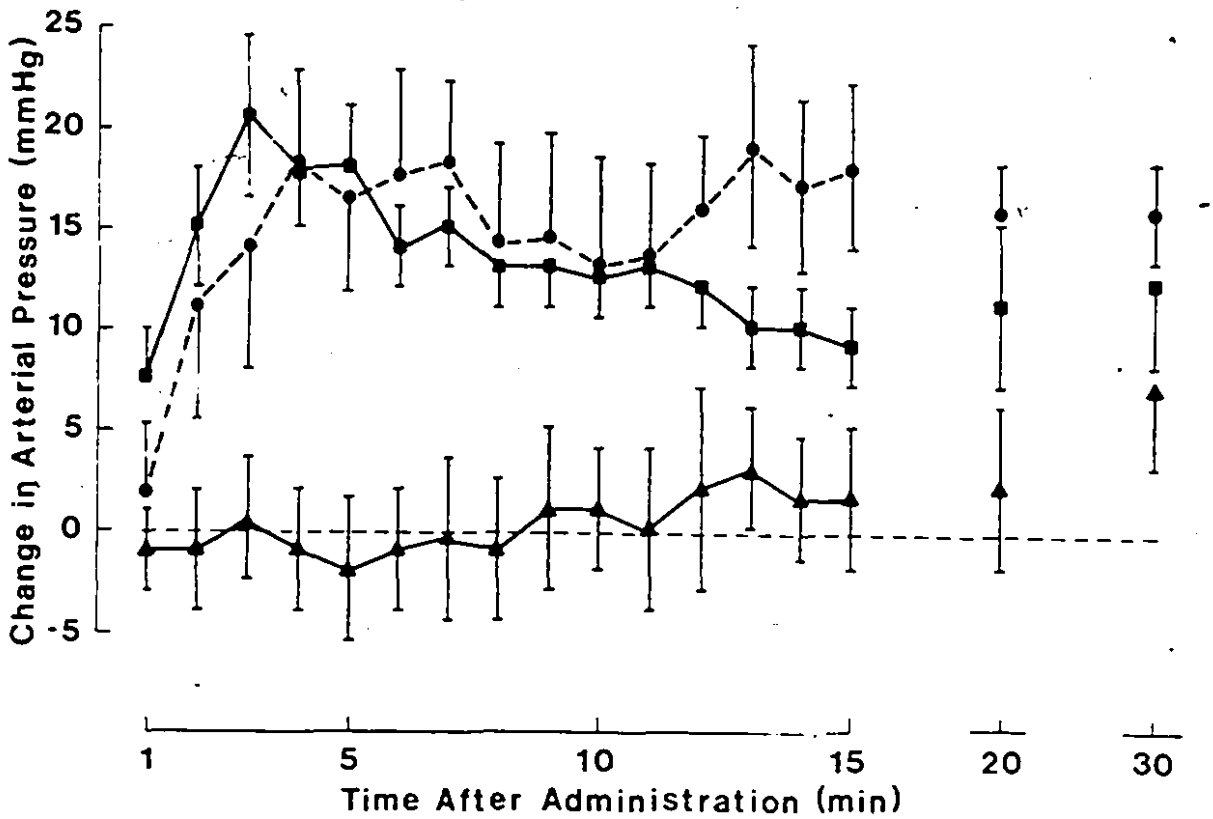

Figure 1. Change in mean arterial pressure induced by substance P injected intrathecally to the ninth thoracic level of the spinal cord of the adrenalectomized (■-----■) or of the sham operated (●- -●) rat. Artificial cerebrospinal fluid (CSF) instead of the substance P solution was given to a third group of animals (▲-----▲). The ordinate shows the mean change in mean arterial pressure as compared to pre-administration values. The abscissa indicates time after administration. Vertical bars each represent one S.E.M.

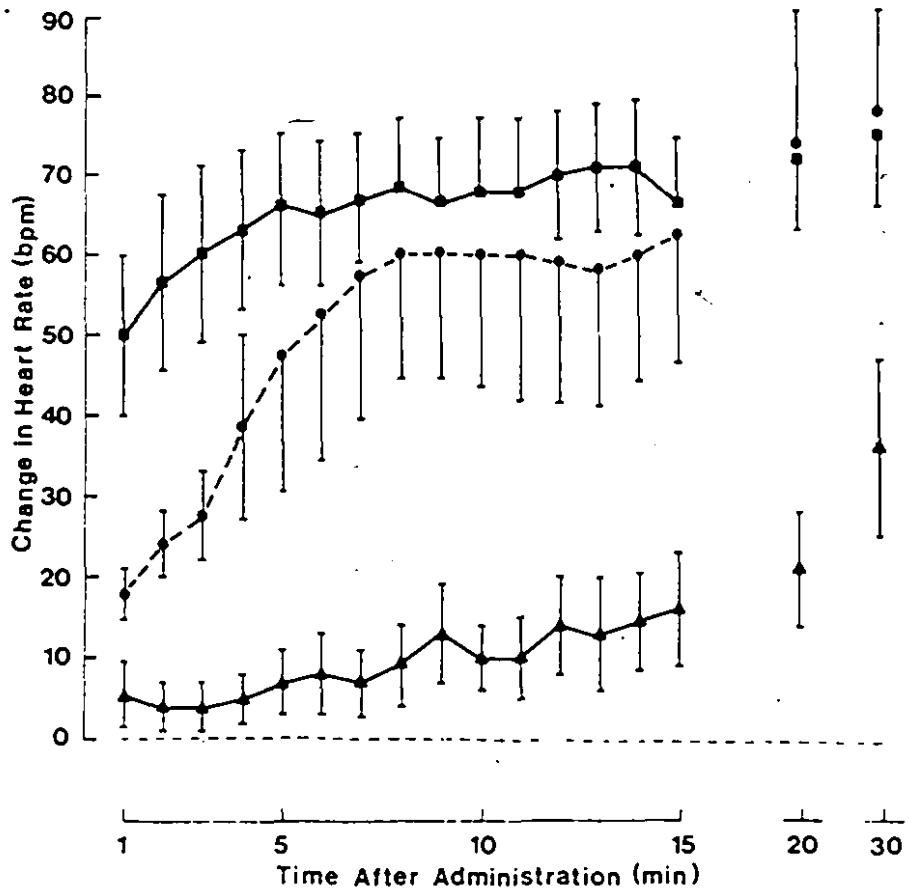

Figure 2. Change in heart rate induced by substance P injected intrathecally in the adrenalectomized (■———■) or in the sham operated (● - - ●) rat. CSF replaced the substance P solution in a third group of rats (▲———▲). Details are otherwise as in the legend to Figure 1.

Table 1. Plasma levels of free adrenaline and noradrenaline (ng/mL plasma) in intact and adrenalectomized animals before and after intrathecal administration of substance P.

	Before Administration	% of preadministration levels 1 min	10 min
Noradrenaline:			
Intact (n=17)	4.8±1.0	153.5±11.8	142.8±10.7
Adrenalectomized (n=12)	2.9±0.5	115.8±22.9	113.5±17.2
Adrenaline:			
Intact	8.3±1.3	154.8±10.8	141.9±12.1
Adrenalectomized	0.5±0.1	91.7±19.0	104.0±11.4

CHAPTER VI

ANGIOTENSIN II STIMULATES SYMPATHETIC OUTPUT BY A DIRECT SPINAL ACTION

Abstract

Angiotensin II, administered intrathecally in a dose of 10 μg to the ninth thoracic segment of the spinal cord in the anesthetized rat, produced a transient increase in systolic and diastolic arterial pressures lasting 1-4 min. Heart rate was also increased, but in this case for more than 30 min. Neither the change in arterial pressure nor the change in heart rate was observed in rats given hexamethonium (10 mg/kg, i.v.) to block nicotinic transmission in sympathetic ganglia, suggesting that the effects were mediated by spinal activation of sympathetic output. When angiotensin II was given i.v., only brief changes in arterial pressure and heart rate occurred. Pretreatment of the rats with [Sar^1,Ile^8]-angiotensin II three min prior to angiotensin II blocked the increase in arterial pressure but not the increase in heart rate. When the antagonist was given 15 min prior to angiotensin II, the full pressor response appeared, suggesting that the antagonist was effective for less than 15 min. In addition, when the antagonist was given 15 min prior to angiotensin II, heart rate increased gradually over the 15 min period, suggesting that it had agonist effects on mechanisms regulating heart rate. These results suggest that angiotensin II activates sympathetic mechanisms by a spinal action and that arterial pressure and heart rate are regulated differentially, arterial pressure via a mechanism in which [Sar^1,Ile^8]-angiotensin II is an antagonist and heart rate via a mechanism in which the analog is an agonist.

INTRODUCTION

Abundant evidence has implicated angiotensin II in central control of the cardiovascular system (for recent references see Corwin et al., 1985; Lappe & Brody, 1984; Guo & Abboud, 1984; Fregly et al.,1984; Scholkens et al., 1982). Although cardiovascular responses to central administration of angiotensin II are apparently mediated at least partly via sympathetic activation (Lappe & Brody, 1984; Unger et al., 1981; Scholkens et al., 1982; Falcon et al., 1978), physiological studies directed at identifying the precise site of action largely overlook the spinal cord as a possible site for these sympathoexcitatory effects (Buckley & Jandhyala, 1977; Ganten et al., 1978; Scholkens et al., 1982).

Recent anatomical studies, on the other hand, have implicated the intermediolateral nucleus (ILN) of the spinal cord as a possible site for the actions of angiotensin II. In an early study using the indirect immunohistochemical method, Fuxe et al.(1976) observed a high density of nerve terminals containing immunofluorescent material located in the ILN in the rat. Ganten et al. (1978) subsequently confirmed this observation and added that this immunoreactivity was not observed in nerve terminals in the ILN one week after transection of the spinal cord. Accumulation of immunoreactive material was observed in descending fibers in the lateral funiculus, although the cell bodies giving rise to these

descending fibers were not identified. In colchicine-treated rats, Brownfield et al.(1982) found that cell bodies containing immunoreactive material were restricted to magnocellular cells in the paraventricular, supraoptic and accessory magnocellular nuclei of the hypothalamus in the rat, suggesting that angiotensin-containing nerve terminals in the ILN are associated with fibers descending from the hypothalamus to directly innervate spinal sympathetic neurons. Radioautographic studies on central binding sites of angiotensin also implicate angiotensin in synaptic transmission in the spinal cord. While the existence of high affinity binding sites for angiotensin in central nervous tissue has been known for some time (Sirette et al.,1977;Bennet & Snyder, 1980; van Houten et al.,1980) Mendelsohn et al. (1984) have recently demonstrated low concentrations of binding sites specifically in the spinal grey matter.

It was with this historical perspective that we initiated the present physiological study. We have been investigating the effects of the intrathecal administration of neuroactive peptides on cardiovascular parameters (Yashpal et al., 1985, 1986a,b), and angiotensin was selected as one of the peptides for study on the basis of the information outlined above. We report here that administration of angiotensin II to the ninth thoracic segment of the spinal cord induces a reproducible, transient increase in arterial pressure and in heart rate which is not mimicked by similar administration of the delivery vehicle. These responses are prevented by

pharmacological blockade of synaptic transmission in sympathetic ganglia. The prior intrathecal administration of the angiotensin II antagonist [Sar^1,Ile^8]-angiotensin II blocks the increase in pressure but not the increase in heart rate. Some of these results have been presented in abstract form (Yashpal et al.,1985b).

METHODS

Male Sprague Dawley rats weighing approximately 350 g were used. They were anesthetized with urethane (2.5 g/kg, i.p.). An intrathecal catheter (Intramedic PE-10) was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the T9 vertebral level, using spinous processes as landmarks. In preliminary experiments the validity of this method for correct positioning of the inner tip of the catheter was confirmed in X-rays of rats implanted with wire-filled catheters (see ref. Yashpal et al.,1985, fig. 1). It was via this catheter that angiotensin II was administered intrathecally.

A second catheter (Intramedic PE-60) was inserted into the left common carotid artery facing the heart for monitoring arterial pressure via a Statham transducer (Gould P23 ID) connected to a Grass polygraph. Heart rate was calculated from this record. Systolic and diastolic pressures were measured from the rateмeter records and mean arterial pressure was

calculated from these measurements. The number of heart beats in a 10 sec period was counted and that number was multiplied by six to obtain heart rate in beats per min.

In experiments where agents were administered intravenously, a third catheter was inserted into the left femoral vein facing the heart.

Animals respired spontaneously throughout the experiments. Rectal temperature was maintained at approximately 37°C by a heating pad.

Experiments with intrathecal administration of angiotensin II

After surgical preparation, a 30 min period was allowed for stabilization. Baseline readings of arterial pressure and heart rate were taken over a five min period and angiotensin II (from Peninsula Laboratories, Belmont, Calif., or from Institut Armand-Frappier, Laval, Quebec) was administered intrathecally in a dose of 10 µg delivered over a period of 15-20 sec in 10 µl of artificial cerebrospinal fluid (CSF; an aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$). After delivery of the peptide the intrathecal catheter was flushed with 10 µl of CSF (the internal volume of the catheter was approximately 8 µl). With zero time being the time of injection of CSF, readings of arterial pressure and heart rate were taken each minute for the next 15 min, and then at 20 and 30 min.

Experiments with intravenous administration of angiotensin II

In view of the possibility that angiotensin II was producing its effects via a peripheral mechanism after passage from the perispinal space into the circulation, the experiments described above were repeated, but angiotensin II was administered intravenously rather than intrathecally. The protocol was the same, with a stabilization period, calculation of baseline values and, after administration of the peptide, calculation of arterial pressure and heart rate at 1-15 min and 20 and 30 min. The peptide was dissolved in 0.5 ml of saline for these experiments and the catheter was flushed with 0.5 ml of saline.

Effects of intrathecal angiotensin II after sympathetic block

As a further check against the possibility that angiotensin II delivered into the intrathecal space was passing to the periphery to exert its effects, the experiments with intrathecal administration were repeated, but in animals which had been given a sympathetic ganglion blocker; the rationale was that the persistence of a response to angiotensin II would suggest that the peptide was indeed having a peripheral effect.

In these experiments, after baseline readings had been taken, hexamethonium was administered via the venous catheter in a dose of 1 mg/100g body weight (concentration of 10 mg/ml in saline). Three more readings were taken at one min intervals, angiotensin II was administered intrathecally as above, and readings were taken each min for the next 10 min.

Intrathecal administration of [Sar^1,Ile^8]-angiotensin II An additional series of experiments was done to determine whether the responses to intrathecal administration of angiotensin II could be blocked by an angiotensin II antagonist. The antagonist chosen was [Sar^1, Ile^8]-angiotensin II; this analog of angiotensin II has been shown to effectively block effects of angiotensin II in the central nervous system (Akaishi et al., 1980). The protocol outlined above was modified so that the analog was administered 3 or 15 min prior to angiotensin II. Thus, in this series of experiments, after baseline readings of arterial pressure and heart rate were taken, the analog was given intrathecally in a dose of 10 µg in 10 µl of CSF, and the catheter was flushed as above. Measurements of arterial pressure and heart rate were taken at one min intervals as before. Then, either 3 or 15 min after this administration of the analog, angiotensin II was given and measurements were taken each min for the next 15 min and at 20 and 30 min.

As a control for [Sar^1, Ile^8]-angiotensin II, vehicle replaced the analog solution.

Statistical analysis. Results from each rat were tabulated as systolic and diastolic arterial pressures and heart rate (for Figs. 1 and 2) and as changes in these parameters from the baseline values determined before intrathecal injection (for Figs. 3-6) . Data for the figures were summarized by taking

the mean ± S.E. of the values from each group of rats at each min following administration. Student's t-test for unpaired data was used for comparison between the two values (angiotensin II vs CSF groups) at each sample time after administration of angiotensin II or of the analog.

RESULTS

Pilot experiments demonstrated that the diffusion of intrathecally administered Fast Green dye was limited to two segments rostral and three segments caudal to the level of injection over a period of 30 min after injection. The rapid onset of the responses in these and in previous experiments (Yashpal et al., 1985, 1986a,b) suggests furthermore that peptides administered intrathecally act at the level of injection rather than at a remote site in the central nervous system. Intrathecal administration of angiotensin II provoked an increase in arterial pressure and in heart rate. There was no effect on respiratory frequency, which remained at a mean value of approximately 100 breaths per minute. In our earliest experiments it was observed that lower doses of angiotensin II than 10ug provoked smaller cardiovascular responses or no response at all. It was also observed that repeated injections of 10ug of angiotensin II, given at 5 min intervals, provoked successively smaller responses.

Effects of intrathecal administration of angiotensin II

In terms of arterial pressure, both systolic and diastolic pressures were increased. The results are illustrated in Figure 1. This effect was transient, peaking at 2 min after administration and being over within five min. Figure 1 also illustrates that the intrathecal injection of the vehicle failed to alter systolic or diastolic pressure. Before administration the mean systolic and diastolic pressures were 110 $\pm$8.6 and 50 $\pm$8.3 mmHg, respectively, in rats given angiotensin II and 113 $\pm$4.9 and 65 $\pm$4.3, respectively, in rats given CSF. At the peak of the response, the systolic pressure had increased by approximately 15 mmHg and the diastolic pressure by approximately 20 mmHg. When the changes in pressure were compared between the peptide- and CSF-treated rats, the changes were different between the two groups at 1-3 min after administration ($p< 0.05$).

The heart rate response followed a different time course. In this case, illustrated in Figure 2, the increase began to plateau at about 4 min and the heart rate remained elevated at the same approximate level throughout the rest of the experimental period, to 30 min after administration. Prior to administration the mean heart rate was 354 $\pm$ 13.4 bpm in rats given angiotensin II and 332 $\pm$15.4 bpm in rats given CSF. Comparison of the changes in heart rate between the two groups

indicated significant differences throughout the 15 min after administration (1min, $p < 0.025$; 2-10 min, $p < 0.005$; 11-14 min, $p < 0.025$; 15 min, $p < 0.05$).

Effects of intravenous administration of angiotensin II

To pursue the possibility that angiotensin II delivered into the intrathecal space might pass into the circulation and express its effects via a peripheral action, these experiments were repeated, except that the same dose of angiotensin II was administered intravenously in a volume of 0.5 ml of physiological saline. Prior to administration the mean systolic and diastolic pressures of the seven animals tested were 123 and 56 mmHg, respectively; changes from these values were 7, 1, -5, -1 and 1 mmHg and 33, 16, 6, -3 and -3 mmHg, respectively, at 1, 2, 5, 10 and 15 min after administration. Similarly, mean heart rate was 390 bpm before administration of angiotensin II and was 66, 66, 42, 18 and 0 bpm greater than this baseline value at 1, 2, 5, 10 and 15 min following administration.

Effect of ganglion block on responses to angiotensin II Results from these experiments are presented in Figure 3. Prior to the administration of 10 mg/kg of hexamethonium i.v., mean baseline systolic and diastolic pressures in the four

animals tested were 130 and 60 mmHg, respectively; the mean baseline heart rate was 353 bpm. Administration of hexamethonium had decreased mean arterial pressure by 17 mmHg and increased heart rate by 32 bpm by the time angiotensin II was given three min later. Figure 3 shows that both mean arterial pressure and heart rate were unaltered by the administration of angiotensin II in these animals pretreated with hexamethonium systemically.

Effects of [Sar^1, Ile^8]-angiotensin II on responses to angiotensin II [Sar^1,Ile^8]-angiotensin II was given at two times prior to angiotensin II to obtain information on whether the response to angiotensin II could be blocked and, when it was found that this occurred, to determine roughly how long the antagonism persists.

The antagonist was given three min prior to angiotensin II in 7 rats. The mean systolic and diastolic arterial pressures prior to administration were 121 and 53 mmHg. The mean heart rate was 325 bpm. Administration of the antagonist had no obvious effect on arterial pressure (Figure 5) and led to a minor increase in heart rate (Figure 6). The subsequent administration of angiotensin II failed to alter arterial pressure. However, heart rate was increased by the administration of angiotensin II. This increase had the same general appearance as that illustrated in Figure 2.

When CSF was given instead of the antagonist there was

no change in either arterial pressure or heart rate. However, when angiotensin II was given to these animals it increased both arterial pressure and heart rate; both responses followed roughly the same time course as that of the responses obtained in the absence of the antagonist (see Figures 1 and 2).

When the antagonist was given 15 min before the angiotensin II, it failed to block the arterial pressure response. This is illustrated in Figure 7. Heart rate showed a very different pattern. The results are illustrated in Figure 8. The antagonist led to a gradual increase in heart rate, and the subsequent administration of angiotensin II had little effect on the slope of this increase.

DISCUSSION

This study has demonstrated that the intrathecal administration of angiotensin II to the ninth thoracic spinal segment increases arterial pressure and heart rate in the rat. These responses fail to occur in animals in which synaptic transmission in sympathetic ganglia is blocked, and therefore the conclusion is reached that the responses observed did not occur by the passage of the peptide from the intrathecal space into the circulation. (This is an important issue because of the possibility that the angiotensin II may have passed into the circulation and then crossed back into the central nervous system at a higher level; Van Houten et al., 1980, 1983.)

Rather, it is suggested that the responses were due to an action of angiotensin II within the spinal cord. This action was probably an excitation of sympathetic preganglionic neurones. As stated above in the Introduction, high densities of nerve terminals containing angiotensin II-like material are found surrounding these neurons (Fuxe et al., 1976; Ganten et al., 1978) and angiotensin II binding sites are found in the spinal grey matter (Mendelsohn et al., 1984). Sensory neurons are probably not involved in mediating the effects reported here because angiotensin II is without effect on dorsal horn neurons in vitro (Miletic & Randic, 1982) and, when angiotensin II is given intrathecally at the lower lumbar level in a dose similar to that used in the present study, it is without effect on tail flick latency (R.A.Cridland & J.L.Henry,unpublished observations).

With the presumption that sympathoexcitatory actions of angiotensin II in these experiments would be on sympathetic preganglionic neurons, it was felt that as no central synapses were involved in mediating the responses observed, these experiments could justifiably be done in anesthetized animals. In fact, with this approach, arterial pressure could be measured reliably and easily by the direct method. In addition, artifactual changes in cardiovascular parameters due to changes in the behavioral states of an awake animal could be avoided. This latter point was considered to be important in view of behavioral responses induced by the intrathecal

administration of some peptides (Yashpal et al., 1982; Hylden & Wilcox, 1981; Seybold et al., 1982; Piercey et al., 1981). We also found in an earlier study (Yashpal et al., 1986a) that anesthesia has no quantitative effect on cardiovascular responses to intrathecal administration of substance P. Therefore, the experiments were all done in the anesthetized rat. Urethane was used because it has less of a disruptive effect on baseline parameters and on cardiovascular reflexes as well as on respiratory parameters than do other anesthetics in the rat (Armstrong, 1981; DeWildt et al., 1983; Sapru & Krieger, 1979).

The possibility was considered that angiotensin II may have diffused to brain stem structures to produce the pressor and cardioacceleratory effects reported here. However, this possibility was considered unlikely for a number of reasons. Injection of dye intrathecally to the ninth thoracic level spreads no farther rostrally than the seventh thoracic level over a 30 min period. Secondly, the time course of both the pressor response, which peaked at 2 min after injection, and the cardioacceleratory response, which had reached a stable level by 4 min, was too rapid for diffusion of the peptide to the brain stem. In addition, in other studies in which substance P was administered at the ninth vs the second thoracic levels, noticeably different responses were observed at the two levels (Yashpal et al., 1986a, b). Finally, the rat is unlike the dog (Ferrario et al., 1972; Gildenberg et al.,

1963; Joy & Lowe, 1970), cat (Ueda et al., 1972) and rabbit (Dickinson &.Yu, 1963; Ferrario et al., 1972; Yu & Dickinson, 1971), in which the area postrema seems to have a prominent role in expressing the pressor effects of angiotensin II; in these other species a greater pressor response to angiotensin II occurs when it is administered into the vertebral rather than into the carotid artery (Dickinson & Yu, 1963; Fukiyama et al., 1971; Sweet et al., 1971). In the rat, the pressor response evoked by administration of angiotensin II into the vertebral artery is significantly less than that to administration of angiotensin II into the carotid artery (Haywood et al., 1980). Rather than the area postrema, it appears that the principal site of the pressor action of angiotensin II is in the periventricular region of the preoptic area of the anterior hypothalamus (Bealer, 1982; Brody, 1981; Johnson et al., 1981).

Another aspect of the present study was the relatively large amounts of angiotensin II used, compared to those used in studies in supraspinal structures. In our experiments we found that 10 µg yielded cardiovascular responses which were clearly different from controls; in the case of intracerebroventricular and intracerebral administration, investigators typically use doses between 10 and 500 µg to

elicit pressor responses in the rat (Akaishi et al., 1980; Falcon et al., 1978; Hoffman & Phillips, 1977; Jones, 1984).

The results with the antagonist are, at face value, unanticipated and perplexing. In the first place, when the antagonist was given three min prior to the administration of angiotensin II it blocked the arterial pressure response, but the heart rate response was essentially indistinguishable from the response when CSF had been given instead of the antagonist. When the antagonist was given 15 min prior to angiotensin II, very different results were obtained: the antagonism of the arterial pressure response was not observed, but there was not the same increase in heart rate that was seen when the antagonist was given three min before angiotensin II.

Several conclusions are offered on the basis of the results from these experiments with the antagonist. Comparison of Figures 5 and 6 demonstrates that the arterial pressure response was blocked but that the heart rate response was not blocked. Figures 6 and 8 demonstrate that the analogue of angiotensin II causes a slow increase in heart rate; such an effect was not oberved on arterial pressure (Figures 5 and 7). These comparisons suggest that

the two cardiovascular responses are mediated via two different types of receptor, the former being mediated via receptors at which [Sar^1,Ile^8]-angiotensin II is an antagonist and the latter being mediated via receptors at which this analogue is an agonist. The suggestion that the receptors mediating the two cardiovascular responses are different is consistent also with the very different time courses of the two responses illustrated in Figures 1 and 2.

Comparison of Figures 5 and 7 demonstrates, furthermore, that the analogue blocked the increase in arterial pressure at three min but that this effect was gone by 15 min. This period of effective blockade is shorter than that observed with another peptide in earlier experiments; an analog of substance P blocked the cardiovascular response to the intrathecal administration of substance P for at least 15 min (Yashpal et al., 1986a).

With regard to the effects of angiotensin II on heart rate illustrated in Figure 8, it is difficult to determine either way whether the analog blocked this response because the increasing variation in the results in the control group made comparisons unreliable.

Our evidence suggests that angiotensin II stimulates sympathetic output via a spinal site of action, presumably by direct excitation of sympathetic preganglionic neurons. In view

of anatomical evidence that angiotensin II is found in nerve terminals surrounding sympathetic preganglionic neurons, our results suggest further that angiotensin II may be an excitatory chemical mediator of synaptic transmission onto these neurons. Thus, in addition to its well documented effects on sympathetic output, via actions at supraspinal sites which have recently been reviewed by Unger et al. (1985), angiotensin II also seems to stimulate sympathetic output via a spinal action.

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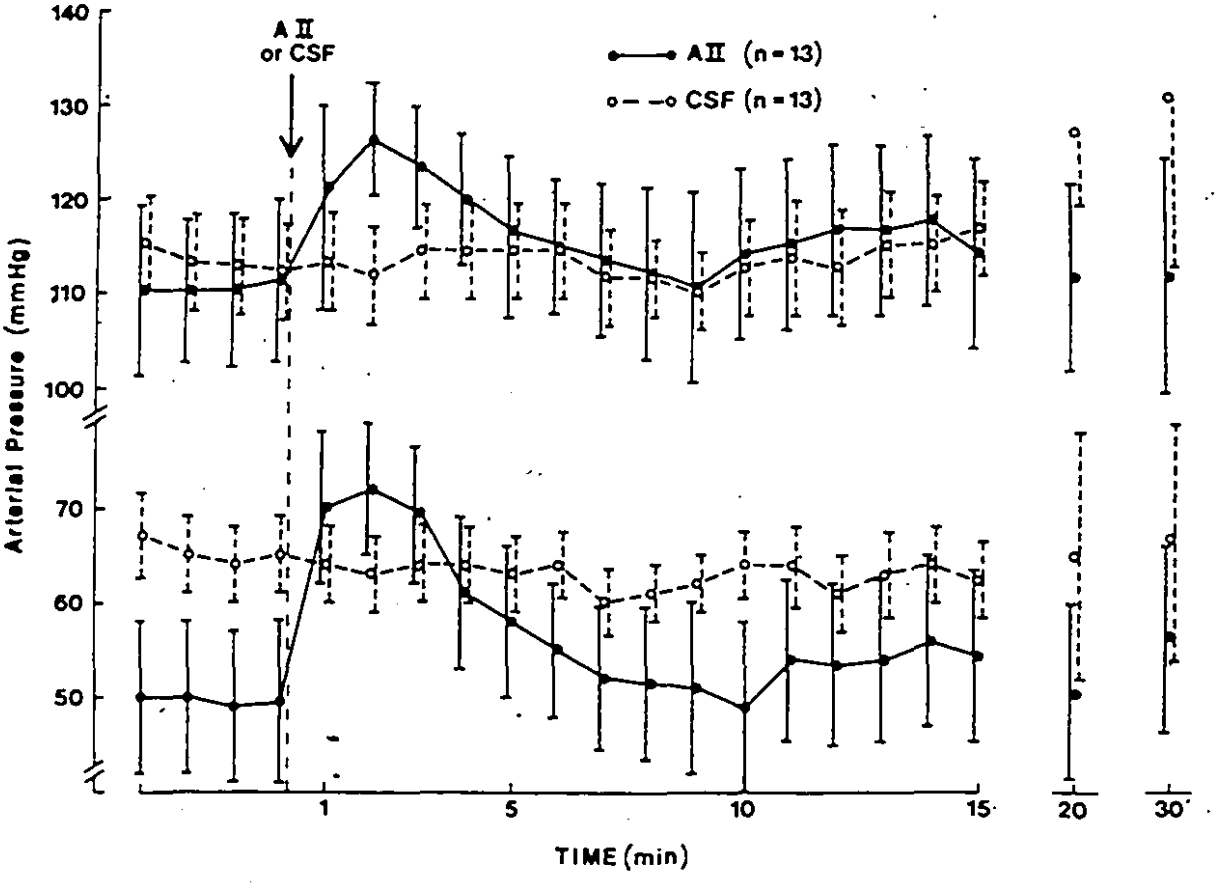

Figure 1. Changes in mean systolic arterial pressure (upper records) and mean diastolic arterial pressure (lower records) induced by intrathecal administration of angiotensin II (A II; 10 µg) or of artificial cerebrospinal fluid (CSF) to the ninth thoracic spinal segment of the anesthetized rat. Angiotensin II was given in a dose of 10 µg dissolved in CSF. The abscissa indicates time after administration. Vertical bars each represent one S.E.M.

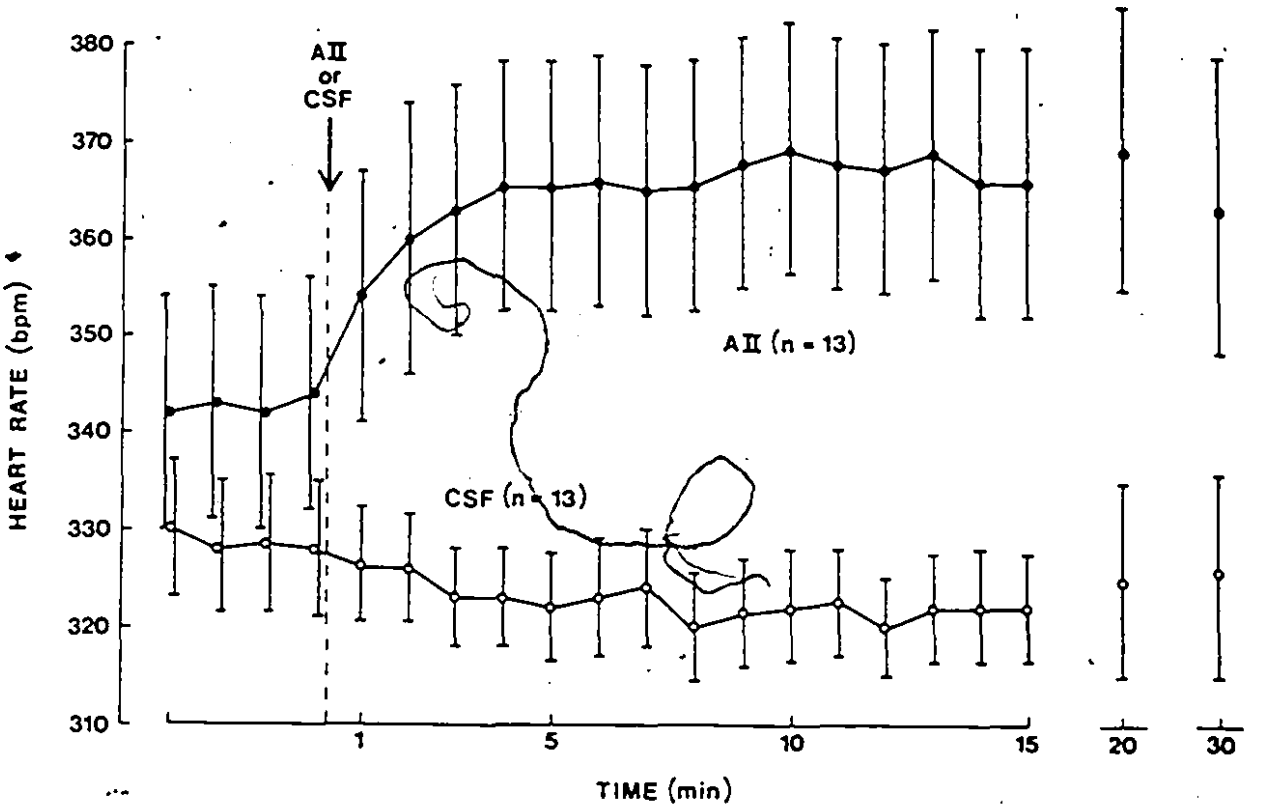

Figure 2. Change in mean heart rate induced by the intrathecal administration of angiotensin II or of CSF to the ninth thoracic segment. Details are otherwise as in the legend to Figure 1.

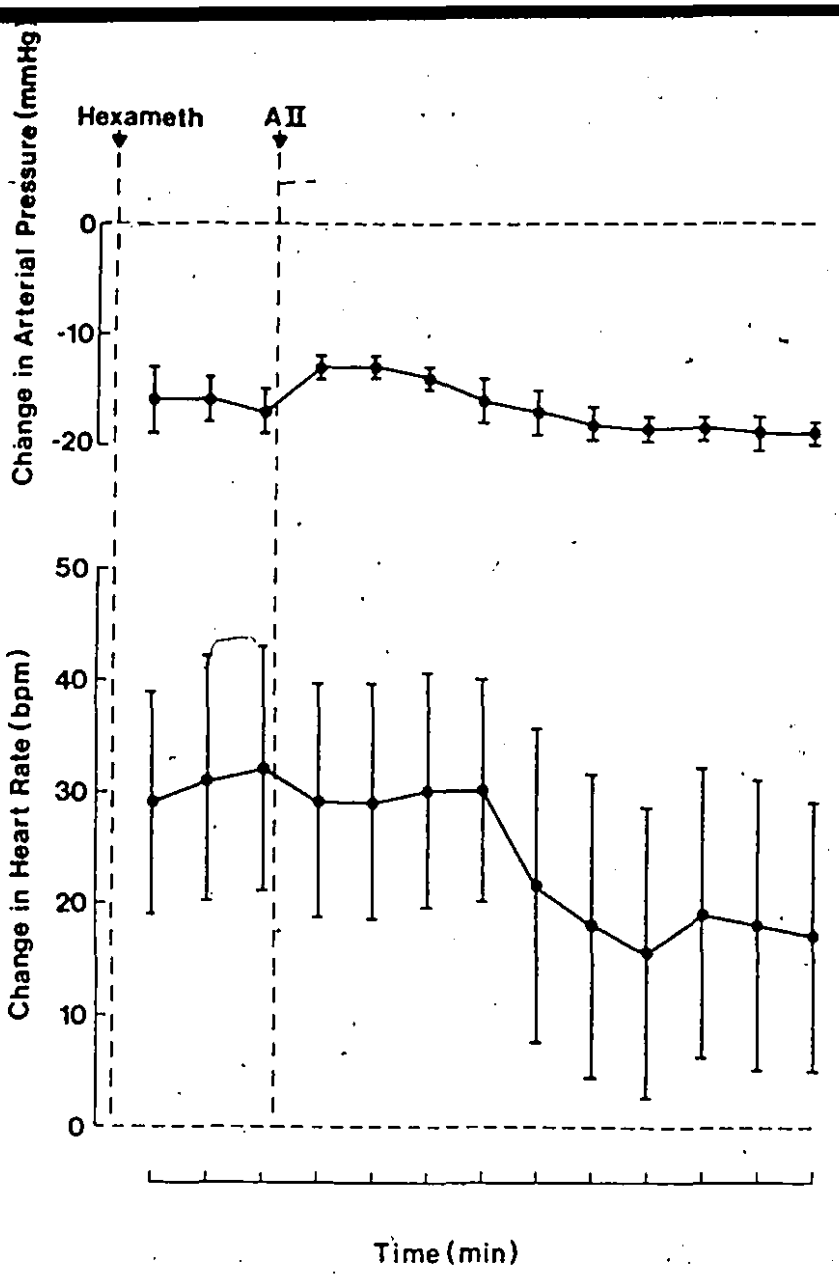

Figure 3. Effects of block of synaptic transmission in sympathetic ganglia on the responses to intrathecal administration of angiotensin II. Hexamethonium (Hexameth) was given in a dose of 10 mg/kg i.v. at the arrow at the left. Values are expressed as changes from baseline arterial pressure (upper graph) and from baseline heart rate (lower graph) determined over the 5 min prior to administration of hexamethonium. Details are otherwise as in the legend to Figure 1.

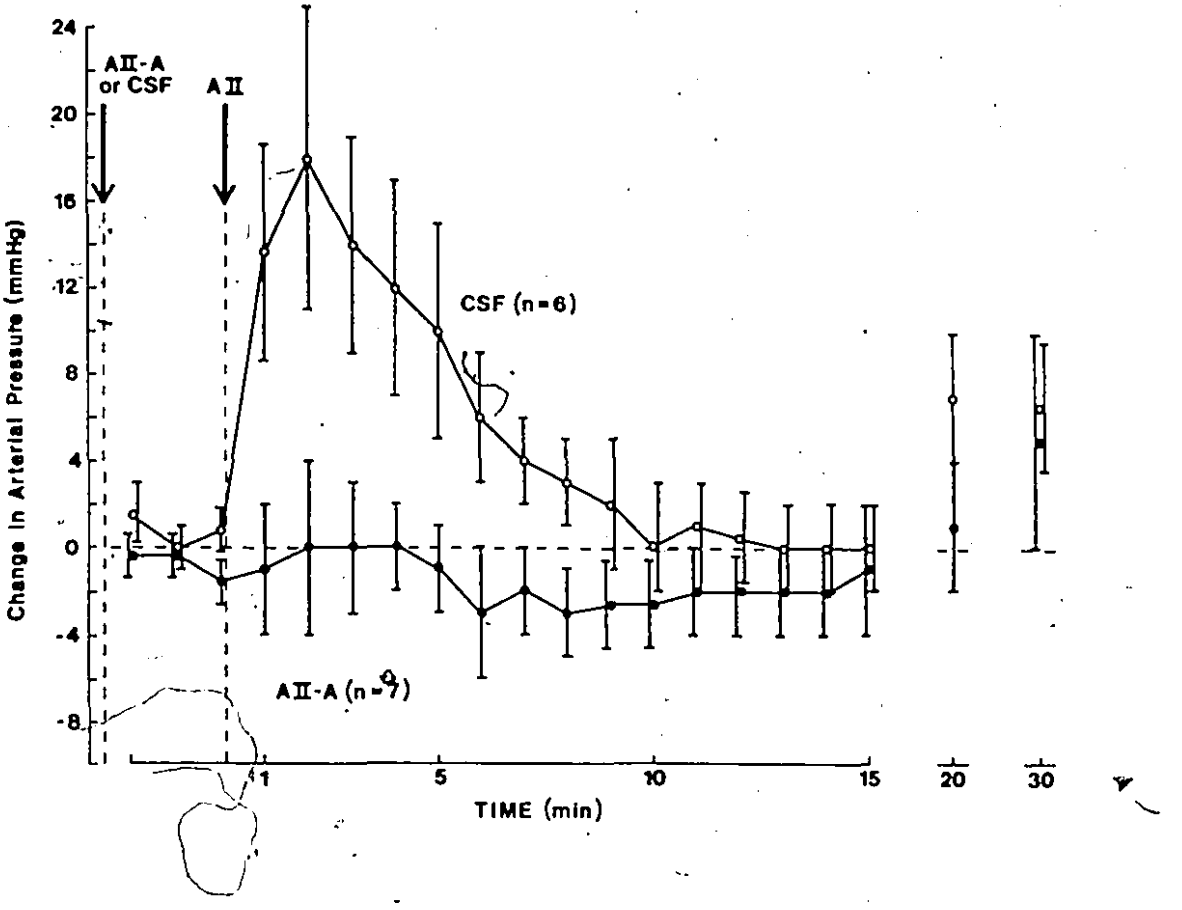

Figure 4. Effects of [Sar^1,Ile^8]-angiotensin II (A II-A) on the pressor response to angiotensin II. At the arrow at the left, either the analog or CSF was administered intrathecally and, after three min, angiotensin II was administered. Values are expressed as changes from baseline arterial pressure determined over the 5 min prior to the first administration. Details are otherwise as in the legend to Figure 1.

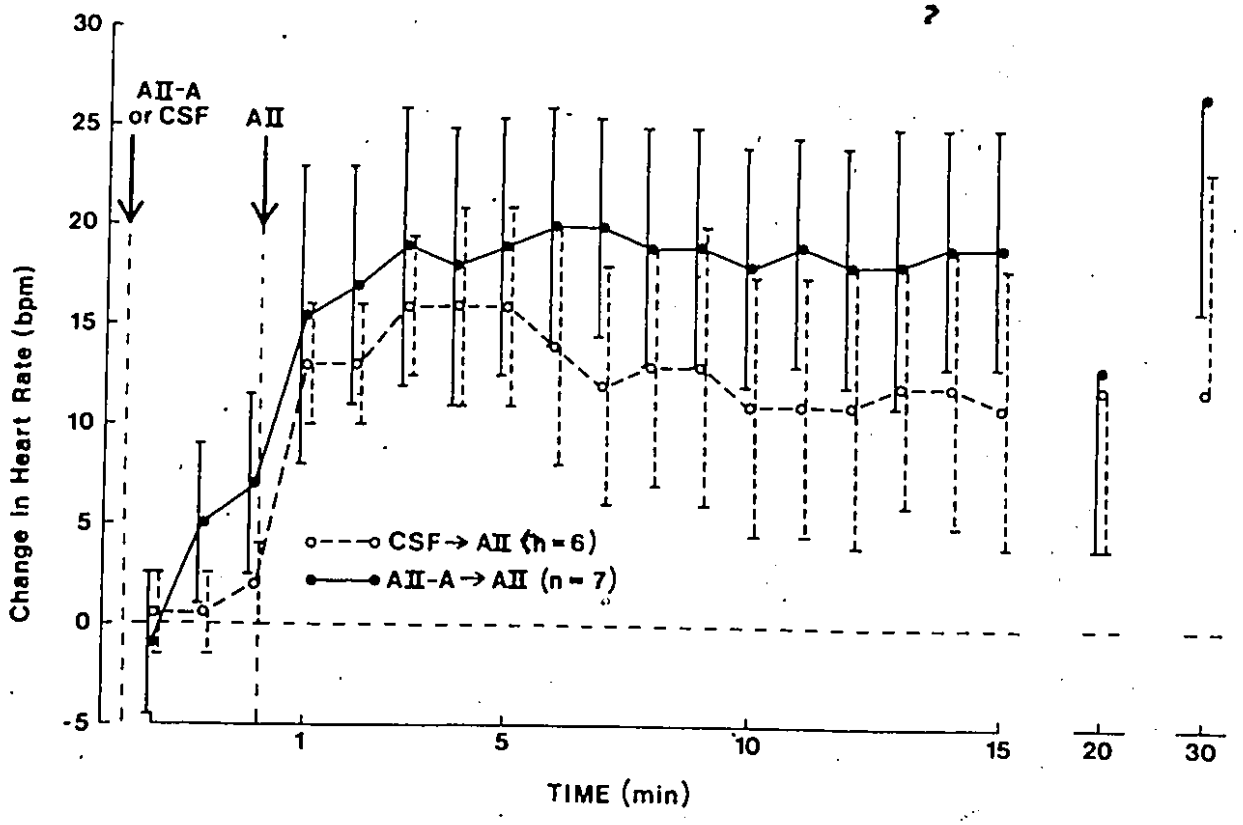

Figure 5. Effects of [Sar^1,Ile^8]-angiotensin II (A II-A) or of CSF on the cardioacceleratory response to intrathecal administration of angiotensin II three min later. Values are expressed as changes from baseline heart rate determined over the 5 min prior to the first administration. Details are otherwise as in the legends to Figures 1 and 4.

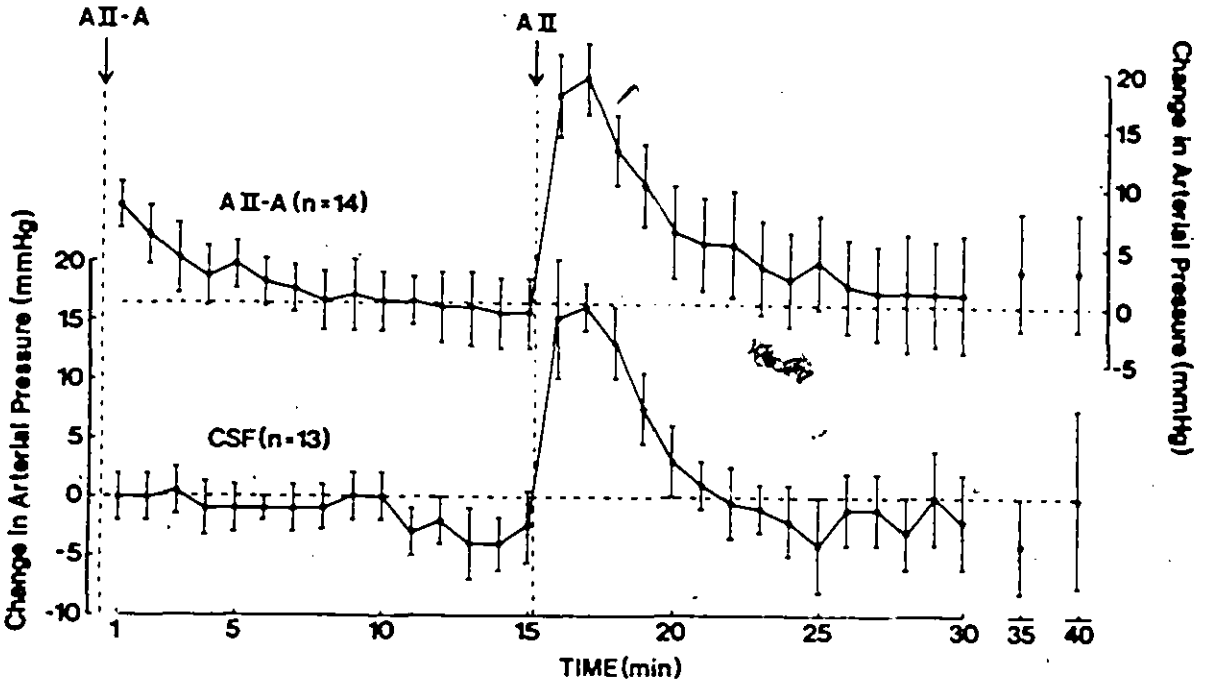

Figure 6. Change in arterial pressure from baseline values induced by intrathecal administration of [Sar^1,Ile^8]-angiotensin II, and of angiotensin II given 15 min later. Details are the same as those given in earlier legends.

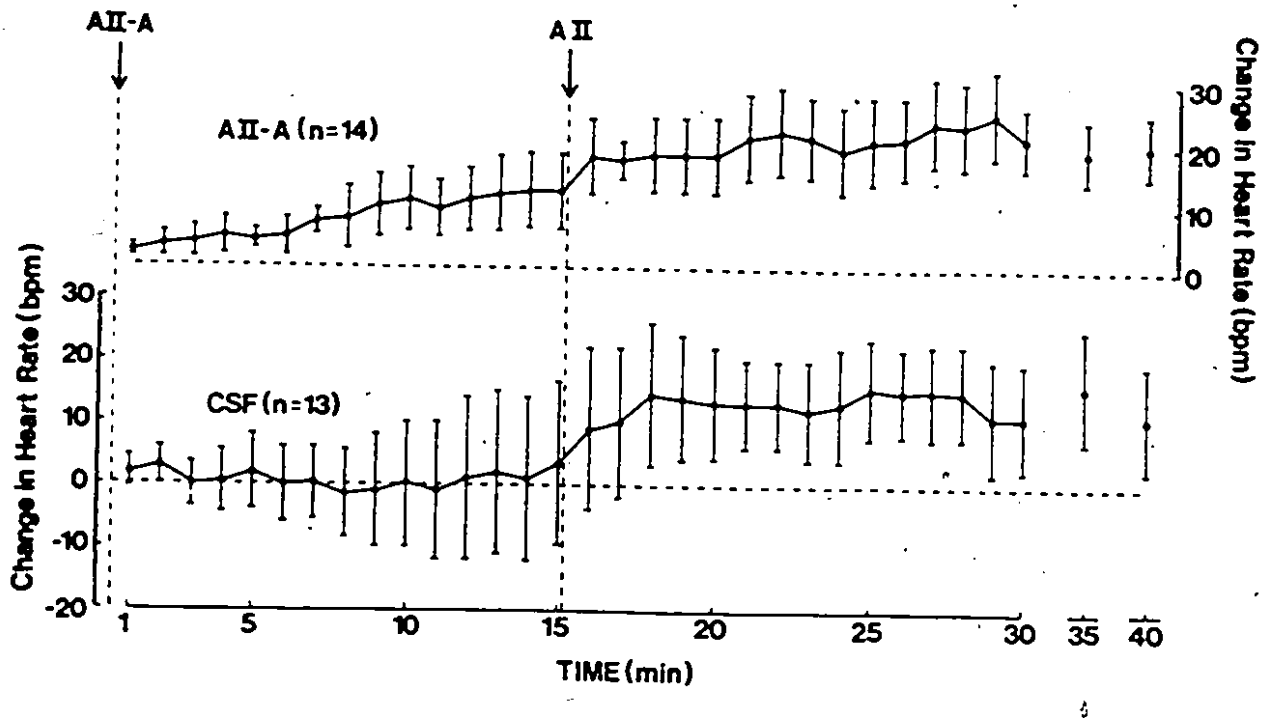

Figure 7. Change in heart rate from baseline values induced by intrathecal administration of [Sar^1,Ile^8]-angiotensin II, and of angiotensin II given 15 min later. Details are the same as those given in earlier legends.

CHAPTER VII

THYROTROPIN-RELEASING HORMONE INTRATHECALLY INCREASES ARTERIAL PRESSURE AND HEART RATE IN THE RAT

ABSTRACT

In view of the presence of TRH-containing terminals around sympathetic preganglionic neurons and the excitatory effects of TRH on these neurons, this peptide was administered in a dose of 6.5 nmoles intrathecally at the T9 and at the T2 spinal levels in the anesthetized rat. At T9, TRH increased arterial pressure and heart rate; these effects peaked at 8 min and decayed over the next 10-15 min. At T2, the pressor effect was absent and the increase in heart rate was smaller. Hexamethonium (10 mg/kg, i.v.) blocked the pressor effect at T9 but did not block the increase in heart rate. In view of the fact that i.v. administration of 6.5 nmoles of TRH failed to alter arterial pressure or heart rate, it is suggested that the effects observed were due to an action of TRH in the spinal cord, that the pressor effect is mediated in the sympathetic ganglia via nicotinic transmission and that the increase in heart rate is mediated at least partly via non-nicotinic transmission. Our results support the suggestion that TRH is a chemical mediator of synaptic transmission onto sympathetic preganglionic neurons and lead to the further suggestion that it participates at least in pathways regulating arterial pressure and heart rate.

INTRODUCTION

Although thyrotropin-releasing hormone (TRH) is best known for its involvement in neuroendocrine regulation of the pituitary-thyroid axis, its extensive distribution throughout the central nervous system (Brownstein et al., 1974; Jackson & Reichlin, 1974; Oliver et al., 1974; White et al., 1974; Winokur & Utiger, 1974) has generated considerable interest in other possible functions of this peptide. A large body of evidence has accumulated implicating TRH in a number of central functions, including regulation of the cardiovascular system. For example, TRH has been found to reverse cardiovascular shock (Holaday et al., 1981; Feuerstein et al., 1983; Lux et al., 1983; Holaday & Faden, 1983; Amir et al., 1984). Furthermore, TRH has been reported to alter arterial pressure and heart rate when given intracisternally (Beale et al., 1977) or intraventricularly (Delbarre et al., 1977; Eriksson & Gordin, 1981; Koivusalo et al., 1979; Holaday & Faden, 1983; Somiya & Tonoue, 1984; Tsay & Lin, 1982; Brown, 1981), or more specifically into the medial preoptic nucleus of the hypothalamus (Feuerstein et al., 1983; Diz & Jacobowitz, 1984).

The focus of the present study is the possible involvement of TRH in regulation of arterial pressure and heart rate at the spinal level. Little physiological

evidence has accumulated on its possible role in spinal autonomic pathways. This is surprising because substantial concentrations of TRH are found in the spinal cord, including moderate amounts in fibers and terminals around sympathetic preganglionic neurons in the intermediolateral nucleus (Gilbert et al., 1982; Hokfelt et al., 1975; Lechan et al., 1983). Besides anatomical evidence, Backman and Henry (1984) have reported that the iontophoretic application of TRH onto single sympathetic preganglionic neurons in the upper thoracic spinal cord induces a slow and prolonged excitatory effect.

The present experiments were therefore done to determine the effects on arterial pressure and heart rate of intrathecal administration of TRH at the second and ninth thoracic spinal segments in the rat.

METHOD

Animal preparation.

Male Sprague Dawley rats weighing approximately 350 g were used. They were anesthetized with urethane (2.5 g/kg, i.p.). An intrathecal catheter (Intramedic PE-10) was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the second or ninth thoracic vertebral

level (T2 and T9, respectively) using spinous processes as landmarks. In preliminary experiments, the validity of this method for correct positioning of the inner tip of the catheter was confirmed in X-rays of rats implanted with wire-filled catheters (Yashpal et al.,1985, Fig.1). It was via this catheter that TRH was administered intrathecally.

A second catheter (Intramedic PE-60) was inserted into the left common carotid artery facing the heart for monitoring arterial pressure via a Statham transducer (Gould P23 ID), connected to a Grass polygraph. Heart rate was calculated from this record. Systolic and diastolic pressures were measured from the ratemeter records and mean arterial pressure was calculated from these measurements. The number of heart beats in a 10 sec period was counted and that number was multiplied by six to obtain heart rate in beats per min.

In experiments where agents were administered intravenously, a third catheter (Intramedic PE-60) was inserted into the left femoral vein facing the heart.

Animals respired spontaneously throughout the experiments. Rectal temperature was maintained at approximately 37^{o}C by a heating pad.

Peptide Administration

After surgical preparation, a 30 min period was allowed for stabilization. Baseline readings of arterial pressure and heart rate were taken over a five min period and TRH (from Peninsula Laboratories, Belmont, Calif.) was administered intrathecally at either the T2 or the T9 level in a dose of 6.5 nmoles delivered over a period of 15-20 sec in 10 µl of artificial cerebrospinal fluid (CSF; an aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$).

In preliminary experiments at T9, 6.5 nmoles were found to be the minimum dose which would reliably alter arterial pressure and heart rate. As one of the objectives of the study was to compare the relative potency of TRH at T9 and at T2, this was the only dose used in this study. Doing so has allowed comparison on the basis of magnitude of change. After delivery of the peptide the intrathecal catheter was flushed with 10 µl of CSF (the internal volume of the catheter was approximately 8 µl). With zero time being the time of injection of CSF, readings of arterial pressure and heart rate were taken each minute for the next 15 min and then at 20 and 30 min.

Block of ganglionic nicotinic transmission

To determine the involvement of ganglionic nicotinic transmission in mediating any changes in arterial pressure and heart rate, the experiments with intrathecal administration were repeated but in animals which had been given hexamethonium; the rationale was that the persistence of a response to TRH would suggest either that the effects were mediated at least partly via non-nicotinic ganglionic transmission or that the peptide was passing into the circulation and having a peripheral effect. In these experiments, after baseline readings had been taken, hexamethonium bromide was administered via the intravenous catheter in a dose of 10 mg/kg body weight (concentration of 10 mg/ml in saline). Three more readings were taken at one min intervals, TRH was administered intrathecally as above, and readings were taken each min for the next 10 min.

Effects of i.v. administration of TRH

Given the possibility that TRH was producing its effects via a peripheral mechanism after passage from the perispinal space into the circulation, the experiments described above were repeated, but TRH was administered intravenously rather than intrathecally. The protocol was otherwise the same as above. The peptide was dissolved in 0.5 ml of saline for these experiments and the catheter was flushed with 0.5 ml of saline.

Statistical analysis

Results from each rat were tabulated as systolic and diastolic arterial pressures and heart rate. Changes in these parameters from the baseline values were also determined. Data for the figures were summarized by taking the mean ± S.E. of the values from each group of rats at each min following administration. Student's t-test for unpaired data was used for comparison between the two values (TRH vs CSF groups) at each sample time after intrathecal administration.

RESULTS

In preliminary experiments in which 10 ul of Fast Green, followed by 10 µl of CSF, was injected via the intrathecal catheter it was observed that over the 30 min period of the experiment diffusion of the dye was limited to two segments rostral and three segments caudal to the level of injection. In addition, the rapid onset of the responses in these and in previous experiments in our laboratory (Yashpal et al., 1982, 1985) supports a local action near the site of injection rather than a more remote action elsewhere in

the central nervous system.

Intrathecal administration of TRH had no effect on respiratory frequency, which remained at a mean value of approximately 100 breaths per minute.

Effects of TRH at the T9 level

Intrathecal administration of TRH at the T9 level increased both systolic and diastolic pressures. This effect was transient, peaking at about 4 min after administration. Similar injection of the vehicle failed to alter systolic or diastolic pressure. The mean values of diastolic and systolic pressures in the 14 TRH- and in the 13 CSF-treated rats at each sample time are illustrated in Figure 1. When the mean value at each sample time was compared to the mean value before administration, the maximum change in systolic pressure was +16.6 $\pm$ 3.5 mm Hg and occurred at 4 min; when the changes in systolic arterial pressure were compared between the two groups, these differences were statistically significant at 3 to 10 min after administration (3-8 min, $p < 0.005$; 9 min, $p < 0.01$; 10 min, $p < 0.025$). With regard to diastolic pressure, the maximum change was +19.3 $\pm$ 4.3 mm Hg and occurred at 4 min; the respective values of the two groups of animals were statistically different at 2 to 15 min (2-13 min, $p < 0.005$; 14 min, $p < 0.05$; 15 min, $p <$

0.025).

Heart rate was also increased by administration of TRH at the T9 level. The results are summarized in Figure 2. In this case the increase occurred more slowly, reaching the maximum change, +43.6 ± 11.9 bpm, at 7 min. Prior to administration the mean heart rate was 332.2 ± 8.0 bpm in TRH-treated rats and 332.8 ± 15.4 bpm in CSF-treated rats. When the changes from preadministration values were compared they were significantly different between the two groups of rats at 1 to 11 min (1 min, $p < 0.025$; 2-7 min, $p < 0.005$; 8 min, $p < 0.01$; 9-10 min, $p < 0.025$; 11 min, $p < 0.05$).

Effects of TRH at the T2 level

With the same dose as used above, the effects of TRH at the T2 level were quantitatively different from those at the T9 level.

The effects on arterial pressure are summarized in the graphs of Figure 3. Systolic pressure in TRH-treated rats (n=11) was increased from 3 to 7 min after administration (3 min, $p < 0.025$; 4-7 min, $p < 0.05$). In this case, the maximum change was +7.6 ± 3.5 mm Hg at 3 min, while at this time the change in CSF-treated animals (n=14) was -1.6 ± 4.9 mm Hg. Diastolic pressure was not altered ($p > 0.05$ for the difference between the two groups at each

postadministration time). In the TRH-treated group the greatest change from the mean preadministration diastolic pressure was +12.5 ± 3.5 mm Hg at 6 min after administration of the peptide. In the CSF-treated group the mean change at 6 min was +12.9 ± 6.3 mm Hg.

Heart rate was increased significantly. Figure 4 shows the changes from baseline heart rate for the TRH- and CSF-treated groups. The values were different between the two groups during the period 1 to 20 min after administration (1 min, $p < 0.01$; 2-3 min, $p < 0.005$; 4-11 min, $p < 0.025$; 12-20 min, $p < 0.05$). During this period the greatest change in the TRH-treated group was +32.5 ± 8.7 bpm, at 3 min. At this time the same change in the CSF-treated group was +4.1 ± 2.0 bpm.

Effect of nicotinic block on responses to TRH

These experiments were done following the same general procedure as above, administering TRH at the T9 level, except that 3 min prior to administration of TRH intrathecally, hexamethonium was given in a dose of 10 mg/kg i.v. to block nicotinic transmission in sympathetic ganglia. Mean baseline systolic and diastolic pressures in the 6 animals tested were 129 ± 4.5 mm Hg and 52.0 ± 0.9 mm Hg, respectively. Administration of hexamethonium decreased these

pressures by about 50 and 15 mm Hg, respectively. Subsequent administration of TRH at the T9 level failed to alter systolic or diastolic pressure. These results are illustrated in Figure 5 along with results obtained from 6 control animals in which CSF replaced the TRH solution.

Mean baseline heart rate in TRH-treated rats was 384.7 ± 19.3 bpm. After administration of hexamethonium heart rate increased by about 8 bpm. After subsequent administration of TRH intrathecally at the T9 level, heart rate increased gradually throughout the following 8 min. Changes in heart rate after administration of CSF followed a different pattern: while the effects of hexamethonium were similar, after CSF was given heart rate did not change. The results obtained in these experiments are summarized in Figure 6.

Effects of intravenous administration of TRH

In view of the fact that TRH increased heart rate in rats treated with hexamethonium, the possibility was considered that TRH delivered into the intrathecal space might have passed into the circulation and expressed its effects, at least on heart rate, via a peripheral action. Therefore, the earlier experiments were repeated, except that the

same dose of TRH was administered intravenously in a volume of 0.5 ml of physiological saline. Prior to administration of the peptide the mean systolic and diastolic pressures of the 4 animals used were 124.5 ± 11.2 and 62.0 ± 6.3 mm Hg, respectively. As can be seen in the data summarized in Figure 7, administration of TRH fails to change either systolic or diastolic pressure.

The results with heart rate are illustrated in Figure 8. The mean heart rate before administration of TRH was 347.3 ± 9.0 bpm. Administration of TRH failed to change heart rate.

DISCUSSION

This study has indicated that the intrathecal administration of 6.5 nmoles of TRH to the ninth thoracic spinal segment increases arterial pressure and heart rate in the rat. Interestingly, the administration of a similar quantity of TRH at the second thoracic level increased heart rate but had a relatively minor effect on arterial pressure. Systemic administration of a similar dose changed neither arterial pressure nor heart rate.

The arterial pressure responses elicited at T9 were greatly reduced or failed to occur altogether in animals in which nicotinic transmission in sympathetic ganglia had been blocked, and therefore the conclusion

is reached that these responses were mediated primarily or exclusively via a nicotinic mechanism in the sympathetic ganglia.

On the other hand, hexamethonium had no effect on the heart rate response. The response was roughly similar in time course and magnitude in rats with normal ganglionic transmission and in those in which nicotinic transmission in sympathetic ganglia had been blocked. Thus, the mechanisms increasing heart rate and those increasing arterial pressure must be different. While it is possible that TRH increased heart rate via a peripheral action, this seems unlikely because the peptide had no effect on heart rate when the same dose was given intravenously. This lack of effect upon systemic administration is supported by the study of Koskinen and Bill (1984), in which even a much higher dose, 2mg/kg i.v., failed to produce an effect on heart rate in the rabbit. An increase in heart rate has been reported with i.v. administration of TRH in the Sprague Dawley rat (Holaday & Faden, 1983) but in this case 4 mg/kg was used, a dose 1400 times greater than was used in our study.

In view of the existence of non-nicotinic transmission in sympathetic ganglia (Ashe & Libet, 1981; Blumberg & Janig, 1983; Brown, 1967; Emmelin & MacIntosh, 1956; Nishi & Koketsu, 1968; Jan & Jan,

1982; Bachoo et al., 1986) it is proposed that the increase in heart rate produced by intrathecal administration of TRH is due to the release by the sympathetic preganglionic neurons of a transmitter besides acetylcholine. It is unfortunate for comparative purposes that in previous studies in which heart rate increases were observed in response to electrical stimulation of the intermediolateral nucleus (Henry & Calaresu, 1972; Faden et al., 1978) effects of ganglion block were not studied.

On the basis of this evidence, then, it is suggested that the cardiovascular responses produced by intrathecal administration of TRH were due to an action within the spinal cord. This action was probably an excitation of sympathetic preganglionic neurons for several reasons. TRH is contained in fibers and terminals around sympathetic preganglionic neurons in the intermediolateral nucleus (Gilbert et al.; 1982; Hokfelt et al., 1975; Lechan et al., 1983) and iontophoretic application of TRH onto single sympathetic preganglionic neurons induces a slow and prolonged excitation (Backman & Henry, 1984). Little evidence exists for a possible action on sensory neurons in the dorsal horn. The TRH levels in this region are relatively low compared to the lateral and ventral horns (Kanazawa et al., 1979; Kardon et al.,

1977) and TRH-containing fibers and terminals are not found there (Gibson et al., 1981; Gilbert et al., 1982; Hokfelt et al., 1975).

TRH has been suggested to play a role in regulation of somatic motor output at the spinal level (Hokfelt et al., 1975; Nicoll, 1977; Cooper & Boyer, 1978; Lighton et al., 1984), and this must be considered in the interpretation of the results of the present study. However, such a mechanism can be excluded because motor effects have been observed only with much larger doses (Cooper & Boyer, 1978). Furthermore, in the present study blockade of nicotinic transmission in sympathetic ganglia abolished the pressor effects; if the responses had been elicited via an action on the somatic motor system this manoeuver would have been without effect.

Additional evidence excluding a somatic motor mechanism lies in the fact that motor effects were not observed in these experiments except for a mild tremor which was seen on some occasions. Finally, in another experimental paradigm in unanesthetized rats, a similar dose of TRH is without effect on reaction time in tail flick test (R.A. Cridland and J.L. Henry, unpublished observations), a test which involves a local motor response.

With regard to the difference in the effects of

TRH at the ninth and second thoracic segments, the data suggest that there is a greater preponderance of TRH receptors at the lower level and therefore that there is a differential regulation of sympathetic output at the spinal level, at least as far as control via TRH-containing pathways is concerned.

This study was done in anesthetized animals for several reasons. As delivery of the peptide was at the spinal level, few central synapses were involved. In fact, if TRH acted upon sympathetic preganglionic neurons, as suggested above, then no central synapses would be involved. Furthermore, with this approach, arterial pressure could be measured reliably and easily by the direct method, and artifactual changes in cardiovascular parameters due to changes in the behavioral states of an awake animal could be avoided. This latter point was considered to be especially important in view of the behavioral responses induced by the intrathecal administration of some peptides (Hylden & Wilcox, 1981; Dobry et al., 1981; Seybold et al., 1982; Yashpal et al., 1982). Urethane was used as the anesthetic because it has a less disruptive effect on baseline cardiovascular parameters and reflexes as well as on respiratory parameters than do other anesthetics in the rat (Armstrong, 1981; De Wildt et al., 1983; Sapru & Krieger, 1979).

The possibility was considered that TRH may have diffused to brain stem structures to produce the cardiovascular effects reported here. However, this possibility was considered unlikely for a number of reasons. If the action had been in the brain stem, one would have expected a greater effect at the second than at the ninth thoracic level, and the opposite was the case. Secondly, TRH administration, either intracisternally (Myers et al., 1977) or selectively into the nuclei of the tractus solitarius (Carter & Lightman, 1985; Feuerstein et al., 1983), induces hypotension and either cardioacceleration (Feuerstein et al., 1983) or no change in heart rate (Carter & Lightman, 1985). In addition, in our own experiments injection of dye intrathecally to the ninth thoracic level spreads no farther rostrally than the seventh thoracic level over a 30 min period. Furthermore, the time course of both the pressor response, which peaked at 4 min after injection, and the heart rate response, which peaked at 7 min, was faster than might be anticipated for a mechanism which required diffusion of the peptide to the brain stem. A similarly rapid onset of effect on adrenal output of catecholamines has been reported when substance P is administered at the ninth thoracic segment (Yashpal et al., 1985).

In summary, our results support evidence implicating TRH as a chemical mediator of synaptic transmission in descending inputs to sympathetic preganglionic neurons (Gilbert et al., 1982; Hokfelt et al., 1975; Lechan et al., 1983; Backman & Henry, 1984) especially in lower thoracic segments. The effects of TRH are central since intravenous administration of this peptide fails to produce any effect on arterial pressure and heart rate. The results with hexamethonium lead to the suggestion that the arterial pressor responses are mediated via predominantly nicotinic transmission in the sympathetic ganglia and that the increase in heart rate is mediated largely via non-nicotinic transmission. The results from this study prompt the further suggestion that TRH is involved in functional pathways which include at least those regulating arterial pressure and heart rate.

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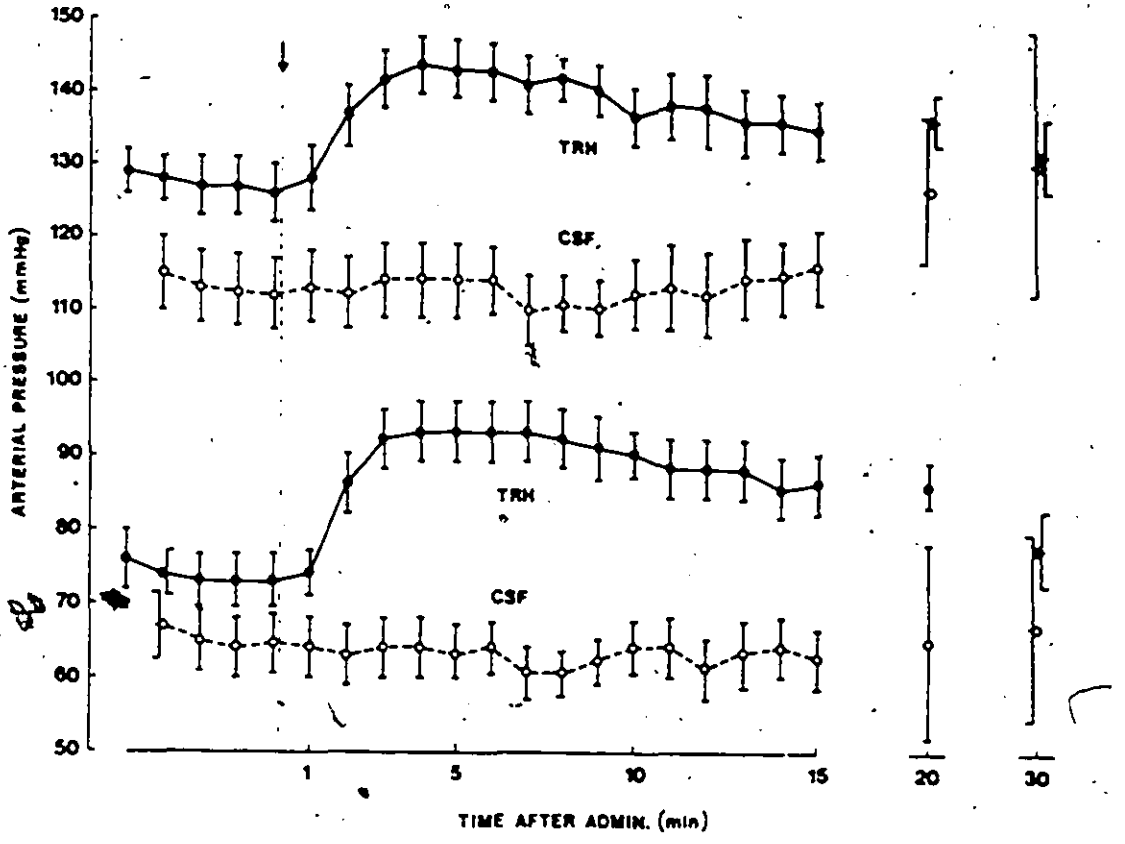

Figure 1. Time-effect curve for the effects of the intrathecal administration of TRH (6.5 nmoles; n = 14; ●——●) or of artificial cerebrospinal fluid (CSF; n = 13; o - - o) on mean systolic and mean diastolic arterial pressures in the rat. Administration was at the ninth thoracic spinal level. The abscissa shows time after administration. Vertical bars each represent one S.E.M.

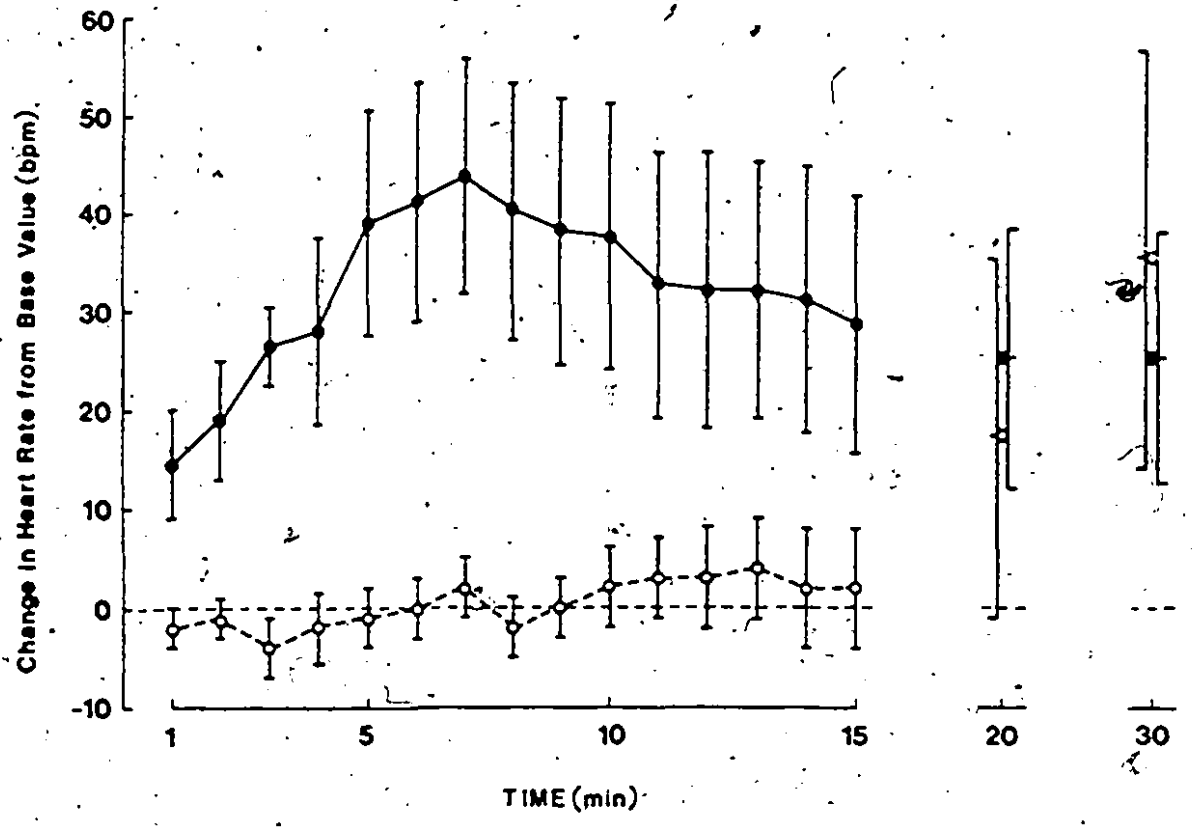

Figure 2. Time-effect curve for the change in mean heart rate induced by TRH or CSF administered to the ninth thoracic level. Details are otherwise the same as those in Figure 1.

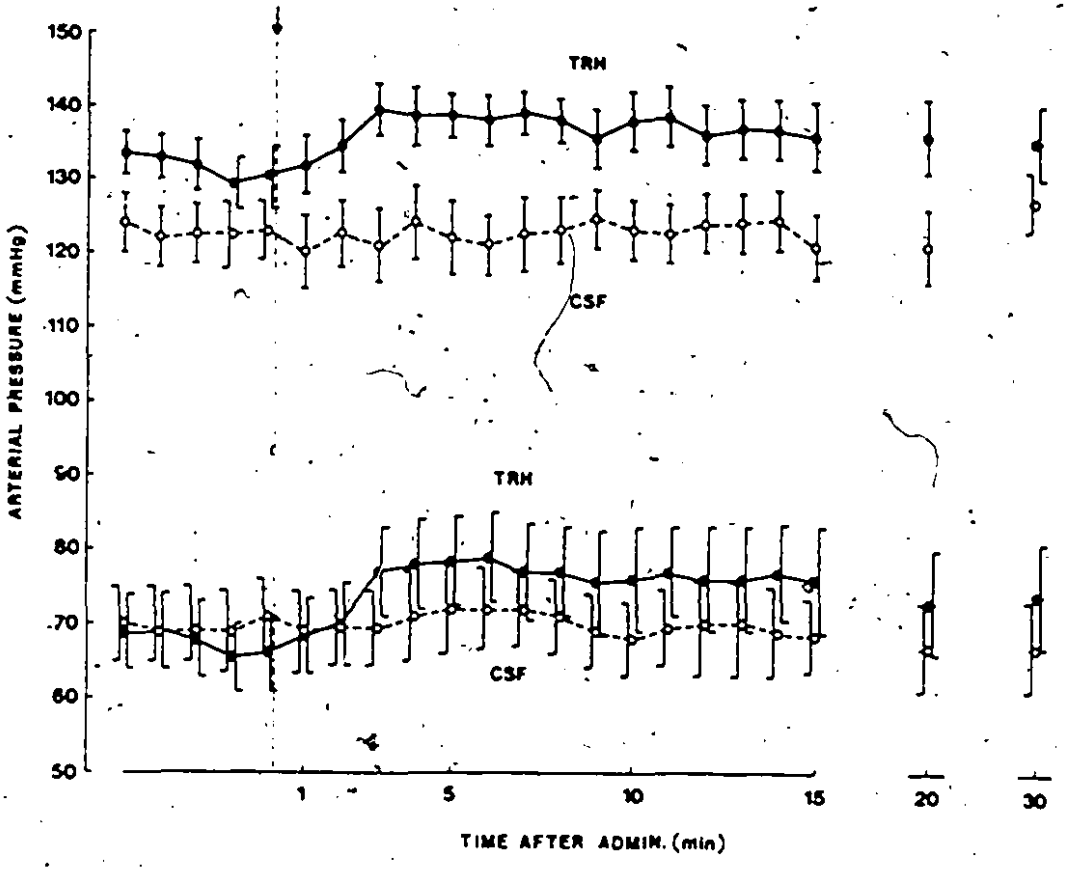

Figure 3. Mean systolic and diastolic pressures in rats given TRH (n = 11) and CSF (n = 14) intrathecally at the second thoracic level. Details are otherwise the same as those in Figure 1.

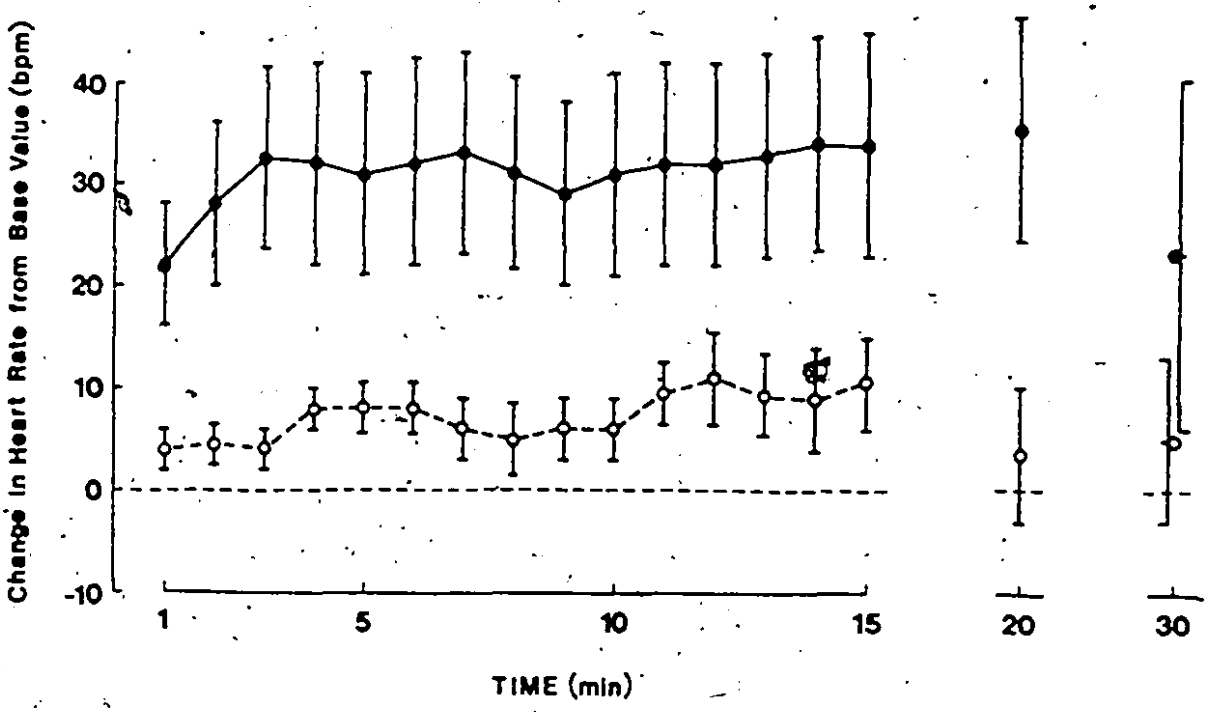

Figure 4. Change in heart rate induced by administration of TRH or CSF at the second thoracic level. Details are otherwise the same as those in Figure 1.

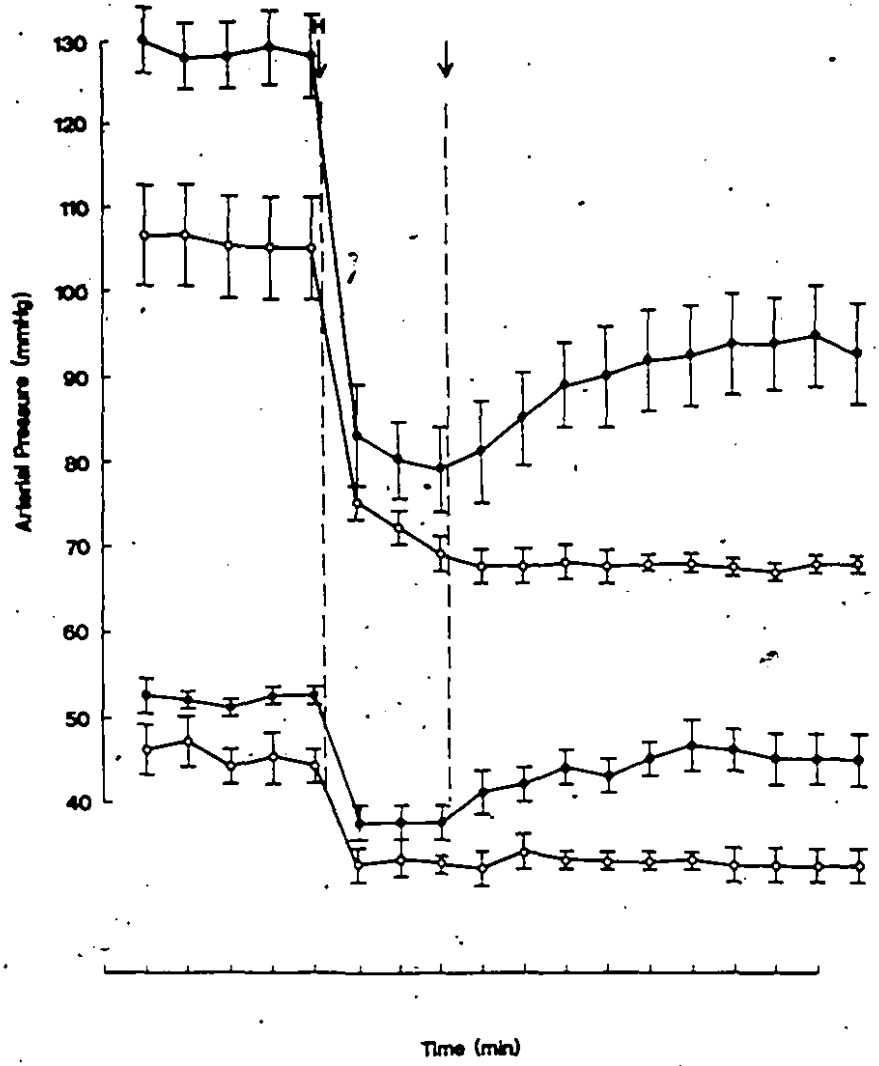

Figure 5. Effects of i.v. administration of hexamethonium (H; 10 mg/kg) on pressure responses to intrathecal administration of TRH (●——●;n = 6) or of CSF (o- - -o;n = 6) at the ninth thoracic level.

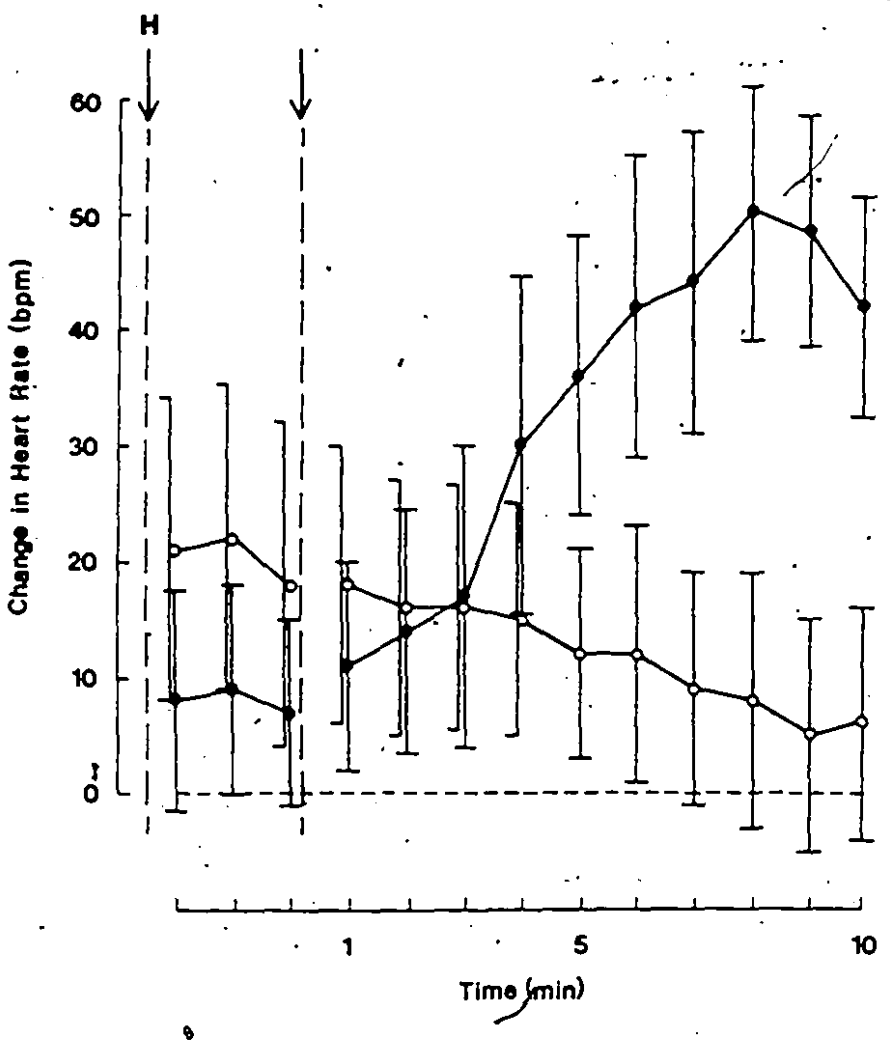

Figure 6. Effects of i.v. administration of hexamethonium on heart rate response to intrathecal administration of TRH or of CSF at the ninth thoracic level. Details are otherwise the same as those in Figure 5.

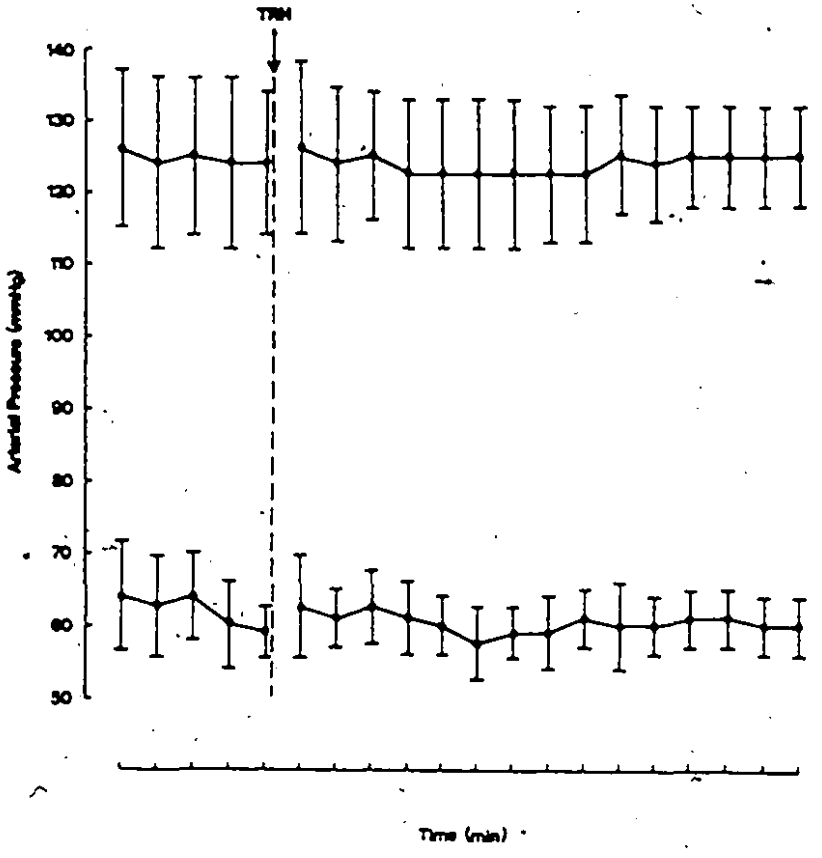

Figure 7. Effects of i.v. administration of TRH (6.5 nmoles; n = 4) on mean systolic and on diastolic pressures.

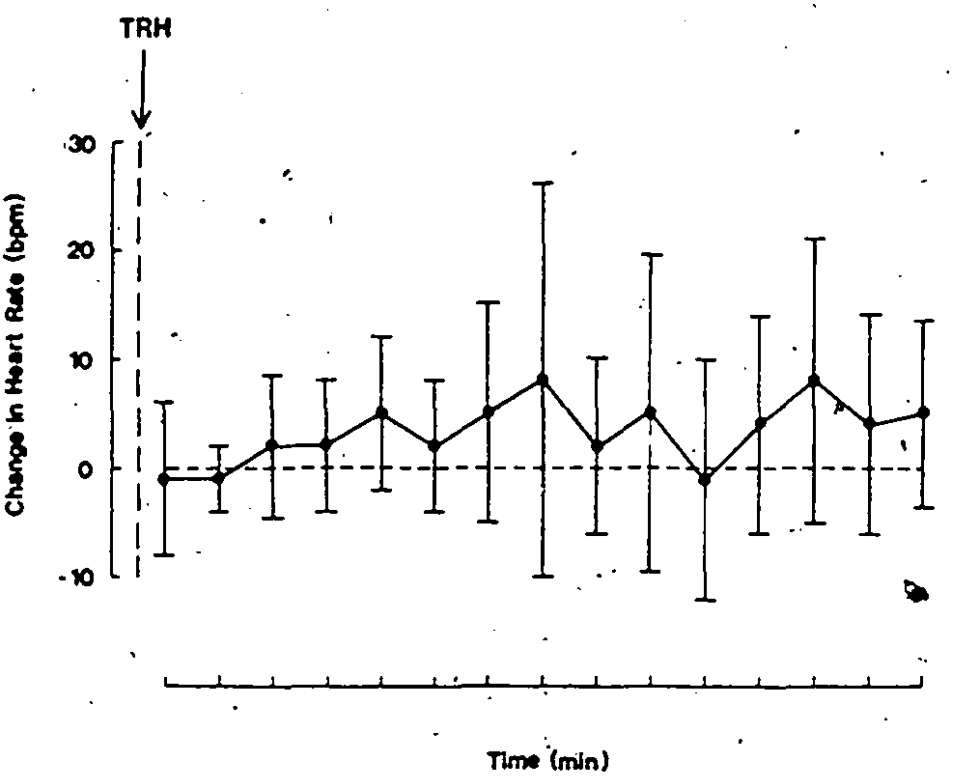

Figure 8. Effects of i.v. administration of TRH on mean heart rate (n = 4).

CHAPTER VIII

EFFECTS OF SPINAL ADMINISTRATION OF SOMATOSTATIN ON ARTERIAL PRESSURE AND HEART RATE IN THE RAT

ABSTRACT

Administration of 6.5 nmoles of somatostatin intrathecally to the spinal T9 level of the anesthetized rat provoked a transient increase in systolic and diastolic pressures lasting from 1 to 5 min after administration. Heart rate was depressed; this effect began at 5-10 min and lasted until 15-20 min after administration. At T2 there was a biphasic effect on both pressure and heart rate, consisting in each case of an initial increase followed by a decrease below control levels. The time course of these changes resembled that of the changes at T9. Adrenalectomy did not abolish either pressure or heart rate responses to administration of somatostatin at T9, suggesting that the adrenals are not necessary for the expression of the responses. In fact, the pressor effect was potentiated in adrenalectomized rats and there was an initial cardioacceleration resembling that elicited from T2. When given i.v., somatostatin failed to have similar effects on either arterial pressure or heart rate, suggesting that the effects of intrathecal administration were due to an action in the spinal cord. Blockade of nicotinic transmission in sympathetic ganglia with 10 mg/kg of hexamethonium bromide i.v. decreased dramatically the pressure effects but the heart rate response was less affected; these results suggest

that the pressure responses were mediated by nicotinic ganglionic mechanisms and that the effects on heart rate were mediated at least partly by non-nicotinic ganglionic mechanisms. Our results support evidence implicating somatostatin as a chemical mediator of synaptic transmission in descending pathways to sympathetic neurons in the spinal cord but demonstrate that the adrenals are not necessary for the expression of the effects of somatostatin in the spinal cord.

INTRODUCTION

The extrahypothalamic presence of somatostatin in the central nervous system has prompted investigations into its possible functional roles in the central nervous system. The total spinal cord content of somatostatin is the second highest in the nervous system, being lower only than that of the hypothalamus (Patel & Reichlin, 1978). Somatostatin in the spinal cord is concentrated mainly in the dorsal horn (Hokfelt et al, 1976; Johansson et al, 1984; Stine et al, 1984) and here it may be largely of primary afferent origin (Hokfelt et al, 1976; Stine et al, 1982), although some is also apparently of intrinsic origin (Burnweit & Forssman, 1979; Dalsgaard et al, 1981; Forssman, 1978). In addition, somatostatin content in the spinal cord decreases caudal to a transection, indicating the additional supraspinal origin of some spinal somatostatin (Stine et al, 1982).

Somatostatin positive terminals can be found in areas ventral to the dorsal horn (Burnweit & Forssman, 1979; Forssman, 1978), including the thoracolumbar intermediolateral nucleus (DiTirro et al, 1983; Holets & Elde, 1982; Massari et al, 1983; Schoenen et al, 1985). In particular, somatostatin is present in descending fibers to the spinal cord (Holets & Elde, 1982) which

innervate predominantly sympathetic preganglionic neurons projecting to the adrenal medullae (Hancock, 1982; Holets & Elde, 1982).

While physiological studies at the spinal level have implicated somatostatin in sensory mechanisms (Hylden & Wilcox, 1981; Randic & Miletic, 1978; Miletic & Randic, 1982; Murase et al., 1982) little is known about its involvement in regulation of sympathetic output. Elde et al. (1984) have demonstrated that somatostatin immunoreactive fibers preferentially occupy the neuropil surrounding sympathoadrenal neurons, and on this basis they have suggested a specific role for somatostatin-containing fibers in regulating sympathetic output to the adrenal medulla. The present experiments have attempted to investigate, in physiological experiments, the possible role of somatostatin in regulation of sympathetic output.

Some of the data in this manuscript have been presented in abstract form (Yashpal et al, 1986).

METHOD

Animal preparation.

Male Sprague Dawley rats, weighing approximately 350 g were anesthetized with urethane (2.5 g/kg, i.p.) and implanted with an intrathecal catheter (Intramedic

PE-10) which was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the ninth or the second thoracic vertebral level (T9 and T2, respectively). Spinous processes were used as landmarks; in preliminary experiments the validity of this method for correct positioning of the inner tip of the catheter was confirmed in X-rays of rats implanted with wire-filled catheters (Yaksh et al, 1985, fig. 1). It was via this catheter that somatostatin was administered intrathecally.

A second catheter (Intramedic PE-60) was inserted into the left common carotid artery for monitoring arterial pressure via a Statham transducer (Gould PE23 ID), connected to a Grass polygraph. Heart rate was calculated from this record. Heart rate was measured as the number of beats in a 10 sec period multiplied by six to obtain the rate in beats per min.

In experiments in which agents were administered intravenously, a third catheter (Intramedic PE-60) was inserted into the left femoral vein.

Animals respired spontaneously throughout the experiments. Rectal temperature was maintained at approximately 37°C by a heating pad.

Preparation of adrenalectomized animals.

In view of the evidence cited in the Introduction implicating somatostatin specifically in regulation of sympathetic output to the adrenals, the experiments described above were repeated in adrenalectomized rats. Thus, in addition to the preparation above, some animals were adrenalectomized surgically. A midline incision was made in the abdomen and each adrenal gland was exposed. It was separated from its surrounding tissues and removed manually using a pair of forceps. Sham operated control rats were treated in the same manner except that the adrenal glands were not removed.

Peptide administration.

A 30 min period was allowed for stabilization after surgical preparation of the animals. Baseline readings of arterial pressure and heart rate were taken over a five min period and somatostatin (from Peninsula Laboratories, Belmont, Calif.) was administered intrathecally at either the T9 or the T2 level in a dose of 6.5 nmoles, delivered over a period of 15-20 sec in 10 µl of artificial cerebrospinal fluid (CSF; an aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$). After delivery of the peptide the intrathecal catheter was flushed with 10 µl of CSF (the internal volume of the catheter was approximately 8 µl).

With zero time being the time of injection of the flushing solution, readings of arterial pressure and heart rate were taken each minute for the next 15 min and then at 20 and 30 min.

Block of ganglionic nicotinic transmission.

To determine the degree of involvement of nicotinic transmission in sympathetic ganglia in the mediation of cardiovascular changes observed, experiments with intrathecal administration of somatostatin were repeated but in animals which had been given hexamethonium bromide (1 mg/100 g body weight i.v.; concentration of 10 mg/ml in saline). Thus, the persistence of a response to somatostatin would suggest either that the effects were mediated via non-nicotinic ganglionic transmission or that the peptide was passing into the circulation and having a peripheral effect.

Experiments with i.v. administration of somatostatin.

In view of the possibility that somatostatin might have been producing its cardiovascular effects by passing into the general circulation and thereby expressing its effects by a peripheral action, the experiments were performed as usual except that somatostatin was administered intravenously rather than intrathecally. The protocol was otherwise the same,

except that the same dose of the peptide was dissolved in 0.5 ml of physiological saline for these experiments and the catheter was flushed with 0.5 ml of saline.

Statistical analysis.

Results from each rat were tabulated as systolic and diastolic arterial pressures and heart rate and as changes in these parameters from the baseline values determined before intrathecal injection. Data for the figures were summarized by taking the mean ± S.E. of the values from each group of rats at each min following administration. Student's t-test for unpaired data was used for comparison between the two values (somatostatin vs CSF groups) at each sample time after intrathecal administration.

RESULTS

Preliminary studies demonstrated that the diffusion of 10 µl of Fast Green dye, followed by 10 µl of CSF, over 30 min was limited to 2 segments rostrally and 3 segments caudally to the site of injection. In addition, the rapid onset of the response to intrathecal injection of peptides in these and in earlier studies (Yashpal et al, 1982, 1985) suggests that the action of somatostatin was local, rather than at a remote site.

Intrathecal administration of somatostatin had no effect on respiratory frequency in these experiments; this frequency remained at a mean value of approximately 100 breaths per minute.

Effects of somatostatin at T9

Administration of 6.5 nmoles of somatostatin intrathecally at the T9 level transiently increased both systolic and diastolic arterial pressures. Initial experiments were done using larger doses, which produced a biphasic effect, consisting of an increase followed by a decrease, the latter being such a profound and prolonged depression of pressure that animals expired. Therefore all the following data were obtained using 6.5 nmoles. Figure 1 shows the mean values for systolic and diastolic pressure at each min throughout the experimental period, for each of the two groups of rats (somatostatin-treated, n = 10; CSF-treated, n = 13). Mean values for these pressures during the period just prior to peptide administration were 122.2 ± 5.2 and 67.5 ± 5.6 mm Hg, respectively, in the 10 animals tested. In the control animals, in which CSF alone was administered, these respective values were 112.8 ± 4.9 and 64.8 ± 4.0 mm Hg. In each of the two groups of animals the changes from baseline values were compared between the two groups of animals; systolic pressure showed a significant

increase over controls in somatostatin-treated animals at 1-2 min (1 min, $p < 0.025$; 2 min, $p < 0.01$) and a decrease at 10-15 min (10 min, $p < 0.05$; 11 min, $p < 0.01$; 12-15 min, $p < 0.001$); diastolic pressure showed an increase at 1-6 min (1-3 min, $p < 0.01$; 4 min, $p < 0.005$; 5 min, $p < 0.01$; 6 min, $p < 0.025$) and a decrease at 9 min ($p < 0.05$). In somatostatin-treated animals the maximum increase in systolic pressure was $+14.3 \pm 4.8$ mm Hg, which occurred at 2 min after administration and the largest decrease was -16.2 ± 2.9 mm Hg at 14 min; the maximum increase in diastolic pressure was $+17.0 \pm 5.8$ mm Hg, which occurred at 3 min, and the largest decrease was -9.1 ± 5.9 mm HG, at 15 min.

Effects on heart rate are illustrated in Figure 2. Baseline heart rate in somatostatin-treated rats was 341.6 ± 11.5 bpm and was 332.0 ± 15.4 bpm in CSF-treated rats. Somatostatin administration did not change heart rate initially, during the period in which arterial pressure was increased. However, 10 min after the administration of somatostatin there was a decrease which occurred 11-15 min after administration (11-13 min, $p < 0.025$; 14-15 min, $p < 0.05$).

Effects of somatostatin at T2

Baseline systolic and diastolic arterial pressures were 130.9 ± 3.5 and 62.5 ± 2.6 mm Hg,

respectively, in somatostatin-treated rats (n = 11) and 121.1 ± 4.2 and 69.4 ± 5.3 mm Hg, respectively, in CSF-treated rats (n = 14). The curve of the readings obtained when the same dose of somatostatin was administered at the second thoracic level (shown in Figure 3) resembled that obtained at the ninth thoracic level. Thus, there was an initial increase in the mean systolic pressure at 1-3 min ($p < 0.05$) and in mean diastolic pressure at 1-2 min ($p < 0.01$). Maximum increases were: systolic, 11.6 ± 4.5 mm HG at 2 min; diastolic, 11.5 ± 4.1 mm Hg at 2 min. In each case the increase was followed by a decrease, systolic pressure at 7-15 min (7 min, $p < 0.025$; 8 min, $p < 0.005$; 9-15 min, $p < 0.001$; largest decrease of -31.9 ± 5.8 mm Hg at 13 min), diastolic pressure at 5-13 min (5 min, $p < 0.005$; 6-8 min, $p < 0.001$; 9-12 min, $p < 0.025$; 13 min, $p < 0.05$; largest decrease of -10.3 ± 3.7 mm Hg at 12 min). By 20 min both systolic and diastolic pressures had returned to normal and were similar in the somatostatin- and CSF-treated groups.

Mean baseline heart rate was 313.8 ± 8.8 bpm in somatostatin-treated and 361.5 ± 15.5 bpm in CSF-treated rats. The experimental results are summarized in Figure 4. From 2-7 min after administration there was an increase in mean heart rate, but the changes in somatostatin-treated rats were not significantly

different from the changes in CSF-treated rats. At 3 min after somatostatin had been administered mean heart rate had increased by 30.9 ± 15.4 bpm. At 8 min heart rate decreased past control levels and there followed a period of depressed heart rate, reaching a level 13.8 ± 4.4 bpm below the preadministration level (11-12 min, $p < 0.005$; 13 min, $p < 0.001$; 14 min, $p < 0.01$; 15 min, $p < 0.025$). By 20 min, heart rate had returned to control levels.

Effects of adrenalectomy on responses at T9

The biphasic appearance of the cardiovascular responses to intrathecal administration of somatostatin and the prolonged nature of the second phase suggested the possible mediation of a humoral mechanism, and the adrenals were the most likely source of such a mechanism, particularly in view of the evidence cited above that somatostatin-containing nerve terminals are found distributed preferentially around sympathetic preganglionic neurons to the adrenals. Therefore the experiments at T9 were repeated in adrenalectomized rats to determine whether the configuration of the responses would be different from those derived from rats with intact adrenals.

Baseline systolic and diastolic arterial pressures before and after adrenalectomy are given in Table I. Administration of somatostatin had biphasic

effects on these pressures, similar to the effects in intact rats. Figure 5 illustrates the results. As before, the effects were biphasic. In this case the initial increases in systolic and diastolic pressures were greater than those in rats with intact adrenals; the maximum increases occurred at one min after peptide administration, with systolic pressure increasing by a mean of 22.3 ± 3.3 mm Hg and diastolic pressure by 25.8 ± 3.6 mm Hg. The increase in systolic pressure in somatostatin-treated rats (n=15) was significantly different from the change in CSF-treated rats (n=8) at 1-3 min after administration (1 min, $p < 0.001$; 2-3 min, $p < 0.05$); the subsequent decrease was significant at 9-13 min (9 min, $p < 0.025$; 10-12 min, $p < 0.01$; 13 min, $p < 0.025$). The greatest decrease was 21.7 ± 4.3 mm Hg at 11 min. Similarly, diastolic pressure was increased at 1-2 min (1 min, $p < 0.001$; 2 min, $p < 0.005$) and was decreased at 6-13 min (6 min, $p < 0.05$; 7-13 min, $p < 0.025$); the greatest increase was 25.8 ± 3.6 mm Hg, at 1 min, and the greatest decrease was 18.9 ± 3.9 mm Hg, at 11 min.

Heart rates before intrathecal administration are also given in Table I. The effects of administration, illustrated in Figure 6, resembled those observed when somatostatin was administered at T2 in intact rats, rather than those when it was administered at T9; after the peptide was given heart rate increased gradually over

4 min, peaking at a mean maximum change of +33.6 ± 9.7 bpm ($p < 0.05$). From this time heart rate declined to a nadir at 12 min, but this decrease was not statistically different from the values from CSF-treated rats.

Effects of nicotinic block on responses at T9

Further experiments were done following the same general procedure, administering somatostatin at the T9 level, except that prior to administration of the peptide intrathecally, hexamethonium was given intravenously in a dose of 10 mg/kg to block nicotinic transmission in sympathetic ganglia.

Mean baseline systolic and diastolic pressures in the 6 animals tested were 128.0 ± 8.8 and 59.3 ± 5.0 mm Hg, respectively. Intravenous administration of hexamethonium decreased these pressures to means of 78.3 ± 1.8 and 41.6 ± 3.0 mm Hg, respectively. After subsequent intrathecal administration of somatostatin systolic pressure rose slightly to a maximum of 87.5 ± 4.1 mm Hg and diastolic pressure to a maximum of 53.3 ± 5.0 mm Hg. The full results are illustrated in Figure 7 along with results obtained from the 6 control animals in which CSF replaced the somatostatin solution.

Mean baseline heart rate in somatostatin-treated rats was 356.7 ± 16.7 bpm. After administration of hexamethonium heart rate decreased by 2.3 ± 13.7 bpm.

Following subsequent administration of somatostatin intrathecally heart rate increased to peak at a mean of 16.3 ± 16.4 bpm above preapplication levels. Changes in heart rate after administration of CSF followed a different pattern: while the effects of hexamethonium were similar, after CSF heart rate did not change. The results obtained in these experiments are summarized in Figure 8.

Effects of intravenous administration of somatostatin

As a means of pursuing further the possibility that somatostatin might be expressing its effects via a peripheral action an additional series of experiments was done in which the same protocol was followed except that somatostatin was administered intravenously. The effects on arterial pressure are illustrated in Figure 9. Prior to administration the mean systolic and diastolic pressures of the 4 animals used were 134.0 ± 9.8 and 58.3 ± 13.2 mm Hg. While there may have been a minor increase in arterial pressure at one to 2 min after peptide administration there was not the type of response observed upon intrathecal administration.

Similarly, when somatostatin was administered i.v. it had no effect on heart rate except for a gradual but continuous increase which did not resemble the time courses of the changes in heart rate observed when the

peptide was given intrathecally (see Figure 10). Baseline heart rate was 376.5 ± 36.1 bpm in these animals.

DISCUSSION

An overall view of the results of this study indicates that the intrathecal administration of 6.5 nmol of somatostatin to the spinal cord has a biphasic effect on arterial pressure and heart rate. As stated above, this dose was the maximum which led to the data presented here because higher doses produced such a profound hypotension that many of the animals expired before the end of the experiment. The full biphasic nature of these changes was not fully manifested at the upper or lower thoracic segments, but became more apparent when the data are considered together. Thus, at T9 somatostatin induced a short time course increase in arterial pressure but a slower decrease in heart rate. When somatostatin was given at the T9 level in adrenalectomized animals the pressor effect was observed but this was followed by a period of depression, particularly of diastolic pressure. More strikingly, heart rate in these animals was increased, following roughly the same time course as the pressor effect. On the other hand depression of the

heart rate was not apparent in these animals. When somatostatin was given at T2, the pressor effects were smaller in magnitude, although the depressor response and the changes in heart rate followed the same time course.

The probable site of action of somatostatin in eliciting the responses observed is the spinal cord. In the first place, systemic administration of the peptide failed to mimick the responses observed when it was given intrathecally. In addition, the possibility was considered that the injected peptide diffused or was carried rostrally to the brain stem; this is a particularly important possibility in view of the recent interesting observation that microinjection of somatostatin and related peptides into the nuclei of the tractus solitarius induces changes in arterial pressure and heart rate (Koda et al, 1985). However, there is evidence to suggest that the effects at the spinal level are mediated via different mechanisms from those provoked from the nuclei of the tractus solitarius, and therefore that the effects observed in the present study were not due to passage of the peptide to the brain stem. In the first place, the effects of somatostatin administration into the nuclei of the tractus solitarius were only hypotension and bradycardia while those observed in the present study were biphasic, consisting of initial pressor and tachycardic effects, followed by depressor

and bradycardic effects. In addition, diffusion of dye in the intrathecal space never exceeded two segments rostrally throughout 30 min, the period of the experiments. Therefore, it is suggested that the effects observed in our study were due to a spinal action.

Anesthetized animals were used in this study for specific reasons. As delivery of the peptide was at the spinal level few central synapses could have been involved in mediating the effects observed. In fact, if somatostatin acted upon sympathetic preganglionic neurons, a possibility in view of the fact that at least some types of these neurons are surrounded by nerve terminals containing somatostatin (Holets & Elde, 1982), then no central synapses would be involved. Furthermore, in anesthetized animals, artifactual changes in cardiovascular parameters due to changes in the behavioral state of an awake animal could be avoided; this point is considered important in view of the behavioral responses induced by the intrathecal administration of some peptides (Hylden & Wilcox, 1981; Dobry et al, 1981; Seybold et al. 1982; Yashpal et al, 1982). Urethane was used as the anesthetic because it has a less disruptive effect on baseline cardiovascular parameters and reflexes, as well as on respiratory parameters than do other anesthetics in the rat (Armstrong, 1981; De Wildt et al, 1983; Sapru & Krieger,

1979) and because this was the anesthetic used in the study cited above in which somatostatin was injected into the nuclei of the tractus solitarius (Koda et al, 1985).

Adrenalectomy did not abolish the effects of administration of somatostatin at the T9 level, and therefore it is concluded that the adrenals are not necessary for the full response seen when the peptide was administered at this level in intact animals. These observations are not consistent with the possibility suggested by Elde et al. (1984) of a specific role for somatostatin containing fibers in regulating sympathetic output to the adrenal medulla. In fact, the increases in arterial pressure and in heart rate were greater in adrenalectomezed than in intact animals, and there may be a depressor and cardioinhibitory agent released from the adrenals, in addition to the catecholamines.

Hexamethonium reduced the pressure responses to the point that it seems likely that they are mediated primarily if not exclusively via nicotinic transmission in the ganglia. After hexamethonium there was a mean increase in heart rate of 15 bpm; this was greater than in the intact rat and less than in the adrenalectomized rat. In view of the existence of non-nicotinic transmission in sympathetic ganglia (Ashe & Libet, 1981; Bachoo et al., 1986; Blumberg & Janig, 1983; Brown, 1967;

Jan & Jan, 1982; Nishi & Koketsu, 1968) it is suggested that the cardioacceleration may have been mediated at least partly via non-nicotinic transmission in sympathetic ganglia.

The basis for the biphasic nature of the effects observed in this study is not readily apparent. It does not seem to be due to mixed central and peripheral actions because systemic administration of somatostatin did not mimick either phase of the responses. It similarly does not seem to be due to mixed neural and humoral mechanisms because both phases were present in adrenalectomized rats. It is suggested, then, that the biphasic nature of the responses observed may have been due to two actions of somatostatin in the spinal cord, one of which would most likely be due to a direct excitatory action on sympathetic output. (Electrophysiological studies have shown somatostatin to have postsynaptic effects on spinal neurons; Miletic & Randic, 1982.) One possibility to account for the depression of arterial pressure and heart rate could be an inhibition of a tonic excitatory descending input to sympathetic preganglionic neurons (Henry & Calaresu, 1974).

In intact rats the effects at T2 are greater than those at T9. However, the effects at T9 in adrenalectomized rats are greater than the effects at T2, suggesting that effects at T2 may have been greater only

because there was less activation of adrenal output. This raises the interesting possibility that a cardioinhibitory factor may be released from the adrenals upon sympathetic activation.

In summary, our results support evidence implicating somatostatin as a chemical mediator of synaptic transmission in descending pathways to sympathetic preganglionic neurons in the intermediolateral nucleus of the spinal cord. The effects of somatostatin are probably within the spinal cord because intravenous administration of this peptide fails to produce similar effects on arterial pressure and heart rate. As the responses could be elicited from adrenalectomized animals, it is suggested that the adrenals are not necessary for expression of the effects. Results with hexamethonium suggest that the arterial pressure responses are mediated via predominantly nicotinic transmission in the sympathetic ganglia and that the increase in heart rate is mediated largely via non-nicotinic transmission.

ACKNOWLEDGEMENTS

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Table I. Values of mean systolic (SAP) and diastolic (DAP) arterial pressures (in mm Hg) and of mean heart rate (HR; in bpm) in adrenalectomized rats. Pre-adrenalectomy values were calculated over a five min period just before adrenalectomy. Post-adrenalectomy values were calculated over the five min period after the stabilization period but immediately prior to intrathecal administration. (Somatostatin, n = 15; CSF, n = 8)

	Somatostatin	CSF
Pre-adrenalectomy:		
SAP	126.1 ± 3.5	118.0 ± 6.1
DAP	59.1 ± 2.1	69.5 ± 0.8
HR	317.9 ± 9.8	324.9 ± 8.3
Post-adrenalectomy:		
SAP	117.4 ± 4.9	105.6 ± 3.7
DAP	68.2 ± 3.9	66.5 ± 2.1
HR	354.8 ± 16.0	325.1 ± 12.4
1 min after admin.:		
SAP	139.6 ± 4.6	104.5 ± 3.6
DAP	97.0 ± 5.2	65.6 ± 3.4
HR	366.9 ± 15.0	321.0 ± 11.3
10 min after admin.:		
SAP	94.0 ± 4.2	105.0 ± 3.5
DAP	49.6 ± 2.7	67.5 ± 3.6
HR	359.9 ± 11.1	313.0 ± 15.1

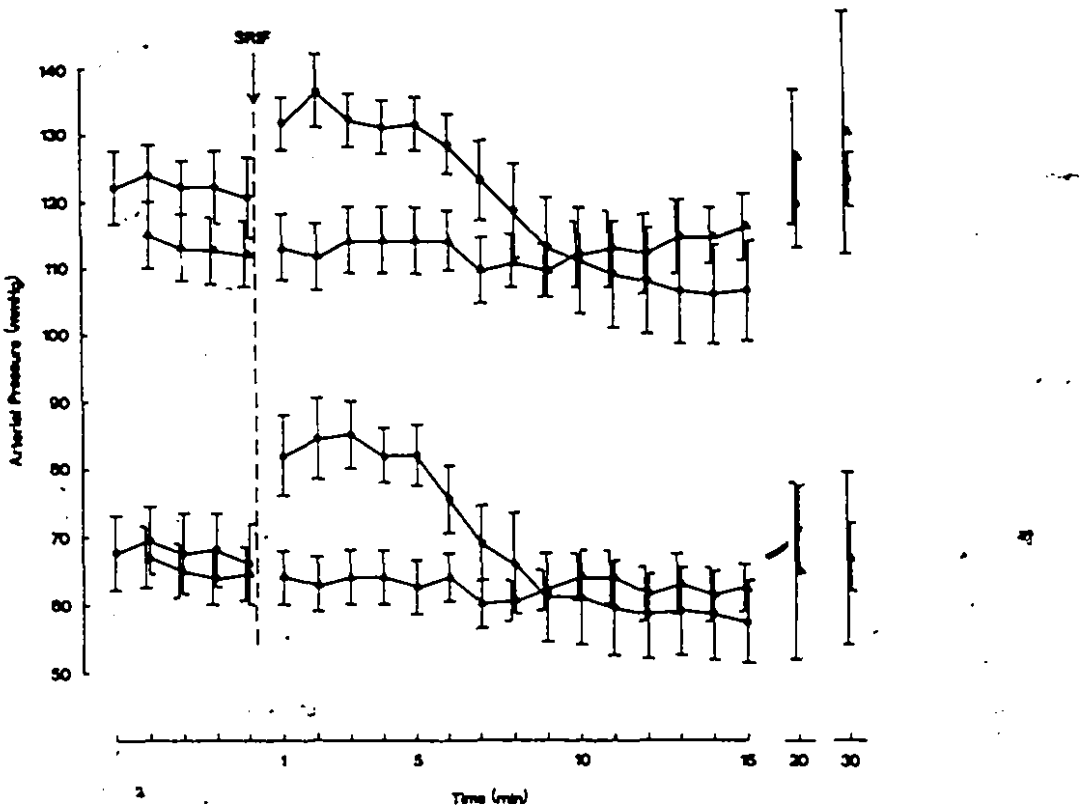

Figure 1. Time-effect curve for the effects of intrathecal administration, at the ninth thoracic level, of somatostatin (6.5 nmoles; ●——●; n = 10) and of artificial cerebrospinal fluid (▲——▲; n = 13), on mean systolic (upper curves) and diastolic arterial pressures in the rat. The abscissa is the time after administration in min. Administration was at the arrow. The vertical bars each represent ± 1 S.E.M.

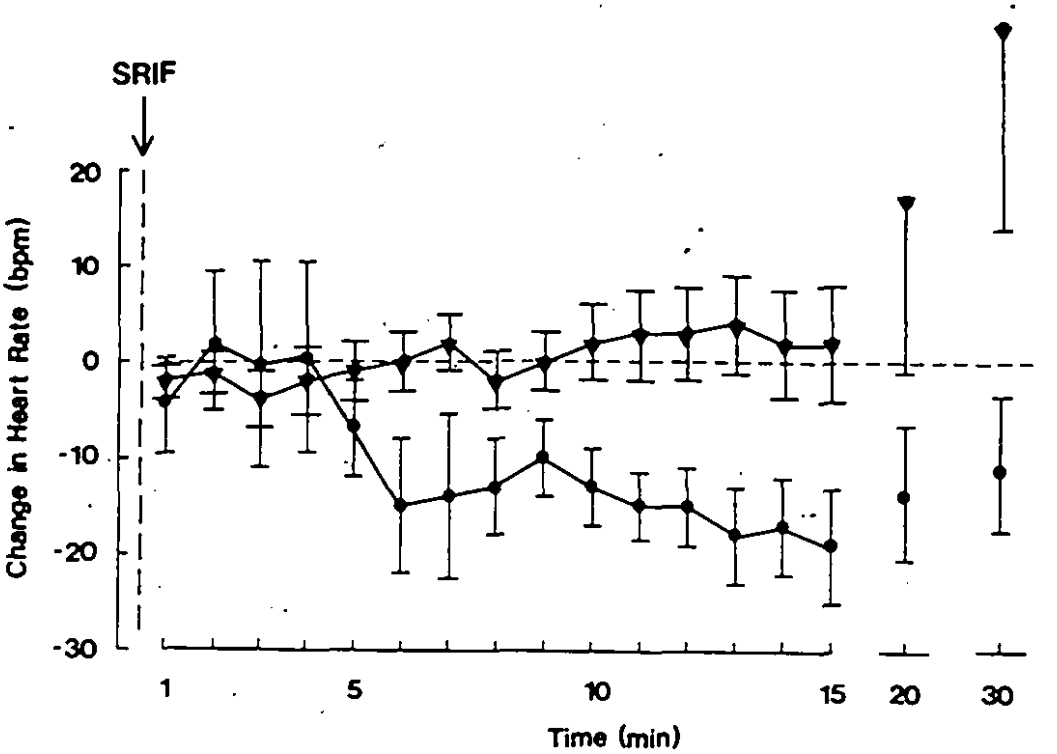

Figure 2. Change in mean heart rate from baseline values after intrathecal administration of somatostatin and of CSF at the ninth thoracic level in the rat. Details are otherwise the same as those in Figure 1.

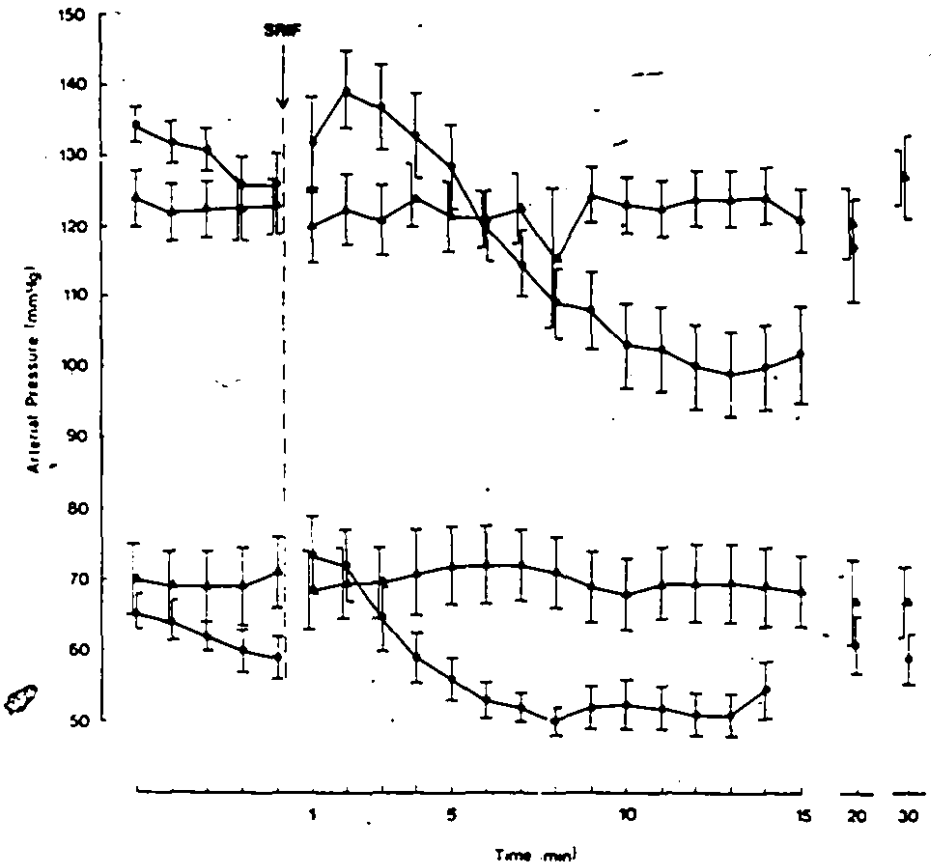

Figure 3. Effects of somatostatin (6.5 nmoles; n = 12) and of CSF (n = 14), administered at the second thoracic level, on systolic and diastolic arterial pressures. Other details are the same as in Figure 1.

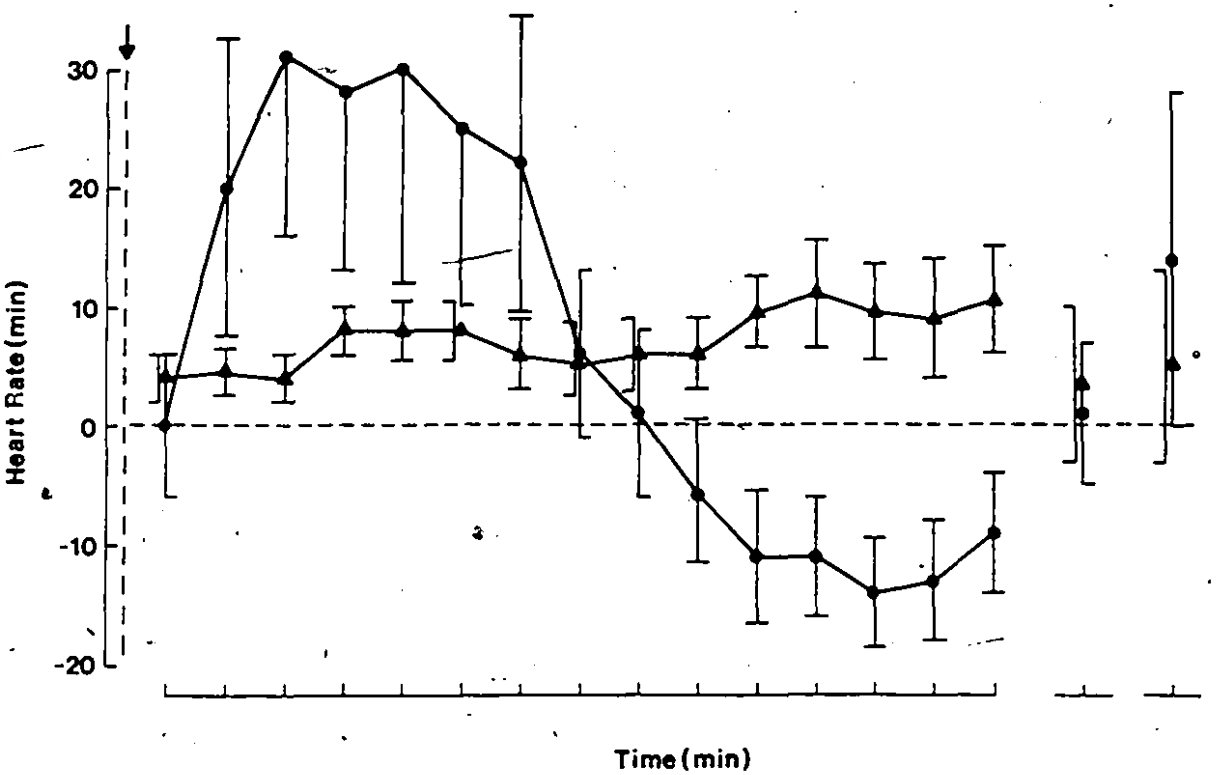

Figure 4. Changes in mean heart rate from baseline values after administration of somatostatin and of CSF at the second thoracic level. Other details are the same as those in Figure 1.

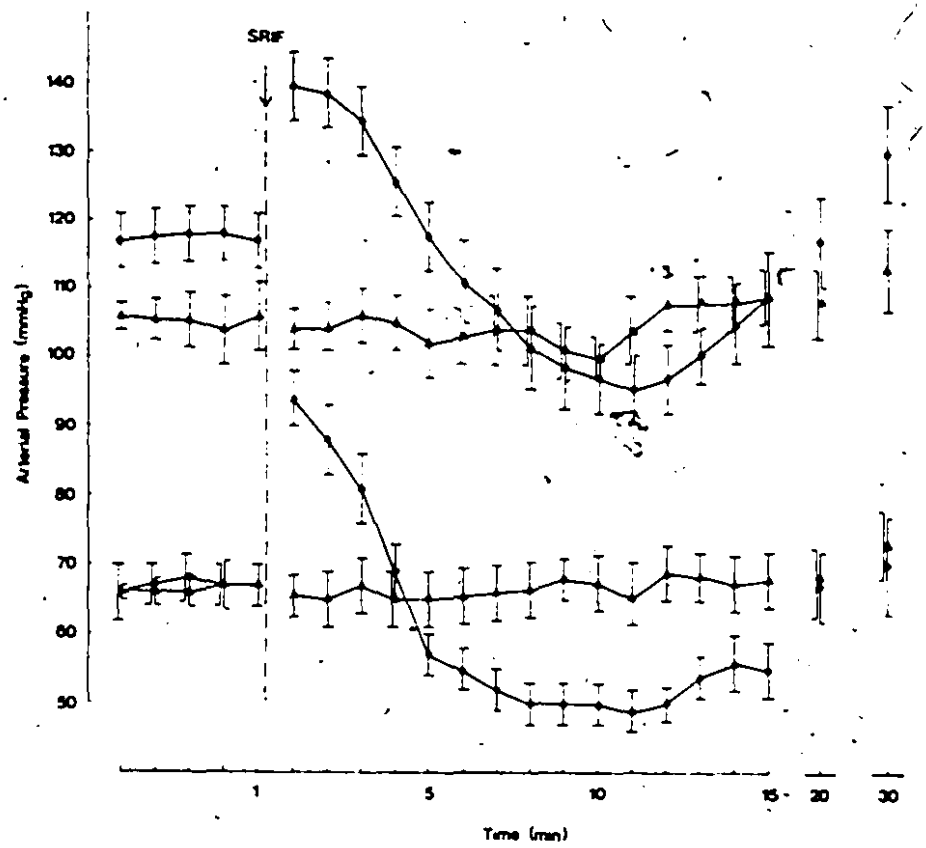

Figure 5. Effects of adrenalectomy on pressure responses to intrathecal administration of somatostatin (n = 15) and of CSF (n = 8) at the ninth thoracic level. Other details are the same as those in Figure 1.

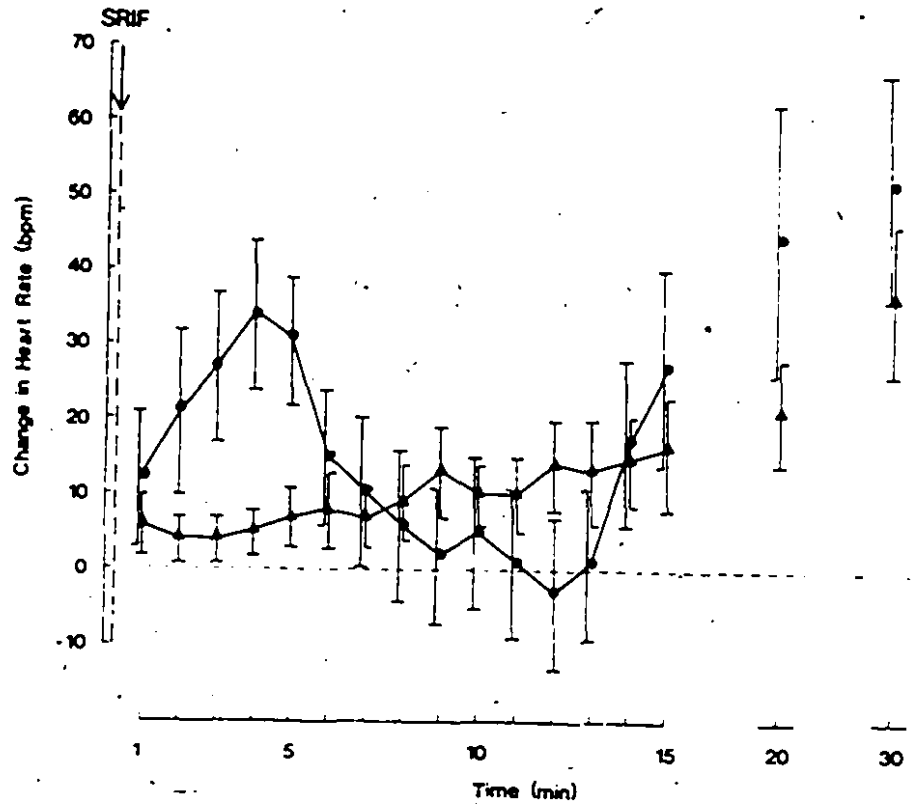

Figure 6. Effects of adrenalectomy on heart rate response to intrathecal administration of somatostatin at the ninth thoracic level. Values for CSF-treated animals are also presented. Other details are the same as those in Figure 1.

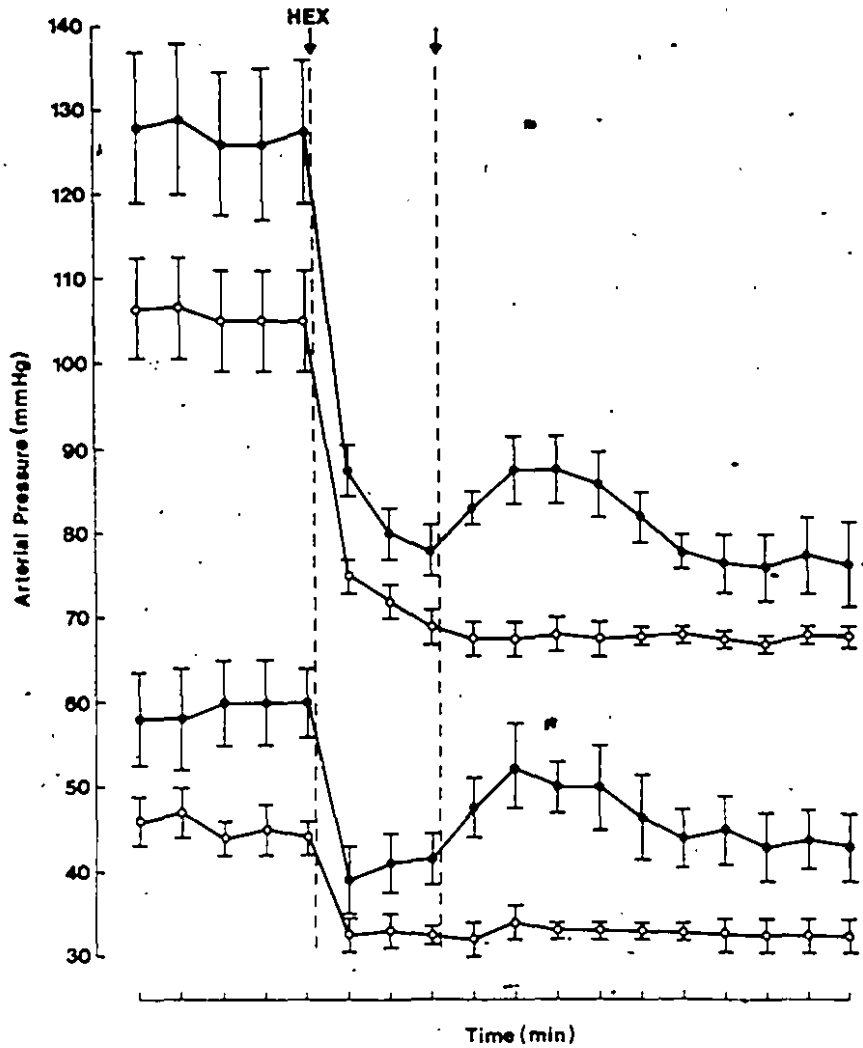

Figure 7. Effects of i.v. administration of hexamethonium (Hex; 10 mg/kg) on arterial pressure responses to intrathecal administration of somatostatin (n = 6) and of CSF (n = 6) at the ninth thoracic level. Other details are the same as those in Figure 1. Somatostatin is in filled circles, CSF is in open circles.

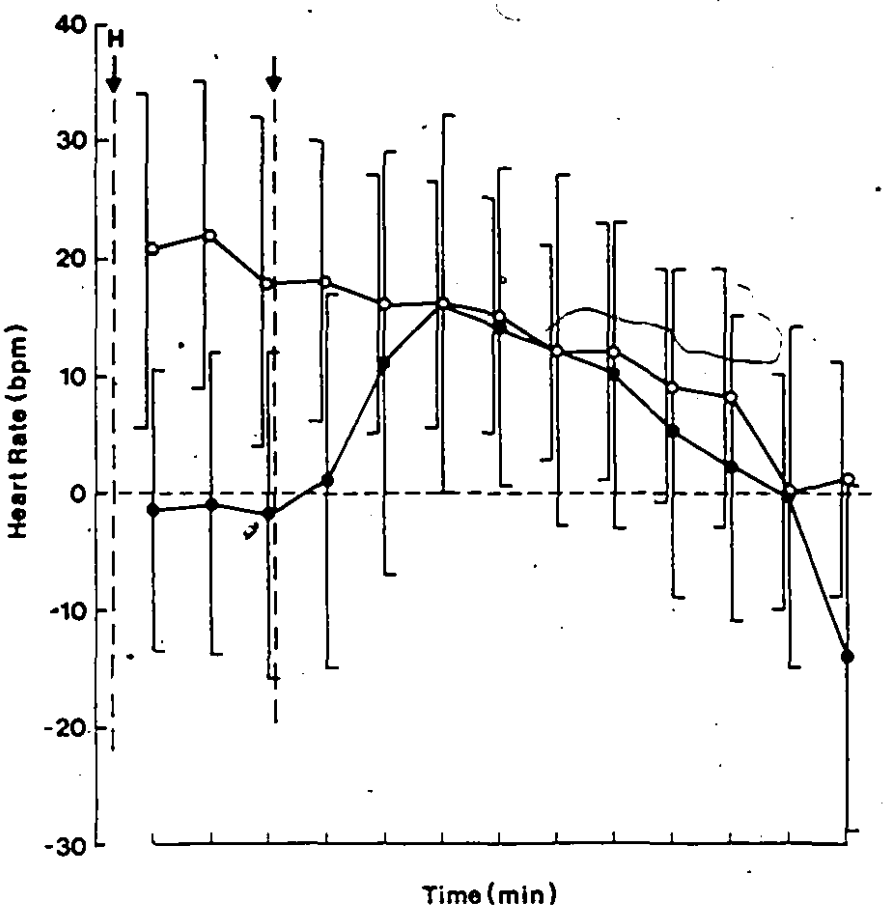

Figure 8. Effects of i.v. administration of hexamethonium on heart rate response to intrathecal administration of somatostatin at the ninth thoracic level. See legend to Figure 1 for other details. Somatostatin is in filled circles, CSF is in open circles.

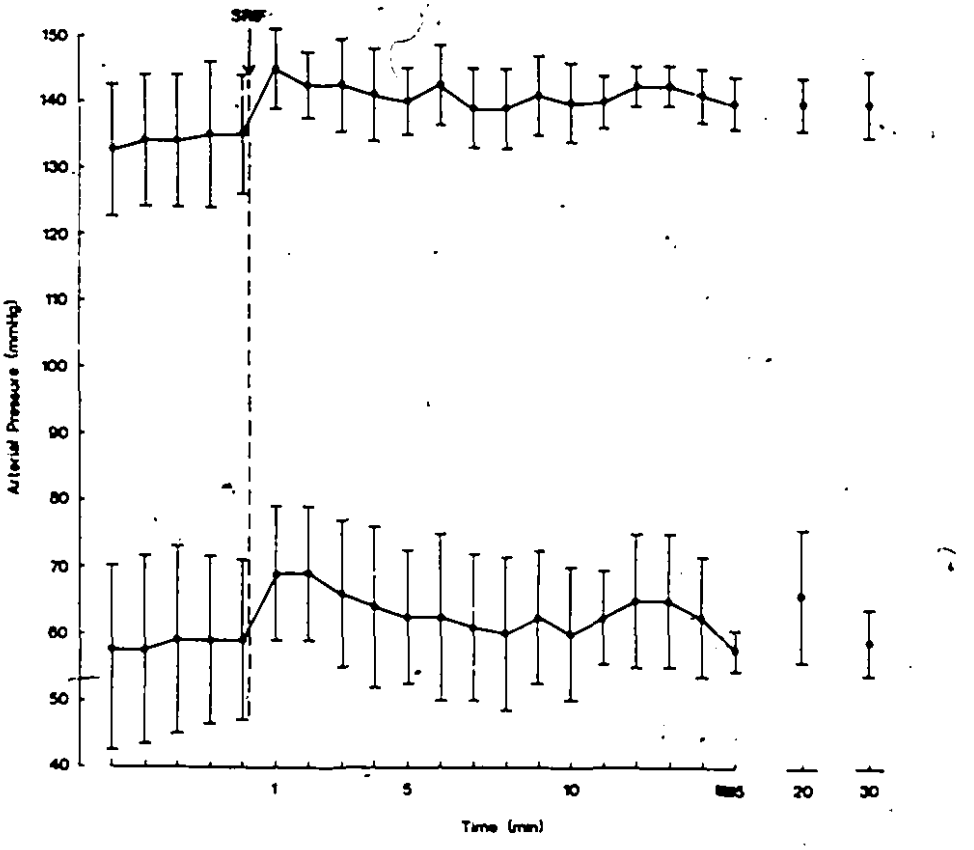

Figure 9. Effects of i.v. administration of 6.5 nmoles of somatostatin (n = 4) on systolic and diastolic pressures.

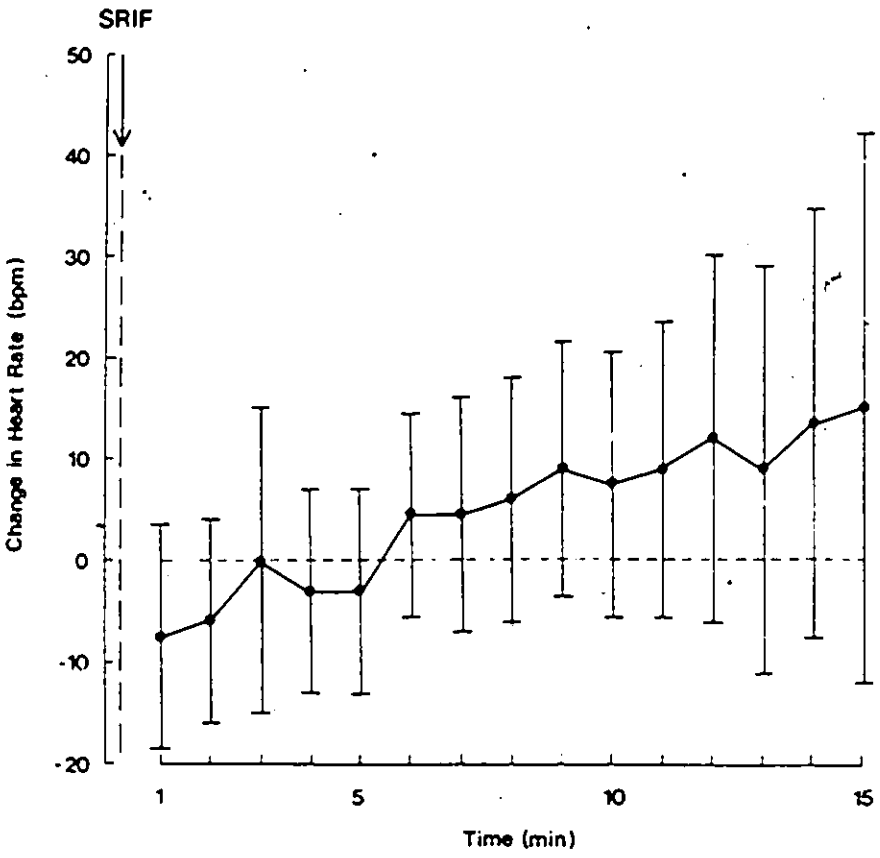

Figure 10. Effects of i.v. administration of somatostatin on heart rate.

CHAPTER IX

OXYTOCIN ADMINISTERED INTRATHECALLY

SELECTIVELY INCREASES HEART RATE NOT ARTERIAL PRESSURE

IN THE RAT

Abstract

Oxytocin, administered at a dose of 6.5 nmoles at the ninth or second thoracic level, increased heart rate but had no effect on arterial pressure. The increase in heart rate began within 1-5 min and reached a peak at 10-30 min; the maximum increase, at 15 min after administration at the ninth thoracic level was about 40 bpm and when it was given at the second thoracic level this change was about 65 bpm. Administration of hexamethonium to block nicotinic transmission in sympathetic ganglia prevented the cardioacceleration in response to intrathecal administration of oxytocin. When 6.5 nmoles of oxytocin were administered i.v., there was a brief decrease in heart rate by about 40 bpm and an increase in diastolic pressure. It is concluded that oxytocin increased heart rate via an action in the spinal cord, presumably on sympathetic preganglionic neurons. Our results are consistent with earlier suggestions that oxytocin may be a chemical mediator of synaptic transmission onto sympathetic preganglionic neurons and provide the further evidence that oxytocin may be involved specifically in pathways regulating heart rate rather than arterial pressure in the rat.

Introduction

Oxytocin has been implicated as a chemical mediator of synaptic transmission onto sympathetic preganglionic neurons in the intermediolateral nucleus of the spinal cord, in particular from terminals of fibers originating in the paraventricular nucleus of the hypothalamus. It has been known for some time from anatomical studies that inputs to sympathetic preganglionic neurons include fibers originating from the paraventricular nucleus. Orthograde labelling of terminals in the intermediolateral nucleus occurs following injection of tritiated amino acids or lectin into the hypothalamus (Saper et al., 1976; Luiten et al., 1985) and retrograde labelling of neurons in the paraventricular nucleus occurs following injection of fluorescent dyes or HRP into the thoracic dorsolateral funiculus (Hancock, 1976; Hosoya & Matsushita, 1979; Kuypers & Maisky, 1975; Nance, 1981; Ono et al., 1978; Saper et al., 1976; Swanson & Kuypers, 1980). The evidence from these studies has been corroborated by more recent electrophysiological evidence (Caverson et al., 1984; Yamashita et al., 1984). Some of the descending fibers from the paraventricular nucleus display immunoreactivity for oxytocin and its neurophysin, (Buijs, 1978; Nilaver et al., 1980;

Sawchenko & Swanson, 1982; SofroniеW, 1980; Sofroniew & Weindl, 1978; Swanson, 1977; Swanson & McKellar, 1979; Swanson & Sawchenko, 1980).

In turn, at least some of these oxytocinergic descending fibers are likely to project specifically to sympathetic preganglionic neurons. After injection of True Blue into the spinal cord, labelled neurons are found in the paraventricular nucleus and these neurons also contain oxytocin-like material (Sawchenko & Swanson, 1981, 1982). Lesions of the paraventricular nucleus lead to a decrease in oxytocin content in the spinal cord (Lang et al., 1983; Millan et al., 1984; Hawthorn et al., 1985) and electrical stimulation of the paraventricular nucleus stimulates oxytocin release from the spinal cord (Pittman et al., 1984). The greatest concentration of oxytocin and of its neurophysin in the spinal cord is in the intermediolateral nucleus, particularly at the ninth thoracic level (Jenkins et al., 1984). Fibers and terminals containing oxytocin and its neurophysin are scattered throughout the intermediolateral nucleus (Swanson, 1977; Swanson & McKellar, 1979), especially around neurons which are not labelled after injection of True Blue or Fast Blue into the adrenal medullae (Holets & Elde, 1982). In electrophysiological experiments, iontophoretic application of oxytocin

causes excitation of sympathetic preganglionic neurons (Backman & Henry, 1984).

In view of this abundant evidence implicating oxytocin as a chemical mediator of synaptic transmission onto sympathetic neurons in the spinal cord, the present study was done in an attempt to identify the functional type of pathway in which oxytocin is involved. Thus, experiments were done to determine the effects on arterial pressure and heart rate of the intrathecal administration of this peptide at the ninth and at the second thoracic spinal segments in the rat.

Materials and Methods

Animal preparation

Male Sprague Dawley rats, weighing approximately 350 g were anesthetized with urethane (2.5 g/kg, i.p.) and implanted with an intrathecal catheter (Intramedic PE-10), which was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the ninth or the second thoracic vertebral level. Spinous processes were used as landmarks; in preliminary experiments the

validity of this method for correct positioning of the inner tip of the catheter was confirmed in X-rays of rats implanted with wire-filled catheters. It was via this catheter that oxytocin was administered intrathecally.

A second catheter (Intramedic PE-60) was inserted into the left common carotid artery for monitoring arterial pressure via a Statham transducer (Gould PE23 ID), connected to a Grass polygraph. Heart rate was calculated from this record by counting the number of beats in a 10 sec period and multiplying this number by six to obtain the rate in beats per min.

In some experiments a third catheter was inserted into the right femoral vein for the intravenous infusion of drugs.

Rectal temperature was maintained at approximately 37^0C by a heating pad. Animals respired spontaneously throughout the experiments.

Peptide administration

Following surgical preparation, a 30 min period was allowed for stabilization. Baseline readings of arterial pressure and heart rate were taken over a five min period and oxytocin (from Peninsula Laboratories, Belmont, Calif.) was administered intrathecally at

either the ninth or the second thoracic level in a dose of 6.5 nmoles. It was delivered over a period of 15-20 sec in 10 µl of artificial cerebrospinal fluid (CSF; an aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$, and 1.4 mM $CaCl_2$). Following delivery of the peptide solution the intrathecal catheter was flushed with 10 µl of CSF, the internal volume of the catheter being approximately 8 µl. With zero time being the time of injection of the flushing solution, readings of arterial pressure and heart rate were taken each minute for the next 15 min and then at 20 and 30 min.

Block of ganglionic nicotinic transmission

To determine the degree of involvement of nicotinic transmission in sympathetic ganglia in the mediation of the cardiovascular changes observed, experiments with intrathecal administration of oxytocin were repeated but in animals which had been given hexamethonium bromide intravenously in a dose of 10 mg/kg and at a concentration of 10 mg/ml in physiological saline. Thus, the persistence of a response to subsequent administration of oxytocin would suggest either that the effects were mediated via non-nicotinic ganglionic transmission or that the

peptide was passing into the circulation and expressing its effects via a peripheral action.

Experiments with i.v. administration of oxytocin

In view of the possibility that oxytocin might have been producing its cardiovascular effects by passing into the general circulation, the experiments were performed as usual except that oxytocin was administered intravenously rather than intrathecally. The protocol was the same as before, except that the same dose of the peptide was dissolved in 0.5 ml of physiological saline for these experiments and the catheter was flushed with 0.5 ml of saline.

Statistical analysis

Results from each rat were tabulated as systolic and diastolic arterial pressures and heart rate, and also as changes in these parameters from the baseline values determined before intrathecal injection. Data for the figures were summarized by taking the mean ± S.E. of the values from each group of rats at each min following administration. To determine statistical significance, Student's *t*-test for unpaired data was applied for comparison between

the two values (oxytocin vs CSF groups) at each sample time after intrathecal administration.

RESULTS

Intrathecal administration of oxytocin had no effect on respiratory frequency in these experiments; this frequency remained at a mean value of approximately 100 breaths per minute.

Effects of oxytocin at the ninth thoracic level

Administration of 6.5 nmoles of oxytocin intrathecally at the ninth thoracic level transiently increased heart rate. The results are summarized in Figure 1. Baseline heart rate in the 9 oxytocin-treated rats was 335.1 $\pm$ 13.0 bpm; in the 13 CSF-treated rats it was 332.2 $\pm$ 15.4 bpm. Administration of oxytocin induced a slow but progressive increase in heart rate which continued throughout the 30 min period of the experiment. Similar administration of CSF had no effect on heart rate. When the changes from preadministration values were compared between the two groups they were different from 5 to 15 min ($p < 0.025$)

Arterial pressure was unaffected by administration of oxytocin and of CSF. Mean values of systolic and diastolic pressures at each sample time are presented in Figure 2. Comparison of the changes from preadministration values revealed that differences between the two groups at all sample times were not significant.

Effects of oxytocin at the second thoracic level

Administration of the same 6.5 nmole dose of oxytocin at the second thoracic level had a qualitatively similar effect as it had at the ninth thoracic level. However, the onset of the response in the upper thoracic cord was faster and the change was greater at any given time during the 15 min after administration. The effects of this administration at the second thoracic level are presented in Figure 3. Baseline heart rate in the 14 oxytocin-treated rats was 380.4 ± 10.3 bpm and in the 14 CSF-treated rats it was 361.4 ± 13.2 bpm. The changes from preadministration values were different between the two groups of animals from 1 to 30 min (1 min, $p < 0.005$; 2-30 min, $p < 0.001$).

Arterial pressure was unaffected by administration at this level. Figure 4 shows the results obtained.

Effect of nicotinic block on responses to oxytocin

These experiments were done following the same general procedure as above, administering oxytocin at the ninth thoracic level, except that 3 min prior to administration of the peptide intrathecally, hexamethonium was given in a dose of 10 mg/kg i.v. to block nicotinic transmission in sympathetic ganglia. Mean baseline heart rate in the 4 oxytocin-treated rats was 381.0 ± 10.0 bpm and in the 6 CSF-treated rats it was 320.2 ± 13.8 bpm. After administration of hexamethonium mean heart rate was 397.5 ± 9.9 bpm in rats given oxytocin and was 338.0 ± 17.2 bpm in rats given CSF. The mean changes in heart rate for the two groups of animals are illustrated in Figure 5. After administration of oxytocin there was a small but insignificant increase in heart rate, to a maximum of 405.0 ± 14.3 bpm. In CSF-treated animals, there was a slow, progressive decrease in heart rate. At no time during the sample period was there a significant difference between the changes induced in the two groups of animals.

Following stabilization from administration of hexamethonium, there was no change in arterial pressure in the two groups of animals upon administration of oxytocin or CSF (see Figure 6). Mean baseline systolic and diastolic pressures before hexamethonium were 118.7

± 11.1 and 51.2 ± 4.3 mm Hg, respectively, in oxytocin-treated rats and 105.5 ± 6.0 and 45.0 ± 2.4 mm Hg, respectively, in CSF-treated rats. Three min after i.v. administration of hexamethonium (ie. just prior to intrathecal administration) these respective values were 80.0 ± 6.2 and 41.2 ± 2.7 mm Hg in oxytocin-treated rats and 69.2 ± 1.7 and 32.5 ± 1.2 mm Hg in CSF-treated rats.

Effects of intravenous administration of oxytocin

In view of the fact that oxytocin caused a small, though insignificant, increase in heart rate in rats treated with hexamethonium, the possibility was considered that oxytocin delivered into the intrathecal space might have passed into the circulation and expressed its effects, at least on heart rate, via a peripheral action. Therefore, the earlier experiments were repeated, except that the same dose of oxytocin was administered intravenously in a volume of 0.5 ml of physiological saline. Prior to administration of the peptide the mean heart rate in the 4 animals tested was 421.5 ± 34.9 bpm. As can be seen in Figure 7, i.v. administration of oxytocin induced an immediate decrease in heart rate, by about 40 bpm. There was a slow recovery of the heart rate over the next 10-15 min toward preadministration levels.

In terms of the effects on arterial pressure, i.v. administration of oxytocin had little or no effect on systolic pressure but had a transient pressor effect on diastolic pressure, increasing it by about 35 mm Hg at one min after administration. From this time diastolic pressure decreased until at 5-10 min after injection the pressure had returned to preadministration levels.

DISCUSSION

This study has demonstrated that the intrathecal administration of 6.5 nmoles of oxytocin to the ninth and to the second thoracic segments increases heart rate but not arterial pressure in the rat. This effect was quantitatively greater at the second thoracic level. The cardioacceleratory response did not occur in animals which had been pretreated with a blocker of nicotinic transmission in sympathetic ganglia, and the cardiovascular effects of oxytocin were qualitatively different when it was given intravenously.

In view of the fact that hexamethonium blocked the increase in heart rate induced by intrathecal administration of oxytocin, it is concluded that the responses were mediated primarily or exclusively via a ganglionic nicotinic mechanism and

therefore that oxytocin must have induced an activation of sympathetic preganglionic neurons via an action in the spinal cord. This action may have been directly on the sympathetic preganglionic neurons themselves because iontophoretic application of oxytocin to these neurons causes excitation (Backman & Henry, 1984; the depression reported by Gilbey et al, 1983, was artifactual due to application of current itself) and because nerve terminals containing oxytocin-like material can be observed surrounding at least a subpopulation of these neurons (Swanson, 1977; Swanson & McKellar, 1979; Holets & Elde, 1982).

A spinal site of action is supported by the failure of intravenous administration of oxytocin to mimick the effects observed when the peptide was given intrathecally. In fact, the effects were opposite in direction.

The possibility was considered that oxytocin may have expressed its effects via an action in the brain stem, after diffusion to that level. However, this possibility is unlikely because intracisternal administration of oxytocin in the rat fails to alter heart rate (Petty et al., 1985). In the anesthetized dog such administration increases arterial pressure but again has no effect on heart rate (Tran et al., 1982). This is very different from the present results in which an increase in heart rate but no change in

arterial pressure was observed. In addition, microinjection of oxytocin into the nuclei of the tractus solitarius, which also receives oxytocin-containing fibers (Buijs, 1978; Sawchenko & Swanson, 1982; Sofroniew & Schrell, 1981), has no effect on either heart rate or arterial pressure (Vallejo et al., 1984).

This study was done in anesthetized animals for several reasons. As delivery of the peptide was at the spinal level, few central synapses were involved. In fact, if oxytocin acted upon sympathetic preganglionic neurons, as suggested above, then no central synapses would be involved. Furthermore, with this approach, arterial pressure could be measured reliably and easily by the direct method and artifactual changes in cardiovascular parameters due to changes in the behavioral state of an awake animal could be avoided. This latter point was considered to be especially important in view of the behavioral responses induced by the intrathecal administration of some peptides (Hylden & Wilcox, 1981; Dobry et al., 1981; Seybold et al., 1982; Yashpal et al., 1982). Urethane was used as the anesthetic because it has a less disruptive effect on baseline cardiovascular parameters and reflexes as well as on respiratory parameters than do other anesthetics in the rat (Armstrong, 1981; De Wildt et al., 1983; Sapru & Krieger, 1979).

In summary, our results support evidence implicating oxytocin as a chemical mediator of synaptic transmission in descending inputs to sympathetic preganglionic neurons. Our data suggest that oxytocinergic inputs are associated specifically with pathways regulating heart rate rather than arterial pressure.

ACKNOWLEDGEMENTS

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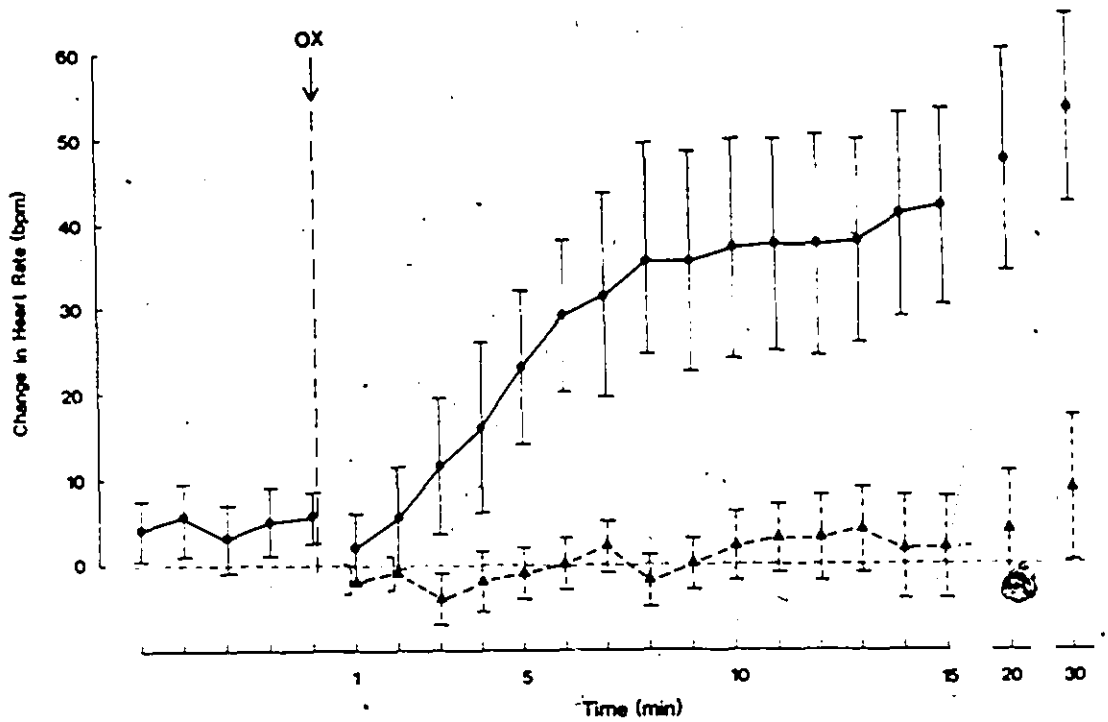

Figure 1. Time effect curve for the effects of the intrathecal administration of oxytocin (6.5 nmoles; n = 9; ●——●) or of artificial cerebrospinal fluid (n = 13; ▲- - -▲) on the change in mean heart rate from the baseline level, determined just before administration, in the rat. Administration was at the ninth thoracic spinal level. The abscissa shows time after administration. Vertical bars each represent ± one S.E.M.

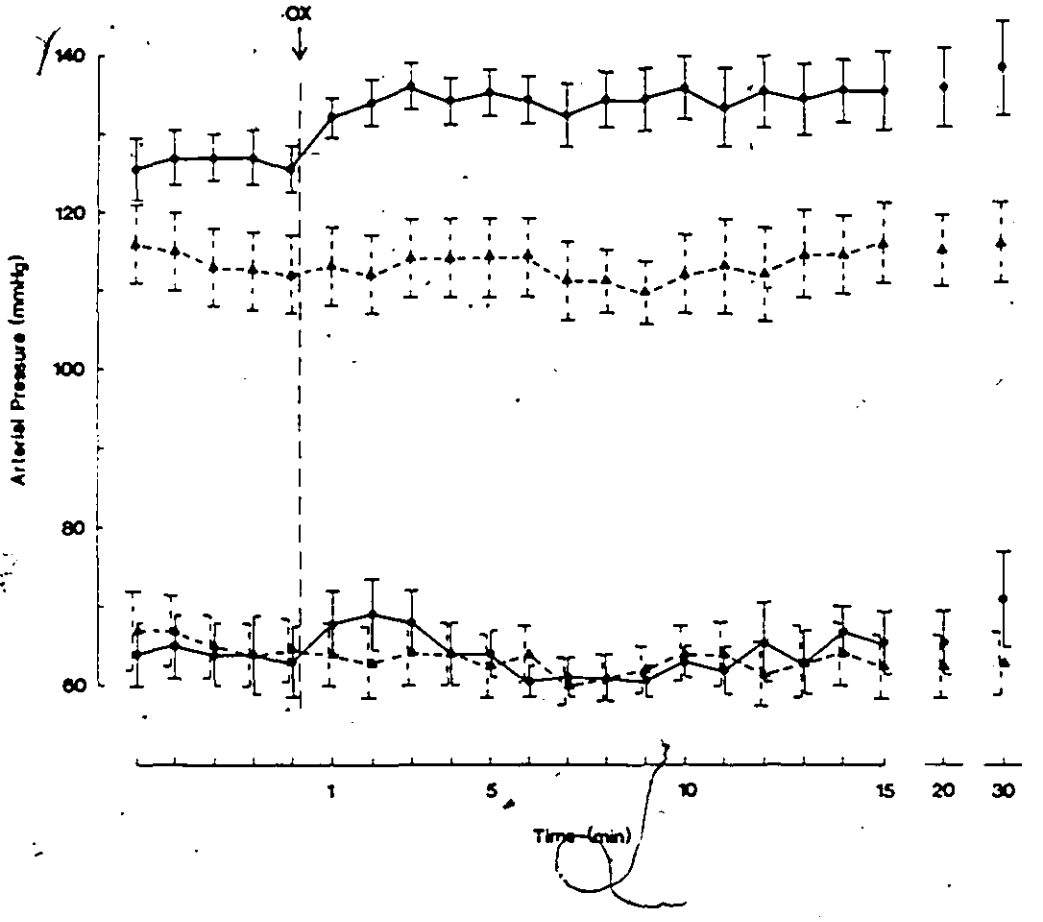

Figure 2. Effects of oxytocin on mean systolic and diastolic pressures, administered at the ninth thoracic level. Other details are the same as in Figure 1.

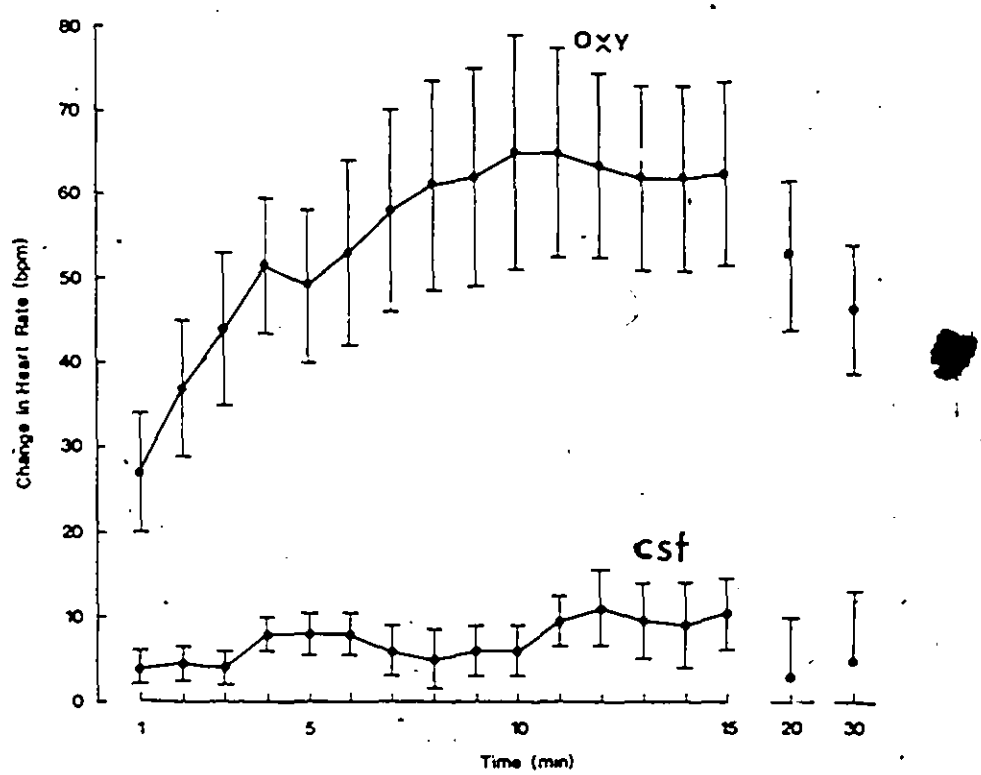

Figure 3. Effects of intrathecal administration of oxytocin (n = 14) or of CSF (n = 14) at the second thoracic level on change in mean heart rate from the baseline level. Details are otherwise the same as those in Figure 1.

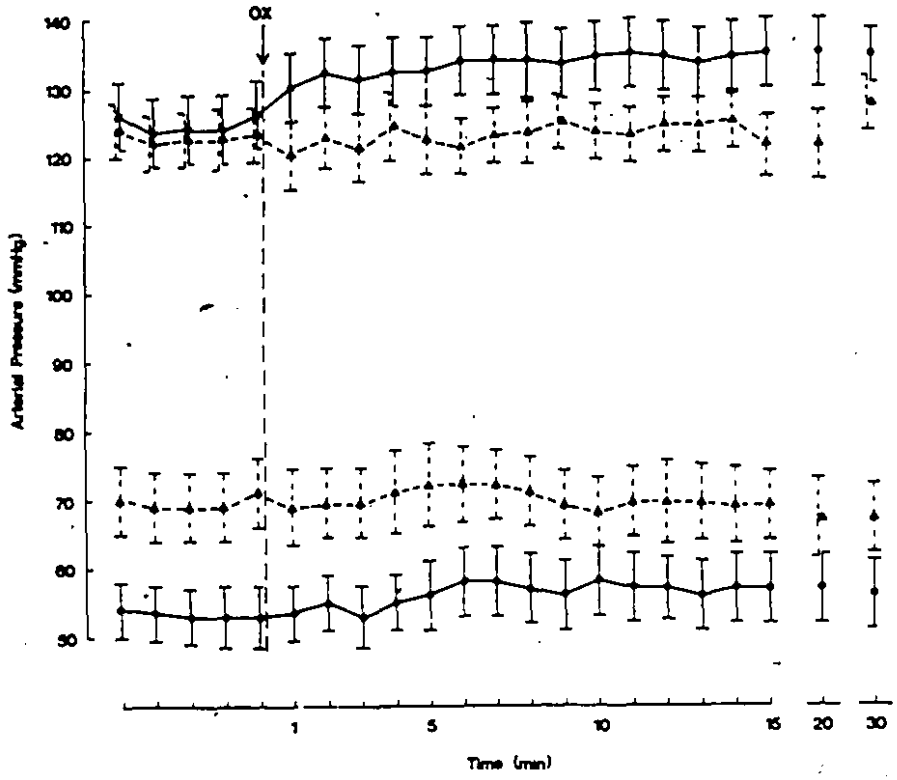

Figure 4. Effects of intrathecal administration of oxytocin and of CSF at the second thoracic level on mean systolic and diastolic pressures. Details are the same as those in Figure 1.

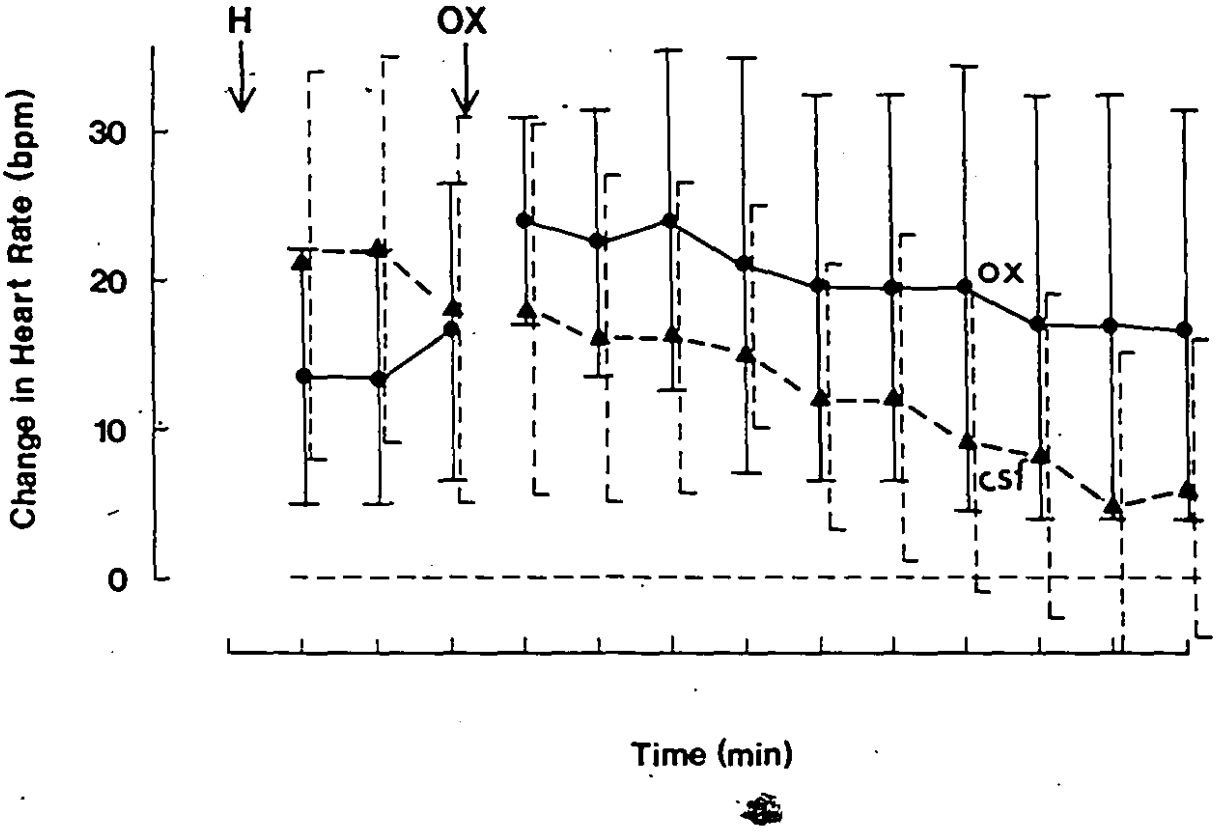

Figure 5. Effect of i.v. administration of hexamethonium (H; 10 mg/kg) on the change in heart rate upon intrathecal administration of oxytocin (n = 4) and of CSF (n = 6) at the ninth thoracic level.

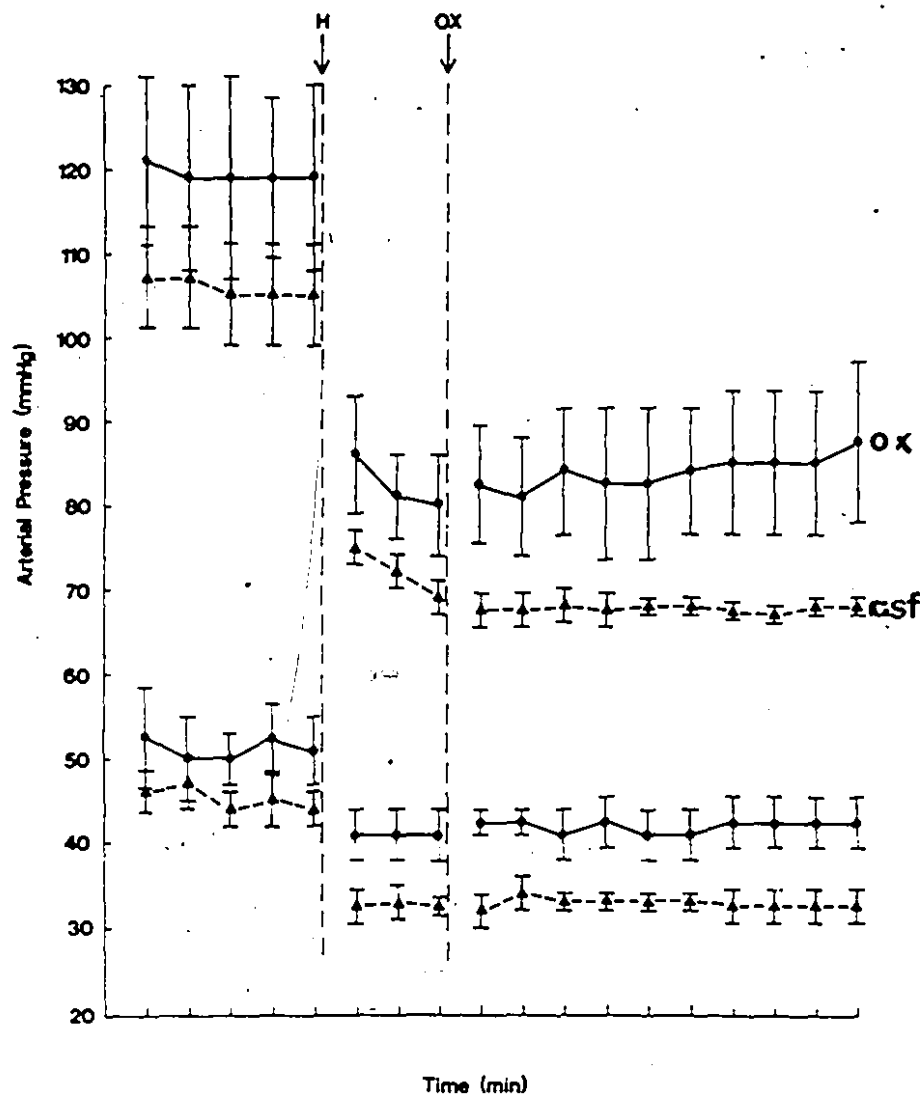

Figure 6. Effects of intrathecal administration of oxytocin and of CSF on systolic and diastolic pressures after prior i.v. administration of hexamethonium.

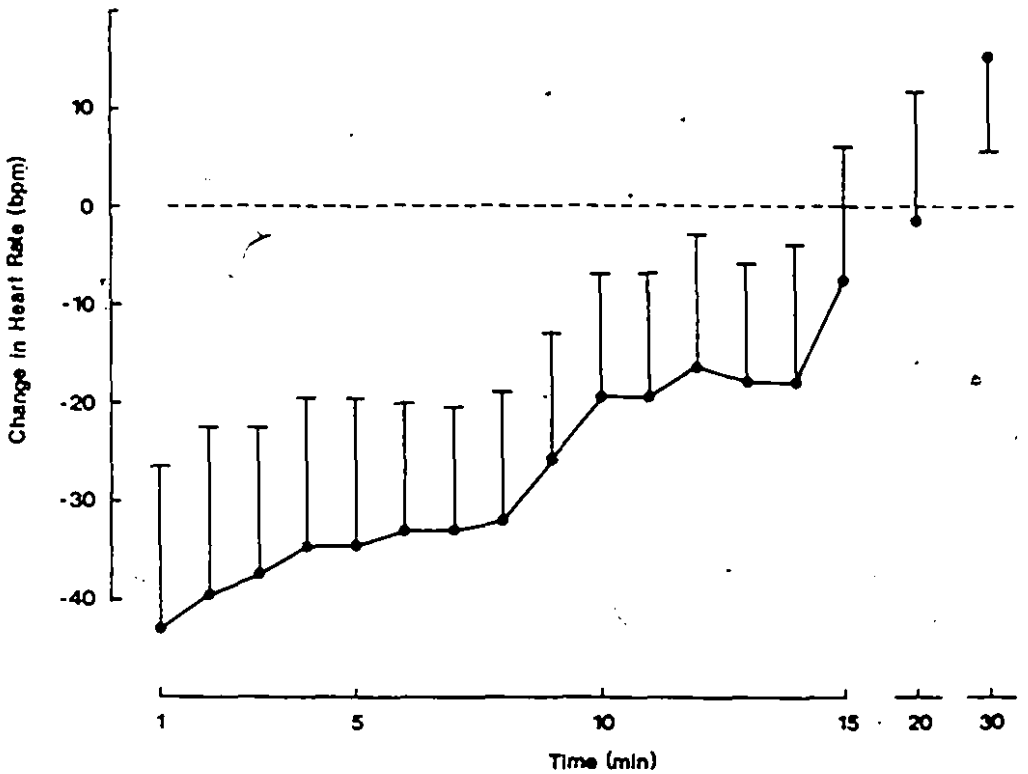

Figure 7. Effects of i.v. administration of 6.5 nmoles of oxytocin (n = 4) on heart rate.

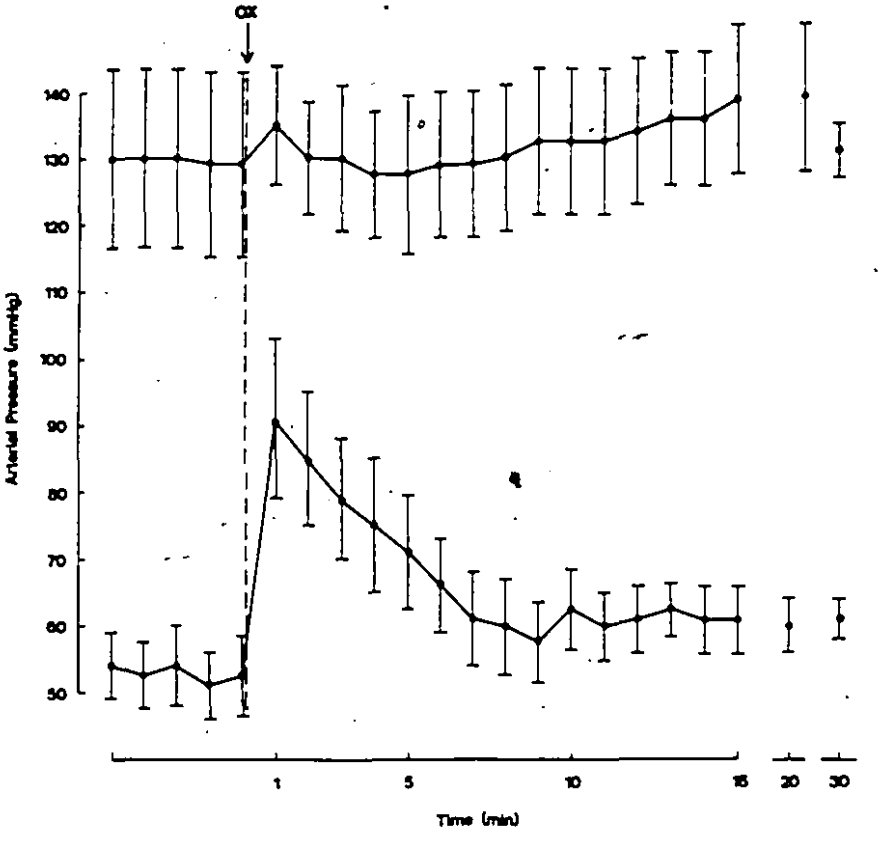

Figure 8. Effects of i.v. administration of oxytocin on systolic and diastolic pressures.

V CLAIMS OF ORIGINALITY

All the findings in this thesis are original. To the best of my knowledge after an extensive survey of the literature, there are no published reports in which the peptides used in the experiments reported here have been applied intrathecally in studies on spinal mechanisms controlling sympathetic output regulating heart, blood vessels and adrenal medulla. All the relevant literature which I have found and which pertains to the subjects included in this thesis has been cited.

The principal original findings which have been made from the experiments are as follows:

(1) The rostro-caudal diffusion of radiolabelled substance P is localized primarily to the region of the spinal cord where the tip of the catheter is placed.

(2) The rostro-caudal distance over which radiolabelled substance P penetrates into the spinal cord is restricted to one cm around the tip of the catheter

(3) Radiolabelled substance P reaches the lateral horn within one min of administration.

(4) Passage of the label into the blood is less than 1% of the amount given.

(5) Intrathecal administration of substance P to the spinal T9 level of the anaesthetized rat increases plasma levels of free noradrenaline and of free adrenaline. These increases are seen as early as one min after administration of the peptide and persist for at least 30 min. Intrathecal administration of a substance P antagonist 15 min prior to administration of substance P blocks these increases.

(6) Intrathecal administration of substance P to the spinal T9 level of the awake or of the anaesthetized rat increases arterial pressure and heart rate. These responses begin within 1-2 min of administration, peak at 4-9 min and persist for 15 min. These responses are blocked in animals which received an earlier intrathecal injection of a substance P antagonist.

(7) These cardiovascular effects of intrathecal administration of substance P are prevented by systemic administration of a blocker of nicotinic transmission in sympathetic ganglia.

(8) Bilateral adrenalectomy fails to abolish these cardiovascular responses.

(9) Intrathecal administration of substance P at the T2 level reveals a more potent effect on heart rate than occurs at T9.

(10) Intrathecal administration of substance P at the T2 level induces a smaller increase in plasma levels of both catecholamines than does administration at T9.

(11) Angiotensin II given intrathecally at the T9 and the T2 level increases arterial pressure and heart rate.

(12) An analogue of angiotensin II which has antagonistic properties in peripheral tissues blocks the angiotensin II-induced increase in arterial pressure but not the increase in heart rate. The analogue itself has no effect on arterial pressure but induces a slow increase in heart rate.

(13) Both responses are blocked in animals pretreated with systemic administration of a nicotinic antagonist.

(14) Thyrotropin-relaeasing hormone given at T9 increases arterial pressure and heart rate.

(15) At the T2 level the pressor effect is absent and the increase in heart rate is smaller.

(16) Systemic administration of an antagonist of nicotinic transmission in sympathetic ganglia blocks the pressor effect at T9 but does not block the increase in heart rate.

(17) When somatostatin is given at T9 it increases arterial pressure but depresses heart rate.

(18) At the T2 level it has a biphasic effect on arterial pressure and on heart rate; in each case the response consists of an initial increase followed by a decrease.

(19) Adrenalectomy does not abolish the arterial pressure or the heart rate response to administration at T9. In fact, the pressor effect is potentiated and the heart rate response resembles that at T2.

(20) Blockade of nicotinic transmission in sympathetic ganglia decreases the pressor effect of administration at T9; the heart rate response is only partially decreased.

(21) Oxytocin administration to the T9 or to the T2 level increases heart rate but has no effect on arterial pressure;

(22) The heart rate response to administration at the T9 level is greater than that at the T2 level.

(23) The heart rate response to administration at the T9 level is prevented by block of nicotinic transmission in sympathetic ganglia.

The principal original conclusions which have been drawn from these findings are as follows:

A. Upon intrathecal administration of radiolabelled substanc P the label remains in the general vicinity of the tip of the catheter, it penetrates the grey matter to the presumed site of action in the lateral horn and it does not pass into the general circulation in quantities sufficient to account for the physiological responses observed.

B. Substance P excites spinal sympathetic neurones involved in pathways regulating heart rate, arterial pressure and adrenal medullary output of catecholamines.

C. The greater heart rate response at T2 than at T9 is most readily accounted for by either the greater preponderance of cardioacceleratory neurones at the upper spinal level or by a greater number of substance P receptors at the upper thoracic level.

D. The greater increase in catecholamine output induced by substance P at the T9 level than at the T2 level is most readily accounted for by the greater preponderance of sympathoadrenal neurones at the lower level; this, in turn, supports the former possibility, raised above in "B", that the greater response at T2 is due to the greater number of cardioacceleratory neurones at that level.

E. As the cardiovascular responses can be elicited in adrenalectomized animals it can be concluded that these responses are elicited at least partially via neuronal rather

than humoral (via adrenal output of catecholamines) mechanisms. From this it can in turn be suggested that the substance P-containing pathways regulating sympathetic output controlling cardiovascular parameters do so mainly via direct neural connections rather than via humoral mechanisms.

F. As the cardiovascular responses were totally blocked by systemic administration of a nicotinic antagonist it is concluded that the sympathetic pathways mediating these responses included a nicotinic link at the synaptic junction between the preganglionic and postganglionic neurones.

G. While angiotensin II has been shown to induce changes in arterial pressure and heart rate when given into the central nervous system, this is the first study to demonstrate cardiovascular effects upon administration at the spinal level. Arguements are presented in the text of this thesis that the action of spinally administered angiotensin II is in the spinal cord rather than either in the periphery or in supraspinal structures.

H. Therefore, it can be concluded that angiotensin II excites spinal sympathetic neurones involved in pathways regulating arterial pressure and heart rate.

I. As hexamethonium blocks both types of cardiovascular response this is taken as support for a spinal action of angiotensin II and it is concluded, furthermore, that synaptic conduction in the sympathetic pathways activated by angiotensin II inputs is nicotinic at the ganglion level.

J. The selective block of the arterial pressure response but not the heart rate response by an analogue with previously reported antagonist properties in peripheral tissues leads to the novel suggestion that two types of angiotensin II receptor exist in the spinal cord. One mediates the increase in arterial pressure. In this case the analogue is an antagonist. The other mediates the increase in heart rate and in this case the analogue is an agonist.

K. Thyrotropin-releasing hormone (TRH) increases arterial pressure and heart rate when it is given intrathecally at the T9 and T2 levels.

L. The greater effects observed when TRH is given at the lower than at the upper thoracic level suggests that there is a longitudinal, or segmental, differentiation of the numbers of TRH receptors, a greater number being at the lower level.

M. It is interesting to contrast these results from experiments with TRH with those from experiments with substance P, in which a preferential effect on heart rate was elicited from the upper thoracic spinal cord. This suggests that different descending pathways, which can be identified on the basis of the chemical mediating the synaptic contact, are involved in differential regulation of sympathetic output and therefore that neurones in one region of the intermediolateral nucleus can be activated without activation of those in other regions; this is different from what one would expect if these neurones all acted as a functional syncitium as suggested by

W.B. Cannon in the 1920's.

N. As the pressor effect was abolished by hexamethonium but was not mimicked by i.v. administration of TRH it is suggested that the pressor effect was due to an action in the spinal cord and that the pressor effect is mediated in the sympathetic ganglia via nicotinic transmission.

O. On the other hand, as the heart rate response was unaffected by hexamethonium, and i.v. administration of TRH also failed to alter heart rate, it is suggested that in this case the effect was also mediated via a spinal action but that the sympathetic pathways activated were non-nicotinic.

P. Somatostatin was the only peptide for which the cardiovascular responses were complex in configuration. In general, considering the results obtained with administration at the two levels as well as those from adrenalectomized animals, intrathecal administration of somatostatin gave rise to biphasic changes in arterial pressure and heart rate, consisting of an initial increase followed by a depression. An explanation for the mechanisms leading to these responses is not readily apparent.

Q. As with TRH, the experiments in which hexamethonium was given before the administration of somatostatin suggest that the sympathetic pathways mediating the arterial pressure response to this peptide are nicotinic but that those mediating the heart rate response are non-nicotinic.

R. In relation to the suggestion in the literature

that somatostatinergic pathways preferentially innervate sympathoadrenal neurones, the results from the present physiological experiments do not provide support for this suggestion. On the other hand, they indicate that the cardiovascular responses were greater in adrenalectomized than in intact animals.

S. These observations lead in turn to the further possibility that there may be a cardioinhibitory and depressor factor released from the adrenals. Considering the experiments with substance P, in which the cardioacceleration occurred more rapidly in adrenalectomized animals than in intact animals, a similar conclusion can be reached.

T. Oxytocin was the only peptide which increased heart rate but had no effect on arterial pressure. This indicates a functional differentiation with respect to at least the oxytocin-containing pathways descending to sympathetic neurones in the spinal cord.

U. This in turn indicates an even more differentiated control of sympathetic output than was indicated in conclusion "L" above. Thus, while both substance P and TRH seem to be involved in regulation of arterial pressure and of heart rate, substance P expresses its greatest effect at the upper thoracic spinal level while TRH expresses its effects primarily at the lower thoracic level. As stated above, this indicates a differentiation in terms of segmental control. On the other hand, the results with oxytocin demonstrate that there is a

selective effect on one cardiovascular parameter (heart rate) but not on the other (arterial pressure) indicating the second type of differentiation, one in terms of functional rather than segmental control.

V. The greater effect of oxytocin at the upper thoracic level than at the lower thoracic level indicates that it also participates in differential segmental control.

W. As hexamethonium blocks the response to oxytocin it is concluded that the sympathetic pathways activated by oxytocinergic inputs are nicotinic.

X. The blockade of the effects of oxytocin by hexamethonium and the failure of systemic administration of oxytocin to mimick the responses elicited from intrathecal administration suggest that the latter responses (ie. those to intrathecal administration) are due to an action within the spinal cord.

Y. The experiments reported here provide overwhelming evidence that the peptides investigated in these studies are involved in regulation of sympathetic output at the spinal level.

Z. They also demonstrate that there is a specific and differential control of this output, and that it may be identified and accounted for on the basis of specific, descending pathways which can be differentiated on the basis of the chemicals mediating their synaptic transmission.