Chemical basis of synaptic transmission in spinal pathways regulating sympathetic output to heart and vessels

by Patricia Dong-Sook Park

A thesis submitted to
the Faculty of Graduate Studies and
Research in partial fulfilment of the
requirements for the degree of
Master of Science.

Department of Physiology
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©October 1993

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To my family

ABSTRACT

In the first study, the effect of intrathecal administration of substance P at T9/T2 on arterial blood pressure and heart rate was studied in acute spinal (T5/T1) transected rats. The results indicate that excitatory effects of substance P on sympathetic output can be elicited in spinal transected rats and that the neural substrate for the effects is intrinsic to the spinal cord. An opioid involvement in suppressing the response to substance P is also confirmed.

In the second study, the role of angiotensin (AII) receptors (AT_1 and AT_2) in mediating the effects of intrathecally administered AII on sympathetic output was investigated using DuP 753 and PD 123319, the AT_1 and AT_2 receptor antagonists, respectively. The results suggest that at the spinal level, AII expresses its effects on sympathetic output to the heart via the AT_1 receptor and to the vessels partly via AT_1 and perhaps also via AT_2 receptors.

RESUMÉ

Dans une première étude, l'effet sur la tension artérielle et al fréquence des pouls de l'administation intrathécale de la substance P (T9/T2) est étudié dans des rats coupés transversalement atteints d'une forte anomalie (T5/T1) de la moëlle épinière. Les résultats indiquent que les effets excitatoires de la substance P sur les éfferences sympathiques peuvent être provoqués dans ces rats et que la cause neurale de ces effets est intrinsèque à la moëlle épinière. On a aussi confirmé le rôle des opiats dans la suppression de la réaction à la substance P.

Dans une deuxième étude, le rôle des récepteurs (AT_1 et AT_2) angiotensins (AII) comme entremises aux effets de l'AII administré de façon intrathécale sur des produits sympathiques a été analysé à l'aide du DuP 753 et du PD 123319, les antagonistes AT_1 et AT_2 respectivement. Les résultats suggèrent qu'au niveau de la moëlle épinière, les effets au coeur du AII sur les produits sympathiques sont démontrés par l'entremise du récepteur AT_1 et que les effets aux vaisseaux sont démontres partiellement part l'entremise du AT_1, et peut-être aussi par l'entremise du récepteur AT_2.

PREFACE

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P.D.S. Park and J.L. Henry. (1992) Differential effects of substance P on arterial pressure

and heart rate in rats acutely spinalized at T5 and T2, *Physiol. Can.* 23: 181 (Abstract).

P.D.S.Park and J.L. Henry. Acute spinal transection: cardiovascular responses to intrathecal substance P in the rat (in preparation).

P.D.S.Park and J.L. Henry. Characterization of the receptors mediating the effects of angiotensin II on sympathetic output: Studies with DuP 753 and PD 123319, (in preparation)

All of the experiments were designed and carried out by the candidate under the supervision of Dr. James L. Henry. The data presented in this thesis are original and have not appeared elsewhere.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Dr. James L. Henry for his encouragement, dedication and constructive advice to my studies. I could not have asked for a better mentor than him.

Thank you to my fellow colleagues, Dr. Kiran Yashpal, Dr. Veeraswamy Radhakrishnan, and Vito Romita, for their invaluable advice.

I am grateful for all the support given by my dear friends.

And finally, but not least, to my family who been my undying supporters for the past two years. I could not have asked for more love and support.

The present work was made possible by a Grant in Aid of Research from the Medical Research Council to Dr. James L. Henry.

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CHAPTER I

LITERATURE REVIEW

A. INTRODUCTION & RATIONALE

Throughout the body, the heart and vascular beds are regulated by neural factors which play a key role in the variety and degree of response which is so much a feature of the cardiovascular system. As early as 1864, Goltz discovered that the integrity of the spinal cord was critical to the maintenance of vasoconstrictor tone. Sherrington (1947) and Yates (1921) not only reaffirmed this observation in chronic spinalized animals but also concluded that the restoration of blood pressure may be mediated via sympathetic preganglionic neurons (SPNs) in the spinal cord. Alexander (1946) implied that sympathoinhibition occurs at a spinal locus since sympathetic nerve discharge increased after cervical spinal transection in which blood pressure and sympathetic discharge was maximally reduced by transecting at the level of the medullary obex. Sympathetic output is controlled by the higher centers of the brainstem as well as other afferent input. From the late nineteenth century, the medulla has been accepted as the site of the major cardioacceleratory/inhibitory centers which control heart rate (Hunt, 1899) and of the centers which regulate vasoconstrictor tone (e.g.vasoconstriction and vasodilation) (Bayliss, 1908). Therefore, in 1929, Cannon provided the idea of the sympathetic outflow from the central nervous system as a widespread diffusion of nervous impulses to the periphery.

It is now well established that the spinal cord represents the final neural site for the generation of patterns of sympathetic activity. The SPN is the final neuronal pool where major integration occurs. Not only is it the final site of integration of all descending control mechanisms but it is also capable of integrating a number of intrinsic spinal systems. Anatomical studies have demonstrated that SPNs, which are a heterogeneous population of cells, are located in several regions in the spinal cord and that these neurons have specific

orientations and dendritic projections. Although SPNs are primarily located in the intermediolateral nucleus (ILp) (Gebber & McCall, 1976b) of the thoracolumbar lateral column, other areas have been implicated in this function, e.g. lateral funiculus (ILf), intercalated nucleus (IC), and other sites. Axons originating from the spinal cord exit from the ventral roots and terminate on postganglionic neurons in sympathetic ganglia. Postganglionic fibers, in turn, project from these ganglia to the neuroeffector organs (e.g. heart and blood vessels and to the secretory chromaffin cells of the adrenal medulla). Information from various brainstem structures, such as the rostral ventrolateral medulla (Ross *et al.* 1984; Loewy *et al.* 1986), caudal raphe nuclei (Henry & Calaresu, 1974 a,b,c,d; Loewy & McKellar, 1981), A5 noradrenergic cell group (Loewy *et al.* 1986), paraventricular nucleus of the hypothalamus (Caverson *et al.* 1984), nucleus of tractus solitarius (Ross *et al.* 1981a) and spinal interneurons (Gebber & McCall, 1976b; Henry & Calaresu, 1974d; McCall *et al.* 1977; Dun & Mo, 1988) participate in modulating the sympathetic discharge regulating cardiovascular function.

Differential distribution of chemically identifiable nerve terminals are found on heterogenous SPNs (Holets & Elde, 1982; Appel & Elde, 1988; Krukoff *et al.* 1985a). These chemical mediators, excitatory and/or inhibitory, such as acetylcholine (Magri & Buccafusco, 1988; Calaresu *et al.* 1990), serotonin (Backman *et al.* 1990; McCall, 1983), excitatory amino acids (Backman & Henry, 1983; Hong & Henry, 1992a,b; Mo & Dun, 1987), substance P (Backman & Henry, 1984a; Yashpal *et al.* 1987b; Dun & Mo, 1988; Keeler *et al.* 1985), thyrotropin releasing hormone (Backman & Henry, 1984a; Yashpal *et al.* 1989b), oxytocin (Backman & Henry, 1984b; Yashpal *et al.* 1987a; Gilbey *et al.* 1982), angiotensin II (Yashpal *et al.* 1989a), glycine (Hong *et al.* 1989) and GABA (via both $GABA_A$ and $GABA_B$ receptors)

(Hong & Henry, 1991a,b,c), have been shown to regulate the excitability of SPNs. Attempts are being made to find functional roles of these putative neurotransmitters in modulating sympathetic discharge.

Two studies were undertaken which involved the intrathecal administration of agonists and antagonists of selected receptors to examine the physiology and pharmacology of the receptors participating in the regulation of arterial pressure and heart rate under normal and abnormal conditions, providing a unique insight into the efferent mechanisms. Spinalized animals, which were used in some experiments, further illustrate the complex integration of information performed by SPNs. Sympathoexcitatory responses (e.g. profuse sweating, increased arterial pressure, tachycardia) are hypersensitive after chronic spinal section and result in abnormal peripheral actions which are potentially life-threatening. Therefore, the first study (Chapter I) examines the effects of substance P on sympathetic output in acute spinalized animals transected at the spinal level of T5 or T1. Angiotensin II plays an important role in maintaining fluid and electrolyte homeostasis and cardiovascular function by both peripheral and central actions. The precise site of action of angiotensin II receptor subtypes have been implicated in many models (Ferguson & Wall, 1992; Ganten *et al.*1978b), but the spinal cord has been largely overlooked. The second study (Chapter II), therefore, examines the effects of the two novel angiotensin II receptor antagonists, DuP 753, an AT_1 receptor antagonist, and PD 123319, an AT_2 receptor antagonist, on the sympathetic responses to spinal administration of angiotensin II.

The present dissertation will focus on the anatomical, neurophysiological and pharmacological findings relevant to spinal SPNs, particularly those involved in regulating the heart and blood vessels, the descending supraspinal influences on the SPNs, and the

mediation of various chemicals involved in synaptic transmission between these descending pathways and the SPNs.

B. SYMPATHETIC PREGANGLIONIC NEURONS

1. Sites of origin

Information from brain structures involved in autonomic regulation is transmitted to the neuronal pools in the spinal cord and, in turn, via sympathetic nerves to the periphery. Identification of SPNs has been based on anatomical (e.g. retrograde transport of horseradish peroxidase along the axons in corresponding sympathetic nerves (Chung *et al.* 1975; Dalsgaard & Elfvin, 1979, 1981; Faden & Petras, 1978)) and electrophysiological (e.g. antidromic activation of SPNs following stimulation of sympathetic nerves (Dembowsky *et al.* 1985; Dembowsky *et al.* 1986; Gebber & McCall, 1976b) studies. SPNs lie in four topographically defined nuclei: 1. the intermediolateral cell column (ILp), 2. the lateral funicular area (ILf), 3. the intercalated cell group (IC) and 4. the central autonomic nucleus (CA).

a. Intermediolateral cell column (ILp)

Gaskell (1886) clearly stated that cell bodies of the SPNs lie mainly in the ILp (or nucleus intermediolateralis thoracolumbalis pars principalis). Other investigators (Onuf & Collins, 1898; Anderson, 1902; Herring, 1903) later confirmed his findings by observing chromatolytic-appearing neurons in lumbar, thoracic or cervical lateral horn following cutting of the white rami. Degenerative studies (Cummings, 1969; Petras & Cummings, 1972a) and retrograde transport studies with horseradish peroxidase into the paravertebral ganglia

(Oldfield & McLachlan, 1981; Petras & Faden, 1978; Chung *et al.* 1975; Chung *et al.* 1979a; Dalsgaard & Elfvin, 1979, 1981; Faden & Petras, 1978; Rando *et al.* 1981) into prevertebral ganglia (Dalsgaard & Elfvin, 1979), lumbar sympathetic/intermesenteric trunk (Hancock, 1982), cervical sympathetic nerve (Murata *et al.* 1982), hypogastric nerve (e.g. bladder control) (Hancock & Peveto, 1979), adrenal medulla (Schramm *et al.* 1975), urinary bladder (Petras & Cummings, 1978), confirmed findings of SPNs in the lateral horn.

b. Lateral funicular area (ILf)

Henry and Calaresu (1972a) suggested that the lateral funiculus contains SPNs due to the similarity of appearance to neurons in the lateral horn. Following thoracic sympathectomy, chromatolytic neurons are found in this region (Petras & Cummings, 1972b; Cummings, 1969). As well, injection of retrograde transport markers (e.g. horseradish peroxidase) into the paravertebral ganglia (Chung *et al.* 1975; Chung *et al.* 1979a; Dalsgaard & Elfvin, 1981; Deuschl & Illert, 1981; Oldfield & McLachlan, 1981; Rando *et al.* 1981), cervical sympathetic nerve (Murata *et al.* 1982) and adrenal medulla (Schramm *et al.* 1975). The area extends laterally and dorsally from the ILp and into the white matter of the lateral funiculus.

c. Intercalated cell group (IC)

Degeneration studies (Onuf & Collins, 1898) illustrated SPNs in the intermediate grey region, a thin band across the grey matter medial to the ILp which forms the intercalated cell group, otherwise known as nucleus intercalatus spinalis (Petras & Cummings, 1972b).

d. Central autonomic nucleus (CA)

SPNs are found clustered just dorsolateral to the central canal (Onuf & Collins, 1898; Chung *et al.* 1975; Cummings, 1969; Schramm *et al.* 1975; Oldfield & McLachlan, 1981). It has been alternatively named the nucleus intercalatus pars paraependymalis (ICpe) (Petras & Cummings, 1972b). The IC also joins this paracentral column of cells near the central canal.

e. Spatial arrangements and conclusions

The longitudinal pattern of arrangement of the SPNs resembles a ladder. In a horizontal or longitudinal section of the spinal cord, the "side supports" of the ladder represent the bilateral columns of the ILp, which contain clusters (frequently termed "nests") of SPNs, ranging from 20-100 neurons. The internest distance in the thoracic spinal cord ranges from 200-500 μm (Oldfield & McLachlan, 1981) but shortens to 100-300 μm in the lumbar sections of the spinal cord (Barber *et al.* 1984; Oldfield & McLachlan, 1981). Transverse sections illustrate the "ladder rungs" which are composed, in part, of the IC neurons and are connected to the "side supports". The inter-rung distances vary among species, differ in frequency within and between spinal segments and have distinctive patterns of afferentation (Berstein-Goral & Bohn, 1988; Davis *et al.* 1984; McLachlan & Oldfield, 1981; Newton & Hamill, 1989; Oldfield & McLachlan, 1981). Continuation of the ladder rung across the midline occurs via the CA neurons.

In general, the major distribution of SPNs among the sub-nuclei is located in the ILp, with the next greater amount in the ILf and not very many in the IC (Leong et al., 1983) and ICpe (Petras & Cummings, 1972b; Deuschl & Illert, 1981; Chung *et al.* 1979a; Dalsgaard

& Elfvin, 1979; Janig & McLachlan, 1986). In conclusion, the general consensus is that the spinal SPNs are mainly located in four regions of the spinal cord, using Petras and Cummings (Petras & Cummings, 1972b) terminology: ILp, ILf, IC and ICpe.

2. Topographical differentiation

SPNs in the various nuclei are distributed bilaterally and in a symmetrical manner. They exhibit a topographical rostrocaudal pattern of efferent connections in the spinal cord. For example, efferent connections at the second thoracic spinal level innervate the heart whereas the ninth thoracic spinal level innervates predominantly the adrenal gland and various other internal organs. Although there is no convincing evidence that sympathetic sub-nuclei specifically innervate particular postganglionic neurons, there have been studies suggesting topographical distribution of preganglionic somata within each sub-nucleus. For example, the thoracic ganglia (Petras & Faden, 1978), stellate ganglion (Chung *et al.*1979a; Oldfield & McLachlan, 1981), cervical sympathetic trunk (Chung *et al.*1975; Oldfield & McLachlan, 1981) and the adrenal medulla (Cummings, 1969; Petras & Cummings, 1972b) appear to be innervated predominantly by cells in the ILp, IC and ICpe. The clearest indication that SPNs are topographically distributed is the studies by McLachlan et al. (1985) and Janig and McLachlan (1986). They concluded that : 1. SPNs close to the lateral edge of the grey matter or the fibre tracts of the white matter lie in the lumbar sympathetic trunk (excluding the splanchic nerves) and extend toward the hind limb; 2. SPNs medial to the lateral border of the grey matter and spread across medially in a transverse band are located in the hypogastic nerve; and 3. SPNs in the lumbar splanchnic nerves, which lie close to the border of the grey matter project towards the colonic nerves. Therefore, there are

characteristic differences existing among the sub-nuclei with regard to the destination of the axons of associated preganglionic somata. While SPNs from several spinal segments contribute to the innervation of a single sympathetic ganglion, the neurons themselves are segmentally organized (Oldfield & McLachlan, 1981; Rubin & Purves, 1980). Serial transverse sections of the cat spinal cord illustrate the topographical distribution of neurons in the spinal cord. SPNs are not present along the entire longitudinal length of the spinal cord but are restricted from the caudal portions of the last cervical segment (e.g. C8) to the upper lumbar spinal segments (L2-3) but some go as far as L5 (Henry & Calaresu, 1972a) and the numerical distribution is between 32,790 to 53,340 neurons (Henry & Calaresu, 1972b). The number of neurons varies from section to section with the greatest number in the regions of T1-2 and L3-4 (Henry & Calaresu, 1972b).

Three efferent pathways have been determined by use of horseradish peroxidase into the stellate ganglion (Dalsgaard & Elfvin, 1981): 1. axons cross from the dorsal part to the lateral border of the ventral horn and then exit the lateral horn, 2. axons medially cross the border of the ventral horn into the lumbar paravertebral ganglia (Deuschl & Illert, 1981), 3. axons course straight through the ventral horn. Upon leaving the spinal cord, the axons assume either a rostral or a caudal trajectory. Only infrequently ($< 2\%$) does an axon split to go in both directions (Forehand & Rubin, 1986), but there are species-specific differences in the spinal locations of SPNs that give rise to this topographical orientation.

3. Somatic and dendritic differentiation of SPNs

Cell bodies of SPNs occur in clusters and vary in size from round to fusiform. There

are at least four different shapes of SPN perikarya (Dembowsky *et al.* 1985; Cabot & Bogan, 1987; Barber *et al.* 1984; Oldfield & McLachlan, 1981; Forehand, 1985). Camera lucida drawings illustrate structural diversity within and across the spinal cord. It is unknown whether functionally related SPNs constitute a single homogeneous class of neurons or whether functionally unrelated SPNs are morphologically distinct. For example, if cardiac SPNs reside in more than one nucleus, it is unknown if the functional organization of the SPNs and the spinal cord anatomy are related.

i. Soma: The soma of the SPN is of perikaryal shape but its shape will not necessarily help distinguish the specific nuclear location in the spinal cord. The dimensions vary among the SPNs (major axes: 15-60 μm; minor axes: 10-40 μm) and are classifed as either small or medium. There are some large multipolar cells but these are less frequent and are localized in the ILf, ILp and IC. Examined by electron microscopy, the smaller neurons contain one or two dendrites while the largest SPNs are multipolar and give rise to two or three large primary dendrites (Chung *et al.* 1980). Fusiform neurons are located caudal to T2 (Oldfield & McLachlan, 1981), multipolar (with less frequent fusiform) are located at T1 and T2 and are oriented obliquely or mediolaterally.

ii. Dendrites: In any section of the spinal cord, dendrites project mediolaterally into the intermediate grey and dorsal horn region and rostrocaudally through the lateral horn. It is very important to define the dendritic orientation of the SPNs because there are few afferent inputs to the SPNs (see section on Afferent Inputs regulating SPNs). Therefore, a majority of the excitation/inhibition occurs within the dendritic trees of the SPNs and so the information on the dendritic arborization is more important than the morphological characterization of cell body, shape and size. Dendrites of multipolar neurons extend

laterally into the white matter and medially into the lateral horn (Deuschl & Illert, 1981).

The principal orientation in the intermediolateral cell column of the thoracic section in the spinal cord is rostrocaudal. The dendritic trajectory extends from 1.5-2.5 mm in the cat (Dembowsky *et al.* 1985), ~0.6 mm in the young rat (Forehand, 1985), and 0.5-1.0 mm in the pigeon (Cabot & Bogan, 1987). Other dendritic projections extend medially into the zona intermedia and overlap laterally-directed dendrites that come from IC groups (Bacon & Smith, 1988; Barber *et al.* 1984; Oldfield & McLachlan, 1981). Less frequent dendritic projections extend laterally into the white matter, dorsolateral along lateral margin of the dorsal horn, and ventrally into the ventral portion of the lateral part of lamina VII (Bacon & Smith, 1988). There is a marked change in dendritic projection in the lumbar sections of the spinal cord. The dominant extensions in the ILp are: mediolateral and dorsoventral (Baron *et al.* 1985; Morgan *et al.* 1986; Torigoe *et al.* 1985). Axons from the intercalated cell groups frequently course either medially towards the central canal or laterally towards the ILp (Bacon & Smith, 1988; Barber *et al.* 1984; Petras & Faden, 1978). The lateral dendrites mingle with the medial projections which arise from the ILp. The medial extensions sometimes penetrate the CA region and occasionally extend across the mid-sagittal plane of the spinal cord. There are also dorsoventral extensions which course obliquely through the transverse plane (Cabot & Bogan, 1987; Forehand, 1985). Little physiology is known about the IC neurons.

iii. Synapses: Rethelyi (1972) described three different types of synapses associated with SPNs in the cat: 1. axo-dendritic terminals containing clear vesicles, 2. large, axo-somatic terminals containing flattened vesicles, and 3. terminals with mixed clear and osmiophilic vesicles. Flattened or pleomorphic vesicles which contain glycine (Matus &

Demison, 1971) or γ-aminobutyric acid (GABA) (Hokfelt & Ljungdahl, 1972) have been associated with inhibition in the spinal cord (Matus & Demison, 1971). Round vesicles, which contain acetylcholine (Marchbanks, 1969), have been associated with excitation (Uchizono, 1967). Cell bodies receive a larger number of terminals with flattened or pleomorphic synaptic vesicles instead of round vesicles, whereas dendrites receive almost the same amount of both types (Chung *et al.*1980). This means that the sympathetic preganglionic somata receive mainly inhibitory inputs whereas the dendrites receive both excitatory and inhibitory inputs and receive more inputs than the cell bodies.

C. AFFERENT INPUTS REGULATING SPNs

A variety of sources and inputs innervate the SPNs and are divided into two main categories: central and peripheral inputs. The central descending excitatory and/or inhibitory pathways such as the medulla, pons and hypothalamus synapse with the SPNs. In this section, the discussion will focus on the central nervous system cell groups that regulate the sympathetic outflow regulating arterial pressure and heart rate.

1. Pons and Medulla

Both anatomical and electrophysiological studies have indicated that a large number of supraspinal structures innervate spinal SPNs and evoke pressor and cardioacceleratory responses. For example, the medulla is considered to be the major site which contains sympathoexcitatory neurons (Calaresu & Yardley, 1988). Electrophysiological experiments by Henry and Calaresu (1974a,b,c,d) explored the brain stem for antidromic activated neurons via electrical stimulation of the ILp in the cat. They found that various nuclei in the

medulla and pons led to an increase in heart rate and blood pressure which suggested that these nuclei are directly involved in sympathetic output and cardiovascular control. Anatomical studies such as retrograde transport with horseradish peroxidase, demonstrated widespread distribution of labelled cell bodies in the brain stem but could not determine which structures specifically innervated SPNs. This was due to diffuse and non-localized horseradish peroxidase injections (Martin *et al.*1979, 1981; Kuypers & Maisky, 1975; Ross *et al.*1981b; Tohyama *et al.*1979; Kneisley *et al.*1978). Anterograde transport of tritiated amino acids into the specific brain stem regions were also used to examine labelled neurons in the spinal cord (Martin *et al.*1979, 1981; Loewy & McKellar, 1981) The nuclei implicated in the cardiovascular control through electrical stimulation were the following: 1 cardioexcitatory responses: nucleus parabrachialis (Mraovitch *et al.*1982), nucleus lateralis reticularis (Henry & Calaresu, 1974a), nucleus parvocellularis (Henry & Calaresu, 1974a), caudal raphe nuclei (Adair *et al.*1977); 2. cardioinhibitory responses: caudal raphe nuclei (Adair *et al.*1977; Henry & Calaresu, 1974a), nucleus paramedium reticular and nucleus medullae oblongata centralis subnucleus ventralis (Henry & Calaresu, 1974a).

The ventrolateral surface of the medulla is the chemosensitive area which has been implicated in cardiovascular control. Responses are evoked either by electrical stimulation (Loescheke *et al.*1970) or injection of kainic acid (McAllen *et al.*1982) and depressed by either lesioning or perfusing with glycine (Guertzenstein & Silver, 1974) or pentobarbitone (Feldberg & Guertzenstein, 1972). Electrical stimulation of the aortic depressor and carotid sinus nerve evoke responses in the nucleus lateralis reticularis, nucleus paramediun reticularis, nucleus medullae oblongatae centralis, nucleus gigantocellularis, nucleus pontis centralis caudalis, nucleus parvocellularis, and raphe nuclei (DeGroat & Lalley, 1974; Lipski

& Trzebski, 1975; Miura & Reis, 1969; Biscoe & Sampson, 1970a,b; Kumada & Nakajima, 1972). These studies show that neurons near the surface area of the ventrolateral medulla are involved in cardiovascular responses. The fact that various central nervous system nuclei project to spinal autonomic neurons attest to the role of the spinal cord in processing information or cardiovascular control. Therefore, these experiments define the descending inputs to the lateral horn from the various nuclei in the pontine and medullary centers.

2. Hypothalamus

Beattie (1930) suggested that there were descending projections from the hypothalamus to the SPNs because electrical stimulation of the hypothalamus produced a pressor response. Ciriello and Calaresu (1977) challenged this hypothesis of a direct input from the hypothalamus to the SPNs. They failed to see any degenerating axons or terminals within the lateral horn following electrolytic lesioning of sites within the posterior and ventrolateral hypothalamic regions in the cat. But others have reported otherwise. Anterograde labelling of tritiated amino acids into the terminals in lateral horn have reported a direct connection to SPNs from the hypothalamus (Saper *et al.* 1976). Retrograde labelling with horseradish peroxidase or fluorescent dyes have also produced similar results from the the paraventricular nucleus to the thoracic dorsolateral funiculus in the rat, thereby suggesting a descending hypothalamo-spinal pathway (Saper *et al.* 1976; Kneisley *et al.* 1978; Kuypers & Maisky, 1975; Swanson & Kuypers, 1980; Swanson *et al.* 1980; Blessing & Chalmers, 1979). The functional role of the hypothalamus is unclear but the paraventricular nucleus has been implicated in a variety of cardioregulatory reflexes. For example, a pressor response is elicited by electrical stimulation in a vagotomized cat (Ciriello & Calaresu, 1980).

As well, the paraventricular nucleus receives a direct projection from the nucleus tractus solitarius (Ricardo & Koh, 1978), which is the main site of termination of the buffer nerves.

3. Raphe nuclei

Stimulation (electrical or chemical) of the medullary raphe nuclei results in a decrease in blood pressure accompanied with or without bradycardia and/or an inhibition of sponateous sympathetic nervous discharge (Wang & Ranson, 1939; Henry & Calaresu, 1974b; Coote & Macleod, 1974a; Coote & Macleod, 1974b; Gilbey *et al.* 1981; Morrison & Gebber, 1982; McCall & Humphrey, 1985). Discrepancies result when stimulating the raphe nuclei due to the possible heterogenity of raphe neurons e.g. SPN discharge, arterial pressure and heart rate may be depressed upon stimulation of the nuclei (Cabot *et al.* 1979), however, pressor responses (McCall, 1984; Haselton *et al.* 1988) and both pressor and depressor responses have been observed (Koss & Wang, 1972; Adair *et al.* 1977; Yen *et al.* 1982).

Type I neurons (sympathoinhibitory) in the raphe nuclei have demonstrated its axons descending to the SPNs in the IML (Morrison *et al.* 1984; Morrison & Gebber, 1985) and has been found to be excited when sinus pressure is elevated. A third type neuron has also been found to extend its projections to the IML (McCall & Clement, 1989) and is thought to modulate the level of excitability of the SPNs (McCall, 1988). Data indicate that sympathoinhibition and sympathoexcitation of type I and III neurons in the raphe nuclei participate with GABA and 5-hydroxytryptamine (5-HT) mechanisms (McCall, 1984; Moore, 1981; McCall & Humphrey, 1985), respectively which is consistent with the observation that a GABAergic descending pathway to the spinal cord from the raphe nuclei has been reported (Millhorn *et al.* 1987; Blessing, 1990).

4. Spinal Sympathetic interneurons

Spinal sympathetic interneurons have been implicated in conveying information from at least some supraspinal networks and afferent nerves to the SPNs. For example, lesions of vasoactive sites in the hypothalamus, or in descending sympathetic pathways in the cervical spinal cord (Chung *et al.* 1979b; Rethelyi, 1974) or in dorsal root ganglionectomy (Petras & Cummings, 1972a) found no degeneration in the intermediolateral nucleus (Ciriello & Calaresu, 1977; Smith, 1965). These anatomical studies indicate that information from supraspinal structures and afferent nerves to the SPNs is relayed over polysynaptic circuits. Therefore, the inclusion of more interneurons in spinal autonomic circuits allows an additional site for integration of inputs to sympathetic nerves which control the cardiovascular system. Electrophysiological studies (McCall *et al.* 1977; Gebber & McCall, 1976a) provided direct evidence for the existence of spinal sympathetic interneurons in both sympathoexcitatory and sympathoinhibitory networks. The first study concluded that it is possible that descending inhibitory influences on SPNs may be mediated directly as well as indirectly via depression of activity of a sympathoexcitatory interneuron (Gebber & McCall, 1976a). The second study identified spinal interneurons which were involved in the sympathoinhibitory network to be located in the medial zone of the intermediate grey region of the upper thoracic spinal cord (McCall *et al.* 1977). They concluded that supraspinal medullary input involved in the baroreceptor reflex circuit projected towards sympathoinhibitory interneurons. Recent experiments in spinal cord slices have shown hyperpolarization and IPSPs in a number of SPNs but these results are not considered to be a direct action because the results are abolished in low Ca^{2+} or tetrodoxin-containing solution or in strychnine (Dun & Mo, 1988). It has been suggested that substance P activates

inhibitory interneurons in the region of SPNs releasing glycine-like material (Dun & Mo, 1988; Backman & Henry, 1984a). This elicits a hyperpolarizing response and IPSPs in SPNs. As well, stimulation of the dorsal root in neonatal rat spinal cord slices evoke spontaneous IPSPs and IPSPs which are blocked by low Ca^{2+}, tetrodoxin and strychnine, suggesting that glycine is release presynaptically (Dun & Mo, 1989). GABA is also released, besides glycine, because some of the SPNs are strychnine resistant and bicuculline-sensitive (Dun & Mo, 1989). Therefore, these studies indicate that spinal autonomic circuits contain spinal interneurons which are involved in mediating influences onto SPNs from vasopressor (or vasodepressor) sites and from visceral and somatic afferents.

D. RECEPTOR DISTRIBUTION AND CHEMICAL CONTROL OF SPNS

A large number of chemicals have been discovered in the central nervous system which has opened up possibilities in deciphering many unknown mechanisms of central nervous regulation, including control of the cardiovascular system. Various substances have been implicated as neurotransmitters, for example, substance P and angiotensin II, which modulate the responses onto SPNs in the spinal cord. These two agents are the focus of this thesis.

1. SUBSTANCE P

a. Historical background

Von Euler and Gaddum (1931) discovered substance P when looking for acetylcholine in liver, spleen and intestinal smooth muscle. The extract stimulated contractions of the isolated jejunum and caused a transient hypotension after i.v. administration. The substance

was subsequently categorized as a peptide when it was unable to be inactivated by trypsin preparations (von Euler, 1936). But purification attempts met with limited success until Leeman and her co-workers (Chang *et al.* 1971) determined the chemical structure of substance P as an undecapeptide: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2. Eventually, substance P was synthesized (Tregear *et al.* 1971) and antibodies were produced (Powell *et al.* 1973) for immunoassays and histochemical assays.

b. Distribution of substance P

Substance P is widely distributed in the spinal cord. Bioassays indicate large amounts of substance P in dorsal roots (Amin *et al.* 1954; Lembeck, 1953; Pernow, 1953) and that more substance P is found in dorsal roots than in ventral roots (Amin *et al.* 1954). The highest levels are located in the dorsal horn and lateral horn (ILp). Localizations of substance P in the ILp have been found in the rat (Holets & Elde, 1982; Hökfelt *et al.* 1977), in pigeon (Davis & Cabot, 1984), in the oppossum (Ditirro *et al.* 1981), in the cat (Holets & Elde, 1983; Krukoff *et al.* 1985a,b) and in man (De Lanerolle & LaMotte, 1983) and, in the ICpe (Davis *et al.* 1984; Krukoff *et al.* 1985a,b). The majority of substance P fibres of the ventral horn and lateral horn seem to be from supraspinal structures (De Lanerolle & LaMotte, 1983; Helke *et al.* 1982; Hokfelt *et al.* 1977b; Kanazawa *et al.* 1979) whereas the dorsal horn substance P is located in the small diameter dorsal root sensory neurons (Cuello *et al.* 1977; Cuello & Kanazawa, 1978; Hokfelt *et al.* 1977b). In the ILp, the greatest accumulation of substance P fibres is found in the T3-5 and L2-4 regions (McLachlan *et al.* 1985).

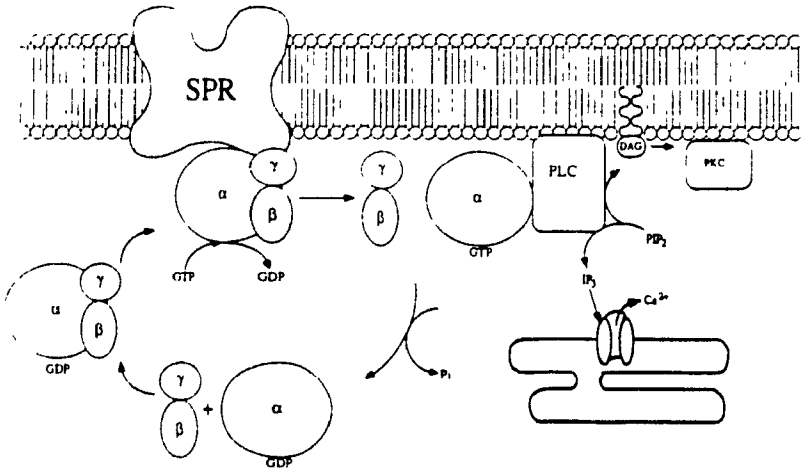
c. Afferent substance P-containing inputs to SPNs

Afferent inputs, such as the RVLM (in the nucleus interfascicularis hypoglossi and the nucleus paragigantocellularis pars lateralis) (Helke *et al.*1982) are implicated in the regulation of SPNs in the lLp (Henry & Calaresu, 1974a,b,c,d; Helke *et al.*1982; Helke *et al.*1986). Anterograde and retrograde transport studies have also proposed the caudal pontine medullary raphe complex as the origin to substance P nerve terminals to SPNs (Gilbert *et al.*1982; Johansson *et al.*1981). Co-existence with serotonin has been shown in raphe neurons (Chan Palay *et al.*1978; Chan Palay, 1979) and projections from the raphe nuclei extend towards the spinal cord (Bowker *et al.*1981). Injections of kainic acid into the raphe nuclei elicit an increase in arterial pressure and heart rate which is blocked by a substance P antagonist (Loewy & Sawyer, 1982). Immunoreactive substance P material is also in terminals of intraspinal neurons in cervical transected animals (Davis *et al.*1984; Davis & Cabot, 1984) indicating a more complex spinal mechanism in regulation of sympathetic output.

d. Substance P receptor

The mammalian tachykinin receptors are classified as NK-1 (substance P receptor), NK-2 (neurokinin A receptor), and NK-3 (neurokinin B receptor) (Henry, 1987). These receptors have a common carboxyl-terminal sequence which allows for receptor interaction and activation: Phe-X-Gly-Leu-Met-NH_2 whereas the amino terminal domain dictates receptor subtype specificity. Concentrating specifically on the NK-1 receptor, evidence of substance P binding sites in the spinal cord shows ^{3}H-substance P in rat spinal cord crude membrane preparations (Greiner, 1985; Siemion *et al.*1990) in spinal cord cultures (Hosli & Hosli,

1985), and in thoracic spinal cord slice (Maurin *et al.*1984). At least two distinct binding sites for substance P-like peptide have been reported in rat cervical spinal cord (Ninkovic *et al.*1984). Substance P binding sites are found in the dorsal horn, lamina X, ventral horn, and the ILp (Ninkovic *et al.*1984; Charlton & Helke, 1985). Molecular studies have proposed a mechanism for substance P receptor activation (Krause *et al.*1992):



Stimulation of the substance P receptor results in the activation of the G-protein cascade. GDP (guanine diphosphate) → GTP (guanine triphosphate) interchange occurs on the Gα subunit. The Gα subunit dissociates itself from the β and γ subunits and activates a specific isoform of phospholipase C (PLC) by a pertussis-toxin insensitive mechanism. PLC then breaks down phosphatidylinositol biphosphate (PIP_2) to i) inositol trisphosphate (IP_3) which causes release of Ca^{2+} from intracellular stores and ii) diacylglycerol (DAG) which activates protein kinase C (PKC) (Berridge & Irvine, 1989; Nishizuka, 1988). Therefore, the second messenger system is activated by ligand/receptor interaction, which plays some role in receptor regulation (Krause *et al.*1992).

e. Substance P as a neurotransmitter

Upon identification, substance P has been implicated as a neurotransmitter in several spinal cord neuronal systems. Lembeck (1953) first postulated that substance P was acting as a mediator of signals from primary sensory afferents due to the independent finding of relatively high amounts of substance P in dorsal roots. Subsequently, strong support for this hypothesis came from the observations of concentrated amounts of substance P in synaptosomes (Lembeck & Holasek, 1960; von Euler & Lishajko, 1961; Inouye & Kataoka, 1962; Ryall, 1962; Otsuka *et al.* 1972a,b).

Substance P is known to have an excitatory effect in the dorsal horn. It produces a slow depolarization mediated via a decrease in K^+ conductance (Krnjevic & Lekic, 1977; Nowak & MacDonald, 1981; Murase *et al.* 1982; Murase & Randic, 1984). It has been shown that substance P causes an increase in a Ca^{2+}-sensitive inward current (Murase *et al.* 1986). The peptide is released from spinal tissue following potassium depolarization (Gamse *et al.* 1979; Jessell & Iversen, 1977), during electrical stimulation of the dorsal roots (Otsuka & Konishi, 1976), and activation of nociceptive afferents (Yaksh *et al.* 1980). Substance P is stored in secretory granules of sensory neurons and released upon axonal stimulation (Pernow, 1983). In rat spinal slice preparations (Kronheim *et al.* 1980) substance P is released by veratrine, Ca^{2+}, high K^+ concentration or low Na^+ concentration, and in isolated neonatal rat spinal cord there is an increase in substance P release with capsaicin or high K^+ concentration (Akagi *et al.* 1980). There is also evidence that substance P relays nociceptive information from the sensory fibres to the spinal cord and brain stem (Hokfelt *et al.* 1975a,b; Hokfelt *et al.* 1977a; Randic & Miletic, 1977) and that substance P is probably released from primary afferent neurons (Backman & Henry, 1984a). Substance P antagonists

block the slow nociceptive response in dorsal horn (Radhakrishnan & Henry, 1991; De Koninck *et al.* 1992) and ventral roots (Otsuka & Yanagisawa, 1988); they also change autonomic functions (Cridland *et al.* 1987; Yashpal *et al.* 1987b) thus indicating that substance P is involved in regulation of SPNs (Backman & Henry, 1984a). Therefore, based on these findings, substance P may be implicated as a neurotransmitter which is involved in spinal regulation of autonomic function and nociception.

f. Effects of substance P on sympathetic output

Evidence shows a higher binding of ^{3}H-substance P in the intermediolateral region in hypertensive rats versus normotensive rats (Takano *et al.* 1985). It was concluded that the regulation of the amount of substance P and substance P receptors on sympathetic neurons will determine the degree of excitation and sympathetic outflow. Von Euler and Gaddum showed that i.v. administration of substance P is able to decrease the blood pressure of an anaesthetized rabbit. Central administration (i.c.v.) of substance P mediated by the sympathetic nervous system (Petty & Reid, 1981; Unger *et al.* 1981) evoked a pressor response (Fuxe *et al.* 1981; Haeusler & Osterwalder, 1980; Unger *et al.* 1981). In intact animals, intrathecal administration of substance P at the levels of T2 or T9, increased arterial pressure and heart rate; similar responses are evoked in adrenalectomized animals (Yashpal *et al.* 1985, 1987b). An analgesic response is produced in the tail-flick test via an adrenal mechanism (Cridland & Henry, 1988). Therefore, these results implicate both a neural mechanism and a humoral mechanism. Substance P may be having a direct action on the SPNs via activation of spino-bulbo-spinal reflex pathway or via an intrinsic spinal reflex mechanism initiated from the dorsal horn.

Chapter I reports the effects of substance P in animals transected at various spinal levels. Transection of the spinal cord leads to an accumulation of substance P rostral and caudal to the section, presumably from the caudal medulla as indicated above but also from ascending fibres. Changes in the level of substance P at the injury site itself have led to the suggestion that it may play a role in the secondary pathophysiological response following spinal trauma and lesion of the descending neurons which contain substance P (using a 5-HT neurotoxin) retards neurological recovery following spinal transection. Significantly for the present experiments, thoracic transection induces an increase in substance P receptors both above and below the level of the lesion, indicating the possible involvement in both the recovery of normal arterial pressure and in autonomic hyperreflexia.

g. Conclusions

In conclusion, substance P has been implicated in various studies as a neurotransmitter, including sympathetic output. Though the exact origin of substance P fibres to the sympathetic preganglionic fibres are argumentative, there is sufficient support that there is a descending pathway from the supraspinal structures projecting towards the SPNs and ultimately regulating sympathetic output to the heart and vessels.

2. ANGIOTENSIN II

a. Central and peripheral roles

The renin-angiotensin-aldosterone system exerts a vital role in blood pressure regulation and electrolyte homeostasis. Angiotensin II, the primary effector of this system, maintains its role via both peripheral and central actions. Synthesis of angiotensin II is well-

characterized in the body. The polypeptide precursor, α-globulin angiotensinogen, (Campbell *et al.*1984; Printz & Lewicki, 1977), produced by the liver (Richoux *et al.*1983), is converted to the inactive decapeptide, angiotensin I, via the action of renin (released by the kidney) (Ganten & Speck, 1978; Hirose *et al.*1978; Samani & Swales, 1991; Schunkert *et al.*1991). Angiotensin converting enzyme (Saavedra & Chevillard, 1982; Yang & Neff, 1972), also known as kininase II, originates from the kidney, lungs and other sites and cleaves the C-terminal dipeptidyl fragment from angiotensin I to form the octapeptide (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), angiotensin II.

In peripheral tissues, angiotensin II is a powerful vasoconstrictor which acts directly on blood vessels to cause an increase in peripheral resistance and thereby increasing blood pressure. At the same time, angiotensin II stimulates the adrenal glands to release aldosterone, which acts on the kidney for sodium retention, thereby counteracting the decrease in blood pressure. Angiotensin II binds to specific receptors that mediate these biological actions via different signal transduction pathways: Ca^{2+} mobilization through stimulation of PLC (through a G protein), production of IP_3 (Haeusler & Osterwalder, 1980) and DAG, activation of Ca^{2+} channels (Hausdorff & Catt, 1988), mobilization of intracellular Ca^{2+} (Carini *et al.*1990) and inhibition of adenylate cyclase via a pertussis toxin-sensitive G-protein (Pobiner *et al.*1985). Angiotensin II also elicits suppression of adenylyl cyclase activity in permanently expressing cells presumably through the intermediacy of the G_i-protein (Inagami *et al.*1993).

Aside from its peripheral effects, angiotensin II is known to have central effects on vasopressin, oxytocin and ACTH release, thirst response, natriuresis, arterial pressure and sympathetic nerve activity (Reid, 1977; Falcon *et al.*1978; Severs & Daniels Severs, 1973).

Studies have focused on angiotensin II receptors, enzymes and physiological functions responsible for the central actions of this peptide due to its powerful ability to increase blood pressure and as a possible source of hypertension. Therefore, this review concentrates on the role of angiotensin II as a chemical mediator of synaptic transmission in the central nervous system, specifically looking at the cardiovascular effects elicited from the spinal cord.

b. Distribution of angiotensin II in the central nervous system

i. Brain

Immunocytochemical studies have shown the presence of fibers or cells staining for angiotensin II-like material in the brain, particularly those involved in regulation of pituitary and autonomic function, e.g. supraoptic nucleus and paraventricular nucleus in the hypothalamus (Phillips *et al.* 1979). Angiotensin II was found in cultured brain neurons of 21 day old rats (Weyhenmeyer *et al.* 1980) and was confirmed by immunoreactivity (Raizada *et al.* 1984). All the components for the renin-angiotensin-aldosterone system are found in neuroblastoma cells (Fishman *et al.* 1981; Inagami *et al.* 1982). Angiotensin II has been quantified and characterized in the brain using high performance liquid chromatography (Hermann *et al.* 1984; Phillips & Stenstrom, 1985).

Central binding studies have implicated angiotensin II receptors in membrane brain fractions (Bennett & Snyder, 1980; Harding *et al.* 1981; Sirett *et al.* 1977). Co-localization of angiotensin II with noradrenaline or adrenaline has been described in nerve terminals within the cardiovascular regions such as the circumventricular organs and at sites near the blood brain barrier, such as the medial preoptic nucleus, paraventricular nucleus (Fuxe *et*

*al.*1982), lateral parabrachial nucleus, spinal trigeminal nucleus, bed nucleus of the stria terminalis and nucleus of the solitary tract (Phillips *et al.*1993). High concentrations of angiotensin II are also located in the area postrema (Ferrario *et al.*1979) and moderate amounts in the organum vasculosum of the lamina terminalis.

ii. Spinal cord

Although many studies have been dedicated to the effects of angiotensin II in the brain, little is known about how angiotensin II affects spinal cord neurons. Electrophysiological experiments have indicated that angiotensin II depolarizes ventral and dorsal roots of the amphibian spinal cord (Konishi & Otsuka, 1974; Phillis & Kirkpatrick, 1979). Angiotensin II is also present in the remaining gray matter of the lumbar spinal cord of the rat and densely concentrated in the superficial layers of the dorsal horn and few (White *et al.*1988), therefore indicating a role in modulating both sensory and motor functions of the spinal cord

Angiotensin II-like material has also been located in varicose nerve terminals using immunohistochemistry in the spinal cord, with the highest density in the ILp (Fuxe *et al.*1976; Lind *et al.*1985). The fibres appear to project from the lateral funiculus to the ILp, suggesting a supraspinal origin of the angiotensin II innervation (Fuxe *et al.*1976). As well, there is evidence for the co-existence of angiotensin II and noradrenaline in the A1 and C1 cell groups in the medulla, which are involved in cardiovascular and neuroendocrine regulation (Covenas *et al.*1990). It has been suggested that the axons forming the angiotensin II-LI synapses originate from the perikarya localized in different higher areas of the central nervous system or are coming from the periphery through the dorsal roots (Galabov, 1992). Results of angiotensin II-LI immunoreactive neurons in the brain stem and in the spinal

ganglia but not in the spinal cord support this hypothesis (Fuxe *et al.*1976; Richoux *et al.*1983). The peptide has also been found in the substantia gelatinosa, the spinal trigeminal nucleus and, in low concentrations, in the grey matter of the spinal cord (Mendelsohn *et al.*1984). Electron microscopy has shown both thoracic and sacral intermediolateral columns contain a considerable number of angiotensin II-LI fibers and terminals (Galabov *et al.*1990) in the guinea pig. However, it is still unclear whether angiotensin II acts as a conventional neurotransmitter or modulates the action of another substance, which may be excitatory or inhibitory in action.

c. Receptor subtypes

Receptor subtypes have been distinguished via non-peptide receptor antagonists (Rowe *et al.*1991). Recently, two distinct angiotensin II receptor subtypes were identified in the adrenal glomerulosa using nonpeptide and peptide antagonists (Whitebread *et al.*1989; Smith *et al.*1992; Rowe *et al.*1992). Since several publications have appeared using different classifications for the two receptor subtypes, an ad hoc nomenclature committee was set up by the American Heart Association Council for High Blood Pressure Research They decided that the angiotensin receptor would be classified as AT and their respective subclasses were to be distinguished with a subscript 1,2,3 and so on. Therefore, the two receptors are distinguished as AT_1 and AT_2 (Bumpus *et al.*1991). Most known effects of angiotensin II are functionally coupled to the AT_1 receptor subtype (Timmermans *et al.*1991; Herblin *et al.*1991; Phillips *et al.*1993), whereas the AT_2 subtype has yet to be clearly functionally defined. These receptor subtypes can be distinguished by their differential sensitivity to the sulfhydryl reducing agent dithiothreitol (Chiu *et al.*1989; Speth *et al.*1991)

and by their ability to selectively bind the non-peptidic ligands, losartan (AT_1) and PD 123319 (AT_2). A third receptor subtype, AT_3, has been recently cloned and expressed (Sandberg *et al.* 1992; Inagami *et al.* 1993), but the functional significance is unknown as of yet.

i. Angiotensin receptor subtype 1 (AT_1)

Although both angiotensin II receptors have been detected, the angiotensin type 1 receptor (AT_1) predominates (Chiu *et al.* 1992; Smith *et al.* 1992; Bauer *et al.* 1991; Murphy *et al.* 1991). It has recently been cloned from bovine adrenal tissue (Sasaki *et al.* 1991a) and rat vascular smooth muscle (Murphy *et al.* 1991; Sasaki *et al.* 1991b) and it has been suggested that its expression is developmentally regulated. The AT_1 receptor is proposed to be a member of the 'G' protein coupled family of receptors (Sasaki *et al.* 1991a). AT_1 predominates in virtually all vascular tissue, in adrenal cortex, and in specific brain regions such as the subfornical organ, paraventricular nucleus, nucleus tractus solitarius and area postrema that are involved in cardiovascular regulation and body fluid homeostasis (Song *et al.* 1991; Speth *et al.* 1991). A recent immunohistochemical study (Phillips *et al.* 1993) has shown immunoreactivity in the paraventricular and supraoptic nucleus, along the lamina terminalis in the organum vasculosum lamina terminalis, the medial preoptic nucleus and extending to the medial septal region. In the brain stem, the cardiovascular control areas include the nucleus rostroventral lateral area, the C1 area, the nucleus tractus solitarius, dorsal motor nucleus of the vagus, the 12th nerve nucleus and the nucleus ambiguus (Phillips *et al.* 1993; Ruggiero *et al.* 1990). The C1 area ventral to the nucleus ambiguus is the site of synaptic relay, which through projections from the nucleus tractus solitarius and dorsal motor nucleus of the vagus to the spinal cord, influences control of sympathetic outflow

(Ruggiero *et al.*1990). A similar distribution of receptors has been shown based on ^{125}I-angiotensin II autoradiography, using specific receptor antagonists (Gehlert *et al.*1991; Song *et al.*1992).

It has been suggested that the AT_1 receptor has its own additional multiple subtypes, AT_{1A} and AT_{1B} from bovine, rat and human tissues and it has been postulated that the receptor subtypes have opposite reactions: AT_{1A} is down-regulated and not mediated by protein kinase C (Murphy *et al.*1991; Makita *et al.*1992) and AT_{1B} is up-regulated (Sasaki *et al.*1991a,b). Receptor subtypes of AT_1 have been cloned and analysis reveal that AT_{1A} is expressed in aortic vascular smooth muscles cells, lung and ovary; AT_{1B} is expressed in adrenal, uterus, and anterior pituitary; both these mRNAs are expressed in liver, kidney, aorta, uterus, adrenal gland, ovary, lung, heart and spleen at similar levels (Kakar *et al.*1992a,b; Sasaki *et al.*1991b; Sandberg *et al.*1992). The functional role of AT_{1B} is yet to be defined in the kidney. A third receptor subtype, AT_{1C} has been identified (Hahn *et al.*1993), but its functional significance is unknown as of yet. mRNAs for the AT_{1C} is weakly expressed in brain and kidney and are abundant in cultured vascular smooth muscle and mesangial cells; they are also expressed in the liver, lungs and spleen (Hahn *et al.*1993).

ii. Angiotensin receptor subtype 2 (AT_2)

The subtype AT_2 receptor is sensitive to the agonists, PD 123319, CGP42112A, PD 123177 and *p*-aminophenylanaline (Whitebread *et al.*1989; Timmermans *et al.*1991; Chiu *et al.*1989; Speth *et al.*1991; de Gasparo *et al.*1990). The AT_2 receptor is reported to have a protein tyrosine-phosphatase action in the pheochromocytoma cell line PC 12W implying a possible role in cellular growth and development (Bottari *et al.*1992). AT_2 receptors

predominate in the adrenal medulla, and specific brain regions such as the thalamus and midbrain involved in sensory integration and in some limbic structures such as the lateral septal nuclei (Song *et al.*1991; Speth *et al.*1991). The AT_2 receptor mediates inhibition of guanylyl cyclase and regulation of potassium channels (Sumners *et al.*1991; Bottari *et al.*1992; Kang *et al.*1992). While the function of AT_2 receptors remains unclear, their distribution suggests a role that is distinct from that of AT_1 receptors.

iii. Angiotensin receptor subtype 3 (AT_3)

Recently, a third angiotensin II receptor subtype, the AT_3 receptor, has been cloned and expressed in the adrenal cortex and pituitary (Sandberg *et al.*1992; Inagami *et al.*1993) and is pharmacologically distinct from the other two receptor subtypes and is not suppressed by GTP analogs (Chaki & Inagami, 1992b). This receptor stimulates soluble guanylate cyclase by an nitric oxide mediated process (Chaki & Inagami, 1992a). Otherwise, no information is available regarding its functional role in the central nervous system.

d. Angiotensin II receptor antagonists

The introduction of angiotensin II receptor antagonists has been the most direct way to interrupt the RAS, most specifically the effector molecule of the system, angiotensin II, for the treatment of hypertension and congestive heart failure (Cody, 1986; Williams, 1988). The first peptidic angiotensin II receptor antagonist was [Phe_4, Tyr_8] angiotensin II which was specific in certain isolated tissues (Marshall *et al.*1970). Various substitutions led to high affinity angiotensin II analogs and specific antagonists in vitro and in vivo (Pals *et al.*1971;

Streeten & Anderson, 1984). But due to poor bioavailability and residual agonistic activity of the antagonists, chronic therapeutic use was not possible. Furukawa et al. (Furukawa *et al.*1982) reported that benzylimidazole analogs inhibited the functional responses to angiotensin II and were shown later to be weak, but selective angiotensin II receptor antagonists. Therefore, the AT_1 prototype, DuP 753, was synthesized and characterized (Chiu *et al.*1990) and found to antagonize the AT_1 receptor. The recent identification of nonpeptide angiotensin II receptor antagonists that lack agonist activity provides the most direct approach to study of the involvement of the RAS in central nervous system function, including blood pressure regulation (Chiu *et al.*1988; Wong *et al.*1988; Chiu *et al.*1989). More receptor antagonists have been published/patented (see table I).

Recently, Zhou *et al.*(1993) showed that losartan had a higher affinity for AT_{1A} receptor and AT_{1B} also displayed a strong affinity for PD 123319. They suggested that one or more G proteins may modulate receptor interactions (Ernsberger *et al.*1992). They propose that the AT_{1A} receptor subtype is coupled to the adenylyl cyclase inhibition because losartan blocks the effect of angiotensin II (Zhou *et al.*1993; Crawford *et al.*1991; Bauer *et al.*1991) in the liver. Zhou *et al.*(1993) also suggest that the AT_{1B} is coupled to the effects associated with the AT_1 receptor, e.g. inhibits the activity of forskolin-stimulated adenylyl cyclase. Losartan, PD 123319, and CGP 42112A all antagonized angiotensin II induced inhibition of adenylyl cyclase. PD 123319 a potent antagonist of calcium response to angiotensin II in mesangial and renal epithelial cells, implying AT_{1B} to be coupled with signalling pathways regulating intracellular calcium (Madhun *et al.*1992). But PD 123319 also shows agonist actions when administered alone. Therefore, AT_{1A} has a higher affinity for losartan and AT_{1B} for PD 123319; both appear to be coupled to adenylyl cyclase through

the G_i protein (Zhou *et al.* 1993).

Table I
Angiotensin II receptor subtypes

	AT_{1A}	AT_{1B}	AT_{1C}	AT_2	AT_3
agonist	AII	AII	AII	AII	AII
antagonist	DuP 753 losartan MK-954	PD 123319		PD 123177 EXP655	
	DuP 532			PD 123319	
	DuP 3892			CGP 42112A	
	EXP 3174				
	BIBS 39				
	BIBS 222				

e. Effects on sympathetic output at the spinal level

Previous experiments done in our laboratory has shown that angiotensin II when administered intrathecally caused an increase in arterial pressure and heart rate; suggesting that angiotensin II activates sympathetic mechanisms by a spinal action (Yashpal *et al.* 1989a). As well, the data proposed that the effects of angiotensin II may be mediated via two different mechanisms. Therefore, it was proposed that two types of angiotensin II receptor may have been mediating the respective reactions. The precise site of action of angiotensin II receptor subtypes have been implicated in many models (Ferguson & Wall, 1992; Ganten *et al.* 1978a), but little in the spinal cord. Since cardiovascular responses are partly activated by sympathetic activation, Chapter III shows the effect of DuP 753, an AT_1 receptor antagonist, and PD 123319, an AT_2 receptor antagonist, on the cardiovascular responses

when angiotensin II is administered intrathecally to the ninth thoracic segment of the spinal cord. Blocking AT_1 receptors with losartan i.c.v. has been shown to prevent centrally mediated angiotensin II-induced pressor response (Kirby *et al.* 1992; Toney & Porter, 1993).

E. CONCLUSIONS

In conclusion, the complex organization of spinal autonomic networks is indicated by the spinal interneurons within sympathoexcitatory and sympathoinhibitory pathways, the various projection from supraspinal regions to spinal autonomic pathways and the chemical mediation of various peptides and amino acids. Therefore, the traditional concept that the spinal cord serves solely as a relay station for transmitting information from the medulla to postganglionic neurons innervating the cardiovascular system is no longer plausible. The compiled information in this literature review indicates the complexity of the spinal cord role in the central nervous system and its modulatory capacity in the periphery.

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CHAPTER II

ACUTE SPINAL TRANSECTION: CARDIOVASCULAR RESPONSES TO INTRATHECAL SUBSTANCE P IN THE RAT

ABSTRACT

Park, Patricia D.S. and James L. Henry. *Acute spinal transection: cardiovascular responses to intrathecal substance P in the rat.* - Previous studies have shown that intrathecal administration of substance P to the awake or the urethane anesthetized rat produces increases in arterial pressure and heart rate. As the effects could be elicited via spino-bulbo-spinal pathways, this study investigated the effects of substance P in acutely (2h) spinal transected rats. Spinal cords of male Sprague-Dawley rats (300-375g) were transected at thoracic T5 or T1 under urethane anesthesia. Substance P (6.5 nmol; n=9) administered at T9, caudal to the transection, increased arterial pressure (systolic arterial pressure, SAP ± S.E.M., by 18.4 ± 3.6 mmHg, diastolic arterial pressure, DAP ± S.E.M., by 15.0 ± 4.1 mmHg) without changing heart rate. Administration of substance P (3.25 nmol; n=7) at T2 (rostral to the transection) increased both arterial pressure (SAP: 11.1 ± 2.0 mmHg, DAP: 7.0 ± 3.2 mmHg) and heart rate (29.8 ± 2.9 bpm). Similar administration of CSF, the vehicle, had no effect (n=11 at T9; n=8 at T2). The maximum responses occurred at 3-4 min and 8-10 min when substance P was given at T9 vs T2, respectively. In animals transected at T1 (n = 8), substance P administration at T9 increased arterial pressure (SAP: 31.5 ± 6.4 mmHg, DAP: 29.5 ± 7.1 mmHg) and heart rate (47.6 ± 9.7 bpm). In similar animals, CSF had no effect (n=11). In animals transected at T5, naloxone (10mg/kg, i.v.; n=5) increased the maximum cardioacceleratory effect of administration of substance P at T2 by about 50 bpm but the arterial pressure response was not potentiated. The data indicate that excitatory effects of substance P on sympathetic output can be elicited in spinal transected rats and therefore that the neural substrate to express the effects of substance P is

intrinsic to the spinal cord.

Substance P, Spinal Cord, Spinal Transection, Thoracic, Sympathetic, Autonomic, Heart Rate, Arterial Pressure, Intrathecal, Naloxone, Spinal Injury, cardiovascular regulation

INTRODUCTION

The capacity of the spinal cord to regulate autonomic function has been known since the time of Yates (43), who first demonstrated that a recovery of near normal arterial pressure occurred over several weeks in chronically transected cats. The exact mechanism of this restorative process is unknown but alterations in chemical mechanisms regulating spinal sympathetic neurons may provide one possible explanation. One of the chemicals which has been implicated in excitatory effects on spinal sympathetic neurons is substance P. In relation to the present report, it has been shown that intrathecal administration of substance P increases sympathetic output to the adrenal medullae (4,40) and to the vessels and the heart in the awake and in the anesthetized rat (19,29,39). While abundant evidence exists to support an action of substance P directly on sympathetic preganglionic neurons (*vide infra*), thereby prompting the possibility that the increase in sympathetic output after intrathecal administration of substance P is due to an action directly on these neurons, other possibilities remain. For example, substance P also has an excitatory effect on dorsal horn neurons (17), particularly on nociceptive neurons (15,26,28,30,37). This raises the alternative possibilities that substance P increases sympathetic output via activation of spino-brain stem-spinal reflex pathways or via activation of intrinsic spinal reflex pathways initiated from the dorsal horn. In fact, one report indicates that the heart rate component of the cardiovascular response to intrathecal administration of substance P at the lower thoracic level is lost in freely moving rats transected acutely at the cervical level (13), indicating that a neuronal pathway between the spinal cord and higher centers mediates this cardioacceleratory component. However, preliminary experiments as part of a larger study

indicated that cardioacceleration could be elicited in acutely spinalized, anesthetized rats. Therefore, experiments were undertaken to examine the cardiovascular responses to intrathecal administration of substance P given at various levels of the spinal cord in rats transected at upper or mid-thoracic levels. A preliminary report of some of the results has been presented in abstract form (25).

METHODS

In all cases, the guidelines described in The Care and Use of Experimental Animals, of the Canadian Council of Animal Care, Vols. I and II, were strictly followed. The experimental protocols followed were also reviewed and approved by the McGill University Animal Care Committee.

Animal Preparation

Adult male Sprague-Dawley rats (300-375g) were anesthetized with urethane (1.5g/kg, i.p.). A polyethylene catheter (Intramedic PE-50), filled with heparinized saline (75 IU/ml in 0.9% NaCl), was inserted into the left common carotid artery toward 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 by measuring the number of beats in 10 s intervals and multiplying this number by 6 to obtain the rate in beats per min (bpm). In some experiments, an additional catheter (Intramedic PE-50) was inserted into the right femoral vein for intravenous (i.v.) injection of drugs. Rectal temperature was maintained at approximately 37°C with a heating pad. Each rat was used in only one experiment.

The fifth and sixth thoracic, or seventh cervical and first thoracic vertebrae were exposed via a dorsal midline incision and the dorsal arches of the vertebrae were chipped away with rongeurs. A small slit was made in the dura at the respective level and a fine polyethylene catheter (Intramedic PE-10) was advanced caudally or rostrally under the dura so that the inner tip lay at the ninth thoracic (T9) or second thoracic (T2) spinal level using spinous processes as landmarks (40). It is via this catheter that agents were delivered intrathecally. The correct positioning of the catheter tip was confirmed by post-mortem examination after the experiment.

The spinal cord was then cut with scissors at T1 or T5 between the respective exposed thoracic spinal vertebrae; immediately prior to cutting, 0.01 ml of lidocaine (1%) was injected to prevent spinal shock and respiratory distress. This was followed by suction of spinal tissue about 2 mm rostrally and caudally to the cut, using a blunt 21-gauge hypodermic needle connected to a vacuum pump. Bleeding was minimal and usually stopped within 20-30s. The completeness of the transection was verified by careful inspection of the lesion with the aid of a high-intensity fiber optic lamp and a dissecting Zeiss-Jena stereomicroscope. A complete transection was confirmed in all rats reported here.

Experimental Protocol

After surgical preparation, the animals were allowed to stabilize for 2 hours. To establish baseline values, five readings of arterial pressure and heart rate were taken over a period of 10 min. Agents were administered intrathecally or i.v. with zero time being the end of drug administration. After administration, readings of arterial pressure and heart rate were taken each minute for 15 min and then at 20 and 30 min.

Intrathecal administration of substance P

Substance P was purchased from Institute Armand-Frappier (IAF, Montreal; lot #340913); the purity as assessed by HPLC was 98.8% and the peptide content by amino acid analysis was 81%. The substance P solution was measured into 100 μl aliquots of 6.5 nmol and 3.25 nmol and kept frozen until used. During the experiment, the aliquot of substance P solution was stored in ice.

The peptide was delivered over a period of 15 s at a dose of 10μg (6.5nmol as determined from the peptide content) dissolved in 10 μl of artificial cerebrospinal fluid (CSF; an aqueous solution (mM) of 128.6 NaCl, 2.6 KCl, 1.0 $MgCl_2$ and 1.4 $CaCl_2$; pH adjusted to 7.33). Following delivery of the peptide, the catheter was flushed with 10 μl of CSF (catheter volume was 6-8 μl). This procedure has been shown to result in penetration of radiolabelled substance P into the grey matter of the thoracic cord, limited to 1 cm rostrocaudally from the level of administration (5). In control experiments, 10 μl of CSF replaced the substance P solution.

Intravenous administration of naloxone

In appropriate groups, naloxone (10 mg/kg) was injected via the i.v. catheter 5 min prior to peptide administration. It was injected in 0.3 ml of 0.9% NaCl and the catheter was flushed by 0.3 ml of 0.9% NaCl.

Statistical Analysis

Results from each rat were tabulated as the change in systolic and diastolic arterial pressures and heart rate. Data were calculated as the change from the mean baseline values.

Data for the figures were summarized by taking the mean ± S.E.M. of the values from each group of rats at each sample time following administration. These changes were analyzed by a *t*-test analysis (non-pairwise) comparison using SigmaStat. The level of statistical significance adopted was $p < 0.05$ and the confidence level was 90%.

RESULTS

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

Acute spinal transection lowered arterial pressure in all animals. To ensure reliable results, data were collected only from animals in which cardiovascular parameters were within a physiologically acceptable range; the baseline pressures and heart rates of the animals used are presented in Table I.

Effects of intrathecal administration of substance P at T9 in rats transected acutely at T5

Effects on systolic and diastolic arterial pressures observed with administration of 6.5 nmol of substance P at T9 in rats transected acutely at T5 ($n = 9$) are illustrated in figure 1. Both pressures exhibited a significant increase, starting within 1 min of administration of substance P (increases were 12.9 ± 5.2 and 10.0 ± 5.5 mmHg, respectively) and reaching an initial peak at 3-4 min (at 18.4 ± 3.6 and 15.0 ± 4.1 mmHg, respectively). From this time, pressure fell gradually toward preadministration levels until at 20 min no effect remained on either systolic or diastolic arterial pressure. On the other hand, in transected rats given CSF instead of the substance P solution ($n = 11$), there was no change in either

systolic or diastolic arterial pressure. The *t*-test analysis revealed that the data from the two groups of rats were significantly different, systolic pressure at 1-15 min ($p < 0.01$), diastolic pressure at 1-13 min (1-10 min, $p < 0.01$, 11-13 min, $p < 0.05$).

Figure 2 shows the effects on heart rate in the same groups of animals. In this case, however, neither group showed any change from preadministration levels and there was no difference between the two groups at any time after administration ($p > 0.05$).

Effects of intrathecal administration of substance P at T2 in rats transected acutely at T5

As the lack of an effect on heart rate in the previous series of experiments might be explained as due to spinal shock, an additional series was done in rats transected at T5, but with administration at the T2 spinal level. Thus, figure 3 illustrates the effects on systolic and diastolic arterial pressures observed with administration of 3.25 nmol of substance P at T2 in rats transected acutely at T5 (n = 7). In this case, a lower dose of substance P was used because in earlier experiments on intact rats (42) it was found that the cardiovascular changes induced from this spinal level were greater than those elicited from T9. However, responses roughly similar in magnitude could be elicited by administration of 6.5 nmol at T9 and of 3.25 nmol at T2. Therefore, in the present study, as magnitudes of responses are to be compared, in the group of rats given substance P at T2 the lower dose was used.

When substance P was given at T2, both systolic and diastolic pressures exhibited an initial decrease, with an abrupt reversal to a significant increase, starting at 5-6 min after administration of substance P and reaching a peak at 8-10 min (at 11.1 ± 2.0 and 7.0 ± 3.2 mmHg, respectively). From this time, the pressures fell gradually; systolic pressure remained elevated for the duration of the experiment, diastolic pressure returning to

preadministration levels by 20 min. In the group of rats given CSF instead of the substance P solution ($n = 8$), neither systolic nor diastolic arterial pressure showed any change from preadministration levels. The *t*-test analysis revealed that the two groups differed: systolic pressure at 5-30 min ($p < 0.01$) and diastolic pressure at 6-10 min ($p < 0.05$).

The effects on heart rate in the animals transected at T5 administered 3.25 nmol of substance P at T2 are illustrated in figure 4. In this case, there was a marked increase in heart rate, starting at 1 min after administration (4.1 $\pm$ 2.5 bpm), reaching a peak at 10-15 min (28.0 $\pm$ 8.8 bpm), and remaining at that peak level for the duration of the experiment. Again, the *t*-test analysis revealed that there was a significant difference between this group and the group given CSF at T9, at all times (1 min, $p < 0.05$, 2-30 min, $p < 0.01$).

Effects of intrathecal administration of substance P at T9 in rats transected acutely at T1

The data in the two preceding series of experiments could not alone eliminate either a supraspinal loop or intrinsic spinal mechanisms to account for the observations regarding effects on heart rate. Therefore, an additional series of experiments was done, giving substance P at T9 in animals with acute high thoracic spinal section, at T1. Figure 5 illustrates the effects on systolic and diastolic arterial pressures observed with administration of 6.5 nmol of substance P at T9 in rats transected acutely at T1 ($n = 8$). Both pressures exhibited a significant increase, starting within 1 min of administration of substance P (values were 17.3 $\pm$ 5.0 and 15.6 $\pm$ 6.0 mm Hg, respectively, at 1 min) and reaching an initial peak at about 4 min (at 31.5 $\pm$ 6.4 and 29.5 $\pm$ 8.2 mm Hg, respectively). From this time, the pressures fell gradually, yet showed a second phase of the response, peaking at about 11-13 min (at 22.8 $\pm$ 5.7 and 19.9 $\pm$ 7.3 mm Hg, respectively). Then, the pressures returned

toward preadministration levels until at 30 min no effect remained. In the group given CSF (n = 11), there was no change in either systolic or diastolic arterial pressure. The *t*-test analysis revealed that the data from the two groups of rats were significantly different at all times, except at the 30 min reading (systolic pressure, 1-20 min, $p < 0.01$, diastolic pressure, 1-9 min and 11-14 min, $p < 0.01$ and 10, 15 and 20 min, $p < 0.05$).

Figure 6 shows the effects on heart rate. In this case, heart rate showed a marked, rapid increase, starting at 1 min after administration, peaking at 3 min (at 47.6 ± 9.7 bpm) and showing a slow return to preadministration levels at 15 min. In the group given CSF, the readings did not differ from preadministration levels. The *t*-test analysis revealed that the data from the two groups of rats were significantly different (1-11 min, $p < 0.01$, 12-15 min, $p < 0.05$).

Effects of naloxone on responses to substance P given at T2 in rats transected at T5

Data in figure 3 indicated a transient decrease in arterial pressure when substance P was given intrathecally in rats transected at T5. Such a response has not been seen in any other paradigm, yet the effect was seen consistently in the transected rats. As a first step in identifying mechanisms which underlie this effect, the experiments were repeated to determine the effects of naloxone on this initial, transient decrease in arterial pressure. In addition, in a previous study naloxone was found to facilitate the rate of development of the increase in heart rate in response to administration of substance P at the T9 spinal level (42). Thus, a similar issue was raised in the present study, in this case because of the surgical separation of the T2 level from mid- and lower thoracic spinal cord sympathetic mechanisms which have been implicated in sympathetic output to the adrenal medullae. Administration

of 10 mg/kg of naloxone i.v. produced a marked potentiation of the cardioacceleratory response to administration of 3.25 nmol of substance P at the T2 level (n = 5). The data are presented in figure 8. The two groups given naloxone differed at all time points. In the group given substance P heart rate increased by 20.0 ± 7.9 bpm at one min. Heart rate continued to increase until it reached a plateau at about 15 min, at 75.6 ± 13.8 bpm. Even at 30 min there was no sign of a trend for the heart rate to return toward preadministration levels. The pressor responses were not potentiated by naloxone (figure 7).

DISCUSSION

The results of the present study indicate that intrathecal administration of substance P at the ninth thoracic spinal level produces increases in arterial pressure and heart rate in animals acutely transected at the upper thoracic level. Therefore, the actions of substance P are mediated at least partly via spinal mechanisms and do not require supraspinal structures, as suggested previously for mechanisms involved in eliciting the heart rate response (13). As diastolic arterial pressure and systolic arterial pressure showed similar changes in response to substance P administration, it may be suggested that the effects may have been primarily on resistive than on capacitative vessels.

As the heart rate response can be elicited in adrenalectomized rats (13,41,42) and are therefore mediated via neural not humoral mechanisms, these data are consistent with the earlier suggestion of Faden and co-workers, on the basis of anatomical (10) and physiological (9) evidence, that long intraspinal sympathetic preganglionic pathways exist. Thus, our data are consistent with the idea that neurons projecting in these long intraspinal pathways from

the region of the ninth thoracic spinal cord are involved in expressing the effects of substance P on heart rate.

There appear to be two principal intraspinal mechanisms to account for the effects of substance P on arterial pressure and heart rate. The more obvious is a direct effect on sympathetic preganglionic neurons. Substance P excites sympathetic preganglionic neurons *in vivo* (1,12) and *in vitro* (8). Nerve terminals forming synaptic contacts are found apposed to sympathetic preganglionic neurons (2,36) and these terminals appear to arise from the caudal brain stem (14,21). From these brain stem sites, neuronal activation provokes the release of substance P from the spinal cord (34) as well as increases in heart rate and arterial pressure (16,20) and these responses are prevented by the intrathecal administration of a peptide substance P antagonist (20); however, the neurotoxic effects of peptide antagonists of substance P (3,11,27) suggest that conclusions based on their use in the central nervous system should be guarded. The pressor and cardioacceleratory responses to intrathecal administration of substance P are blocked by systemic administration of hexamethonium (39), indicating mediation via autonomic pathways and thus a central, rather than a peripheral, site of action. Finally, high affinity binding sites for substance P are found in the intermediolateral nucleus (33,38). The second possible intraspinal mechanism to account for the results of this study is that substance P activated intrinsic neurons involved in processing of nociceptive information (15) and that these in turn activated sympathetic output. This experimental paradigm does not lend itself to differentiating between these two possibilities.

The potentiation of the cardioacceleratory response by naloxone is consistent with our earlier observation (42). However, the present study goes further and indicates that, as the response to administration to substance P at T2 was potentiated in rats in which spinal section

eliminated sympathetic output to the adrenals, e.g. section at T5, the mechanisms facilitated by naloxone in the present paradigm are independent of adrenal output. As opioids have been located in nerve terminals in sympathetic ganglia (32) as well as in sympathetic preganglionic neurons (6), in view of our present results, it is suggested that an opioid mechanism depresses the cardioacceleratory response to activation of sympathetic output in normal pathophysiology. In addition, the selectivity of the effects on the cardioacceleratory but not the pressor response indicates that this control mechanism preferentially regulates neural output to the heart and not to the vessels.

Chronically spinal transected animals exhibit restoration of arterial pressure to nearly normal levels (43). Furthermore, spinal transection (22,23) and large lesions within the spinal cord (7,31) do not abolish either spontaneous sympathetic activity (7,22,23,31,35) or spontaneous hypertension (7,24,31). This information, together with our data presented here that substance P-induced activation of sympathetic output to the vessels and the heart does not involve a supraspinal loop, suggests that intrinsic peptidergic spinal mechanisms may contribute to essential hypertension. In addition, after chronic transection, or possibly even spinal trauma, any change in the sensitivity of this system which develops, such as a supersensitivity resulting from interruption of or damage to descending fibers, may contribute to heightened sympathetic reflexes as seen in autonomic hyperreflexia (18,24).

In conclusion, the present study indicates that excitatory effects of substance P on sympathetic output can be elicited in spinalized animals and therefore that supraspinal structures are not necessary for expression of these effects. In addition, however, if a change in sensitivity of substance P receptors results from spinal transection, activation of these receptors by intrinsic substance P-containing neurons might contribute to altered sensitivity

of spinal autonomic reflexes.

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Table I. Baseline values of systolic and diastolic arterial pressures (AP), and heart rate 2h after spinal transection.

Group of rats	n	Systolic AP	Diastolic AP	Heart Rate
SP at T9:				
T5 transection	9	86.5 ± 4.0	41.0 ± 3.9	407.3 ± 18.3
T1 transection	8	78.6 ± 5.0	49.9 ± 4.4	317.7 ± 11.5
SP at T2:				
T5 transection	7	101.5 ± 3.7	51.3 ± 4.5	377.4 ± 21.8
CSF at T9:				
T5 transection	11	93.5 ± 4.1	54.2 ± 2.9	352.7 ± 12.5
CSF at T2:				
T5 transection	8	97.8 ± 4.9	52.1 ± 4.1	363.9 ± 12.5
naloxone/SP	5	99.2 ± 1.5	56.2 ± 1.9	320.1 ± 30.5
naloxone/CSF	3	87.1 ± 7.6	48.3 ± 4.2	303.6 ± 15.6

FIGURES

Fig. 1: Time-effect curve for the change in arterial pressure in response to intrathecal administration of substance P (6.5 nmol) or CSF at T9 in animals transected acutely at T5. Each ordinate represents the mean (± S.E.M.) change from the preadministration values of systolic arterial pressure (SAP, mmHg) and diastolic arterial pressure (DAP, mmHg). o-o CSF (n=11); ●-●, substance P (n=9).

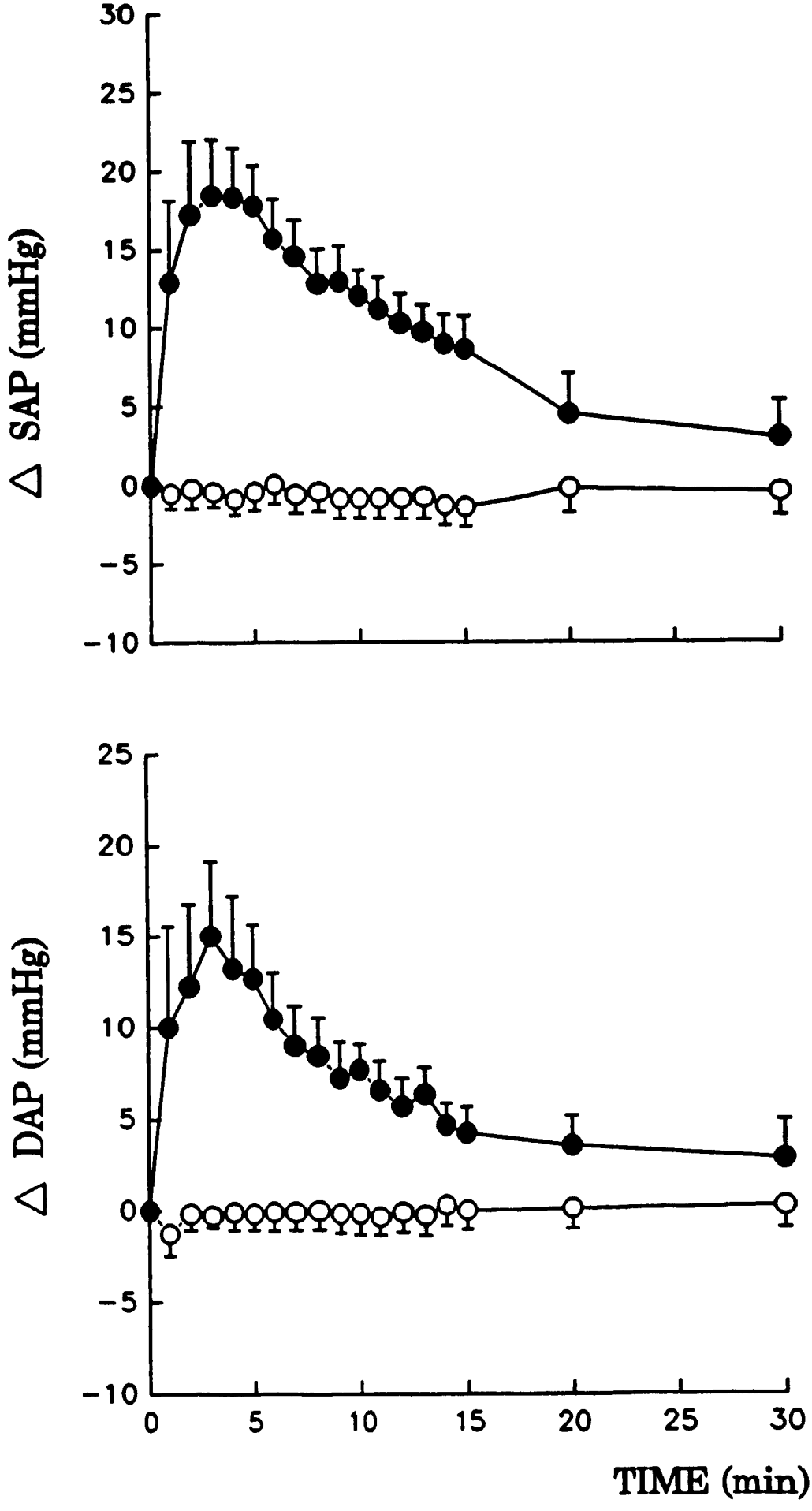

30
25
20
15
10
5
0
−5
−10
Δ SAP (mmHg)
25
20
15
10
5
0
−5
−10
Δ DAP (mmHg)
0
5
10
15
20
25
30
TIME (min)

Fig. 2: Time-effect curve for the change in heart rate in response to intrathecal administration of substance P (6.5 nmol) or CSF at T9 in animals transected acutely at T5. The ordinate shows the mean (± S.E.M.) change in heart rate (HR) from the preadministration level. o-o CSF (n=11); ●-● substance P (n=9).

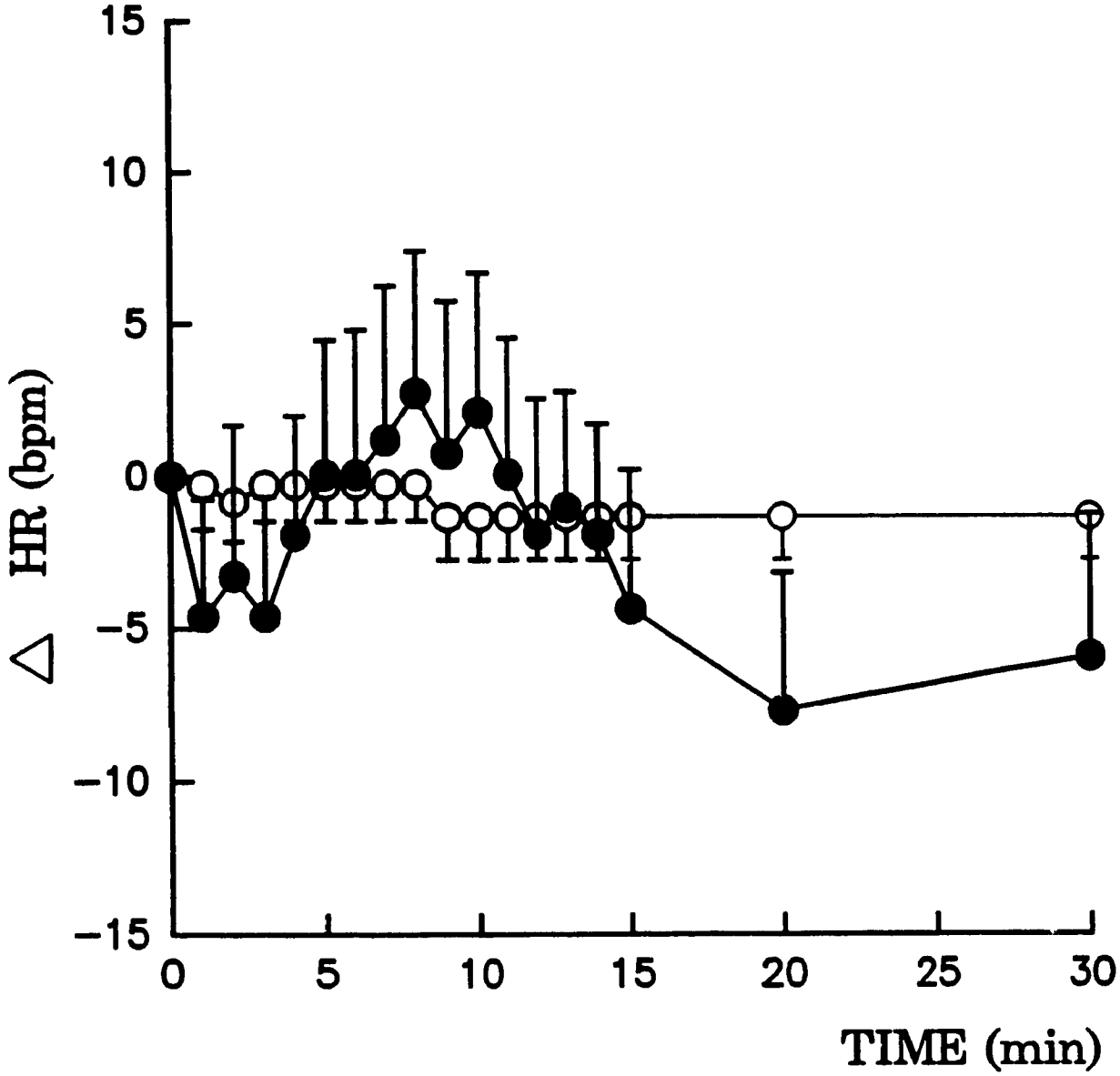

15
10
5
0
−5
−10
−15
Δ HR (bpm)
0
5
10
15
20
25
30
TIME (min)

Fig. 3: Effects on systolic (SAP) and diastolic (DAP) arterial pressures of intrathecal administration of substance P (3.25 nmol) or CSF at T2 in animals transected acutely at T5. ▵-▵ CSF (n=8); ▲-▲ substance P (n=7).

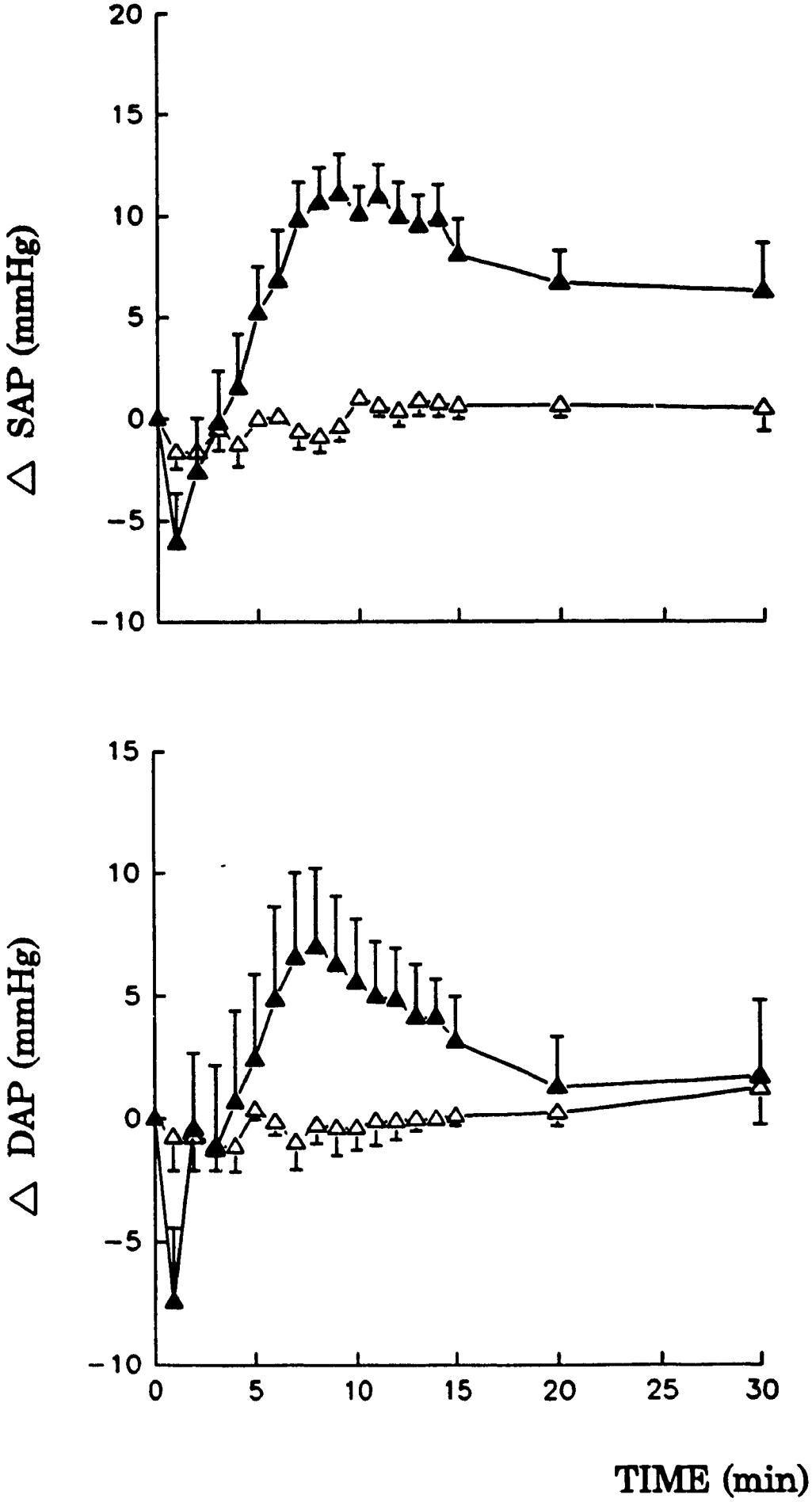

20
15
10
5
0
−5
−10
Δ SAP (mmHg)
15
10
5
0
−5
−10
Δ DAP (mmHg)
0
5
10
15
20
25
30
TIME (min)

Fig. 4: Effects on heart rate (HR) of intrathecal administration of substance P (3.25 nmol) or CSF at T2 in animals transected acutely at T5. △-△ CSF (n=8); ▲-▲ substance P (n=7).

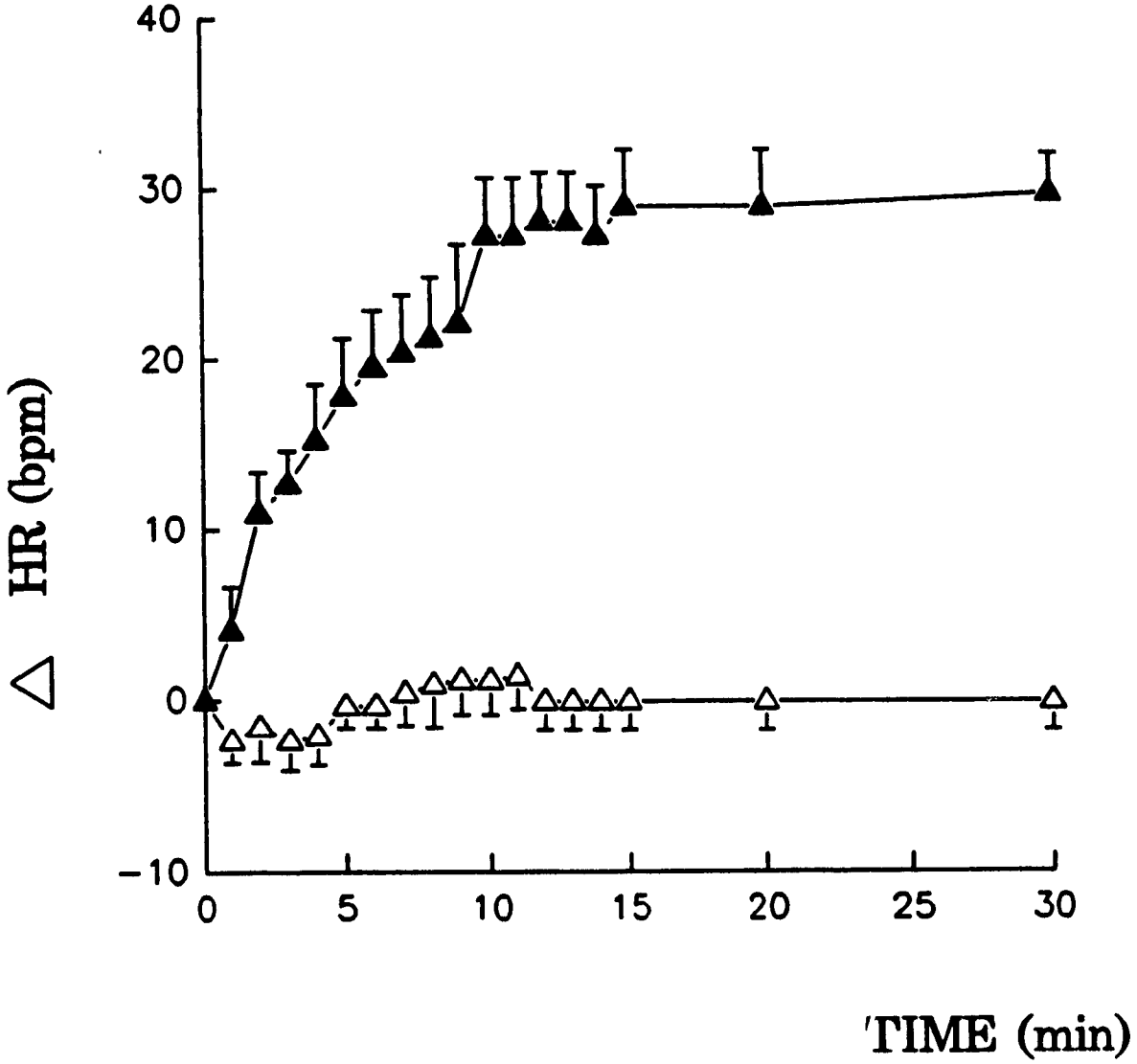

40
30
20
10
0
−10
0
5
10
15
20
25
30
△ HR (bpm)
TIME (min)

Fig. 5: Effects on systolic and diastolic arterial pressures of intrathecal administration of substance P (6.5 nmol) or CSF at T9 in animals transected acutely at T1. ◇-◇ CSF (n=11); ◆-◆ substance P (n=8).

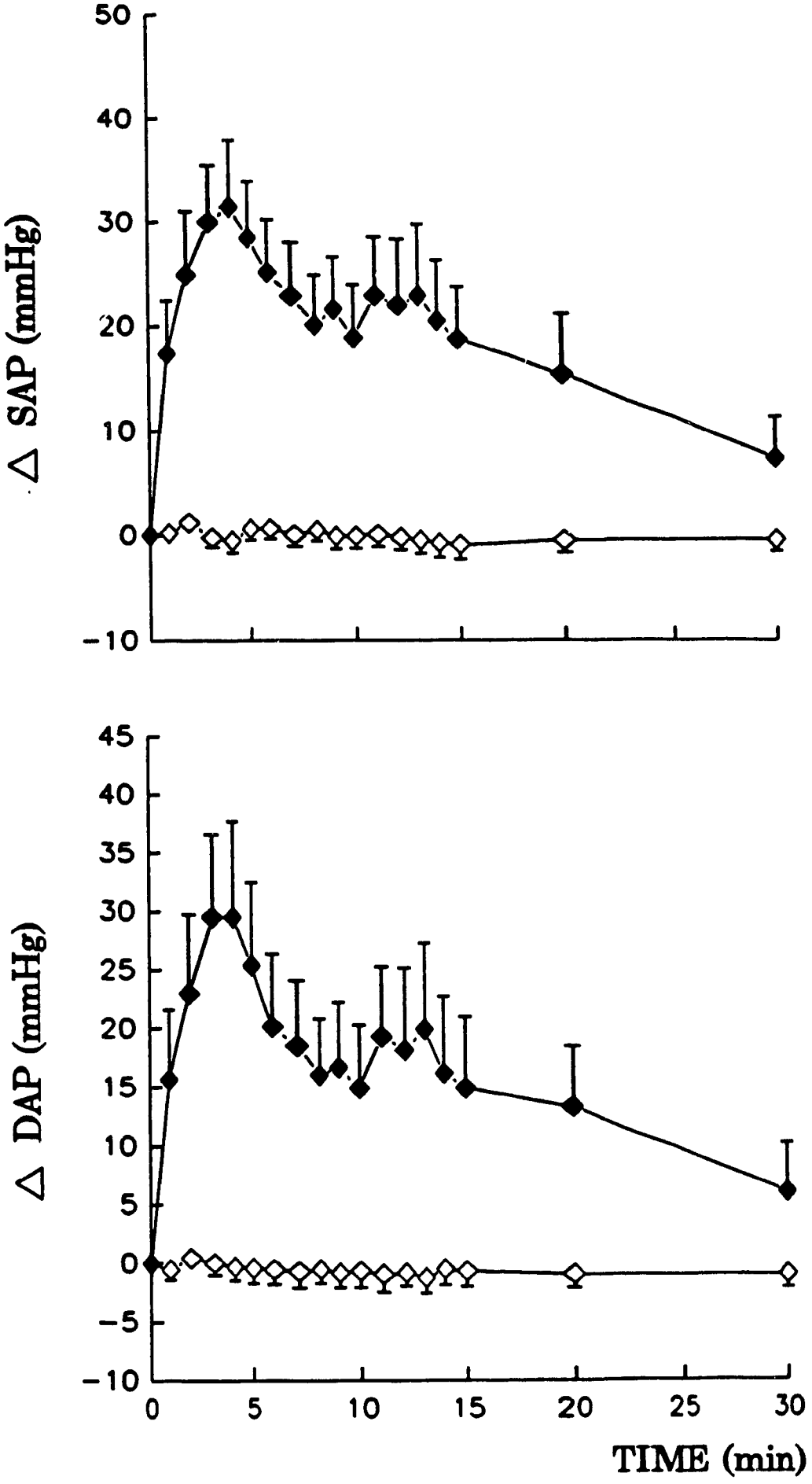
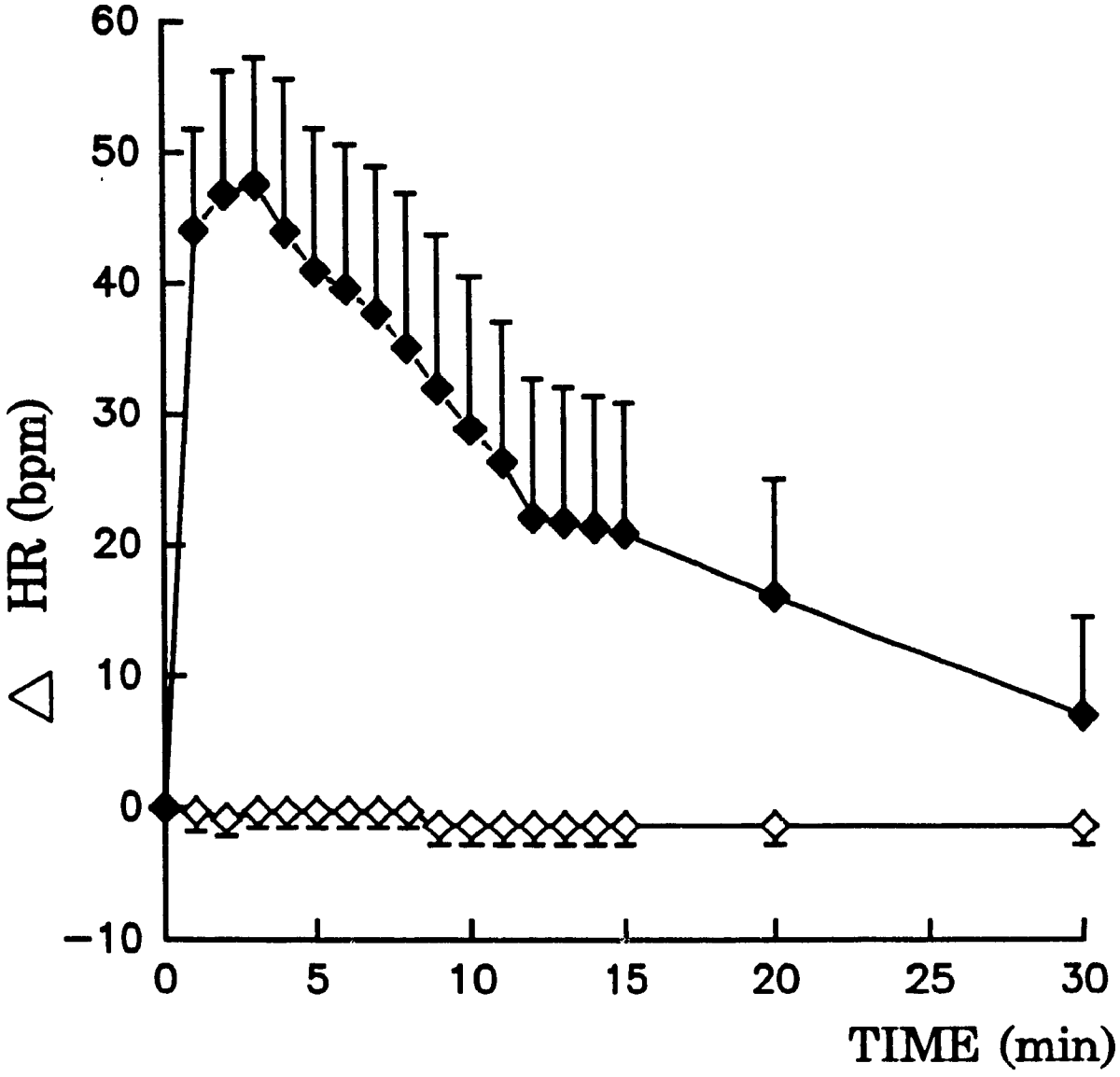

50
40
30
20
10
0
-10
Δ SAP (mmHg)
45
40
35
30
25
20
15
10
5
0
-5
-10
Δ DAP (mmHg)
0
5
10
15
20
25
30
TIME (min)

Fig. 6: Effects on heart rate (HR) of intrathecal administration of substance P (6.5 nmol) or CSF at T9 in animals transected acutely at T1. ◇-◇ CSF (n=11); ◆-◆ substance P (n=8).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50
40
30
20
10
0
−10
△ HR (bpm)
0
5
10
15
20
25
30
TIME (min)

Fig. 7: Effects of naloxone (10 mg/kg, i.v.) on the increases in systolic (SAP) and diastolic (DAP) arterial pressures produced by intrathecal administration of substance P (3.25 nmol) at T2 in rats transected at T5. □-□ control animals given naloxone and CSF (n=3); ■-■ rats given naloxone and substance P (n=5).

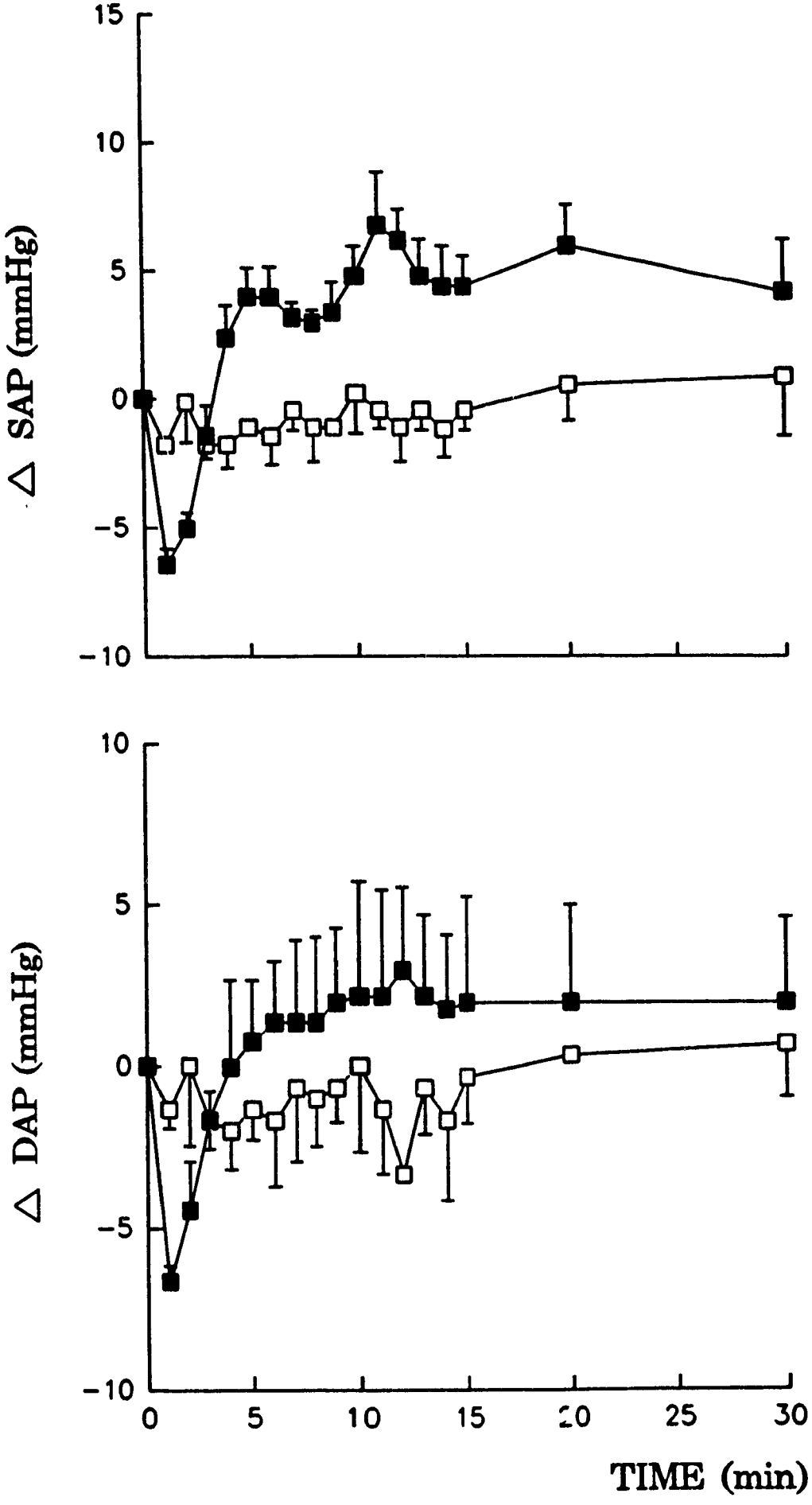
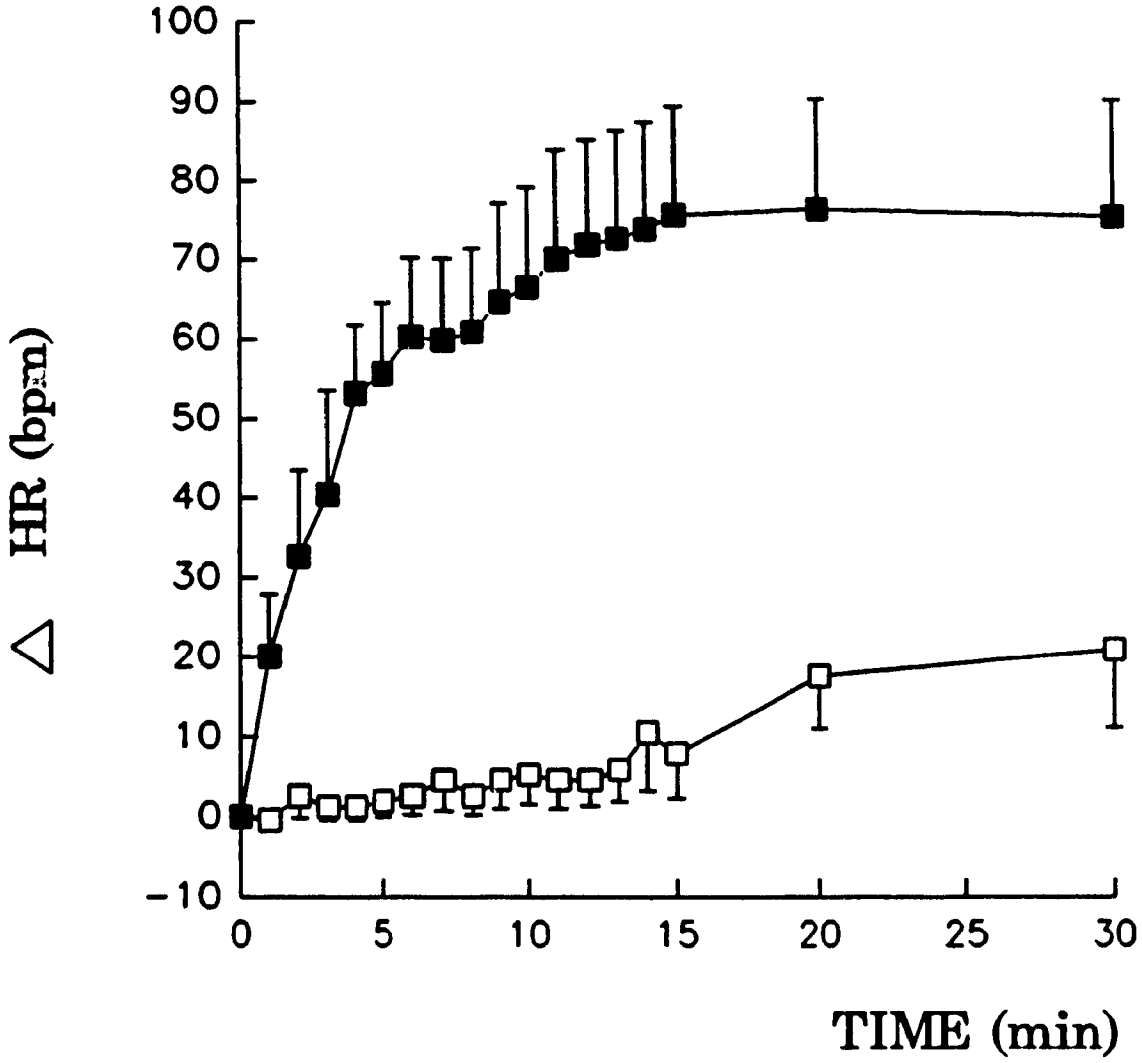

15
10
5
0
−5
−10
Δ SAP (mmHg)
10
5
0
−5
−10
Δ DAP (mmHg)
0
5
10
15
20
25
30
TIME (min)

Fig. 8: Effects of naloxone (10 mg/kg, i.v.) on the increase in heart rate produced by intrathecal administration of substance P (3.25 nmol) at T2 in rats transected at T5. □-□ control animals pretreated with naloxone (n=3); ■-■ rats given naloxone and substance P (n=5).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90
80
70
60
50
40
30
20
10
0
-10
0
5
10
15
20
25
30
△ HR (bpm)
TIME (min)

CHAPTER III

CHARACTERIZATION OF THE RECEPTORS MEDIATING THE EFFECTS OF INTRATHECAL ANGIOTENSIN II ON SYMPATHETIC OUTPUT TO THE VESSELS AND THE HEART: STUDIES WITH DUP 753 AND PD 123319

ABSTRACT

It has previously been shown in this laboratory that intrathecal administration of 10 μg of angiotensin II produces an increase in arterial pressure and heart rate. As two receptor subtypes of angiotensin II, termed AT_1 and AT_2, have been identified recently in central nervous tissue this study examines the effects of selective antagonists on the pressor and cardioacceleratory responses to intrathecal administration of 10 μg of angiotensin II to the ninth thoracic spinal cord. The two non-peptide antagonists were DuP 753, which is selective for the AT_1 receptor, and PD 123319, which is selective for the AT_2 receptor Intravenous administration of DuP 753 blocked both pressor and cardioacceleratory effects of angiotensin II. Intrathecal administration of DuP 753 blocked only the pressor effects, raising the possibility that block of the heart rate response was in the periphery Intrathecal administration of PD 123319 blocked the pressor effect of angiotensin II but had no effect on the cardioacceleratory response. However, by itself the antagonist produced a transient increase in arterial pressure and a slower increase in heart rate, raising the possibility of receptor desensitization as the basis for the block of angiotensin II effects by this antagonist The data support the involvement of the AT_1 receptor in mediating the effects of exogenously administered angiotensin II but also indicate a possible role of AT_2 receptors at the spinal level.

INTRODUCTION

Angiotensin II plays an important role in maintaining fluid and electrolyte homeostasis and cardiovascular function by both peripheral and central actions. Despite some anatomical and morphological evidence that angiotensin II may play a role in regulation of sympathetic output at the spinal level (Galabov, 1992; Fuxe et al. 1976), physiological studies have largely neglected the possibility that angiotensin II can express effects on cardiovascular parameters via actions in the spinal cord (Yashpal, Gauthier and Henry, 1987, Yashpal, Gauthier and Henry, 1989), and in recent reviews on central effects of angiotensin II this central nervous system site has been largely overlooked (Ferguson and Wall, 1992; Ganten et al. 1978). Previous experiments done in our laboratory indicated that angiotensin II, when administered intrathecally in the rat, causes increases in arterial pressure and heart rate which are prevented by block of nicotinic transmission in autonomic ganglia, suggesting that angiotensin II activates sympathetic mechanisms by a spinal action (Yashpal, Gauthier and Henry, 1987; Yashpal, Gauthier and Henry, 1989). As well, the data indicated that the effects of angiotensin II may be mediated via two different mechanisms, because a peptide antagonist, [Sar^1, Ile^8]-angiotensin II, blocked the pressor but not the cardioacceleratory response (Yashpal, Gauthier and Henry, 1989). Recently, two distinct types of angiotensin II receptor have been identified, termed AT_1 and AT_2 largely on the basis of studies using selective antagonists (Chiu et al. 1990; Smith et al. 1992; Timmermans et al. 1992; Timmermans et al. 1991). Therefore, the present study was undertaken to determine, using two newly developed non-peptide antagonists of angiotensin II, DuP 753 and PD 123319, whether two types of angiotensin II receptor mediate the respective responses and which type

of receptor is involved in expressing effects of angiotensin II on heart rate versus on arterial pressure. In addition, it was important to re-run the experiments with non-peptide antagonists in view of possible non-specific effects of [Sar^1, Ile^8]-angiotensin II in the central nervous system (Gruber, Callahan and Eskridge-Sloop, 1992).

METHODS

Surgical Preparation

Adult male Sprague-Dawley rats (275-350 g), obtained from Charles River Canada, St. Constant, Quebec, were anesthetized with urethane (1.5 g/kg, i.p.). An intrathecal catheter (Intramedic PE-10) was passed through a slit in the dura at the atlanto-occipital junction and was positioned so that the inner tip lay at the ninth thoracic (T9) spinal level, corresponding to the principal level of sympathetic neurons to the adrenals (Cummings, 1969, Backman, Sequeira-Martinho and Henry, 1990). Spinous processes were used as landmarks Post mortem examination determined correct positioning of the catheter tip.

A second polyethylene cannula (Intramedic PE-50) was filled with heparinized saline (75 IU/ml in 0.9% NaCl) and inserted into the left common carotid artery with the tip positioned approximately at the level of the aortic arch. This catheter was then connected to a Statham transducer (Gould PE 23 ID) which was attached to a Grass P5 polygraph to monitor arterial pressure and heart rate. Heart rate was determined in beats per min (bpm) by counting the number of beats in a 10 s period and multiplying by 6. In some experiments, a third catheter (Intramedic PE-50) was inserted into the right femoral vein for intravenous (i.v.) injection of various drugs.

After surgical preparation and a period of 30 min to allow the animals to stabilize, five readings of arterial pressure and heart rate were taken over a period of 10 min and averaged to give baseline values. Agents were then administered intrathecally or intravenously with zero time being the end of administration of angiotensin II. Readings of arterial pressure and heart rate were taken each minute for 15 min and then at 20 and 30 min.

Rectal temperature was maintained at approximately 37°C with a heating pad. Each rat was used in only one experiment.

Intrathecal administration of angiotensin II

Angiotensin II (Human) was purchased from Peninsula Laboratories Inc. (Lot #022661). The peptide was delivered over a period of 30-50 s at a dose of 10 μg, dissolved in 10 μl of artificial cerebrospinal fluid (CSF; an aqueous solution, in mM, of 128.6 NaCl, 2.6 KCl, 1.0 $MgCl_2$ and 1.4 $CaCl_2$; pH adjusted to 7.33). Following delivery of the peptide, the catheter was flushed with 10 μl of CSF (catheter volume was 6-8 μl). In control experiments, 10 μl of CSF replaced the angiotensin II solution.

Intravenous administration of DuP 753

The first series of pharmacological experiments with antagonists involved the use of the AT_1 receptor antagonist, DuP 753 (losartan; MK 954; 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1*H*)-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole, potassium salt). It was provided by DuPont Pharmaceuticals, Wilmington, Delaware. The antagonist was injected via the i.v. catheter, at a dose of 10 mg/kg, in a volume of 0.3 ml, 5 min prior to

intrathecal administration of angiotensin II (10 μg). The catheter was then flushed with 0.2 ml of saline (0.9% NaCl). The intravenous route was selected first because DuP 753 has been reported to have access to the central nervous system upon systemic administration (Li, Bains and Ferguson, 1993; Song, Zhuo and Mendelsohn, 1991); this point is covered further in the Discussion.

Intrathecal administration of DuP 753

In an attempt to determine whether effects of DuP 753 were expressed at the spinal level, a further series of experiments was done giving the compound intrathecally. Thus, the AT_1 receptor antagonist was given 2 min prior to injection of 10 μl of either CSF or angiotensin II (10 μg). The procedures were otherwise the same as above.

Intrathecal administration of PD 123319

In the final series of experiments the AT_2 receptor antagonist, PD 123319-0121K, 1-[[4-(Dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid, ditrifluoroacetate, dihydrate, was given intrathecally. This compound was provided by Parke-Davis Pharmaceutical Research Divisions, Warner-Lambert Co. This AT_2 antagonist was given 2 min prior to injection of 10 μl of either CSF or angiotensin II as above.

Statistical Analysis

Results from each rat were tabulated as the change in systolic and diastolic arterial pressures and heart rate. Data were calculated as the change from the mean baseline values.

Data for the figures were summarized by taking the mean ± S.E.M. of the values from each group of rats at each sample time following administration. These changes were analyzed by a *t*-test analysis (non-pairwise) comparison using SigmaStat. The level of statistical significance adopted was $p < 0.05$ and the confidence level was 90%.

RESULTS

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

Effects of intrathecal administration of angiotensin II

Angiotensin II delivered at a dose of 10 μg to the ninth thoracic (T9) spinal level 2 min after intrathecal administration of CSF (n = 10) produced an increase in both arterial pressure and heart rate. Systolic and diastolic arterial pressures both increased rapidly, to peak at 2-3 min after administration (at 26.5 ± 5.2 and 26.2 ± 6.2 mmHg, respectively). By 15 min, the pressures had returned to preadministration levels (Fig. 1). Heart rate also increased rapidly following administration, but in this case the effect peaked at about 5 min. In addition, the response persisted throughout the experiment (Fig. 2). In rats given only CSF (n = 6), there was no significant change in systolic or diastolic pressure or in heart rate. The *t*-test analysis revealed that the data from the two groups of rats were significantly different: systolic pressure at 1-15 min (8-11, 14 and 15 min, $p < 0.05$, 1-7 min. $p < 0.01$), diastolic pressure at 1-10 min (9 and 10 min, $p < 0.05$, 1-8 min, $p < 0.01$). Heart rate was different between the two groups at 2-30 min (2 and 6-15 min, $p < 0.05$, 3-5, 20

and 30 min, $p < 0.01$). These effects are the same as those found in our earlier experiments (Yashpal, Gauthier and Henry, 1987; Yashpal, Gauthier and Henry, 1989) and thus confirm the reliability of angiotensin II administration for the following experiments.

Effects of intravenous administration of DuP 753 on responses to angiotensin II

As DuP 753 has been reported to have access to central nervous system tissue from the circulation, experiments were begun with a study to determine the effects of systemic administration of the antagonist on the effects of intrathecal administration of angiotensin II on systolic pressure, diastolic pressure and heart rate. Figures 1 and 2 illustrate the effects of angiotensin II given 5 min after i.v. administration of 10 mg/kg of DuP 753 (n = 6). In this case, the responses to angiotensin II administration were blocked by intravenous administratin of DuP 753. The *t*-test analysis between the control angiotensin II group and the group pretreated with DuP 753 were significantly different: systolic pressure was different at 1-30 min (14-30 min, $p < 0.05$, 1-13 min ($p < 0.01$), diastolic pressure was different at 1-13 min (1 and 9-13 min, $p < 0.05$, 2-8 min, $p < 0.01$) and heart rate was different at 1-30 min (1 min, $p < 0.05$, 2-30 min, $p < 0.01$).

In a control group in which DuP 753 was given i.v. followed 5 min later by intrathecal administration of CSF (n = 6), there was no significant change in arterial pressure or heart rate from preadministration values (Fig. 1 and 2).

Effects of intrathecal administration of DuP 753 on responses to angiotensin II

As systemically administered DuP 753 could have expressed its effects via peripheral or spinal actions, an additional series of experiments was run, giving the antagonist

intrathecally rather than i.v. Figures 3 and 4 illustrate the effects of intrathecal administration of DuP 753 on the cardiovascular responses to intrathecal administration of angiotensin II. Administration of 10 μg of DuP 753 two min before angiotensin II was given (n = 2) blocked the pressor responses to angiotensin II; there was no significant difference between this group and the group given CSF alone.

However, the cardioacceleration induced by angiotensin II was not as obviously blocked; the increase in heart rate was delayed in onset compared to the response to angiotensin II following CSF administration. In this case, though, while the maximum change in heart rate was similar to that in the group given angiotensin II following CSF administration, the *t*-test revealed that administration of angiotensin II in the group pretreated with DuP 753 did not differ from the group given CSF alone.

To determine whether the increase in heart rate in this case was due to angiotensin II or to DuP 753, a fourth group of rats was run in which DuP 753 was given followed by CSF (n = 3). This group was not different from the group given CSF alone.

Effects of intrathecal administration of PD 123319 on responses to angiotensin II

Intrathecal administration of PD 123319 produced a rapid and transient increase in arterial pressure, as shown with the group given PD 123319 followed by intrathecal administration of CSF (n = 8; Fig. 5). In the group given angiotensin II two mins after PD 123319 was given (n = 7), the pressure change was identical to the previous group. Thus, the *t*-test analysis showed that there was no difference between the two groups. However, there was also no difference between both groups given PD 123319 and the group described above which was given two intrathecal injections of CSF.

Heart rate was affected differently from arterial pressure. The group given PD 123319 and then CSF showed a gradual increase in heart rate. However, the antagonist did not block the increase in heart rate produced by angiotensin II administration.

DISCUSSION

The present data confirm excitatory effects of angiotensin II at the spinal level on sympathetic output to the vessels and to the heart. However, they also indicate a rather complex control of sympathetic output involving both AT_1 and AT_2 angiotensin II receptors in the spinal cord. Thus, while systemic administration of the AT_1 receptor antagonist, DuP 753, blocked the pressor and cardioacceleratory responses to intrathecal administration of angiotensin II, it blocked only the pressure effects when it was given intrathecally. Therefore, the block of the heart rate response by systemic administration could be due to a peripheral effect. A central site of action in the antagonism of the pressor response is consistent with the observation that DuP 753 does not inhibit pressor responses to sympathetic nerve stimulation in the pithed rat (Ohlstein et al. 1992).

A central site for the block of the pressor response is supported by evidence which suggests that peripherally administered DuP 753 has access to central neurons. It has been claimed that DuP 753 does not cross the blood-brain barrier because dipsogenic and pressor responses to i.c.v. administration of angiotensin II are not blocked by systemic administration of DuP 753 (Bui, Kimura and Phillips, 1992; Wong et al. 1990). However, this contradicts other evidence that systemic administration of DuP 753 does block the dipsogenic effects of angiotensin II given i.c.v. (Fregly and Rowland, 1991). Further supporting the ability of

DuP 753 to cross the blood-brain barrier is recent evidence that systemic administration of DuP 753 inhibits the responses of paraventricular nucleus neurons to local application of angiotensin II and to electrical stimulation of the subfornical organ in the rat (Li, Bains and Ferguson, 1993). Finally, i.v. administration of DuP 753 inhibits subsequent binding of [Sar^1, Ile^8]-angiotensin II in circumventricular organs, paraventricular hypothalamus, median preoptic nucleus and nucleus of the tractus solitarius (Song, Zhuo and Mendelsohn, 1991). Thus, multidisciplinary evidence supports the possibility that systemically administered DuP 753 in the present study blocked the pressor effect of angiotensin II by an action in the spinal cord.

The effects of DuP 753 are similar to those reported earlier from experiments using the peptide antagonist [Sar^1, Ile^8]-angiotensin II (Yashpal, Gauthier and Henry, 1989). This antagonist, when given intrathecally, also blocked the pressor but not the cardioacceleratory effects of angiotensin. However, it caused a gradual increase in heart rate without altering baseline arterial pressure when it was administered alone intrathecally. In addition, the peptide antagonist did not block the cardioacceleratory response to angiotensin II administration (Yashpal, Gauthier and Henry, 1989). Therefore, the present data show that DuP 753 and [Sar^1, Ile^8]-angiotensin II differ somewhat in their properties; this is significant in view of the claim that in supraspinal structures the peptide antagonist occupies AT_1 receptors (Rowe, Saylor and Speth, 1992)

Our data from experiments with the AT_2 receptor antagonist, PD 123319, indicate that it blocks the pressor response, because there was no difference between the groups administered the antagonist followed by either CSF or angiotensin II. On the other hand, the cardioacceleration resulting from intrathecal administration of angiotensin II was not blocked.

This suggests that AT_2 receptors are involved in pathways to the vessels but not in those to the heart.

In addition, the antagonist also had effects similar to angiotensin II, in that given alone (in the group given the antagonist followed by CSF) a transient increase in systolic and diastolic pressures was observed along with a slowly developing but sustained increase in heart rate. Therefore, the possibility remains that the "antagonism" of the pressor effects of angiotensin II by PD 123319 were due to desensitization of AT_2 receptors.

In peripheral tissues, AT_1 and AT_2 subtypes seem to mediate the biological actions of angiotensir. II via different signal transduction pathways. AT_1 has been shown to interact with G proteins causing a decrease in cellular cAMP and a stimulation of both inositol 1,4,5-trisphosphate (IP_3) and diacylglycerate (DAG) generation. AT_2, on the other hand, does not require G proteins and has actually been shown to decrease cGMP when stimulated. It is not clear what its action is in the brain, but stimulation of angiotensin II receptors in the brain evokes many autonomic, endocrine and behavioral responses.

The rapid effects of angiotensin II in eliciting pressor and cardioacceleratory effects in the present study is consistent with earlier evidence of a role as a chemical mediator of synaptic transmission in spinal sympathetic pathways. Angiotensin II-like immunoreactive material is found in the thoracic lateral horn (Fuxe et al. 1976), specifically in nerve terminals making synaptic contact with neurons (Galabov, 1992). In addition, angiotensin II-like immunoreactivity is absent in the lateral horn of rats transected spinally one week previously (Ganten et al. 1978) and this is consistent with the suggestion that the source of angiotensin II is descending fibers from magnocellular cells in diencephalic nuclei (Brownfield et al. 1982). Thus, it appears that angiotensin II may be a chemical mediator

of synaptic transmission onto spinal sympathetic neurons in pathways to the vessels and that both AT_1 and AT_2 receptors mediate the effects of angiotensin II on arterial pressure. Finally, in view of the suggestion that DuP 753 may be useful in the treatment of hypertension (DePasquale et al. 1992; Jablonskis et al. 1992; Mizuno et al. 1992; Toney and Porter, 1993), one of the sites at which it acts may be on spinal neurons.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council of Canada. The gifts of DuP 753, from the Du Pont Merck Pharmaceutical Company, Wilmington, Delaware, and of PD 123319, from Parke-Davis, Pharmaceutical Research Division, Ann Arbor, Michigan, are gratefully acknowledged.

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FIGURES

Fig. 1: Time-effect curve for the effects of intravenous adminstration of DuP 753 on the systolic and diastolic arterial pressure responses to intrathecal administration of angiotensin II (10 μg) or CSF at T9. DuP 753 was given at a dose of 10 mg/kg. ●-●, CSF followed by angiotensin II (n=10); ▵-▵ DuP 753 followed by CSF (n=6); ▲-▲ DuP 753 followed by angiotensin II (n=6). Each ordinate represents the mean (± S.E.M.) change from the preadministration values of systolic arterial pressure (SAP) and diastolic arterial pressure (DAP). Zero time was the end of the second administration.

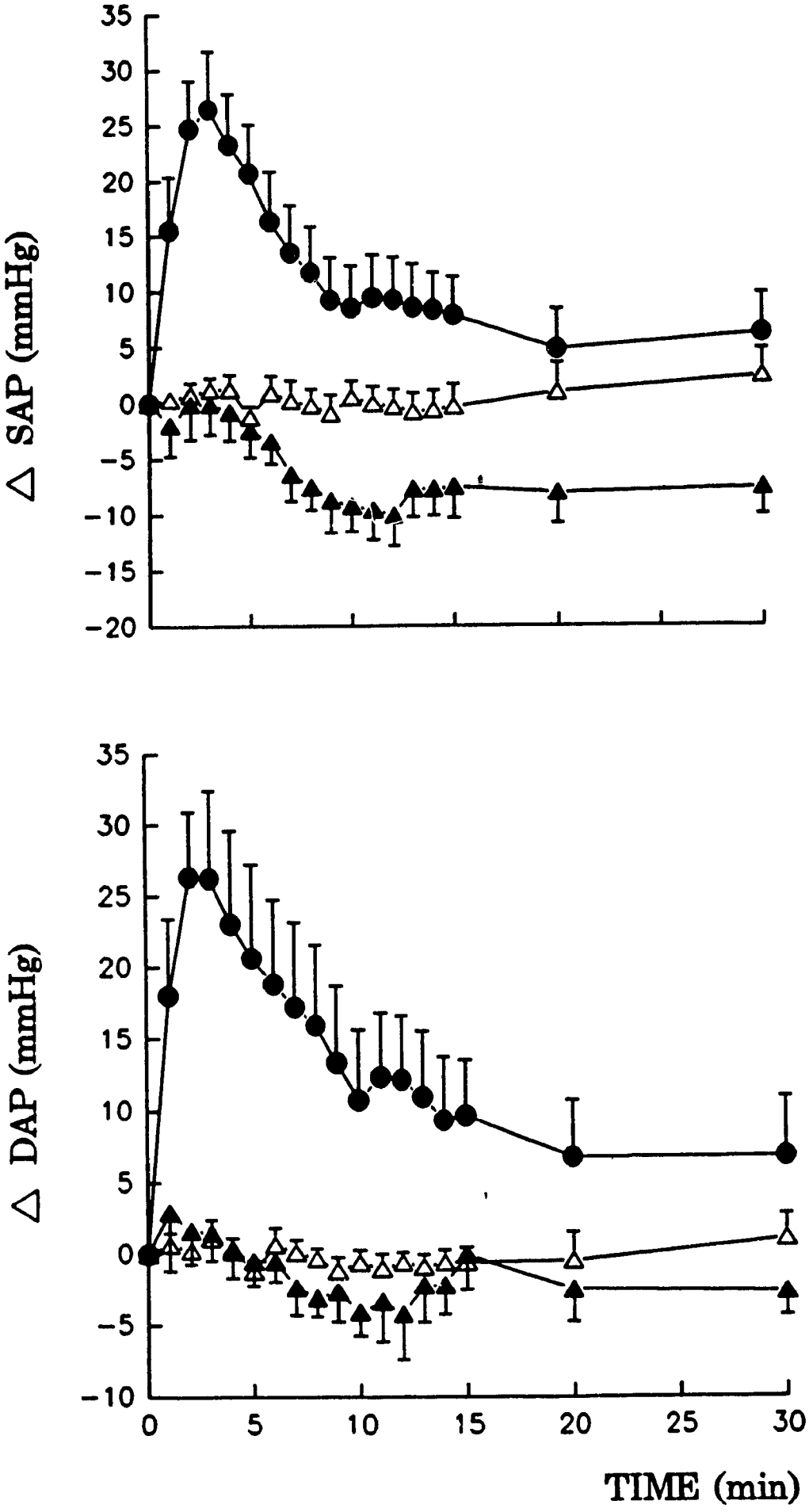

Δ SAP (mmHg)
35
30
25
20
15
10
5
0
−5
−10
−15
−20
Δ DAP (mmHg)
35
30
25
20
15
10
5
0
−5
−10
0
5
10
15
20
25
30
TIME (min)

Fig. 2: Time-effect curve for the effects of intravenous administration of DuP 753 on the heart rate response to intrathecal administration of angiotensin II (10 μg) or CSF at T9. DuP 753 was given at a dose of 10 mg/kg. ●-●, CSF followed by angiotensin II (n=10); ▵-▵ DuP 753 followed by CSF (n=6); ▲-▲ DuP 753 followed by angiotensin II (n=6). Each ordinate represents the mean (± S.E.M.) change from the preadministration values of heart rate (HR). Zero time was the end of the second administration.

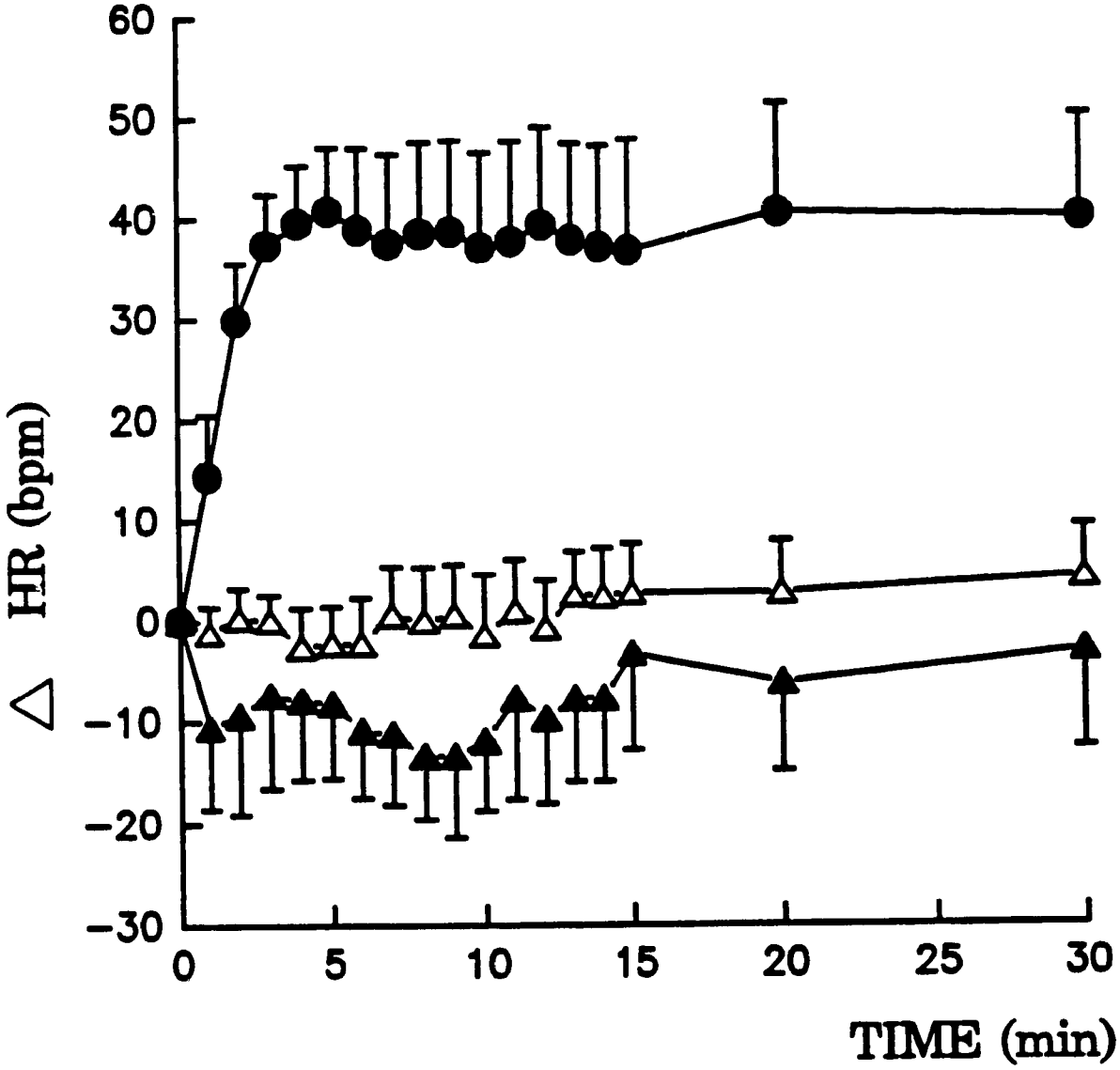
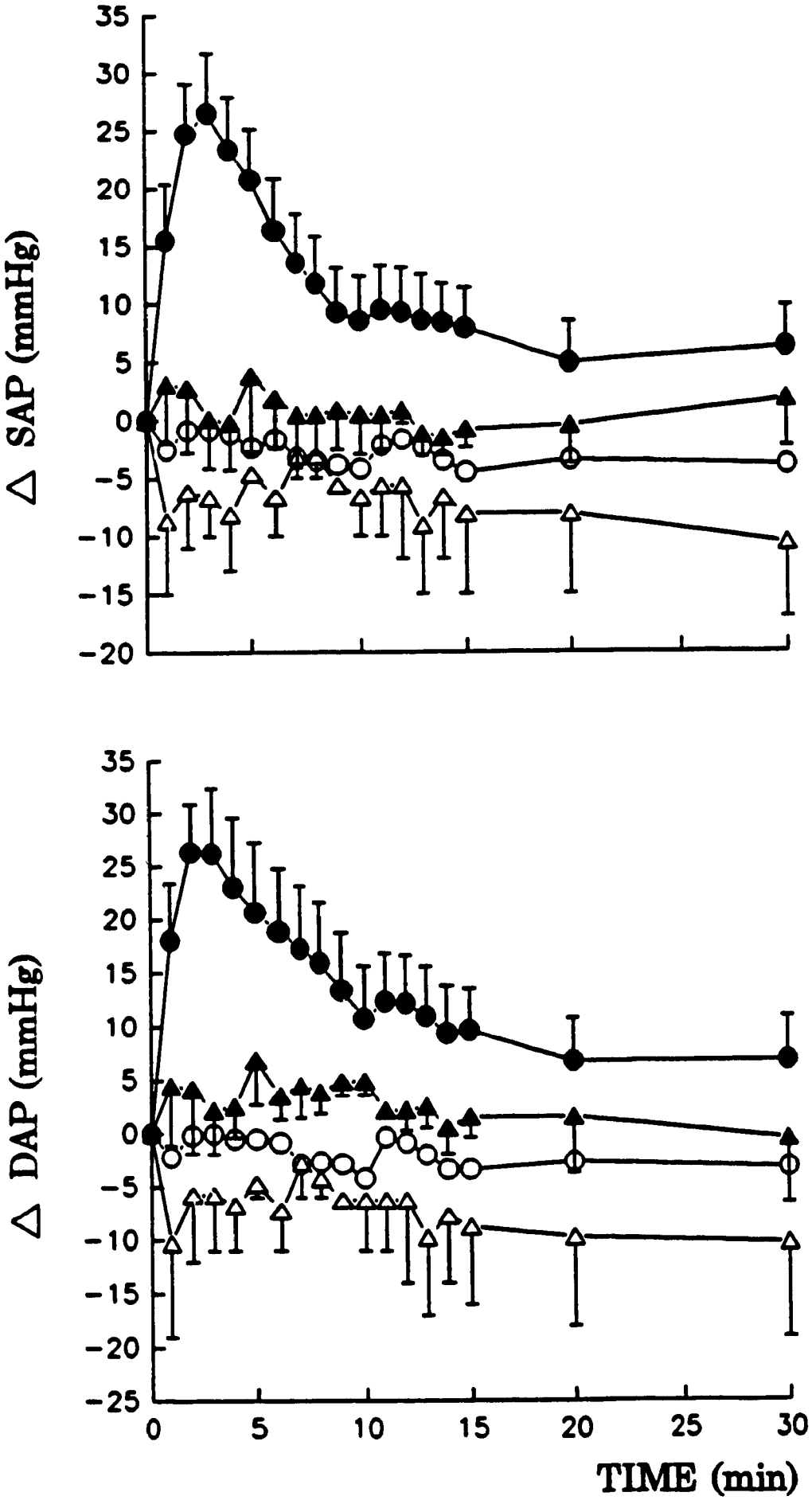

60
50
40
30
20
10
0
−10
−20
−30
△ HR (bpm)
0
5
10
15
20
25
30
TIME (min)

Fig. 3: Effects of pretreated rats with CSF or with DuP 753, given intrathecally at a dose of 10 μg, on the change in arterial pressure in response to intrathecal administration of angiotensin II (10 μg) or CSF at T9. ○-○ CSF (two doses; n=6); ●-●, CSF followed by angiotensin II (n=10); ▵-▵ DuP 753 followed by CSF (n=3); ▲-▲ DuP 753 followed by angiotensin II (n=2). Details are otherwise the same as in Figure 1.


Δ SAP (mmHg)
35
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20
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5
0
−5
−10
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−20
Δ DAP (mmHg)
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30
25
20
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0
−5
−10
−15
−20
−25
0
5
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15
20
25
30
TIME (min)

Fig. 4. Effects of pretreated rats with CSF or with DuP 753, given intrathecally at a dose of 10 μg, on the change in heart rate in response to intrathecal administration of angiotensin II (10 μg) or CSF at T9. ○-○ CSF (two doses; n=6); ●-●, CSF followed by angiotensin II (n=10); ▵-▵ DuP 753 followed by CSF (n=3); ▲-▲ DuP 753 followed by angiotensin II (n=2). Details are otherwise the same as in Figure 2.

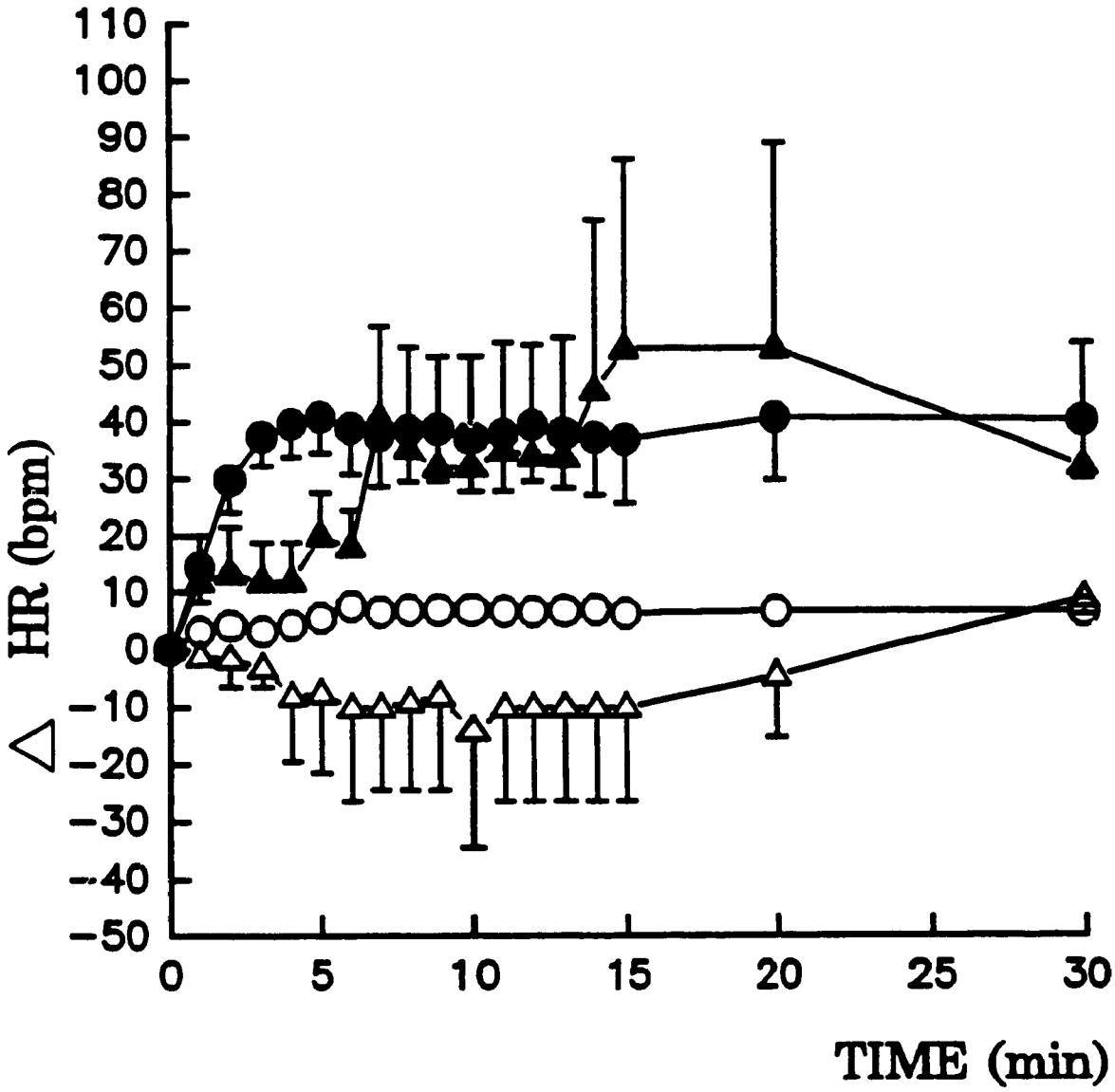
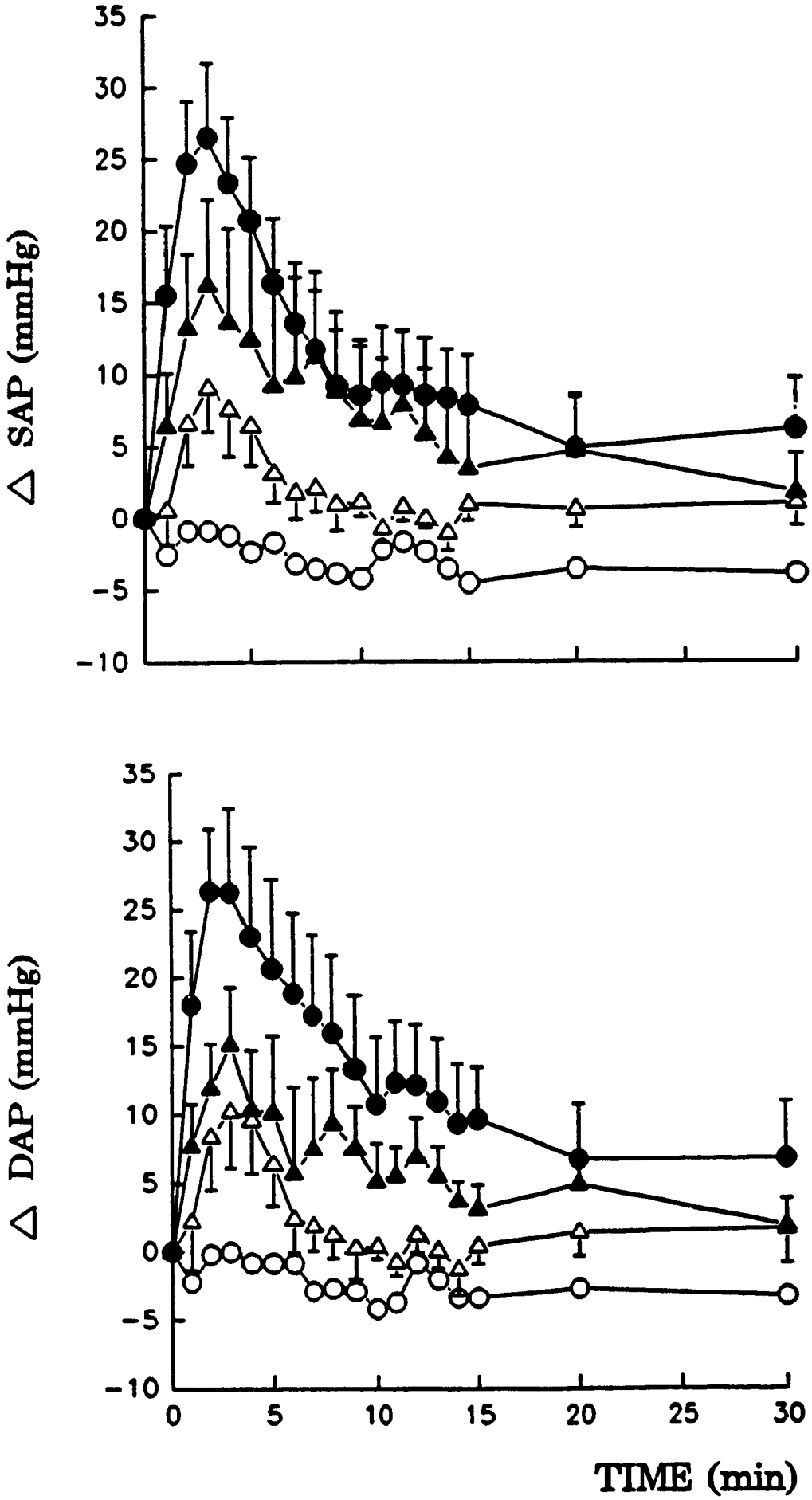

110
100
90
80
70
60
50
40
30
20
10
0
−10
−20
−30
−40
−50
Δ HR (bpm)
0
5
10
15
20
25
30
TIME (min)

Fig. 5: Effects of intrathecal administration of PD 123319 on the systolic and diastolic arterial pressure responses to intrathecal administration of angiotensin II (10 μg) or CSF at T9. PD 123319 was given intrathecally at a dose of 10 μg. o-o CSF (two doses; n=6); ●-●, CSF followed by angiotensin II (n=10); ▵-▵ PD 123319 followed by CSF (n=8); ▲-▲ PD 123319 followed by angiotensin II (n=7). For comparative purposes, the data from Figure 1 representing the group given CSF followed by angiotensin II are included. Details are otherwise the same as in Figure 1.


35
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25
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15
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−5
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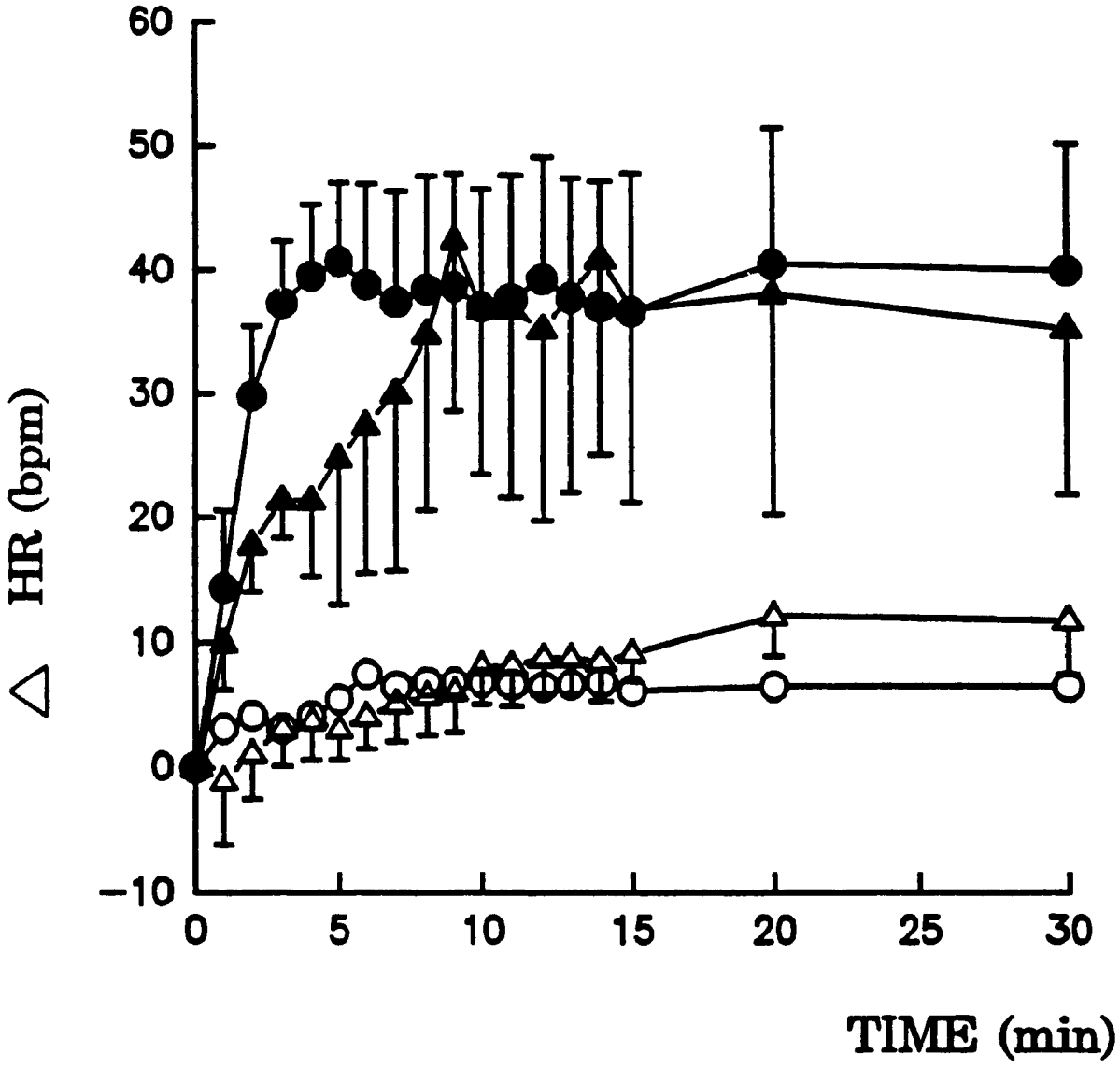
Δ SAP (mmHg)
Δ DAP (mmHg)
0
5
10
15
20
25
30
TIME (min)

Fig. 6: Effects of intrathecal administration of PD 123319 on the heart rate response to intrathecal administration of angiotensin II (10 μg) or CSF at T9. PD 123319 was given at a dose of 10 μg. ○-○ CSF (two doses; n=6); ●-●, CSF followed by angiotensin II (n=10); ▵-▵ PD 123319 followed by CSF (n=8); ▲-▲ PD 123319 followed by angiotensin II (n=7). Details are otherwise the same as in Figure 2.


60
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−10
△ HR (bpm)
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TIME (min)

CHAPTER V

DISCUSSION AND CLAIMS OF ORIGINALITY

V. DISCUSSION AND CLAIMS OF ORIGINALITY

This thesis has examined the roles of chemical neurotransmitters on the effects of sympathetic output to the heart and vessels in normal and abnormal conditions (e.g. spinal transection) using the intrathecal technique. The significance of these results presented in this thesis will be discussed only briefly here as it is discussed in detail in the respective chapters.

A. Substance P

The second chapter illustrates that substance P is involved in regulation of arterial pressure and heart rate in acutely spinalized animals and that it has differential effects on both parameters. Therefore, the results indicate that the action of substance P does not require supraspinal structures in mediating activation of sympathetic output to the heart and vessels and is mediated at least partly by a spinal mechanism. The potentiation of the heart rate response in the presence of naloxone suggests that an opioid mechanism depresses the cardioacceleratory response to SPNs under normal conditions. The data also suggest that an intrinsic spinal mechanism may contribute to essential hypertension in spinalized animals and that substance P may contribute to autonomic hyperreflexia which occurs after spinal transection in humans.

B. Angiotensin II

The third chapter illustrates that there are two different angiotensin II receptor subtypes involved in control of sympathetic output at the spinal level: AT_1, which is specific to the antagonist DuP 753, and AT_2, which is specific to the antagonist PD 123319. The AT_1 antagonist blocked the pressor and cardioacceleratory responses whereas the AT_2 antagonist

had no effect on either parameter. The results indicate that the AT_1 receptor subtype but not the AT_2 receptor subtype is involved in the spinal regulation of sympathetic output.

C. CLAIMS OF ORIGINALITY

The main findings described in this thesis are original. These findings ma be summarized as follows:

1. Intrathecal administration of substance P increases arterial pressure and heart rate in acutely spinalized animals.

2. In T5-transected animals, substance P given at T9 fails to elicit cardioacceleration whereas at T2, substance P increases heart rate indicating that cardioacceleratory mechanisms lie rostral to the T5 level.

3. In T2-transected animals, substance P given at T9 increases heart rate, indicating long intraspinal pathways related to control of heart rate.

4. In naloxone pretreated animals, cardioacceleration is potentiated upon intrathecal administration of substance P, indicating an opioid depression of cardioacceleratory mechanisms.

5. Intrathecal administration of the AT_1 receptor antagonist, DuP 753, but not the AT_2 receptor antagonist, PD 123319, blocked the cardiovascular responses to intrathecal administration of angiotensin II, indicating mediation of the responses by AT_1 receptors.

6. Systemic administration of DuP 753 blocked the cardiovascular responses to intrathecal administration of angiotensin II, indicating that the antagonist crosses the blood-brain barrier.