BRAINSTEM NEURONS INVOLVED IN THE CONTROL OF THE

CARDIOVASCULAR SYSTEM.

by

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SUMMARY

Neurons that lie within the ventrolateral medulla
have been implicated in the tonic and reflex control of
the cardiovascular system. In this thesis the chemical
content of these neurons, their effect on sympathetic
outflow and the release of putative neurotransmitters
from their terminals has been investigated.

Chemical stimulation and inhibition of the
ventrolateral medulla revealed that the changes seen in
blood pressure were associated with parallel changes in
sympathetic nerve activity, suggesting that bulbospinal
sympathoexcitatory pathways were responsible for
mediating these effects. In addition it was found that
the increase in blood pressure that follows anodal
electrolytic lesions of the caudal ventrolateral
medulla is closely associated with an increase in
sympathetic nerve activity, suggesting that it is an
increase in bulbospinal traffic that is responsible for
these changes rather than an increase in plasma
vasopressin.

The importance of serotonergic and
catecholaminergic neurons in the brain in mediating
changes in the activity of sympathetic nerves was
assessed by recording sympathetic nerve activity,
plasma vasopressin, adrenaline and noradrenaline, in

response to intracisternal injections of the neurotoxins 6-hydroxydopamine and 5,7-dihydroxytryptamine. In both cases increases in sympathetic nerve activity were seen, although there was a differentiated response in terms of the changes in the levels of plasma hormones.

In chapters 5,6 and 7 the release of putative neurotransmitters (neuropeptide Y and serotonin) from the spinal cord was used as an index of the activity of spinally projecting pathways that utilise these substances. Two methods were used, firstly the technique of in vivo tissue dialysis was adapted for the measurement of serotonin in the thoracic spinal cord of the rat. Secondly, spinal subarachnoid space perfusion was used in the rabbit in order to measure the release of neuropeptide Y like immunoreactivity. It was found that stimulation of the serotonin-containing cells in the rostral ventrolateral medulla of the rat increased blood pressure and the release of serotonin from the spinal cord. Both the increase in arterial pressure and the release of serotonin were prevented by prior destruction of central serotonergic pathways with 5,7-dihydroxytryptamine. Similarly stimulation of the rostral ventrolateral medulla of the rabbit elicited an increase in arterial pressure and the concentration of neuropeptide Y - like immunoreactivity in the

subarachnoid space. An increase in arterial pressure and an increase in the release of neuropeptide Y- like immunoreactivity was also seen after inhibition of the caudal ventrolateral medulla, suggesting that the pressor and depressor effects that can be elicited from the caudal ventrolateral medulla are due to changes in the activity of spinally - projecting neuropeptide Y - containing neurons of the rostral ventrolateral medulla.

In an attempt to determine whether or not the serotonin or neuropeptide Y containing neurons participate in the baroreflex control of blood pressure, the release of these compounds from the spinal cord was measured before and after acute sinoaortic denervation in urethane anaesthetized rabbits. Although this procedure resulted in an elevation of arterial pressure, there was no increase in the release of either substance. Since on the other hand there was an increase in plasma vasopressin levels that could account for the increase in arterial pressure, it seems likely that acute sinoaortic denervation in this context is not a suitable stimulus of bulbospinal sympathoexcitatory pathways.

Finally, the localization of substance P and catecholamines within neurons of the ventrolateral medulla was examined. It was found in the rat that the

extent of colocalization of substance P with the adrenaline synthesizing phenylethanolamine- N-methyl transferase was small, and dual labelled fibres were seen only rarely in the spinal cord. Similarly, no colocalization of substance P with catecholamine fluorescent neurons was observed in the rabbit.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

P.M. PILOWSKY

PUBLICATIONS

Publications arising from work presented in this thesis:

Pilowsky P.M., West M.J. Chalmers J.P. (1985) Renal sympathetic nerve responses to stimulation, inhibition and destruction of the ventrolateral medulla in the rabbit. Neurosci. Letts. 60: 51-55.

Chalmers J.P., Kapoor V., Macrae I.M., Minson J.B., Pilowsky P.M. West M.J. (1985) New approaches to the study of bulbospinal (B3) serotonergic neurons in the control of blood pressure. J. Hypertension 3: 55-59.

Pilowsky P.M., Kapoor V., Minson J.B., West M.J. Chalmers J.P. (1986) Spinal cord serotonin release and raised blood pressure after brainstem kainic acid injection. Brain. Res. 366: 354-357.

Pilowsky P.M., Morris M.J., Kapoor V., West M.J. Chalmers J.P. (1986) Role of renal nerve activity, plasma catecholamines and plasma vasopressin in cardiovascular responses to intracisternal neurotoxins in the rabbit. J. Autonom. Nerv. Syst. 17: 109-120.

Pilowsky P.M., Minson J.B., Hodgson A.J., Howe P. Chalmers J.P. (1986) Does substance P coexist with adrenaline in neurons of the rostral ventrolateral medulla in the rat? Neurosci. Letts. 71:293-298

Chalmers J.P., Kapoor V., Mills E., Minson J.B., Morris M.J., Pilowsky P.M. West M.J. (1987) Pressor neurones from the ventrolateral medulla containing amines and peptides. Can. J. Physiol. Pharmacol. In press

Morris M.J., Pilowsky P.M., Minson J.B., West M.J. Chalmers J.P. (1987) Microinjections of kainic acid into the rostral ventrolateral medulla causes hypertension and release of neuropeptide Y like immunoreactivity rabbit spinal cord. Clin. Exp. Physiol. Pharmacol. In Press

Pilowsky P.M., Morris M.J., Minson J.B., West M.J., Chalmers J.P., Willoughby J.O. Blessing W.W. (1987) Inhibition of vasodepressor neurons in the caudal ventrolateral medulla of the rabbit increases the release of neuropeptide Y like immunoreactivity from the spinal cord and arterial pressure. Brain Res. submitted.

ACKNOWLEDGEMENTS

Chapter 1: Table of Contents

<u>General Introduction</u>

<u>1 Historical Background</u>

The role of the central nervous system in the   control
of vasomotor tone was established in the latter part of the
nineteenth century, with the observation by Claude   Bernard
and others that spinal transection dramatically lowered
blood pressure (see review by Bard 1930). It was not   until
1870 however, when Dittmar reported that stimulation of the
central end of a cut sciatic nerve elicited a reflex
increase in arterial pressure that was not abolished by
separating the medulla and spinal cord from the rest of the
brain, that that portion of the central nervous system
responsible for tonic vasomotor control was found to be
situated in the medulla (Dittmar 1870).   A more precise
definition of the area responsible for this reflex was
achieved in 1871 by Owsjannikow who sectioned the brainstem
from rostral to caudal until effects on the   sciatic
nerve-blood pressure reflex were observed (Owsjannikow
1871). He reported that the region of the brainstem that
was important in this respect lay in an area one to two
millimeters caudal to the inferior colliculi and four to
five millimeters rostral to the calamus scriptorius.

Following the work of Dittmar (1870) and Owsjannikow

(1871) a number of studies were performed in the first half of the twentieth century, in which the medulla oblongata was stimulated electrically and blood pressure or sympathetic nerve activity recorded (Ranson and Billingsley 1916; Wang and Ranson 1939; Alexander 1946). These studies were made possible by the invention of the Horsley-Clarke stereotaxic apparatus that enabled accurate and reproducible stimulation of centres within the substance of the brain for the first time. Using this technique Alexander (1946) was able to map those areas in the brainstem from which electrical stimulation elicited changes in blood pressure and efferent sympathetic nerve activity. An important drawback of this technique however is its non-specificity as noted by Bard (1930). In this far-sighted review of the field, Bard (1930) states that 'It is possible that instead of being the sites of vasoconstrictor and vasodilator centres, these small areas represent points on the course of tracts connected with the true centre.'. To a limited extent these problems can be overcome by the use of chemical stimulation and inhibition of neurons as will be discussed later. In addition to his descriptions of the effect of electrical stimulation of the medulla oblongata on efferent sympathetic nerve activity, Alexander (1946) also examined the effect of brainstem transections on blood pressure and efferent sympathetic nerve activity. He found, in agreement with the work of Dittmar (1870) and Owsjannikow (1871), that a transection of the brainstem just caudal to the 'pressor area' led to a profound fall in arterial pressure that was

not influenced by further transections. However, he also reported that a transection at this point (caudal to the pressor area) led to a complete abolition of activity in the sympathetic nerve, and that a small amount of nerve activity could be restored by a further transection below the 'depressor area', providing evidence for the existence of a tonically active spinally-projecting depressor centre, a subject which is still a matter of controversy today.

These early studies led to the hypothesis that the centres for the tonic and reflex control of blood pressure lay within a diffuse area of the medullary reticular formation.

The landmark discovery by Dahlstrohm and Fuxe (1964) that under certain conditions of fixation, catechol- and indole- amines could be rendered fluorescent, and their subsequent description of spinally-projecting catechol- and indole- amine containing neurons in the medulla oblongata and pons, suggested a role for these cell groups in the control of blood pressure. The subsequent use of immunohistochemical techniques for the demonstration of putative neurotransmitters and neuron related antigens, has expanded enormously the number of different neuronal populations that may be involved in cardiovascular control. Studies on a possible role for these different cell groups will be discussed below.

2 The neural circuitry of brainstem cardiovascular control

The neural circuitry underlying the control of the cardiovascular system is extremely diverse. Inputs from pulmonary, cardiac, aortic and carotid receptors, relay a variety of information to the nucleus tractus solitarius (NTS), from where integrated information is relayed to all levels of the neuraxis. Efferent pathways include the vagal and sympathetic motorneurons as well as vasoactive substances released from the pituitary. In addition, neurons impinging on these pathways, have the potential for modifying the neural signal at any point along its course. Although the peripheral efferent and afferent systems are well understood, due in large part to their greater accessibility, considerable controversy surrounds the pathways in between.

In this section I will be focussing primarily on afferent, integrative and efferent pathways, their location, projections, inputs, chemical content and relationship to the tonic and reflex control of blood pressure.

2.1 Afferent pathways

2.1.1 Location and projections

The cell bodies of the primary afferent neurons
are situated in the nodose ganglion in the case of the
aortic baroreceptors and pulmonary stretch afferents
(Donoghue et al 1982 a,b), and in the petrosal
ganglion of the glossopharyngeal nerve in the case of
the carotid baro- and chemo- receptors (Macdonald
1983; Donoghue et al 1984). The central projections
of these neurons have been shown to terminate in the
NTS and area postrema by histological (Cottle 1964;
Berger 1979; Wallach and Loewy 1980; Kalia and
Mesulam 1980; Panneton and Loewy 1980; Ciriello and
Calaresu 1981; Ciriello 1983; Seiders and Stuesse
1984) and electrophysiological (Crill and Reis 1968;
Miura and Reis 1969; Lipski et al 1975; Miura and
Kitamura 1979; Ciriello and Calaresu 1981; Donoghue
et al 1982 a,b 1984; Czachurski et al 1982)
techniques. Terminations outside this area have been
proposed to occur in the paramedian reticular
formation (Crill and Reis 1968). Very extensive
lesions of this region have been reported to affect
the cardiovascular responses to electrical stimulation

of the carotid sinus nerve (Miura and Reis 1972). On the other hand Lipski et al (1975), in attempting to replicate the findings of Crill and Reis (1968) reported that the sinus nerve responses obtained by stimulation of the paramedian reticular formation were artefactual. Indeed in a later study by Miura and Kitamura (1979) in which the activity of brainstem neurons was recorded, it was found that the onset latency of post-synaptic potentials of neurons in the paramedian reticular formation was on average greater than four milliseconds suggesting a polysynaptic pathway to this region. It therefore seems likely that the major site of termination of cardiovascular afferent neurons is the NTS, with a small number occurring in the area postrema.

2.1.2 Neurotransmitter candidates

The chemical messenger responsible for the transmission of baroreceptor information is unknown. There are two major candidates, glutamate and substance P.

2.1.2.1 Glutamate

Elucidation of a possible neurotransmitter role for glutamate has been hampered by the lack of a method for the immunocytochemical identification of glutamate-containing neurons. The recent description by Storm-Mathisen et al (1983) of an antibody to glutamate has not so far been followed by a detailed localization of its distribution in fibres and neurons of the medulla.

The biochemical and physiological evidence for glutamate as the transmitter of primary afferent neurons in the baroreflex arc is circumstantial, and at times contradictory. The possibility that glutamate may be the transmitter of baroreceptor afferent neurons was first suggested by Talman et al in 1980, and in subsequent reports from the same group (Perrone 1981; Talman et al 1981b; Reis et al 1981; Granata et al 1983a; Talman et al 1984). Three major lines of evidence were given. First, unilateral nodose ganglionectomy was reported to elicit rapid and selective falls in high-affinity glutamate uptake and glutamate concentrations in the NTS (Talman et al 1980, 1981b, 1984; Reis et al 1981), whilst the levels of glutamine, glycine, gamma-aminobutyric acid (GABA) and aspartate were

unchanged. These experiments were repeated in 1985 by Simon et al, who found that unilateral nodose ganglionectomy had no effect on glutamate uptake. The reason for the discrepancy in these reports is unclear and suggests the need for further studies. Secondly, following pre-loading of the NTS with tritiated amino acids, stimulation of the vagus nerve was found to elicit a release of tritiated glutamic acid and aspartic acid into a push-pull cannula situated in the intermediate portion of the NTS (Reis et al 1981; Granata and Reis 1983; Talman et al 1984). Although this appears to be quite strong evidence, several points need to be borne in mind, as noted by the authors, pre-loading with exogenous compounds carries the risk that these compounds are being subsequently released by neurons that do not normally synthesize them, furthermore, even if they are being released by glutamatergic neurons, these may be interneurons within the NTS, and not the primary afferent neurons. In addition, the finding that aspartic acid is also released needs to be reconciled with the findings from the same group that nodose ganglionectomy elicits falls in glutamate but not aspartate in this region. Finally, since it is tritium rather than glutamate that is being measured, the possibility that the evoked release consists of a metabolic product of glutamate needs

to be considered. The third line of evidence that
has been adduced to support a role for glutamate as
the transmitter of the primary afferent neurons
comprises studies on   the   effects   of   applying
glutamate, its agonists and antagonists, to the NTS
by microinjection and observing their effects on
blood pressure, heart rate and baroreflex function.
When injected into the intermediate portion of the
NTS, glutamate elicits hypotension and bradycardia
(Talman et al 1981b 1984; Reis et al 1981).   This
effect is mimicked by the application of low doses
of kainic acid (Talman et al 1981b) and antagonised
by glutamate diethylester (Talman et al 1981b,
1984; Humphrey and McCall 1984). Although these
findings support the hypothesis that glutamate may
be an integral part of the baroreflex arc, they do
not provide specific evidence that glutamate acts
at the first synapse in the arc.   A further
criticism that may be levelled at these results is
that although glutamate and kainic acid act on cell
bodies, they have also been shown to depolarize
primary afferent neurons (Agrawal and Evans 1986),
raising the possibility that the effects of these
compounds is mediated indirectly by evoking the
release of endogenous neurotransmitter.

Thus, although some evidence exists to support
a role for glutamate as   the   transmitter   of

baroreceptor   afferents, this   is   by   no   means
conclusive.

2.1.2.2 Substance P

The   second   major   candidate   for   the
neurotransmitter   of   the   baroreceptor   afferent
neurons is substance P.   As   with   glutamate,   the
evidence that this   peptide   acts in such a way is
circumstantial. Substance P was first reported as
an unidentified vasodilator substance by von Euler
and Gaddum in 1931.   These authors demonstrated its
presence in a wide variety of tissues, with highest
concentrations in   small   intestine,   brain   and
stomach. The   amino   acid   sequence was determined
fifty years later by Chang et al (1971).

In contrast to glutamate, the localization of
substance   P by immunocytochemistry   has   been
extensively reported (Cuello   and   Kanazawa 1978;
Ljungdahl et al 1978; Maley and Elde 1982;   Kawano
and Chiba 1984;   Gallagher et al 1985;   South and
Ritter 1986),   as   has   the   autoradiographic
localization of substance P receptors (Helke et al
1984).   These reports have shown the presence of
substance P-like immunoreactivity   in the carotid

body (Gallagher et al 1985), in the peripheral
ganglia of the primary afferents histochemically
(Gallagher et al 1985) and biochemically (Gillis et
al 1980), and in the nucleus of the solitary tract
both histochemically (Cuello and Kanazawa 1978;
Ljungdahl et al 1978; Maley and Elde 1982; Kawano
and Chiba 1984; Gallagher et al 1985; Maley 1985)
and biochemically (Gillis et al 1980). In
particular, substance P-like immunoreactivity has
been localized within fibres in those portions of
the NTS (medial, commisural and lateral) where
cardiopulmonary afferents have been shown to
terminate by anterograde tracing techniques (Cottle
1964; Berger 1979; Panneton and Loewy 1980;
Wallach and Loewy 1980; Maley and Elde 1982;
Ciriello 1981, 1983; Maley et al 1983; Lorez et
al 1983; Voorn and Buijs 1983; Kalia et al 1984;
Seiders and Stuesse 1984), although these afferents
have not been shown to contain substance P-like
immunoreactivity in the NTS. Indeed some studies
have suggested that most of the substance P-like
immunoreactivity present in the NTS may be derived
either from trigeminal afferents or intrinsic
systems (Cuello et al 1978; Helke et al 1981;
Lorez et al 1983; South and Ritter 1986)
Interestingly, ultrastructural studies on the
relationship of terminals containing substance
P-like immunoreactivity to neurons in the NTS have

shown that these terminals tend to form synapses with distal dendritic spines rather than with proximal dendrites or cell bodies (Maley 1985). This pattern of innervation has been correlated with a lesser influence of an input than a more proximal innervation (Rall 1967). Thus substance P satisfies some but not all of the histological criteria for the role of the primary afferent transmitter in the baroreflex pathway.

The physiological studies that have been performed in order to test the role of substance P as a transmitter in the baroreflex pathway are similar to those described for glutamate. First, chronic denervation of the afferent fibres of the glossopharyngeal and vagus nerves as they enter the brainstem has been reported to elicit a 45-53% decrease in substance P-like immunoreactivity in the intermediate and commisural portions of the the NTS (Gillis et al 1980), although these authors found no change in the substance P-like immunoreactivity of the corresponding ganglia. In addition, vagotomy and nodose ganglionectomy were found to have no effect on ^{125}I-substance P binding in the NTS. Secondly, pretreatment of animals with capsaicin, a compound that selectively destroys unmyelinated primary afferent fibres (Nagy et al 1981) elicited a reduction in substance

P-like immunoreactivity in some situations (Atkinson and Chaggar 1983; Gamse et al 1986), but not others (Gallagher et al 1985). The effect of such treatment on the baroreflex (Bond et al 1982; Furness et al 1982; Lorez et al 1983; Lembeck and Donnerer 1983) and chemoreflex (Bond et al 1982; Gallagher et al 1985) has been assessed with variable results. In most studies in which the baroreflex has been tested in capsaicin treated animals there was no effect on baroreflex function (Furness et al 1983; Lorez et al 1983; Lembeck and Donnerer 1983). On the other hand, Bond et al (1982) reported a significant attenuation of the baroreceptor reflex in anaesthetized rats following neonatal treatment with capsaicin. These authors found that the pressor response to bilateral carotid occlusion was decreased from about 25 mmHg to about 15 mmHg. No explanation for the discrepancy was offered, however it should be noted that the baseline blood pressures in the two groups were different as were the group variances; neither of these factors appeared to have been taken into account in the statistical analysis of these experiments.

Thirdly, a number of studies have been performed on the effect of applying substance P to the NTS. The first of these studies was reported

by Haeusler and Osterwalder in 1980, who found that
application of substance P to the NTS in rats and
cats elicited a dose-related fall in sympathetic
nerve activity. This effect was also produced by
the first application of capsaicin, with subsequent
applications being ineffective, consistent with its
action of causing release followed by destruction.
These effects were not produced by vehicle alone,
or by vehicle containing luteinizing
hormone-releasing hormone (a peptide that has a
similar molecular weight to substance P),
supporting the view that these effects were
specific to substance P. These findings have been
challenged by Talman and Reis (1981), who reported
that the microinjection of substance P was only
effective when given in volumes large enough to
elicit falls in blood pressure and heart rate
through non-specific actions. A similar lack of
effect of substance P has been reported by others
(Carter and Lightman 1983, 1985). On the other
hand, electrophysiological evidence is available to
support a neurotransmitter role for substance P in
the NTS (Henry and Sessle 1985; Morin-Surin et al
1984), a notion that is supported by biochemical
studies that have shown decreased levels of
substance P in the NTS following prolonged
haemorrhagic hypotension (Feuerstein et al 1984).
Although these studies would, on the whole, suggest

that substance P is not the transmitter of primary
baroreceptor afferents.

2.2 Brainstem interneurons

As discussed in the previous section, baroreceptor
afferent neurons terminate in the NTS. Increases in the
activity of these neurons leads to an increase in the
activity of vagal preganglionic neurons, a decrease in
the activity of sympathetic preganglionic neurons and a
decrease in the activity of vasopressin-containing
neurons. The term 'baroreflex' is therefore intended to
encompass changes in the activity of a number of efferent
mechanisms in response to alterations in arterial
pressure. Since these changes involve both increases and
decreases in the activity of efferent neurons,   the
neuronal circuitry underlying these changes requires the
presence of at least one inhibitory interneuron   to
mediate the decrease in vasopressin release, and the
decrease in the activity of the sympathetic preganglionic
neurons. A large body of evidence exists that suggests
that spinally-projecting neurons whose cell bodies lie in
the ventrolateral and raphe portions of the rostral
medulla mediate changes in the activity of sympathetic
preganglionic neurons.   These neurons will be dealt with
later. Similarly, neurons whose cell bodies are located

in the caudal ventrolateral medulla are thought to be
responsible for mediating changes in the activity of
vasopressinergic neurons of the hypothalamus. In both
cases, these neurons are thought to be excitatory, that
is, increases in their activity leads to increases in
sympathetic activity, and increases in circulating
vasopressin. Thus, the need for an inhibitory
interneuron remains.

2.2.1 Location and projections

In contrast to the baroreceptor afferent neurons,
the location and projections of the second order
baroreceptor interneurons is unknown. This lack of
understanding is due largely to the technical
difficulties of neuroanatomical research in areas such
as the NTS where large numbers of functionally
distinct neurons are intermingled. In a study in the
cat (Lovick 1986), the retrograde tracer horseradish
peroxidase was injected into the rostral ventrolateral
medulla. After processing, retrogradely-labelled
neurons were found in many parts of the medulla
oblongata and pons, particularly the contralateral NTS
and the caudal trigeminal nucleus. Interestingly,
many labelled cells were also reported in the
contralateral ventrolateral medulla, as well as in the

raphe' nuclei.   In contrast, in a   similar   study
performed in the rabbit, Dampney et al (1982) reported
that labelled cells were found mainly in the NTS, and
the parabrachial nucleus of the pons. Similar results
have been reported in the rat (Norgren 1978; Ross et
al 1985) and the cat (Loewy and Burton 1978).

Although there is evidence that these neurons may
form an integral part of the baroreflex arc, it is not
clear whether or not they are the second   order
neurons. If the chemical content of the second order
neuron can be identified, a great deal more progress
is likely.

2.2.2 Neurotransmitter candidates

The difficulties in determining which neurons in
the NTS are responsible for mediating the effects of
changes in the activity of the baroreceptors, combined
with the huge number of putative neurotransmitters
that have been localized in this region, has led to a
plethora of studies on the effects of these compounds
and   their agonists and antagonists   on   various
cardiorespiratory parameters. These compounds include
catecholamines, serotonin, amino acids and peptides.
In this   section   I   will examine some of the evidence

that these putative neurotransmitters serve either an integral or modulatory role in the baroreflex arcs.

2.2.2.1 Catecholamines

Pontomedullary catecholamine cell groups initially described by Dahlstrohm and Fuxe (1964) in the rat, and subsequently by many other workers (Blessing et al 1980, 1981, 1986; McKellar and Loewy 1982; Neil and Loewy 1982; Ross et al 1983, 1984; Everitt et al 1984; Goodchild et al 1984; Kalia et al 1985a,b,c; Sawchenko et al 1985; Lorenz et al 1985; Marson and Loewy 1985; Loewy et al 1986) in a variety of species, are present throughout the brainstem and have been shown to contain either noradrenaline or adrenaline (figure 1.1). The terminology introduced by Dahlstrohm and Fuxe (1964) to describe these cell groups is used throughout this thesis (A=noradrenaline, B=serotonin and C=adrenaline). Some of these neurons (A5,A6,C1) have been shown to project to the spinal cord, suggesting that they have a role in mediating changes in sympathetic activity in response to changes in baroreceptor and other neuronal activity. These neurons will be discussed in section 2.3 of this chapter. Of the remaining

OBEX −1
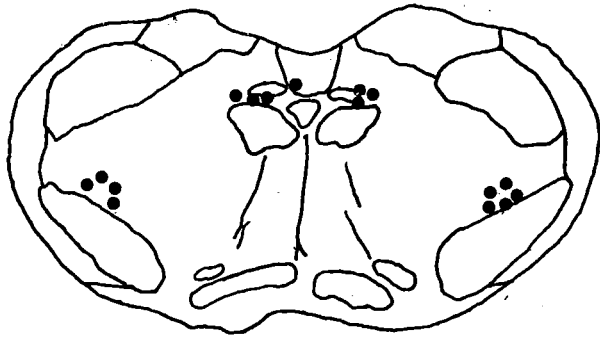
OBEX
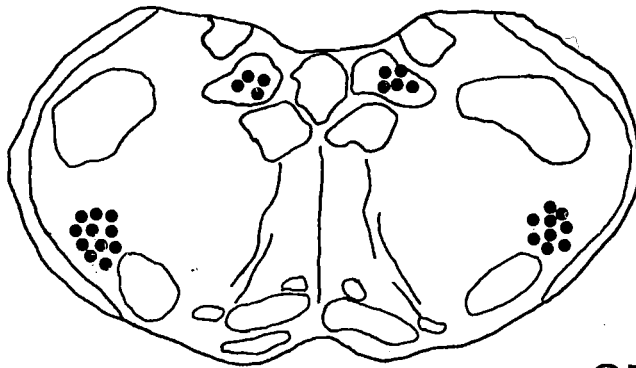
OBEX +2
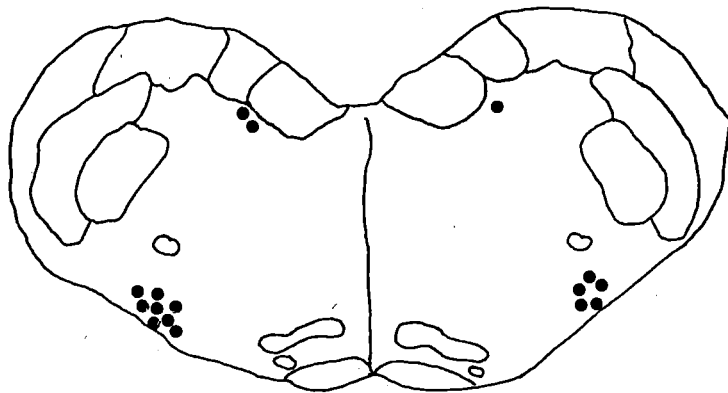
Figure 1.1: Location of catecholamine fluorescent neurons in the brainstem of the rabbit.

groups, the A1 cell group projects mainly rostrally to the hypothalamus, where its fibres innervate the vasopressin-secreting magnocellular neurons in the paraventricular and supraoptic nuclei. Their role in the control of vasopressin secretion will be discussed separately. The C2 and A2 cells also have rostral projections, but their precise pattern of termination is unclear. The terms A3 and A4 apply to small variable groups of cells.

In addition to the presence of cell bodies that contain catecholamines in the medulla, the presence of immunoreactive terminals has been extensively reported. In a recent series of reports (Kalia et al 1985a,b,c), immunoreactive fibres were demonstrated in the region of the NTS, area postrema, dorsal motor nucleus of the vagus, nucleus ambiguus and in the rostral and caudal ventrolateral medulla. These results confirm and extend the findings of previous workers (Takahashi et al 1979; Armstrong et al 1981; Blessing et al 1981). In addition, a number of reports have found evidence for synaptic interactions of catecholamine-containing fibres with axons, dendrites and cell bodies at the ultrastructural level in the NTS, suggesting a role for catecholamines in cardiovascular control (Chiba and Doba 1975, 1976; Sumal et al 1983; Hwang and Wu

1984). The source of these terminals in the NTS is believed to be from the A2 and C2 neurons. These neurons are thus well placed to influence afferent baroreceptor impulses.

This possibilty is supported by studies demonstrating the presence of alpha-2-receptors in the NTS (Dashwood et al 1985), and by pharmacological studies that have shown that microinjection of catecholamines and their agonists into the region of the NTS elicits falls in blood pressure and heart rate (Zandberg and Dejong 1977; Zandberg et al 1979; Kubo and Misu 1981; Wolf et al 1981). Furthermore, electrolytic or specific chemical destruction (with 6-hydroxydopamine), of the A2 neurons results in a short-lived increase in arterial pressure associated with a chronic increase in the lability of arterial pressure, and an attenuation of the bradycardic response to brief increases in arterial pressure (Snyder et al 1978, Talman et al 1980). Interestingly, the effects observed by Snyder et al (1978), involved a reduction in dopamine-beta-hydroxylase activity of only 60% following 6-hydroxydopamine treatment. Although Snyder et al (1978) conclude that catecholamines are modulators rather than mediators of the baroreflex in this region, the possibility of a further attenuation or even abolition of the

baroreceptor heart rate reflex in the presence of a more complete reduction in the number of catecholamine neurons remains. Similar conclusions were made by other workers in studies on the unanaesthetized rabbit, who reported that intracisternal injections of 6-hydroxydopamine facilitated the vagal component of the baroreceptor heart rate reflex immediately after the injections, but inhibited it after two weeks (Korner et al 1978,1984; Head and Korner 1980; Korner and Head 1981). Since these authors have demonstrated that the acute (0-3 hours) effect of 6-hydroxydopamine is to elicit a release of noradrenaline from nerve terminals, whilst the chronic effect is a destruction, they conclude that noradrenergic neurons facilitate vagal motorneurons (Korner and Head 1981). These studies have also suggested a role for spinally-projecting noradrenergic neurons in modulating the activity of sympathetic preganglionic neurons, as will be discussed later.

Another area in the brainstem where catecholamines are thought to exert a modulatory role on sympathetic outflow is the ventrolateral medulla. Microinjection of clonidine into this region exerts a hypotensive effect (Bousquet et al 1984) and an inhibition of the firing of presumptive sympathoexcitatory neurons (Sun and

Guyenet 1986).   Furthermore, these workers have
reported that neurons of the A5 cell group project
to this region (Sun and Guyenet 1986) findings that
are consistent with earlier reports showing a
hypotensive response to the chemical stimulation of
cell bodies in the A5 region (Stanek et al 1984).
A   possible role for the   A5   neurons   in
cardiovascular control will be discussed below.

At present, there is no evidence implicating
adrenaline containing   neurons   as   brainstem
interneurons. The role of the bulbospinal   C1
adrenaline neurons in mediating sympathoexcitatory
impulses will be examined below.

2.2.2.2 Serotonin

Immunohistochemical studies have demonstrated
the presence of serotonin- like immunoreactivity
within neuronal cell bodies and fibres throughout
the brain.   In the medulla, large numbers of
serotonin-containing cell bodies are present in the
raphe nuclei of the medulla and pons (designated B1
to B3 by Dahlstrohm and Fuxe 1964, figure 1.2, 1.3,
1.4) and their lateral extensions (Steinbusch 1978,
1982, 1984a,b; Bowker at al 1981a,b;   Johansson et

OBEX −1

OBEX

OBEX +2
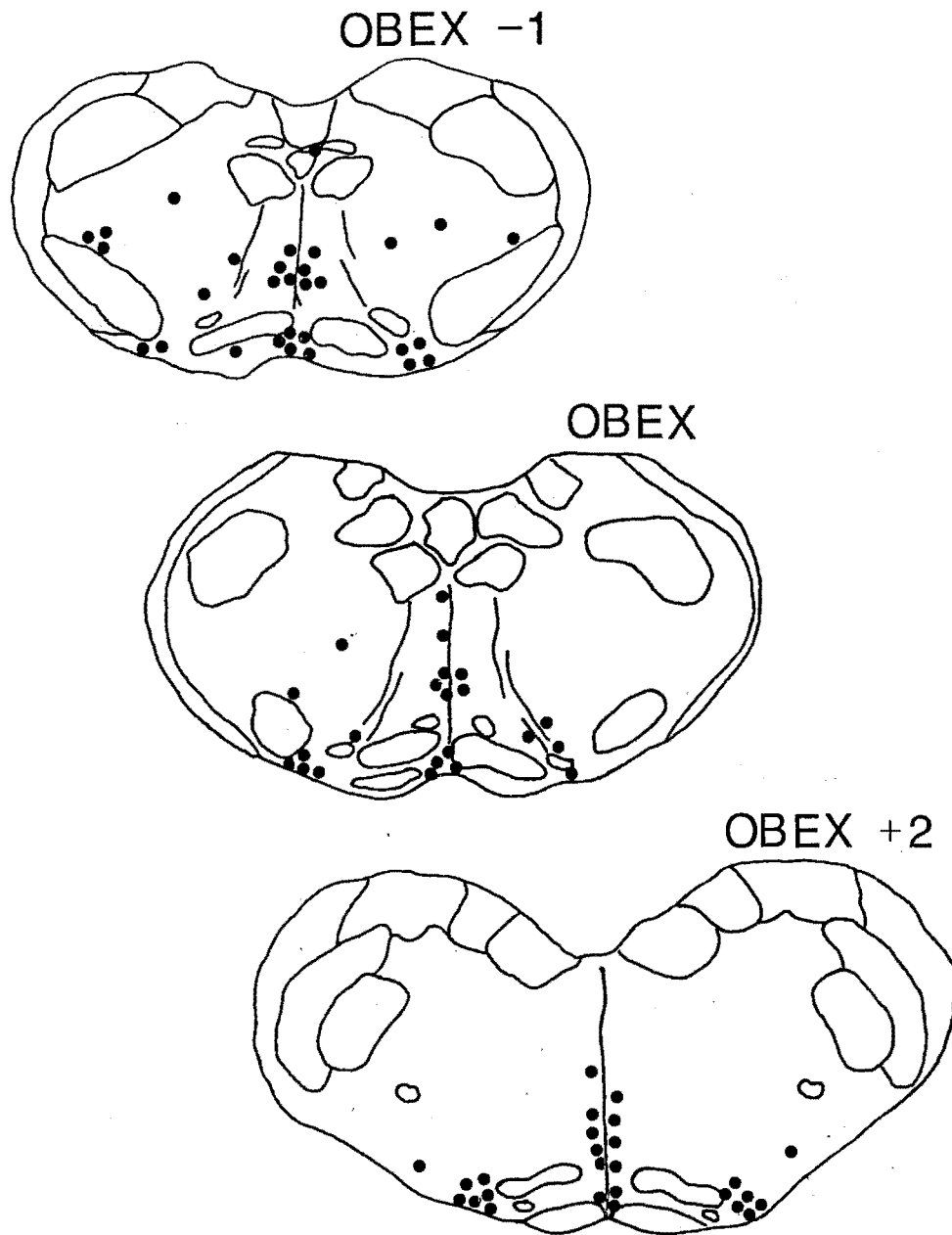
Figure 1.2: Location of serotonin immunoreactive neurons in the brainstem of the rabbit. The cells in the ventrolateral medulla lie more medially than the catecholamine neurons.
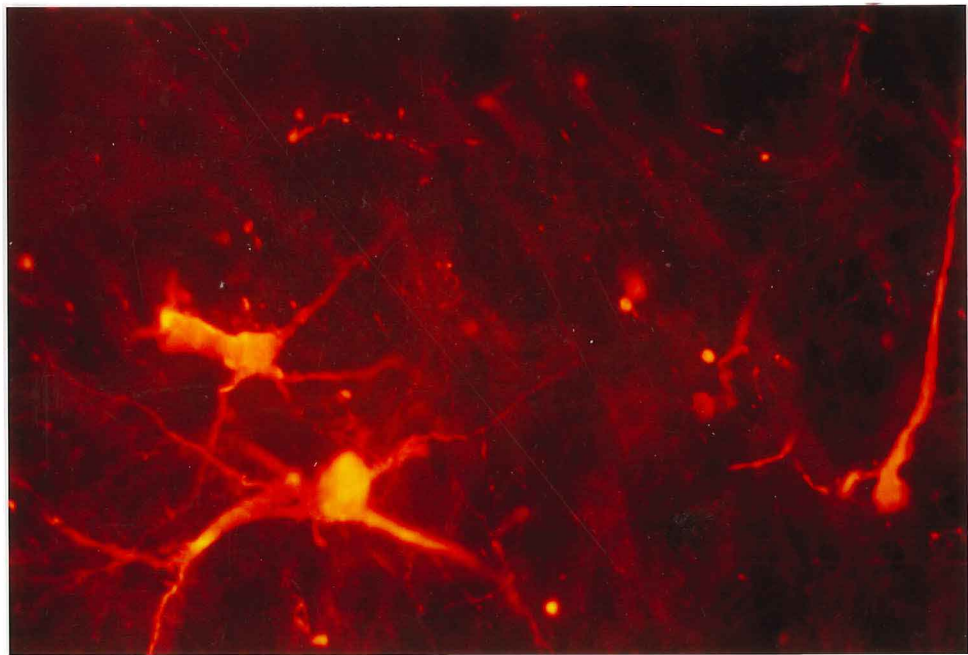
Figure 1.3 Catecholamine fluorescent neurons (upper panel) and serotonin - like immunoreactivity (lower Panel) in the rostral ventrolateral medulla of a rabbit pre-treated with colchicine. There is no coexistence of the two transmitters, however some intermingling of the two groups does occur.
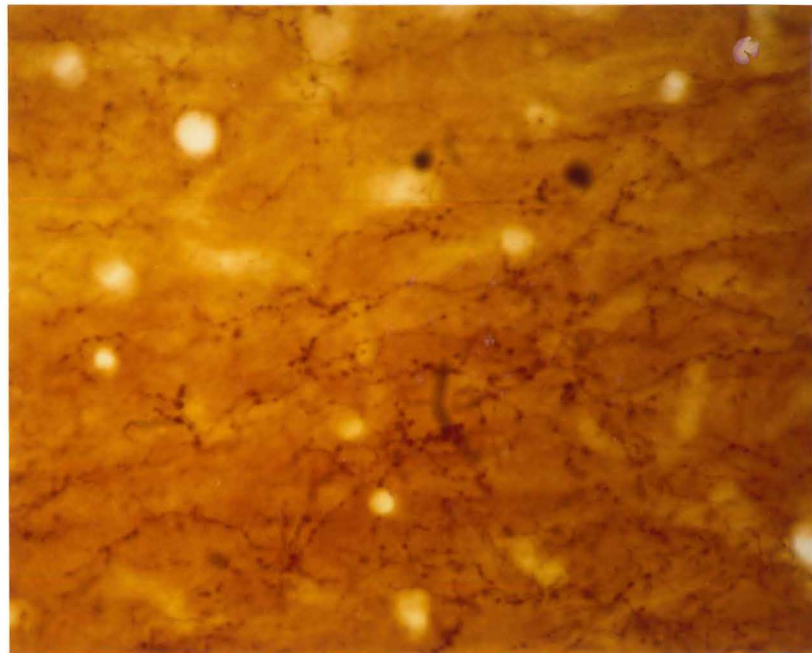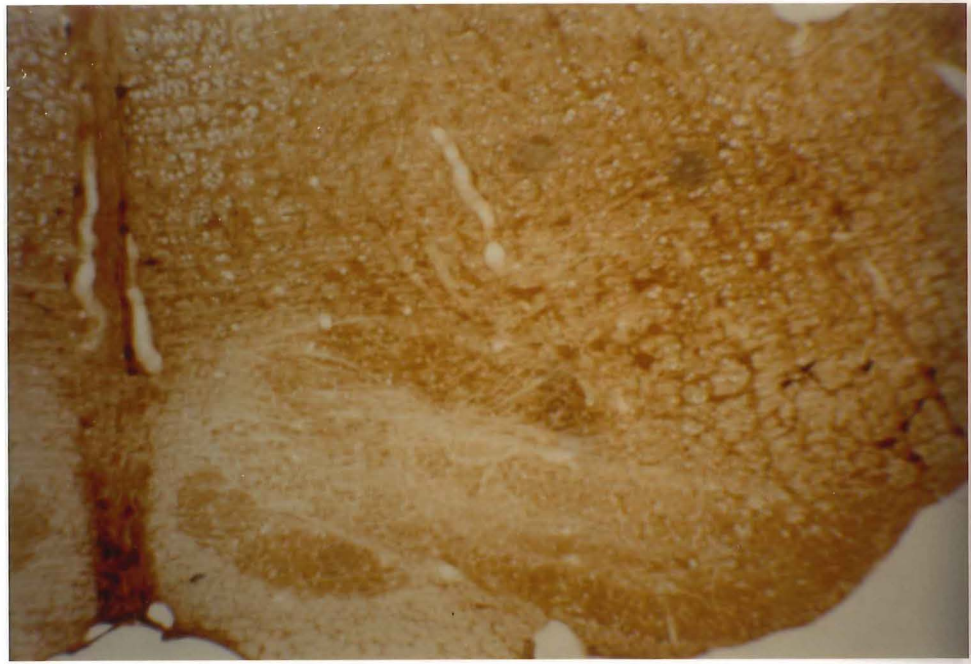
Figure 1.4 The upper panel shows the location of serotonin immunoreactivity in the brainstem of a rabbit. Cell bodies can be seen in the raphe, and laterally adjacent to the pyramidal tracts. The lower panel shows serotonin immunoreactive fibres within the nucleus tractus solitarius.

al 1981;   Hunt and Lovick 1982;   Leger and   Wiklund
1982;   Takeuchi et al 1982, 1983;   Howe   et al
1983b;   Agnati et al   1984;   Jacobs   et al 1984;
Yamada and Sano 1985;   Leger et al 1986).

Large numbers of fibres are present in several
places, but particularly the NTS (figure 1.4),   and
to a lesser extent in   the   ventro- medial   and
lateral, parts of the   medulla.   The   large   fibre
density in the NTS has been further examined at the
ultrastructural level,   and   it has been shown that
the serotonin-like immunoreactivity seen with   the
light   microscope is   contained   in   axons   and
terminals (Maley and Elde 1982;   Pickel   et al
1984). Furthermore,   these   terminals tend to make
synaptic contacts with dendrites rather than   cell
bodies. In   the   report by Pickel et al (1984), it
was demonstrated that   these   synapses   were   often
present   on tyrosine   hydroxylase immunoreactive
neurons. The latter finding provides a   strong
ultrastructural basis   for   a role for serotonin in
the modulation of baroreceptor reflexes,   since   in
an earlier   study   it   had   been   demonstrated that
vagal afferent   fibres   terminate   on   tyrosine
hydroxylase immunoreactive   neurons   in   this   area
(Sumal et al 1983).

In addition   to   these   anatomical   studies,

physiological studies have supported a role for serotonin in cardiovascular control. In a study by Wolf et al (1981), microinjections of serotonin into the NTS elicited increases in blood pressure that were blocked by pretreatment with serotonin antagonists. In contrast, Laguzzi et al (1984) have reported that microinjections of serotonin into the NTS elicits a dose dependent fall in blood pressure that is also blocked by a serotonin antagonist. The true nature of the role of serotonin in this region thus awaits further study. A possible role for spinally projecting serotonin neurons in cardiovascular control will be discussed below.

2.2.2.3 Amino acids

2.2.2.3.1 Gamma aminobutyric acid (GABA)

Immunocytochemical studies have localized GABA- and glutamate decarboxylase- (the GABA synthesizing enzyme) containing cells (Ruggiero et al 1985; Meeley et al 1985; Maley and Newton 1985) in the NTS and ventrolateral medulla. At the present time, the anatomical

projections of these neurons is unclear. In the study by Meeley et al (1985), it was shown that destruction of the NTS had no effect on the GABA or glutamate decarboxylase content of the ventrolateral medulla and that neurons that were retrogradely labelled in the NTS following injections of horseradish peroxidase into the ventrolateral medulla did not appear to be present in the same region as the glutamate decarboxylase positive neurons, suggesting that these neurons do not project to the ventrolateral medulla. A definitive study involving the use of antibodies to GABA itself, combined with retrograde transport, is necessary to resolve this problem.

When applied to neurons, GABA is uniformly inhibitory, a property that has led to its widespread use as a means of inhibiting neuronal activity without affecting fibres of passage. This policy may not be without risks, since GABA has also been shown to depolarize peripheral myelinated nerves as well as primary afferent terminals (Morris et al 1983 a,b), and to elicit a release of excitatory transmitter at the crayfish neuromuscular junction (Finger 1985). With the advent of agonists (e.g. muscimol) and antagonists (e.g. bicuculline and picrotoxin),

the possibility of a neurotransmitter role for
GABA has been explored, with the result that a
number of papers have appeared suggesting a role
for GABA neurons in baroreflex function, and the
control of the cardiovascular system (Antonaccio
and Halley 1975; Antonaccio and Taylor 1977;
Antonaccio et al 1977,1978a,b; Dimicco et al
1979; Sweet et al 1979; Williford et al
1979,1980; Antonaccio and Snyder 1981;
Feldberg et al 1981; Bousquet et al
1982,1984,1985; Gillis et al 1982,1984; Yamada
et al 1982; Willette et al 1983,1984a,b; Segal
et al 1984; Humphrey and McCall 1985; McCall
and Humphrey 1985; Sun and Guyenet 1985,1986;
McCall 1986). The general consensus of these
experiments is that GABA systems are widespread
and subserve an inhibitory function in all of
the systems studied so far. What is far more
difficult to establish is whether or not these
neurons are a link in the baroreflex pathway, or
if they serve to protect the integrity of the
pathway in the same way as the
glycine-containing Renshaw cells of the spinal
cord.

Two studies contrast these views. Firstly,
in the papers by Sun and Guyenet (1985,1986),
single-unit recordings were made from neurons in

the   rostral   ventrolateral   medulla.   These
neurons are believed to be responsible   for   the
excitation of sympathetic preganglionic neurons
on the   grounds that they are antidromically
activated from   the   spinal cord, and that their
activity is inversely   related   to   arterial
pressure. These authors found that application
of GABA significantly decreased the activity of
these units, whilst application of the GABA
antagonist bicuculline significantly increased
their activity.   Furthermore, application of
bicuculline prevented the fall in unit discharge
that normally accompanies increases in blood
pressure, an effect that these authors suggest
is evidence that GABA-containing neurons are an
integral part of the baroreflex   pathway. In
considering these results, the specificity of
the pharmacological agents used needs to be
taken into account. As noted by the authors,
bicuculline is also a potent inhibitor of
acetylcholinesterase in vitro, and has   been
shown to potentiate the actions of acetylcholine
in vivo (Svenneby and Roberts 1973; Miller and
McLennan 1974), indicating that the   effects
observed may be due to the presence of a
cholinergic input. Another interesting feature
of   these   results, is that   despite   quite
substantial overshoots in   arterial   pressure

after releasing the aortic occluder (to produce
transient increases in pressure), no rebound
increases in unit activity can be seen in any of
their published traces.   In addition, in one
trace where arterial pressure was lowered by
intravenous   injection of nitroprusside,   no
increase in unit discharge is seen.   One
possible explanation for this result is that the
reflex increase in sympathetic activity that
accompanies falls in arterial pressure is
mediated by a decrease in the activity of an
inhibitory pathway, distinct from these neurons.
Clearly, this question deserves further study.

In contrast to the findings of Sun and
Guyenet (1985,1986), McCall (1986), in studies
on the cat, has suggested that GABA neurons are
not   involved   in   baroreceptor   mediated
sympathoinhibition. Using the GABA antagonist
picrotoxin, he found that when given in an
intravenous dose that blocked the effect of GABA
on pressure sensitive units in the rostral
ventrolateral medulla, there was no blockade of
the inhibition resulting from increases in
arterial pressure. Despite some deficiencies in
the study by McCall (1986), (such as failing to
demonstrate   that   these   neurons   were
antidromically activated by stimulation in the

spinal cord), and differences (cat as opposed to rat), these results suggest that the role of GABA as an integral part of the baroreflex arc is by no means certain.

2.2.2.3.2 Glutamate

Just as the transduction of increases in baroreceptor activity into decreases in sympathetic outflow requires the presence of an inhibitory link, the increases in vagal activity require an excitatory pathway. If primary baroreceptor afferent neurons synapse directly onto vagal motorneurons (a possibility for which direct anatomical evidence is lacking), then the evidence discussed above supports a role for glutamate in this pathway.

Although microinjection of glutamate has been shown to increase neuronal activity in many areas of the brain (e.g. Freis and Zieglgansberger 1974; Curtis 1979), the evidence that this amino acid functions as a neurotransmitter in any of these systems is not convincing (Curtis 1979).

2.3 Efferent Pathways

After the information from peripheral receptors has been integrated, there are three major efferent pathways; the sympathetic, the parasympathetic, and the vasopressin neurons in the hypothalamus. In this section I will discuss the inputs to the preganglionic sympathetic and parasympathetic neurons, and the vasopressin neurons.

2.3.1 Parasympathetic pathways

Preganglionic parasympathetic motorneurons have their cell bodies in the dorsal motor nucleus of the vagus, and in the nucleus ambiguus, (McAllen and Spyer 1976; Geis and Wurster 1980; Miura and Okada 1981; Hoover and Barron 1982; Jordan et al 1982; Stuesse 1982; Ellenberger et al 1983; Chan et al 1984; Kalia et al 1984), and project to the periphery through the vagus nerve. The viscerotopic organization of these nuclei has been examined in the cat, where it has been shown that most of the cardiac preganglionic neurons arise from the nucleus ambiguus, with a smaller contribution from the dorsal motor nucleus of the vagus. Direct electrical or chemical stimulation of these nuclei elicits hypotension and

bradycardia (Ellenberger et al 1983; Chan et al 1984).

As noted above, the integrity of the baroreceptor heart rate reflex requires the presence of an excitatory link between these neurons and the baroreceptor afferents. At the present time, the number, location, projections, and chemical content of these interneurons is unknown.

2.3.2 Vasopressin neurons

Vasopressin synthesizing neurons are located in the magnocellular portions of the paraventricular and supraoptic nuclei. The axons of these neurons project to the posterior pituitary where they release vasopressin into the circulation. The secretion of vasopressin occurs in response to a variety of stimuli, and the neural pathway that these stimuli traverse is a subject of much controversy. I will examine these pathways and their chemical content below.

2.3.2.1 Location and projections

Inputs to the paraventricular and supraoptic regions of the hypothalamus have been shown to arise from the renal nerve (Day and Ciriello 1985), and from peripheral baro- and chemo- receptors (Kannan and Yagi 1978; Yamashita and Koizumi 1979; Kubo et al 1984; Blessing and Willoughby 1985). Within the central nervous system, major inputs have been demonstrated from the caudal ventrolateral medulla (Swanson and Kuypers 1980; Feldberg and Rocha e Silva 1981; McKellar and Loewy 1981,1982; Blessing et al 1982a,b; Tanaka et al 1984; Blessing and Willoughby 1985; Kubo et al 1985; Tribollet et al 1985), the nucleus tractus solitarius (McKellar and Loewy 1981; Sawchenko and Swanson 1981; Yamane et al 1984; Sawchenko et al 1985; Sved et al 1985b; Tribollet et al 1985), the diagonal band of Broca (Jhamandas and Renaud 1986) and the perinuclear zone dorsal to the supraoptic nucleus (Tribollet et al 1985).

2.3.2.2 Neurotransmitter candidates

A number of putative neurotransmitter candidates have been reported to affect the release of vasopressin from hypothalamic neurons, including noradrenaline (Miller et al 1979; Armstrong et al 1982,1986; Day and Renaud 1984; Blessing and Willoughby 1985; Day et al 1985a,b,c) neuropeptide Y (Day et al 1985a), acetylcholine (Barker et al 1971; Moss et al 1972; Arnauld et al 1983) and GABA (Arnauld et al 1983).

The innervation of these neurons by noradrenaline-containing neurons originating in the caudal ventrolateral medulla (Ricardo and Koh 1978; Sakumoto et al 1978; Swanson et al 1981; Blessing et al 1982; Sawchenko and Swanson 1982; Sawchenko et al 1985) is particularly interesting in view of the effects on vasopressin release caused by alterations in the activity of neurons in the caudal ventrolateral medulla. Stimulation of this region with glutamate increases the release of vasopressin (Kubo et al 1985; Sved et al 1985a), whilst inhibition prevents the baroreceptor mediated increases in vasopressin (Blessing and Willoughby 1985). A decrease in the noradrenergic innervation of these neurons following treatment

with 6OHDA   attenuates the secretion of vasopressin
that follows osmotic stimuli (Miller et   al   1979).
The original finding of Blessing et al (1982) that
A1 neurons of the caudal ventrolateral medulla
tonically   inhibit vasopressin release   because
electrolytic lesions of the caudal ventrolateral
medulla   increased plasma vasopressin has been
attributed by Blessing and Willoughby (1985) to   an
excitatory   effect   of   these   lesions,   since
non-excitatory   inhibition   of   the   caudal
ventrolateral medulla with tetrodotoxin does not
elevate   plasma   vasopressin.   Finally,   in
electrophysiological studies, exogenously applied
noradrenaline has been shown to increase the firing
rate of vasopressin neurons (Randle et al 1986a,b).
Although some controversy still exists,   since in
some early studies, application of noradrenaline to
hypothalamic explants decreased vasopressin release
(Armstrong et al 1982),   it seems likely that in
vivo, noradrenergic inputs increase the release of
vasopressin.

Acetylcholine has been shown to increase the
activity of vasopressin-containing neurons (Barker
et al 1971; Moss et al 1972; Arnauld et al 1983)
and nicotinic acetylcholine receptors have been
demonstrated on hypothalamic neurons (Mason 1985),
strongly suggesting a cholinergic input to these

neurons. Despite this,   the   source   of   this
innervation has not yet been demonstrated.

Similarly, GABA has been shown to inhibit the
firing of vasopressin neurons, but the source of
this input has not been identified.

2.3.3 Bulbospinal pathways

The final common pathway for information that
affects   the   sympathetic nervous system   is   the
sympathetic preganglionic neurons of the spinal cord.
These   neurons   are   mainly   situated   in   the
intermediolateral cell column of the spinal cord, with
smaller   numbers   in   the   nucleus   intercalatus.
Information reaches these neurons from many levels of
the neuraxis, but in this section I will be dealing
mainly   with   pathways   originating   in   the   medulla
oblongata, since these pathways form the primary focus
of this thesis.

2.3.3.1 Location and Projections

Bulbospinal pathways have been extensively mapped by the use of neuroanatomical tract tracing techniques. In most cases this involves the detection of horseradish peroxidase after its injection into the spinal cord. One of the difficulties with these studies is that when large injections are made into the spinal cord, populations of neurons other than those projecting to the intermediolateral cell column may be revealed. This problem was highlighted in a recent paper by Aston-Jones et al (1986), that examined the afferent inputs to the locus coeruleus. With the aid of carefully controlled, restricted iontophoretic deposits into this nucleus they found only a small number of afferent inputs, in contrast to earlier reports (e.g. Cedarbaum and Aghajanian 1978) suggesting a large afferent network. These findings were substantiated by the use of anterograde tracing techniques that confirmed the retrograde labelling studies. In two papers where the microinjections of horseradish peroxidase were carefully confined to the intermediolateral horn of the cat, labelled cells were found in three main locations; the ventrolateral medulla, the raphe area and the dorsomedial medulla (Amendt et al

1979; Miura et al 1983).   The labelled cells were present bilaterally, but   with   an   ipsilateral predominance.

Similar findings have been reported by other groups in other species, where the extent of the injections into the spinal cord clearly exceeds the bounds of the intermediolateral cell column, or is not shown (Blessing et al 1981; McKellar and Loewy 1982; Ross et al 1983,1984; Farlow et al 1984; Goodchild et al 1984).

2.3.3.2 Neurotransmitter candidates

Although the neuroanatomical studies discussed above have provided   evidence   for   inputs   to sympathetic preganglionic neurons from many areas of the medulla, pons, midbrain and hypothalamus, physiological studies have suggested that the area of the medulla that is critical for the maintenance of sympathetic tone, and the   transmission   of baroreceptor   information,   lies   in   the   rostral ventrolateral medulla (Feldberg and Guertzenstein 1972; Guertzenstein 1973; Dampney and Moon 1980; Dampney et al 1982, 1985;   McAllen et al 1982; Goodchild et al 1982, 1984; Granata et al 1983,

1985; Howe   et   al   1983a;   Ross   et   al   1984;

Willette et   al 1984a,b;   Yamada et al 1984;   Sun

and Guyenet 1985,   1986).   A number of putative

neurotransmitter candidates have been localized in

this region, and have been suggested to play a role

in the   transmission   of   sympathoexcitatory

information;   these   include   substance   P,

adrenaline, neuropeptide Y and   serotonin.   In

addition,   colocalization of   some   of   these

substances within   the   same   neuron   has   been

reported.

2.3.3.2.1 Substance P

Substance P containing neurons have been

demonstrated in the raphe nuclei and their

lateral extensions of all species studied so far

(Nilsson et al 1974;   Cuello and Kanazawa   1978;

Ljungdahl et al   1978;   Johansson   et al 1981;

Lovick and Hunt 1983;   Marson and   Loewy   1985).

Many of these neurons have been   shown to be

spinally projecting (Johansson   et   al   1981;

Helke et al 1982).   Furthermore substance P like

immunoreactivity has been demonstrated within

fibres in the intermediolateral cell column, as

have substance P binding sites (Takano and Loewy

1984; Charlton and Helke 1985), raising the possibility that these neurons may subserve a sympathoexcitatory function.

This possibility is   supported   by   the finding   that   intrathecally   administered substance P elicits   a   hypertensive   effect associated with increases in sympathetic nerve activity (Petty and Reid 1981, 1982;   Unger et al 1985).   As noted above, the laterally lying neurons of the rostral ventrolateral medulla are of particular interest, because of the changes in arterial pressure and baroreflex function that result   from   alterations   of   neuronal activity in this area. In a series of studies by Takano et al (1984, 1985a,b), it was found that chemical stimulation of this region with kainic acid elicited increases   in   arterial pressure that were associated with a release of substance P into the spinal sub-arachnoid space. These pressor responses were blocked by the intrathecal administration of   a   putative substance   P   antagonist   (d-Pro2, d-Trp7,9-substance P).   · Furthermore,   in previously untreated rats, this compound has been shown to produce a dose dependent fall in arterial pressure (Loewy and Sawyer 1982). Similar observations have been made by Keeler et

`al (1985)   and Keeler and Helke (1985), who also
reported that   administration   of   a   stable
substance   P   analogue   (pGlu5,   MePhe8,
MeGly9)-substance   P   (5-11),   produced   dose
dependent increases   in   arterial   pressure that
were blocked by another substance P antagonist,
(d-Arg1, d-Pro2,   d-Trp7,9,   Leu11)-substance P.
These authors have suggested on the basis of
these   results   that   substance   P   is   the
transmitter responsible   for   mediating   the
effects discussed   above.   A number of problems
with these studies make these   conclusions   less
convincing. Firstly,   the   antagonists   used in
these experiments were   originally   reported   as
being effective in the periphery (Mizrahi et al
1982).   Studies on their efficacy in the central
nervous system have   found   that   they   are   not
specific antagonists of   substance   P   in this
region (Salt et al 1982).   Secondly, it has been
reported that they exert non-specific neurotoxic
effects at   the   doses   used   in   the   studies
described above   (Hokfelt et al 1981;   Matsumura
et al 1985;   Post and Paulsson 1985).

2.3.3.2.2 Serotonin

The distribution of spinally-projecting serotonin-containing neurons is similar to that of substance P (Bowker et al 1981a,b,1982; Johansson et al 1981; Loewy and McKellar 1981; Hunt and Lovick 1982; Leger and Wiklund 1982; Takeuchi et al 1982,1983; Howe et al 1983b; Gorcs et al 1985; Agnati et al 1984; Jacobs et al 1984; Steinbusch 1984; Marson and Loewy 1985; Yamada and Sano 1985; Brodin et al 1986; Leger et al 1986), and several groups have reported that these compounds coexist in neurons in the medulla (Johansson et al 1981; Lovick and Hunt 1983). When applied to sympathetic preganglionic neurons iontophoretically, serotonin has consistently been excitatory (McCall 1983; Coote et al 1981). Nevertheless, the role of serotonin neurons in the central control of blood pressure has been a subject of much controversy (Neumayr et al 1974; Wing and Chalmers 1974a,b; Baum and Shropshire 1975; Chalmers and Wing 1975; Antonaccio and Cote 1976; Smits and Struyker-Boudier 1976; Ogawa 1978; Coote et al 1978; Wolf et al 1981a,b; Echizen and Freed 1982,1984; McCall and Humphrey 1982; Howe et al 1983a; McCall 1983,1984; Wessendorf and Anderson 1983;

Laguzzi et al 1984; Minson et al 1984;   Wolf

and Kuhn 1984a,b; Elam et al 1985; Kuhn et al

1985), with some groups suggesting an excitatory

role for serotonin, and others an inhibitory

function. These discrepancies may well be a

consequence of the wide distribution of

serotonin, with different pathways having

different roles in cardiovascular control.   In

the study by Howe et al (1983a), it was reported

that electrical stimulation of the serotonin

neurons that lie in the rostral ventrolateral

medulla elicited increases in arterial pressure

that were not seen in animals pretreated with

the   serotonergic   neurotoxin

5,7-dihydroxytryptamine, whilst in the report by

Laguzzi et al (1984),   microinjection of

serotonin into the nucleus tractus solitarius

elicited dose dependent falls in arterial

pressure.   Finally,   the   presence   of

serotonin-containing neurons in the rostral

ventrolateral medulla raises the possibility

that these neurons may play a role in the tonic

and reflex control of sympathetic tone. The

absence of a clear fall in arterial pressure

associated with lesions of central serotonergic

neurons with 5,7-dihydroxytryptamine (Howe et al

1983a; Minson et al 1984) makes it unlikely

that these neurons are solely responsible for

`the maintenance of vasomotor tone, however a modulatory role in this or other sympathetic beds cannot be excluded.

2.3.3.2.3 Adrenaline

The presence of adrenaline-containing neurons in the rat brain was first reported by Hokfelt et al (1974), using immunohistochemical detection of phenylethanolamine-N-methyl transferase the adrenaline synthesizing enzyme. Spinally-projecting adrenaline-containing neurons have since been demonstrated in the ventrolateral medulla of rats and rabbits (Ross et al 1983; Goodchild et al 1984; Blessing et al 1986) at sites where electrical or chemical stimulation elicits large increases in arterial pressure, and inhibition results in falls in pressure (Dampney et al 1982, 1985; Goodchild et al 1982; Granata et al 1983, 1985; Ross et al 1984). Anterograde tracing techniques have demonstrated a preferential input of neurons in this area to the intermediolateral cell column in the rat (Ross et al 1984). These findings have been interpreted to mean that adrenaline is the transmitter responsible for mediating these

effects by exciting sympathetic preganglionic
neurons. Whilst this is a tempting hypothesis,
there is at the present time no direct evidence
to support it. On the other hand, the results
of a number of studies that have examined this
possibility in other ways have suggested with
few exceptions (Liang et al 1982), that
adrenaline either exerts no effect, or is
inhibitory (Bolme et al 1974; Borkowski and
Finch 1977, 1978, 1979; Hahn et al 1983;
Sangdee and Franz 1983; Connor and Drew 1985).
In the report by Connor and Drew (1985) for
example, it was found that the hypertensive
response to stimulation of the rostral
ventrolateral medulla in the region of the C1
neurons was unaffected by intrathecal injections
of propranolol or phentolamine. Furthermore,
electrophysiological studies have demonstrated a
consistently inhibitory effect of adrenaline
when applied iontophoretically to sympathetic
preganglionic neurons (Coote et al 1981;
Guyenet and Cabot 1981; Guyenet and Stornetta
1982). The findings of these experiments do not
discount the possibility that
adrenaline-containing neurons are responsible
for mediating the sympathoexcitatory effects of
stimulating the rostral ventrolateral medulla,
but the evidence that adrenaline is the chemical

'mediator responsible is still lacking.

2.3.3.2.4 Noradrenaline

As noted in section 1.2.2.2.1 above, noradrenergic neurons are present throughout the brainstem. Although some earlier studies suggested that there was a spinal projection from the A1 cell group (Satoh et al 1977), more recent studies have demonstrated that this cell group projects almost exclusively rostrally, innervating the vasopressin synthesizing neurons of the hypothalamus (Swanson et al 1981; Blessing et al 1982). Similarly, the contribution of the A2 cell group to the spinal cord is minimal.

The contribution of suprabulbar noradrenergic cell groups (A5 and A6) is discussed below.

2.3.3.2.5 Neuropeptide Y

Neuropeptide Y-like immunoreactivity has been demonstrated in the majority of the adrenaline-containing neurons of the rostral ventrolateral medulla (Everitt et al 1984; Sawchenko et al 1985; Blessing et al 1986). The possibility that neuropeptide Y may be involved in the pressor responses elicited from the rostral ventrolateral medulla has been investigated by a number of workers. In a report by Petty et al (1984) in the rabbit, intracisternal injections of neuropeptide Y were found to have no effect on blood pressure or heart rate. In a series of studies from another group, neuropeptide Y administered into the spinal subarachnoid space was found to have a hypotensive effect in rats (Fuxe et al 1983, 1984; Harfstrand et al 1984). There have been no reports of a central pressor effect of neuropeptide Y. These studies and those discussed above suggest that if these adrenaline/neuropeptide Y containing neurons are sympathoexcitatory, then the transmitter remains to be identified. One possible candidate is substance P, since in a recent report (Lorenz et al 1985) substance P was reported to be

'colocalized   in   large   numbers   of
spinally-projecting   adrenaline-containing
neurons. This finding is somewhat surprising
since an   earlier   report   by   Ljungdahl et al
(1978) on the relationship of catecholamine
neurons to   substance P neurons had not reported
such a coexistence. This question is examined
in more detail in chapter 8.

2.3.4 Supra-bulbar pathways

The importance of suprapontine pathways in the
tonic and reflex control of the cardiovascular system
was examined in an extensive series of experiments by
Korner et al (1972), who examined the role of vagal
and sympathetic   effector   mechanisms   in   the
baroreceptor-heart period reflex. These authors found
that sectioning the brainstem as low as the inferior
colliculi did not prevent changes in heart rate in
response to   alterations in arterial pressure, nor was
the gain or heart-period range of the reflex affected.
On the other hand, the reflex curve was shifted to the
left, due to a small but significant decrease in the
resting blood pressure and BP50, of the pontine
animals. As a result of these experiments, it was
concluded that although suprapontine pathways exert a

modulatory effect, they are not critical for the
operation of the baroreceptor reflex.

Significant   projections   of
noradrenaline-containing cells to the spinal cord do
occur from pontine regions, including the locus
coeruleus (A6) and the A5 cell group. The role of
these cell groups has been extensively investigated.

A possible role for spinally projecting
noradrenaline containing neurons in baroreflex
function was suggested by Chalmers and Wurtman (1971)
in a study on the effect of acute sino-aortic
denervation on tyrosine hydroxylase activity and
noradrenaline turnover, and by Chalmers and Reid
(1972) in a study on the effect of 6-hydroxydopamine
on the hypertensive response to sino-aortic
denervation. Briefly, it was found that although
endogenous levels of noradrenaline and dopamine were
unchanged following sino-aortic denervation, there was
an increase in the turnover of exogenously
administered, tritiated noradrenaline, as well as an
increase in the tyrosine hydroxylase activity of
thoraco-lumbar spinal cord and hypothalamic
homogenates. These results were interpreted as
providing evidence of a role for descending
sympathoexcitatory noradrenergic. pathways in the
hypertensive response to sino-aortic denervation.

Although such a role is still possible, these results must be considered in the light of later studies which have demonstrated the existence of spinally projecting adrenergic (Hokfelt et al 1974; Ross et al 1984; Goodchild et al 1984) and dopaminergic neurones (Blessing and Chalmers 1979, Bjorklund and Skagerborg 1979). Direct stimulation of the A5 group of catecholamine containing neurons has been reported to elicit both increases and falls in arterial pressure, heart rate and peripheral resistance (Loewy et al 1979; Neil and Loewy 1982; Stanek et al 1984). This diversity of responses illustrates the difficulty in the use of electrical stimulation that affects fibres of passage as well as cell bodies. In the initial report from Loewy et al (1979), electrical stimulation of the A5 cell group elicited a pressor response, whilst in a later series of reports, chemical stimulation with glutamate, which is believed to selectively activate cell bodies, consistently elicited falls in arterial pressure. If these latter responses are due to activation of A5 neurons, and not other neurons in the vicinity, then it seems likely that the A5 cells subserve an inhibitory function. As noted above, these effects are also consistent with an inhibitory pathway from the A5 neurons to the rostral ventrolateral medulla as has been demonstrated by Sun and Guyenet (1986).

In contrast to the A5 group, the evidence implicating the A6 cell group in cardiovascular control is much weaker. In one report, for example, alterations in blood pressure were found to have little effect on the firing rate of A6 neurons (Guyenet and Byrum 1985).

In studies on the effect of noradrenaline on sympathetic preganglionic neurons some groups have suggested a sympathoexcitatory role, (Yoshimura et al 1986; Ma and Dun 1985), whilst an inhibitory role has been suggested by others (Coote et al 1981; Andrade and Aghajanian 1982; Byrum et al 1984; Guyenet 1984; Guyenet and Byrum 1985). It is conceivable that in the intact organism these neurons do have a role in the transmission of cardiovascular information, but there is no clear evidence that a loss of these neurons results in a gross impairment of central cardiovascular control.

3 Plan of study

        The evidence outlined above strongly supports a role
for neurons in the ventrolateral medulla in the central
control of blood pressure.   In this thesis a number of
aspects of this control have been examined. In the first
part, chemical stimulation and inhibition of the caudal and
rostral ventrolateral medulla was used in order to assess
the role of these regions in the control of sympathetic
nerve activity in the rabbit. In addition, the role of the
sympathetic nervous system in mediating the increase in
arterial pressure that follows anodal electrolytic lesions
of the caudal ventrolateral medulla in the region of the A1
group of noradrenaline-containing neurons was examined.
Secondly, intracisternal administration of the noradrenergic
neurotoxin 6OHDA, and the serotonergic neurotoxin 57DHT was
used in conjunction with measurements of renal sympathetic
nerve activity, and plasma adrenaline, noradrenaline, and
vasopressin, to assess the relative roles of these effectors
in mediating the pressor responses evoked by these
neurotoxins. Since these neurotoxins have been shown to
exert their acute effects by eliciting a release of
neurotransmitter from central noradrenaline and serotonin
containing neurons, the results provide information on which
central neurons may be involved   in   mediating
sympathoexcitatory responses. Although these studies are
valuable in determining the location of neurons that are

involved in cardiovascular control (first part), and in a general   way, which neurotransmitters may be important (second part), they do not provide information about the chemical   content of particular neuronal pathways.   In subsequent chapters of this thesis I have focussed on the role of neurons whose cell bodies originate in the medulla and which project to the spinal cord. In order to examine these bulbospinal pathways, it proved useful to devise a means of measuring changes in their activity in the spinal cord.   This was achieved by measuring neurotransmitter release with the recently described technique of in vivo tissue dialysis in the case of serotonin, and by spinal sub-arachnoid space perfusion in the case of neuropeptide Y. Finally, immunohistochemical techniques were used to examine the extent   of   colocalization   of   the   putative neurotransmitter peptide, substance P, with catecholamine synthesizing neurons, so as to establish whether or not there are subpopulations of neurons in the ventrolateral medulla. These studies will form the basis of future physiological investigations into the role of different groups of medullary neurons in the control of the cardiovascular system.

Chapter 2: Table of Contents

General Methods

1 Animals

1.1 Rabbits

Male New Zealand White rabbits weighing 2.0-3.5kg were used. The animals were housed separately and allowed free access to pellets and water.

1.2 Rats

Male Wistar Kyoto rats weighing 250-350g were used. These animals were housed in groups of up to five in a cage with free access to pellets and water.

2 Anaesthesia

2.1 Rabbits

In experiments where the animals recovered, anaesthesia was induced with propranidid (Epontol, Bayer 1.5-2.0ml of a 50mg/ml solution I.V.). During the course of these studies this product was removed from the market. In subsequent studies sodium thiopentone (Pentothal, Abbott 1.5-2.0ml of 25mg/ml solution I.V.) was used. The rabbits were intubated with Cole neonatal endotracheal tubes (Portex, internal diameter of tracheal end 2.5 or 3.0mm). Anaesthesia was maintained with halothane (Fluothane ICI), delivered with room air from a Goldman vaporizer. If muscle relaxation was required, suxamethonium chloride (Scoline, Glaxo 1-2mg every ten to twenty minutes) was used.

In these and other animals, where mechanical ventilation was required, this was achieved with a Harvard ventilator set at a rate of thirty breaths per minute, and a tidal volume set to achieve 250ml/min/kg body weight.

In these experiments, rabbits were able to breathe

spontaneously and sit up within 15-20 minutes following the cessation of anaesthesia.

In experiments where the animals did not recover anaesthesia was induced with urethane (Sigma, 1.2-1.5g/kg delivered by intravenous infusion over thirty to forty minutes using a 50% solution).

Surgical anaesthesia was considered to have been achieved if it was possible to intubate the rabbit and insert a femoral artery catheter without response.   At this point, the rabbit was paralysed with pancuronium bromide (Pavulon, Organon 0.8mg I.V.   followed   by additional doses of 0.4mg as needed) and ventilated. During the course of the experiment, the rabbit was permitted to recover from the neuromuscular blockade in order to determine if supplemental doses of anaesthetic were required.

At the end of these acute experiments the rabbits were killed with an overdose of sodium pentobarbitone (Nembutal, Ceva 150mg I.V.). Sodium pentobarbitone was also used at lower doses, to deeply anaesthetize rabbits prior to fixation by intravascular perfusion.

2.2 Rats

Rats were anaesthetized with urethane (1.0-1.5g/kg
I.P.). Additional doses of 0.1-0.2g/kg were occasionally
required during the course of long experiments.

3 Surgical procedures

3.1 Arterial and venous catheterization

The marginal ear vein of the rabbit was used for
intravenous drug and fluid administration.   This was
catheterized under   local   anaesthesia   (lignocaine
hydrochloride   1%,   subcutaneously) with a disposable
plastic cannula (jelco, Critikon 22 or 24 gauge).

In experiments   where the   rabbits   regained
consciousness, the   central ear   artery was catheterized
for the measurement of arterial pressure. This procedure
was also performed under local anaesthesia.   Plastic
cannulae fabricated from vinyl tubing (Dural SV65, i.d.
0.86mm, o.d.   1.52mm), which was connected to the
transducer, and a polyethylene tip (SP45, i.d. 0.58mm,

o.d.   0.96mm), which was placed in the artery, were used.

In acute experiments in rabbits the right femoral artery was catheterized with vinyl tubing (SV65). In the experiments in rats, the right femoral artery was catheterized with polyetheylene tubing (SP10, i.d. 0.28mm, o.d.   0.61mm), connected to vinyl tubing (SV65).

3.2 Cannulation of the cisterna magna

The rabbit was placed in a stereotaxic frame with the head fully flexed, the atlanto-occipital membrane was exposed by a dorsal midline incision, followed by retraction of the occipital musculature. A hole was made in the membrane with a 21 gauge hypodermic needle, and a sterile vinyl catheter (SV45, i.d.   0.58mm, o.d.   0.96mm) inserted so that the tip lay just within the cisterna (figure 2.1). This was sealed in place with a drop of cyanoacrylate adhesive (Supaglue, Selleys) and secured with a 5/0 silk suture onto the occipital periosteum. The wound was closed and the other end of the catheter sealed and buried subcutaneously between the scapulae.

In acute experiments, the cisterna magna was exposed by incising the atlanto-occipital membrane and reflecting it laterally with two 5/0 silk threads. In the

INTRACISTERNAL CATHETER
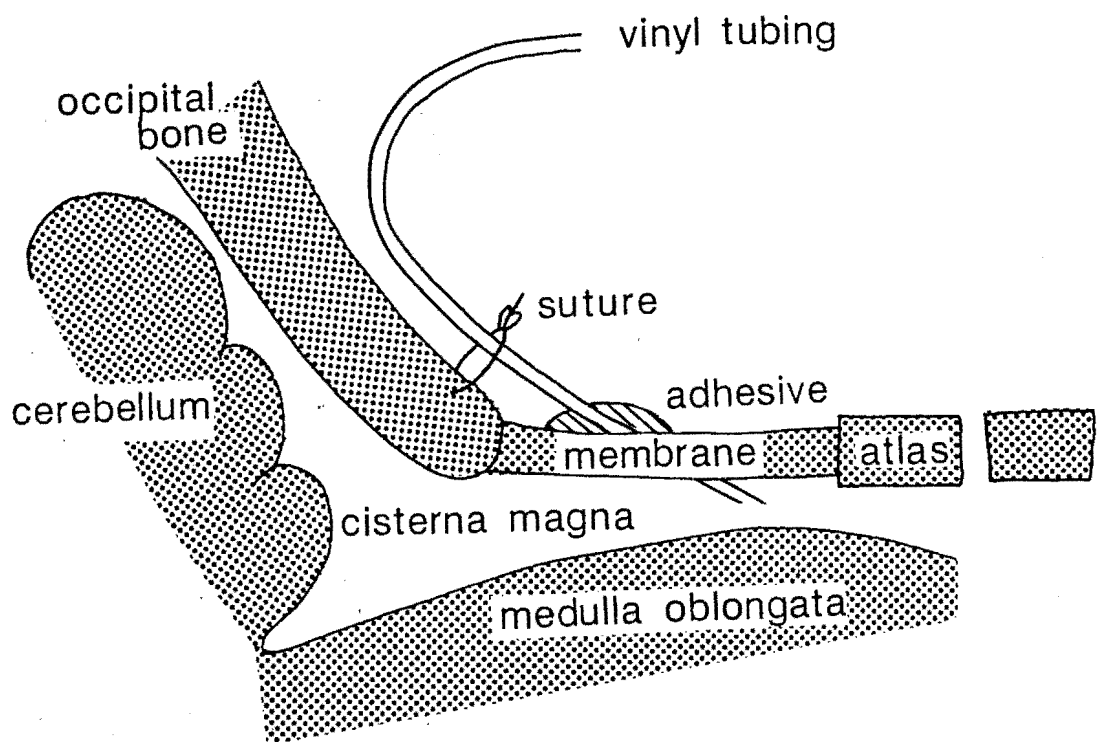
Figure 2.1: A diagram showing the position of a vinyl
catheter in the cisterna magna of a rabbit.

experiments in which kainic acid was injected into the rostral ventrolateral medulla, fluid was collected from the cisterna magna using a polyethylene catheter (SP37 i.d. 0.5mm, o.d. 1.0mm) whose tip lay just within the cisterna. In subsequent experiments, a silastic catheter (Dow-Corning i.d. 0.025in, o.d. 0.047in) was advanced 6cm into the spinal sub-arachnoid space and sealed in place with 5% agar (Bactoagar, Difco).

3.3 Cannulation of the spinal sub-arachnoid space

The spinal cord was approached through a midline incision and the paravertebral musculature retracted laterally. The tissue overlying the spinal column was removed, and a dorsal spinous process removed with rongeurs. Haemostasis was achieved with diathermy and bone wax. A laminectomy was then performed with a high-speed electric jeweller's drill, and the dura incised with a 21 gauge needle. The spinal sub-arachnoid space was then catheterized by passing an 18 gauge epidural catheter cranially into the dural incision. The catheter was sealed in place with cyanoacrylate adhesive, and the retractors removed.

3.4 Implantation of renal nerve electrodes

Renal nerve electrodes were fabricated from two lengths of teflon coated stainless steel wire (Medwire) according to the method of Dorward et al (1985). The construction of this electrode is relatively complicated, and is shown diagrammatically in figure 2.2.

In order to implant this electrode, the rabbit was laid on its right side, and the left renal neurovascular bundle exposed through a loin incision. With the aid of an operating microscope, a renal nerve was isolated for a distance of 10-12mm, taking care to preserve its blood supply and keep it moist at all times. The electrode was then sutured to the adventitia of the renal artery with 7/0 prolene thread. The free nerve was then slipped into the spirals of the electrode, and the entire assembly covered with silicone gel (Silgel 604A,B, Wacker Chemie).

After the gel had set, the electrode plugs were buried sub-cutaneously, and the wound closed. In acute experiments the plugs were led out through the wound. The method of recording and quantitating renal nerve activity is described below.

RENAL NERVE ELECTRODE
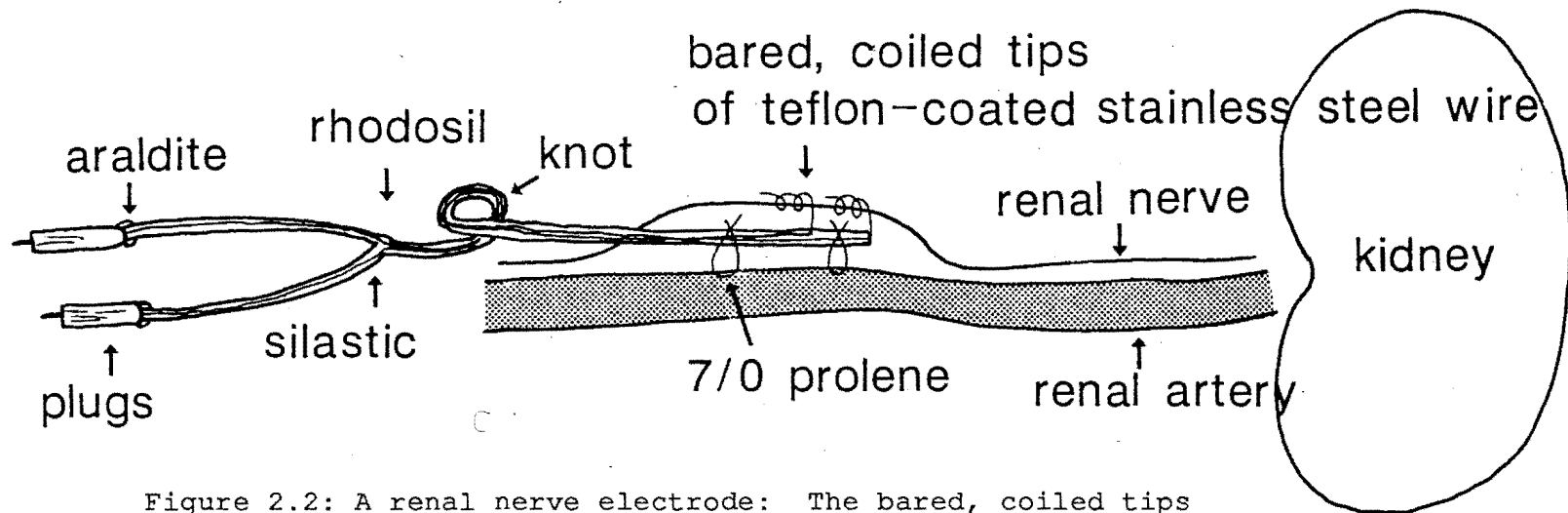
Figure 2.2: A renal nerve electrode: The bared, coiled tips of the electrode that contact the nerve are connected to plugs. The wires are covered in silastic tubing to provide extra strength, and to prevent damage to the teflon coating. All open parts of the silastic tubing, and the points at which the electrode exit from the tubing are coated with liquid silastic (Rhodosil). The probes were sutured to the renal artery with 7/0 prolene, the nerve slipped into the coils, and the entire assembly isolated with silicone gel.

3.5 Implantation of dialysis probes

The dialysis probes used in rats and rabbits were identical and consisted of a single folded tubule from a renal dialyzer as shown diagrammatically in figure 2.3.

For implantation in rats, the head and tail were fixed to the operating table.   The lower thoracic vertebral column was approached from a dorsal incision, and one of the spinous processes retracted rostrally. The spinal cord was exposed by incising the overlying interspinous membrane and dura.   A hole was then made over the right or left dorsolateral funiculus with a 27 gauge hypodermic needle, and the dialysis probe inserted by hand. The wound was covered with silicone gel (Silgel 604A,B) to provide stability, and one limb of the tubule was then connected to an infusion pump (Braund). Perfusions were performed at a rate of 50-100ul/20min with Ringers solution (NaCl 8.6g, KCl 0.3g, CaCl2 0.32g, per 1000ml, pH 6.3) containing zimelidine HCl (0.1g per 1000ml).

For implantation in rabbits, the spinal cord was approached as described above (2.3.3), and the probe inserted in the same fashion as described for the rats.
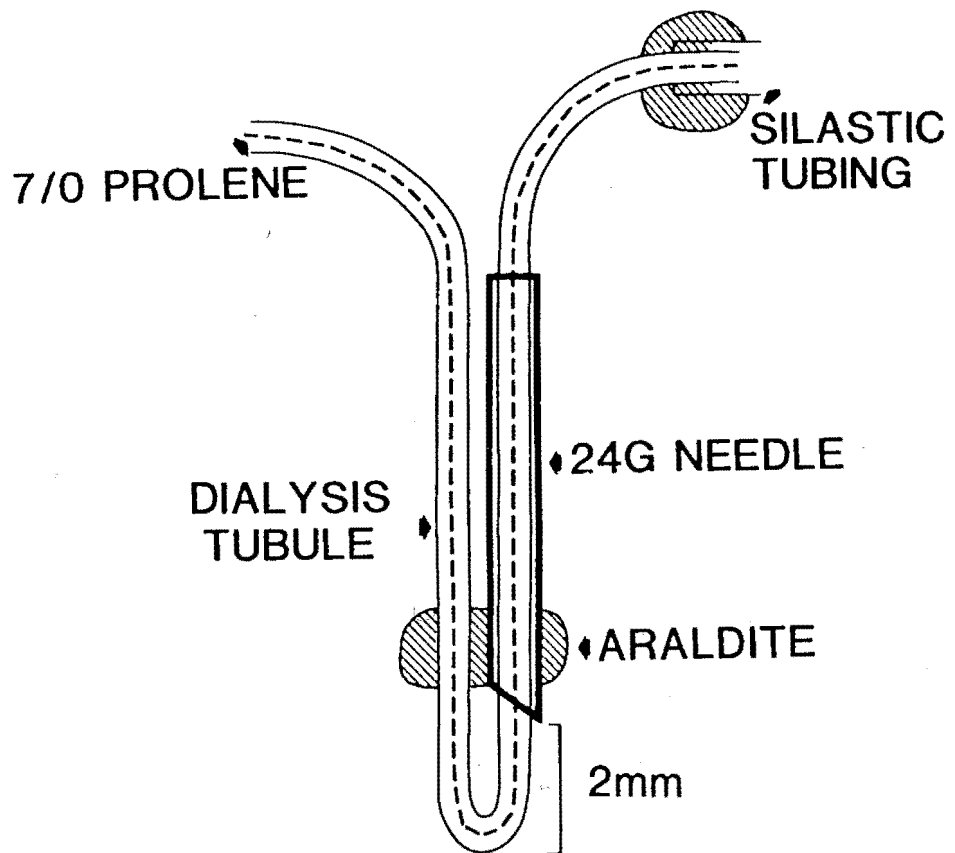
Figure 2.3: A dialysis probe: Single dialysis tubules were threaded with lengths of 7/0 prolene, before being threaded into 24 gauge needles, the hubs of which had been removed. The tubule was then folded over and glued in place with araldite. The two millimetre loop at the tip was inserted into the region of interest in the dialysis experiments.

3.6 Electrolytic lesions

The dorsal surface of the medulla oblongata was exposed as described above (2.3.2). Lesions of the caudal ventrolateral medulla were made using teflon coated stainless steel wire electrodes (MWS wire), the tips of which were ground to a fine point (figure 2.4). The head of the rabbit was adjusted so that the dorsal surface of the medulla was flat. The obex (rostral border of the area postrema in the midline) was used as the reference point. Lesions were performed at three sites on each side of the brainstem as given below.

Rostrocaudal level	lateral(mm)	ventral(mm)
obex	3.4	3.7
obex-1mm	3.1	3.5
obex-2mm	2.8	2.7

In making the lesions, 1.5mAmps of anodal direct current was passed at each site from a Grass constant current lesion maker. One electrode was used for the two obex sites, and one for the four caudal sites. Sham lesioned rabbits were subjected to exactly the same procedure, except that no current was passed.

LESIONING ELECTRODE
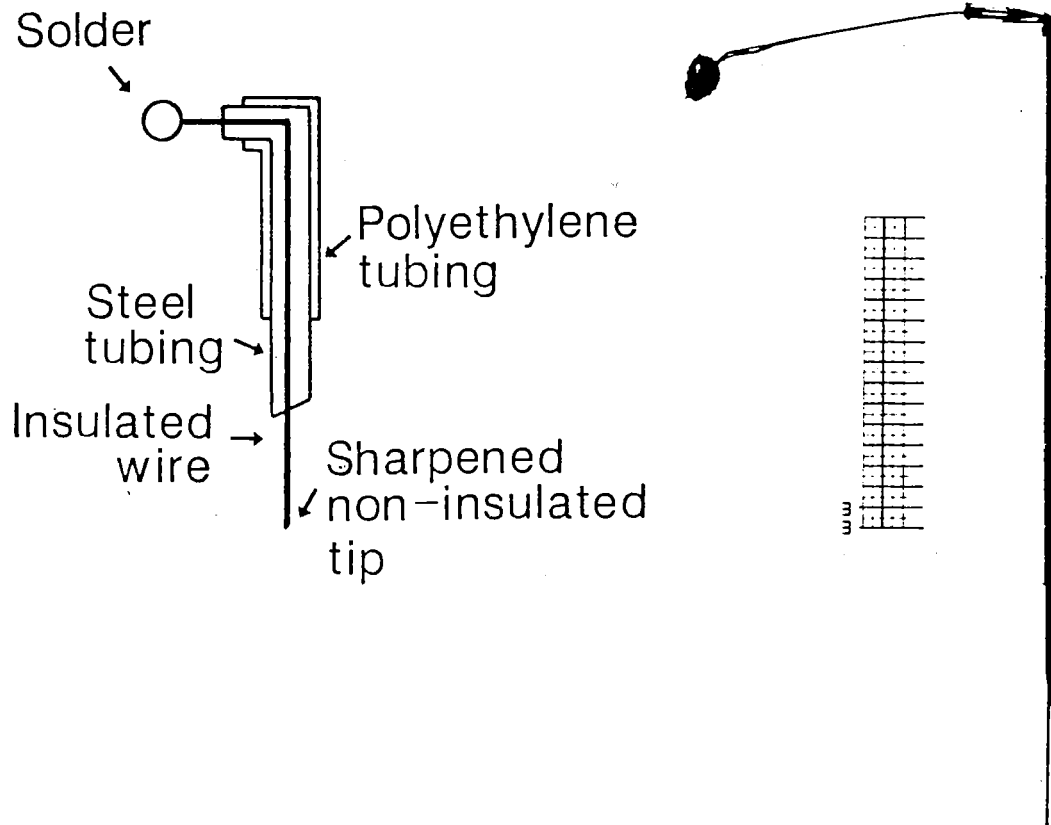
Figure 2.4 Electrode used for making electrolytic lesions of the medulla oblongata.

3.7 Microinjections

Microinjections were made by pressure ejection.   In early experiments a 30 gauge dental needle was used. Subsequently glass micropipettes with a tip diameter of 50 microns were used. The other end of the pipette was connected by tubing to a syringe, and the volume injected was monitored by observing an air-fluid interface in the pipette. The glass pipettes used were Accu-fill 90 micropet disposable pipettes (Clay-Adams).   These pipettes are calibrated by the manufacturer to contain 1ul of fluid in each 1.78cm of tubing, thus by placing a graticule next to the air-fluid interface it was possible to eject volumes as small as 25nl with accuracy (figure 2.5). Injection sites could be localized by the addition of 0.2% pontamine sky blue to the injectate.

Intraventricular injections were made in a similar fashion.
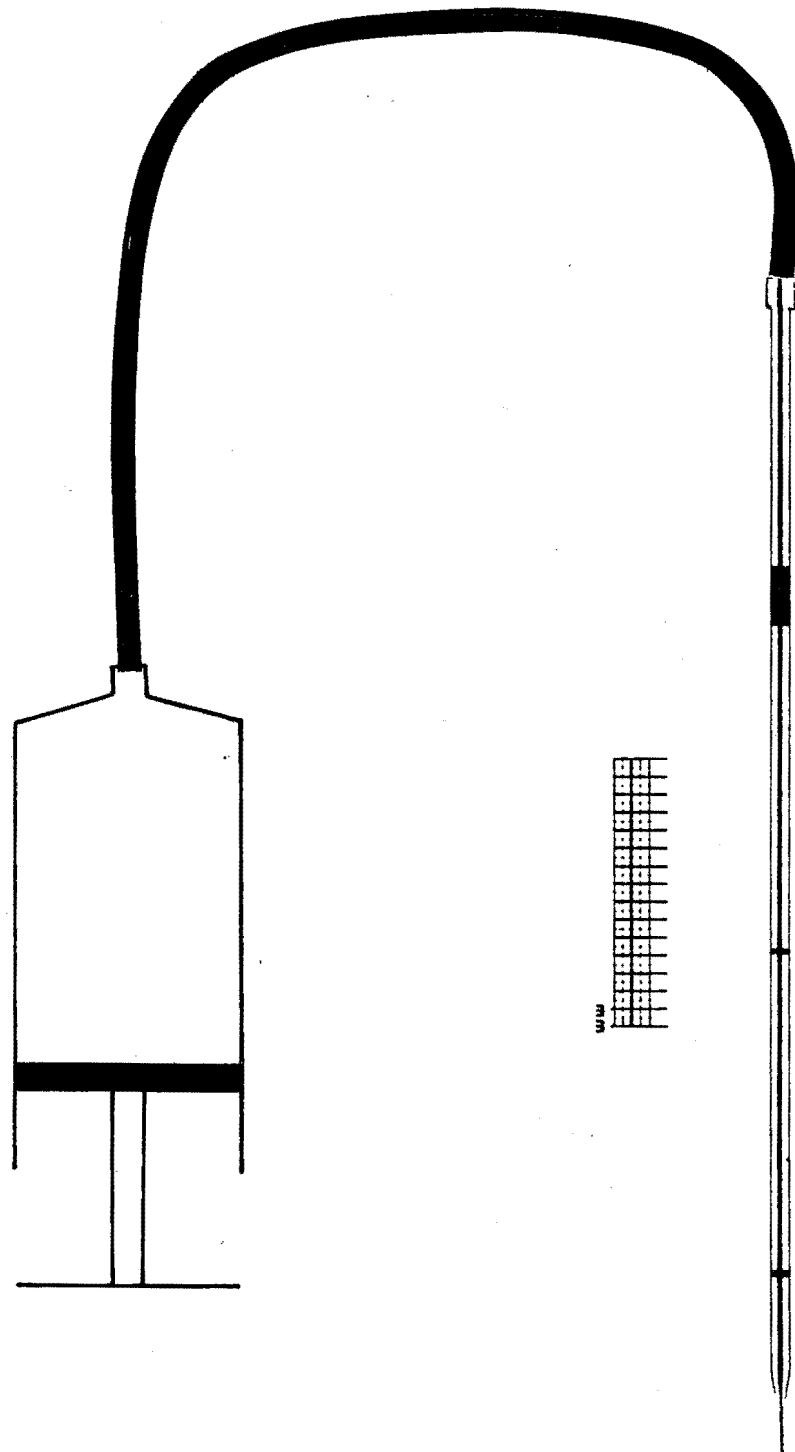
Figure 2.5 A microinjection line: The micropipettes used were calibrated capillary blood sampling pipettes with one microlitre calibrations. A piece of reduced graph paper attached to the side of the pipette enabled accurate ejections of twenty-five nanolitres to be made.

3.8 Sino-aortic denervation

This was accomplished by sectioning the carotid sinus and aortic depressor nerves (figure 2.6). The carotid artery and sinus were approached by a dorsolateral incision. The nerves were identified electrophysiologically by their characteristic pulse related discharge, and cut. This procedure was found to be sufficient to eliminate the bradycardia that accompanies acute elevations in arterial pressure produced by drug administration (noradrenaline or phenylephrine 10ug I.V.). In experiments where the effects of acute sino-aortic denervation were examined, the nerves were identified and isolated on loops of 5/0 silk thread and covered with saline soaked cotton wool. This procedure did not affect the baroreceptor-heart rate reflex. Denervation was accomplished by pulling the loops around the sinus nerve until the nerve broke and by cutting the aortic nerve. This procedure takes one to two minutes.
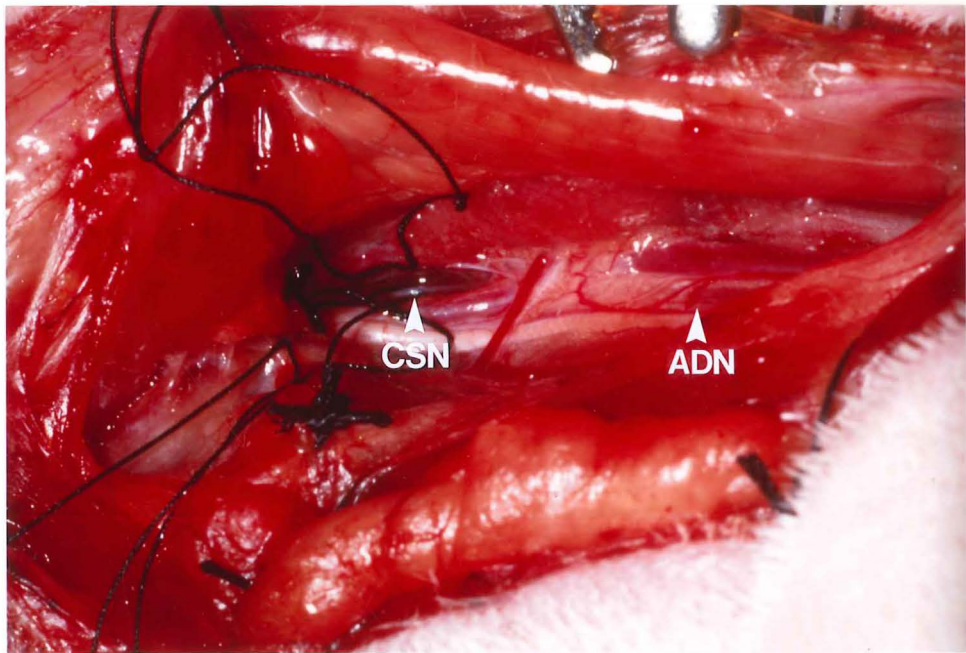
Figure 2.6 Location of the carotid sinus nerve. The aortic depressor nerve lies next to the vagus nerve and common carotid artery, and was found at the point shown by the arrow.

3.9 Fixation

Rabbits were fixed for histological examination of the brainstem by trans- aortic perfusion. The animals were deeply anaesthetized with pentobarbitone, and the abdominal aorta exposed through a midline incision and cannulated. The right atrium was then exposed via a right thoracotomy, and cut. The rabbit was then perfused with 200ml of 0.8% sodium nitrite and 500 units of sodium heparin in 0.01M sodium phosphate buffer (pH 7.0), followed by 2000ml of 4% formaldehyde and 0.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.0) (FAGLU, Furness et al 1977). The perfusion arrangement is shown in figure 2.7. The brainstem was then removed and stored in fixative prior to sectioning.

Rats were perfused transcardially with a needle in the ascending aorta which was inserted via the left ventricle. The rats were perfused with 100ml of 0.1M phosphate buffered saline (pH 7.4, PBS) followed by 500ml of 4% formaldehyde with 0.05% glutaraldehyde and 0.2% picric acid in PBS. The brains were removed and stored in 4% formaldehyde in PBS at 4 degrees C prior to sectioning.

FIXATION APPARATUS
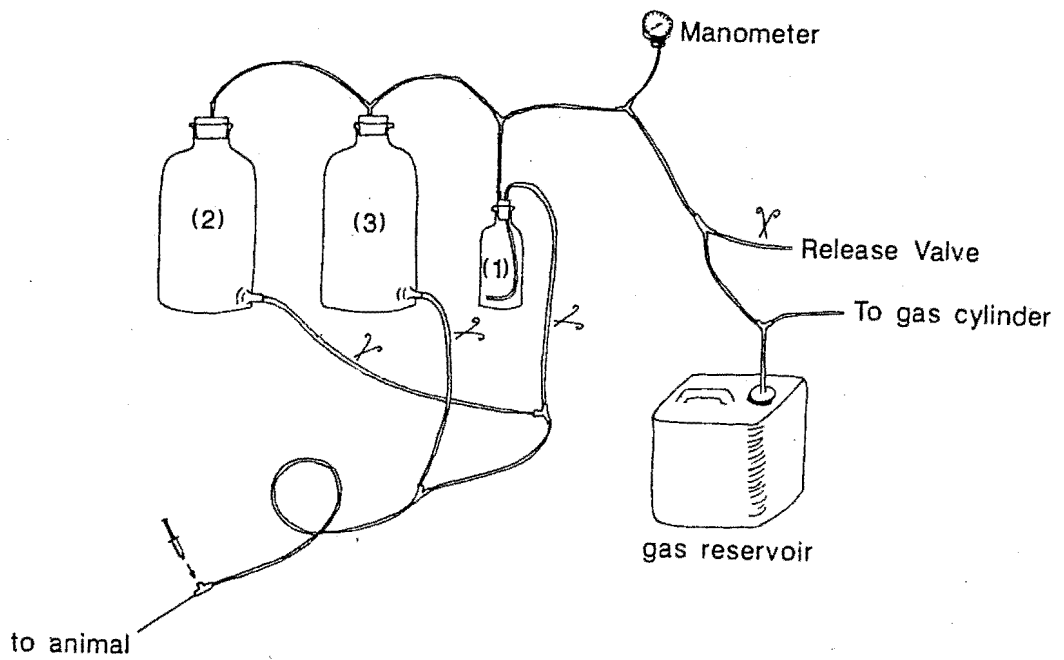
Figure 2.7 The arrangement of the perfusion apparatus. The pressure in the system is monitored with the manometer and controlled by varying the flow rate from the gas cylinder. Bottle (1) contains buffered nitrite, bottle (2) contains fixative, and bottle (3) contains saline.

4 Measurements

4.1 Arterial pressure

Phasic arterial pressure was recorded on a Grass model 7D polygraph with a statham P23Db transducer and a 7P1E preamplifier. The system was calibrated at each session by connecting the transducer to a mercury manometer. Mean arterial pressure was obtained by electronically damping the phasic arterial pressure.

4.2 Heart rate

Heart rate was measured from the phasic pulse interval in all cases where the results were used for statistical analysis. The heart rate was also recorded with a Grass 7P4F tachograph.

4.3 Renal nerve activity

The two renal nerve electrodes were connected to a Tektronix AM502 differential preamplifier. The signal was filtered (low pass:100Hz, high pass:3000Hz), and led to a Grass 7P10 integrator with the threshold set to exclude all noise. This setting remained constant for the duration of each experiment. The integrator was set to full-wave rectify the incoming signal, and integrate over one second periods. Examples of renal nerve activity are shown in figure 2.8, and in chapters 3 and 4. The average height of five one second periods was recorded for the later statistical analysis at each time point. No attempt was made to record nerve activity in volts as this varies up to ten-fold between animals, instead the values obtained for each animal in the pre-intervention period were used as covariates in examining the response after the intervention. The statistical analysis of these results is discussed below.
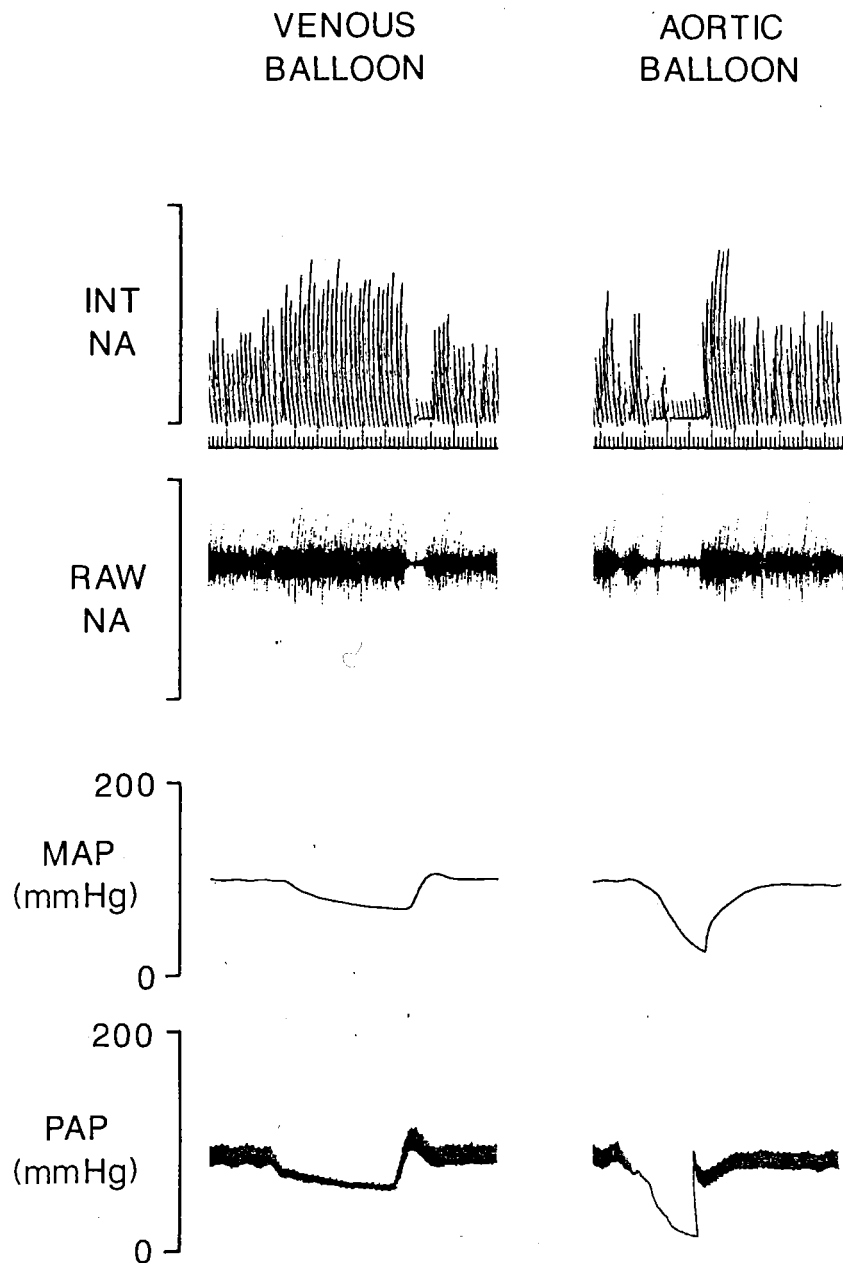
Figure 2.8 This figure shows the effect of occluding the vena cava (left side) or aorta (right side) on renal nerve activity; integrated (INT NA) and raw (RAW NA) on arterial pressure; mean (MAP) and phasic (PAP). Decreasing venous return by occluding the vena cava causes a fall in MAP and a rise in renal nerve activity. Conversely, occluding the aorta causes a fall in nerve activity. In the latter case MAP appears to fall because the catheter was in a femoral artery.

5 Biochemical estimations

The vasopressin and neuropeptide Y assays were performed by Dr M.J. Morris, and the catecholamine and indoleamine assays by Dr V. Kapoor.

5.1 Vasopressin

Plasma vasopressin concentrations were estimated by radioimmunoassay using the method of Sved et al (1985). The vasopressin was separated from the plasma by cation exchange chromatography, and eluted from the columns with 75% ethanol (pH 1.5 with HCl). The eluate was dried in a Savant speedVac sample concentrator, and then reconstituted in assay buffer. The anti-vasopressin antibody was a gift of Dr R. Woods.

5.2 Neuropeptide Y

Neuropeptide Y (NPY) concentrations were measured by direct radioimmunoassay (Morris et al 1986). The antibody was a gift of Dr B. Jarrott (Maccarone and Jarrott 1985). [125-I]NPY labelled with Bolton-Hunter

reagent (2000 Ci/mmol, Amersham) was used as the tracer. Standard curves (figure 2.9) were prepared with synthetic porcine NPY (Sigma). NPY-like immunoreactivity was measured in triplicate in the plasma samples, and singly in the perfusate samples. Tracer bound to antibody was separated   from free tracer by precipitation with polyethylene glycol. Non-specific binding was determined for each rabbit, and the percentage of counts bound to antibody was calculated as (counts/sample - non specific binding counts) divided by total counts originally added to the tube, multiplied by 100. The NPY concentration was then determined from the standard curve (figure 2.9).

5.3 Serotonin

Dialysis samples were collected into Eppendorf microfuge tubes, containing 0.4M HEPES buffer pH 3.5, and stored at -20 degrees C for up to 7 days prior to assay. There was no detectable loss of serotonin in standard samples over this period.

The amount of serotonin in samples of dialysate was measured by electrochemical detection (ECD), after separation by high performance liquid chromatography (HPLC). Chromatography was performed with an O.D.S. II column (Phase separations, 25cm, 4.6mm i.d., 5 micron

NPY-LI STANDARD CURVES IN:
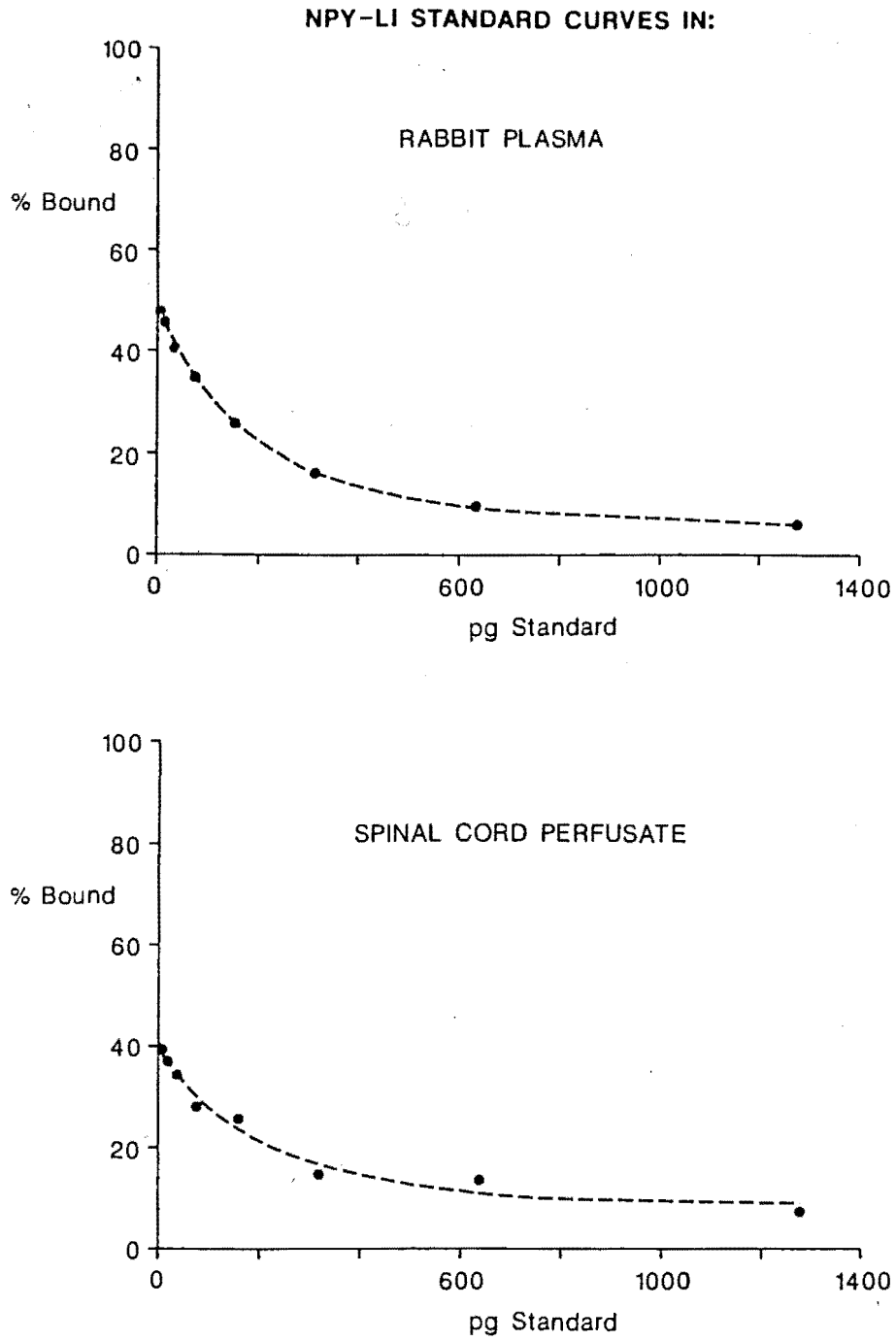
Figure 2.9 Standard curves used in the estimation of of the concentration of neuropeptide Y- like immunoreactivity in plasma and spinal cord perfusates.

particle size). The mobile phase consisted of 0.1M
KH2PO4, 0.3M EDTA, 1mM sodium octyl sulfonate, 14%
methanol at pH 3.9, with glacial acetic acid.
Electrochemical detection was carried out with a glassy
carbon cell (Bio-Analytical Systems) with the working
potential set at +0.65 volts.

5.4 Catecholamines

Plasma concentrations of adrenaline and
noradrenaline were assayed by HPLC with ECD as described
above, after solvent extraction (Smedes et al 1982).

6 Histology

6.1 Catecholamine fluorescence

Fixation with FAGLU as described above results in
the formation of fluorophores from catecholamines
(Furness et al 1977). These were visualized using a
fluorescence microscope (Leitz Ortholux II, Ploem
epi-illumination), in 50 micron Vibratome sections

mounted on glass slides that had been dried with a stream

of hot air from a portable hair dryer, and coverslipped

with Depex mounting medium. An example of FAGLU induced

fluorescence is shown in figures 2.10 and 1.3.

6.2 Immunofluorescence Histochemistry

The   protocol   used   for   double   label

immunofluorescence histochemistry is shown in figure

2.11, and described below.

Fifty micron vibratome sections were washed for

thirty minutes in buffer (NaCl 120mM, KCl 5mM, Na2HPO4

8.5mM, NaH2PO4 1.5mM, Tris base 10mM, sodium   merthiolate

1mM, Triton X100 0.3%, pH 7.4). This buffer was used as

the diluent for all subsequent incubations. The sections

were then incubated for thirty minutes in 10% normal

sheep plasma.   Sections were then incubated for 72 hours

in 10% normal sheep plasma containing either a rabbit

antiserum to phenylethanolamine -N- methyltransferase

(1/2000, a gift of Dr P.R.C. Howe), or a monoclonal rat

antiserum to substance P (seralab, Cuello et al 1979), or

both antisera together, or 10% normal sheep plasma alone.

The sections were then washed three times for twenty

minutes in 1% normal sheep plasma and then incubated for

24 hours in 1% normal sheep plasma containing either
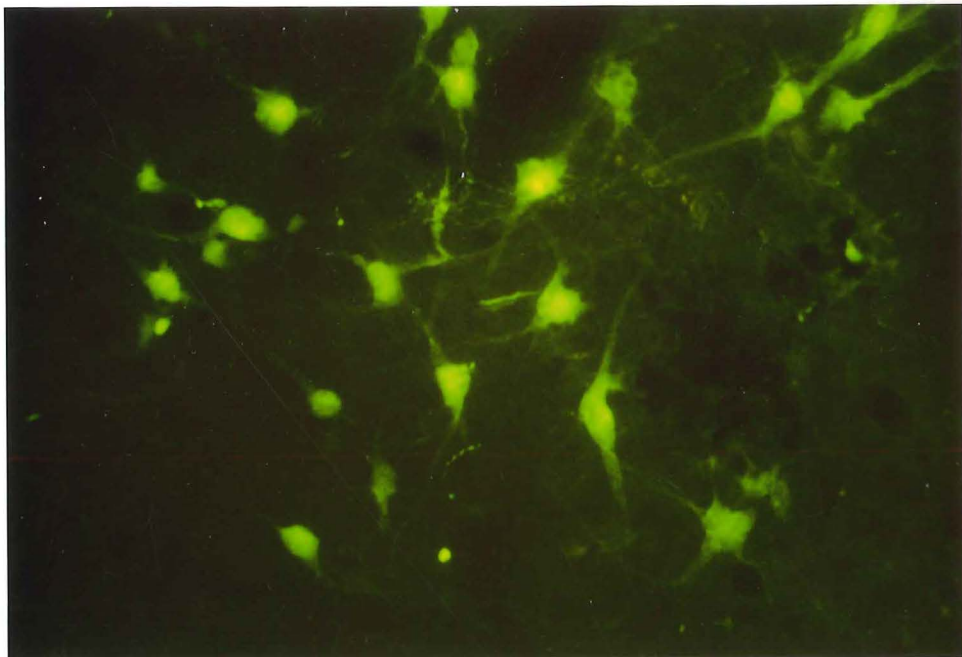
Figure 2.10 FAGLU induced fluorescence in A1 catecholamine
containing cells of the rabbit caudal ventrolateral medulla.

SIMULTANEOUS DETECTION OF SUBSTANCE P - and
PHENYLETHANOLAMINE-N-METHYL TRANSFERASE- LIKE
IMMUNOREACTIVITY

6)   WASH FOR 10' THREE TIMES IN BUFFER MOUNT ON
PLAIN GLASS SLIDES,COVERSLIP

5)   **TRITC-SHEEP**   **FITC-SHEEP**
  ANTI MOUSE 1:50   **ANTI RABBIT 1:50**

(In buffer
containing
1% NSP
24 hrs/23°C)

4)   WASH FOR 10' THREE TIMES IN 1% NSP

3)   **RAT ANTI -**   **RABBIT ANTI -**
  SUBSTANCE P   **PNMT**
    1:1000   **1:2000**

(In buffer
containing
10% NSP
72hrs/23°C)

2)   WASH FOR 10' THREE TIMES IN BUFFER

1) | SUBSTANCE P | | PNMT |

VIBRATOME SECTION
(50 microns)
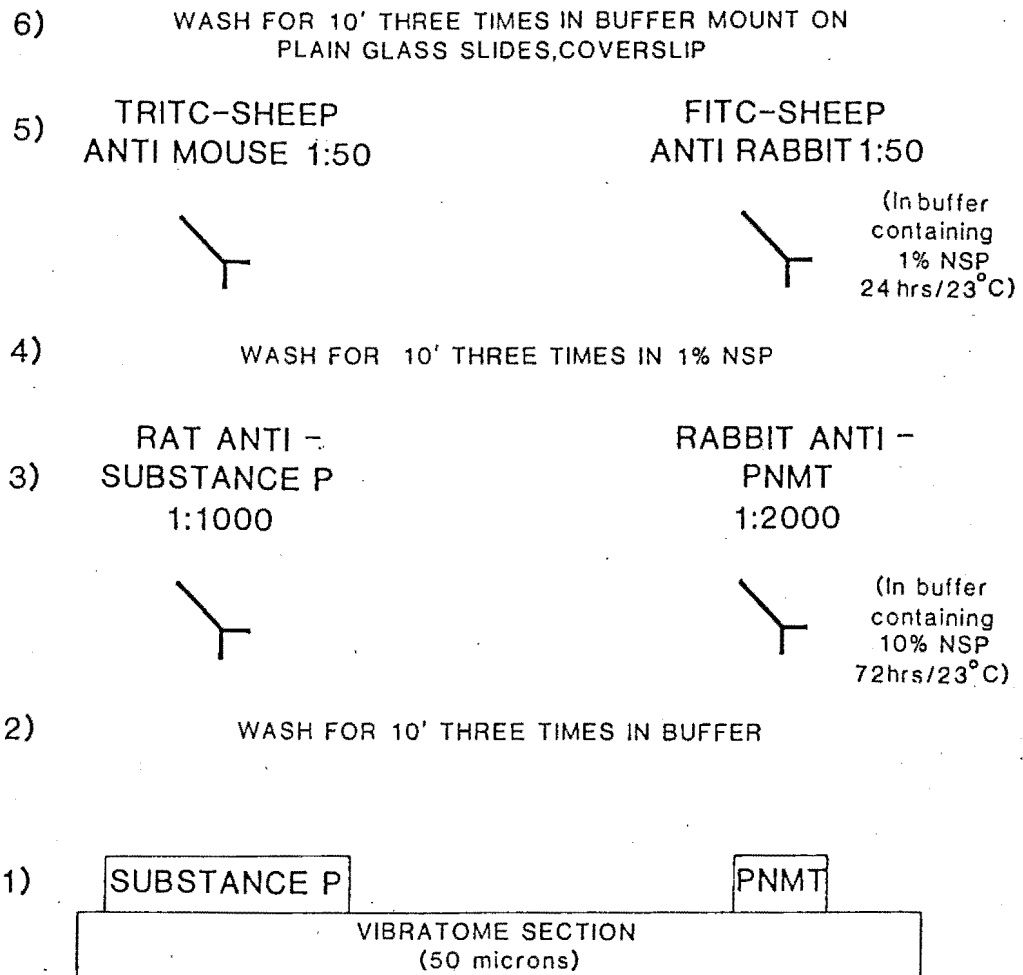
Figure 2.11 Protocol for the simultaneous detection of substance P- and PNMT- like immunoreactivity in vibratome sections of the rat brain. The order of the steps is shown on the left side.

fluoroscein isothiocyanate   (FITC)-conjugated   affinity
purified   sheep   anti   rabbit   antibody   (1/50,   Silenus
Australia) or rhodamine isothiocyanate (TRITC)-conjugated
affinity purified   sheep   anti   mouse   antibody   (1/50,
Silenus),   or   both   antisera together,   or 1% normal sheep
plasma alone. Sections were finally washed   three   times
for twenty   minutes   in   buffer   alone,   mounted on plain
glass slides and coverslipped with   a   glycerol-carbonate
buffer (1:1,   pH   8.6).   The sections were viewed with a
fluorescence microscope.   Filter combinations   L3   (FITC)
and N2   (TRITC)   allowed   absolute discrimination between
the two fluorophores.   Single   label   immunofluorescence
was also   used   in conjunction with FAGLU fluorescence to
demonstrate serotonin cells   and   catecholamine   cells
.(figure 1.3).   Although the catecholamine filter did not
block rhodamine   fluorescence,   the   two   colours   were
clearly distinguishable.

7 Statistical analysis

7.1 Correlation

    Pearson's product-moment correlation coefficient was
used to determine if a significant corelation existed
between two variables.

7.2 Analysis of variance

    7.2.1 Assumptions

    Valid use of analysis of variance requires that
four main assumptions be met. Firstly, the samples
should be drawn at random. Secondly, each sample
should be drawn independently. Thirdly, the variances
should be homogenous. Finally, the variances should
be normally distributed. Failure to ensure that these
assumptions are met can invalidate the analysis.

    In any of the experiments animals were allocated
randomly to each group. In addition to measuring the
effects of different treatments on separate groups of
animals, we also wished to assess the effect of the
different treatments at varying times after each

treatment. The difficulty presented here is that as
stated by Healy (1981), "it is not possible   to
randomize Tuesday to make it equally likely to fall
before or after Monday." This problem was overcome by
using a split plot analysis of variance design as
recommended by Rowell and Walters (1976, 1985) and
described below.

        Similar problems are encountered in ensuring that
the samples are independent. This assumption is one
of the most important in analysis of variance (Evans
1983). This means that no sample should have any
bearing on the measurement of subsequent samples. In
experiments where responses are measured over time it
is clear that the closer one makes one's measurements,
the more likely they are to be the same, a phenomenon
known as serial correlation. Although procedures are
available for testing for the. extent of this
correlation (Rowell and Walters 1985, Hearne et al
1983), the design of the experiments made this
unnecessary (see below).

        The third assumption is that of homogeneity of
variance; this implies that the dispersion around the
mean does not change if the mean changes. Several
methods are available for testing this, of which
Hartleys' Fmax test is the simplest to perform (Sokal
and Rohlf 1981).   However,   since   all   of   the

statistical analyses were performed using the GENSTAT
statistical package (a general statistical package;
Lawes agricultural trust, Rothamstead experimental
station), it proved easier to plot the   residual
against   fitted values to see if there was any
deviation from homogeneity (see figure 2.12). This is
essentially the same procedure as that recommended by
Zivin and Bartko (1976) who suggest examination of a
graph of the means against the standard deviations for
non-linearity, and   hence   homogeneity.   Where
heterogeneity of variance was found to be present, the
data was transformed so as to minimize it.

The final assumption, of normality, was   not
formally tested, since most data from biological
systems is normally distributed, and the analysis of
variance is. relatively insensitive to even quite large
departures from normality (Sokal and Rohlf 1981).

7.2.2 Analysis of variance

Two designs were used in the majority of cases.
Firstly, a simple two-factor analysis of variance was
used to determine whether significant   differences
existed 'between animals' prior to an intervention.
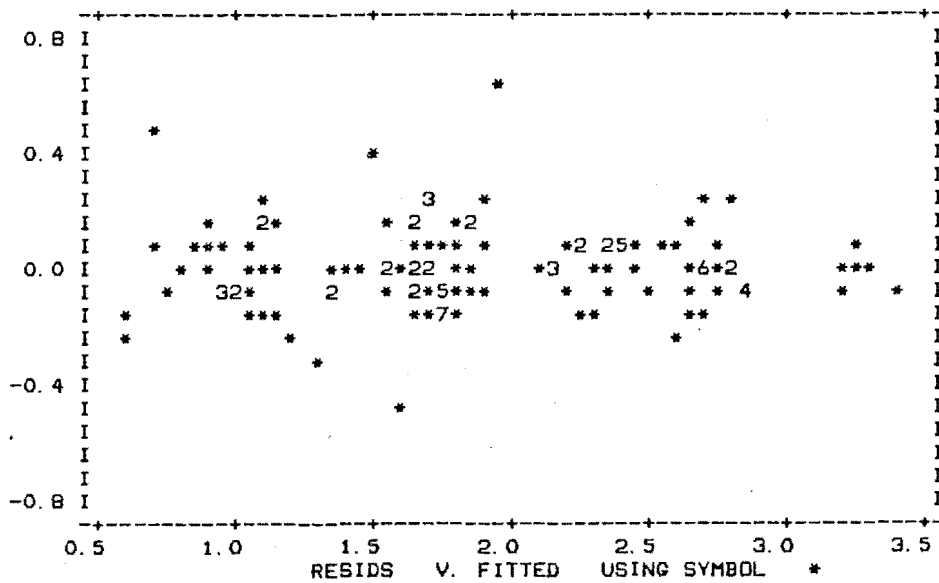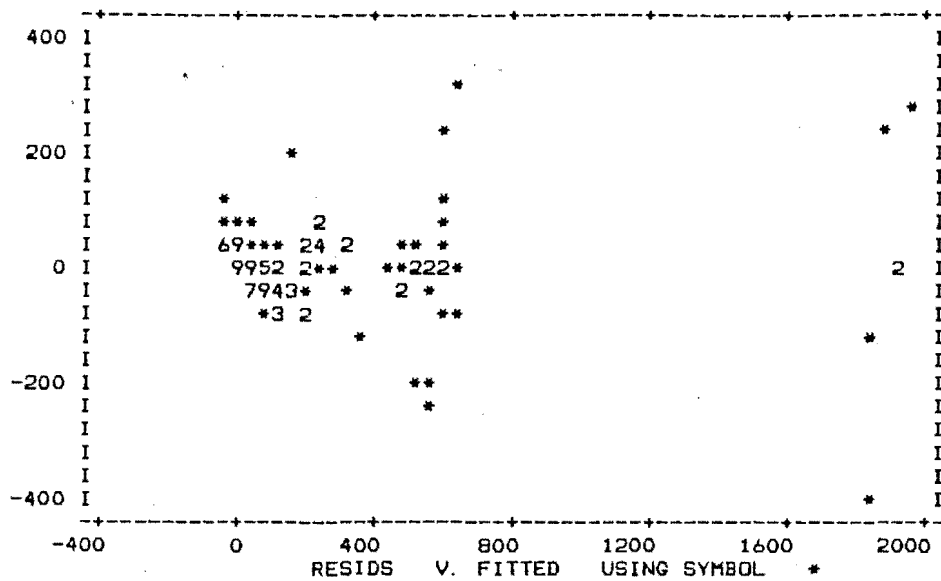An example of this analysis is shown in figure 2.13.

```
      400 I  -+---------+---------+---------+---------+---------+--------+-
          I                                                              I
          I                                                              I
          I                                  .                           I
         'I                               *                              I
          I                                                         *  I
          I                                                           *  I
    - 200 I                                                              I
          I                            *                            *  I
          I                 *                                           I
          I               ***      2        **  *                       I
          I               69*** 24 2      **  *                         I
        0 I                9952 2**     **222*                      2  I
          I                7943*   *      2  *                          I
          I                 *3 2            **                          I
          I                       *          **                         I
          I                                               *          I
     -200 I                              **                             I
          I                              *                              I
          I                                                              I
          I                                                              I
          I                                                              I
     -400 I                                              *             I
          -+---------+---------+---------+---------+---------+--------+-
        -400        0        400       800      1200      1600     2000
                      RESIDS   V. FITTED    USING SYMBOL   *
```

```
    0.8 I  -+---------+---------+---------+---------+---------+--------+-
        I                                                               I
        I                                                               I
        I                                    *                          I
        I                                                               I
        I            *                                                  I
    0.4 I                              *                                 I
        I                                                               I
        I                 *                3    *                        I
        I             *  2*         *  2  *2              *  *           I
        I         *  *** *        ****  *       *2 25* **  *      *      I
    0.0 I       *  *  ***     *** 2*22 **     *3  **  *    *6*2     ***  I
        I        *    32*       2   * 2*5***     *   *   *  * * 4    *   * I
        I   *          ***         **7*          **          **          I
        I   *                    *                *                       I
        I                     *                                          I
   -0.4 I                              *                                 I
        I                                                               I
        I                                                               I
        I                                                               I
   -0.8 I  -+---------+---------+---------+---------+---------+--------+-
          0.5       1.0       1.5       2.0       2.5       3.0      3.5
                      RESIDS   V. FITTED    USING SYMBOL   *
```

Figure 2.12 Plots of residual versus fitted values before (upper panel) and after (lower panel) a logarithmic transformation. Heterogeneity of variance is present prior to the transformation, since the dispersion of residuals increases at higher fitted values. This effect is not present after transformation.

***** ANALYSIS OF VARIANCE *****

VARIATE: X

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	F PR
UNITS STRATUM						
SUBJ	13	536261	82.59	41251	28.525	<.001
TIME	5	19084	2.94	3817	2.639	0.031
RESIDUAL	65	93998	14.48	1446		
TOTAL	83	649342	100.00	7823		
GRAND TOTAL	83	649342	100.00			

***** ANALYSIS OF VARIANCE *****

VARIATE: X

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	F PR
SUBJ STRATUM						
TREAT	1	10767	1.66	10767	0.246	0.629
RESIDUAL	12	525494	80.93	43791		
TOTAL	13	536261	82.59	41251		
SUBJ.TIME STRATUM						
TIME	5	19084	2.94	3817	2.785	0.025
TIME.TREAT	5	11786	1.81	2357	1.720	0.144
RESIDUAL	60	82213	12.66	1370		
TOTAL	70	113082	17.41	1615		
GRAND TOTAL	83	649342	100.00			

Figure 2.13 An example of a two factor (upper panel) and split plot (lower panel) analysis of variance. The tables are part of the GENSTAT output. The data is from an experiment in which animals were observed for a period, and then one group received a treatment and the other a sham treatment. Both of the analyses are on the period prior to the intervention. In the upper panel the analysis shows that there is a significant variation between animals (SUBJ, F13,65 = 28.525, P,0.001). In the lower panel, the analysis shows that there is no difference between the groups (TREAT, F1,12 = 0.246, P=0.629).

If a ` significant between animals variation was found,
then the average value of the pre- intervention period
for each animal was used as a covariate in the
analysis of the treatment effect. Clearly, no
conclusions regarding changes over time can be drawn
from this analysis since this factor is not
randomized.

The second form of analysis employed was a
split-plot analysis of variance. This design is used
where there are repeated measurements on one factor, a
situation that occurred in almost all of the
experiments described in this thesis, since in all
cases the response to an intervention was measured
over time. With this design, the analysis has two
strata, each with its own error term (see figure
2.14). The error term in the upper stratum represents
the variance between groups, whilst the error term in
the lower stratum represents the variance within
groups. It can be seen from the degrees of freedom of
the error term that this procedure is similar to a
comparison between groups at any one time point, and
in fact the results of the analysis are the same if
one uses the means of each animal over the period
being examined. This design is appropriate for
testing the treatment effect using the F-distribution,
but the use of the F-distribution in the sub-plot
portion is generally not justified (Rowell and Walters

1976). Instead, Rowell and Walters (1976) recommend an analysis of contrasts over time.

7.2.3 Analysis of covariance

In most experiments of the kind described in this thesis, the 'resting' or 'pre' values of the animals differ significantly. If this difference is random, it can lead to falsely rejecting a real effect of the treatment. On the other hand, if the difference is systematic, treatment effects may be found where none exist. All of the experiments described here fall into the first (i.e. random) category. An example of the second (i.e. systematic) category might be a comparison of the effect of a drug on blood pressure between two groups of animals where one group had received a prior intervention that lowered (or raised) the mean blood pressure of that group.

This problem has been dealt with by the use of a combination of linear regression and analysis of variance known as analysis of covariance. With this method, the values obtained after the intervention are regressed on the average value before the intervention and the means adjusted prior to analysis. In addition to the assumptions of analysis of variance, analysis

of covariance requires that the slopes of the regression lines of the dependent on the independent variable be homogenous. This was tested in each case where this method was used.

An example of this procedure using data from an experiment in which muscimol was injected into the caudal ventrolateral medulla and neuropeptide Y release from the spinal cord was measured is given below (table 2.14). Without the aid of statistical packages such as GENSTAT these analyses are extremely arduous, and prone to arithmetic errors. In order to simplify matters I have used the means of the post injection values so as to give an analysis that only has one stratum (i.e. groups).

7.2.4 Comparisons of two means

Where the analysis of variance showed that a significant difference was present between groups, and more than one group was present, comparisons were made using students t-test. Since in all cases where this was done, the comparisons were planned, it was not necessary to use the corrections made for post hoc multiple comparisons. This procedure is equivalent to Fisher's (protected) least significant difference

***** ANALYSIS OF VARIANCE *****

VARIATE: Y

SOURCE OF VARIATION	DF(MV)	SS	SS%	MS	VR	F PR
SUBJ STRATUM						
TREAT	1	187609	16.49	187609	2.960	0.111
RESIDUAL	12	760606	66.84	63384		
TOTAL	13	948215	83.33	72940		
SUBJ.TIME STRATUM						
TIME	5	138369	12.16	27674	8.087	<.001
TIME.TREAT	5	27000	2.37	5400	1.578	0.182
RESIDUAL	54(6)	184789	16.24	3422		
TOTAL	64	350159	30.77	5471		
GRAND TOTAL	77	1298374	114.10			

ESTIMATED GRAND MEAN   203.0
TOTAL NUMBER OF OBSERVATIONS   84
NUMBER OF MISSING VALUES   6
MAXIMUM NUMBER OF ITERATIONS   7

UNIT NUMBER	ESTIMATED VALUE
42	83.5
52	387.0
53	396.1
54	423.3
60	336.3
73	132.6

***** ANALYSIS OF VARIANCE *****
(ADJUSTED FOR COVARIATE)

VARIATE: Y

SOURCE OF VARIATION	DF(MV)	SS	SS%	MS	VR	COV EF	F PR
SUBJ STRATUM							
TREAT	1	277394	24.38	277394	10.595	0.980	0.008
COVARIATE	1	472602	41.53	472602	18.051		0.001
RESIDUAL	11	288004	25.31	26182		2.421	
TOTAL	13	1038000	91.22	79846			
SUBJ.TIME STRATUM							
TIME	5	138369	12.16	27674	8.087	1.000	<.001
TIME.TREAT	5	27000	2.37	5400	1.578	1.000	0.182
RESIDUAL	54(6)	184789	16.24	3422		1.000	
TOTAL	64	350159	30.77	5471			
GRAND TOTAL	77	1388159	121.99				

ESTIMATED GRAND MEAN   203.0
TOTAL NUMBER OF OBSERVATIONS   84
NUMBER OF MISSING VALUES   6
MAXIMUM NUMBER OF ITERATIONS   7

UNIT NUMBER	ESTIMATED VALUE
42	83.5
52	387.0
53	396.1
54	423.3
60	336.3
73	132.6

Figure 2.14 An example of a split-plot analysis of variance before (upper panel) and after (lower panel) adjusting for covariates. A detailed description of this analysis is given in the text.

test.

7.2.5 Graphical representation of the data

In cases where no transformation and no adjustment by covariance was made, the points shown in the graphs represent the means at the points shown. Error bars in these cases represent the error at a given time point calculated from the residual mean square in the sub plot stratum of the analysis, but using the degrees of freedom from the main plot portion in order to avoid inappropriately small errors due to correlations over time as discussed above.

In cases where a logarithmic transformation was used the values are plotted on their original scales using the antilogs of the means of the transformed values. In these cases the error bars represent 95% confidence intervals calculated using the standard error of the mean derived as discussed above. These confidence intervals were transformed back to the original scale in the same way as the means, and are therefore asymmetrical.

If covariates were used in the analysis, then the values plotted represent the means adjusted for the

covariates.

TABLE 2.1

AN EXAMPLE OF AN ANALYSIS OF COVARIANCE.

SHAM GROUP		TREATMENT GROUP	
PRE	POST	PRE	POST
253.67	346.00	158.50	384.67
174.83	202.00	220.50	367.23
220.17	99.17	164.50	280.05
48.83	89.33	70.33	174.17
12.17	56.00	25.17	158.83
109.50	242.17	30.67	197.10
56.83	55.25	47.83	189.50

OBTAIN THE SUM OF EACH COLUMN.
S= 876   1089.2   717.5   1751.55

OBTAIN THE SUM OF EACH VALUE SQUARED IN EACH COLUMN.
SS=161141.76   243169.35   109611.32   491577.7

OBTAIN THE SUM OF THE PRODUCTS (PRE.POST) IN EACH GROUP.
S(PRE.POST)=179620.47   219368.63

RECORD THE NUMBER IN EACH COLUMN.
n=   7   7   7   7

OBTAIN THE MEAN FOR EACH COLUMN (S/n)
MEAN=125.14   155.7   102.5   250.22

PRELIMINARY COMPUTATIONS

	PRE	POST

OBTAIN THE SUM OF ALL THE PRE AND POST VALUES.
1.GRAND TOTAL   1593.5   2841.47

OBTAIN THE SUM OF ALL THE PRE AND POST VALUES SQUARED.
2.SUM OF SQUARES   270753.08   734747.05

OBTAIN THE SUM OF GROUP TOTALS SQUARED DIVIDED BY n.
3.SUM OF SQUARES   183168.89   607979.00

CORRECTION TERM - GRAND TOTAL SQUARED DIVIDED BY TOTAL n.
4.CF   181374.45   576710.84

OBTAIN SStot (2-4)
5.SStot   89378.63   158036.21

OBTAIN SSgroups (3-4)
6.SSgroups   1794.44   31268.16

OBTAIN SSwithin (5-6)
7.SSwithin   87584.19   126768.05

TABLE 2.1

PRELIMINARY ANOVA OF THE DEPENDENT VARIABLE- POST

SOURCE OF VARIATION	DF	SS	MS	F	P
GROUPS	1	31268.16	31268.16	2.96	n.s.
WITHIN	12	126768.05	10564.00		
TOTAL	13	158036.21			

THIS ANALYSIS DOES NOT SHOW A SIGNIFICANT DIFFERENCE
BETWEEN THE GROUPS: WE PROCEED TO AN ANALYSIS OF
COVARIANCE.

ANALYSIS OF COVARIANCE

	SHAM GROUP	TREATMENT GROUP

SUM OF THE DEVIATIONS FROM THE MEAN SQUARED

8.PRE	51559.43	36285.429
9.POST	73549.71	53010.857
10.PRE.POST	43365.86	39892.286

CALCULATE REGRESSION COEFFICIENT: b = 10/8

b	.84108	1.09940

SUM OF SQUARES EXPLAINED BY THE REGRESSION $(10)**2/8$

11.	36474.372	43857.673

UNEXPLAINED SUM OF SQUARES 8-11

12.	37075.338	9153.184

MEAN SQUARE 12/n-2

13.	7415.068	1830.637

CALCULATE THE F RATIO   12/13

14.	4.919 n.s.	23.958 p<0.01

REPEAT THE CALCULATIONS TO OBTAIN POOLED WITHIN

	POOLED WITHIN
15.	87844.859
16.	126560.567
17.	83258.146

REGRESSION COEFFICIENT WITHIN GROUPS (b within)

18.	.94779
19.	78910.923
20.	47649.644
21.	4331.78
22.	18.2167

OBTAIN THE F RATIO FOR BETWEEN GROUPS

23.	SUM OF 12.	46228.522
24.	SUM OF n	10
25.	VARIANCE, 23DIVIDE24	4622.852
26.	SSamong slopes, 20-23	1421.122
27.	MSamong slopes	1421.122
28.	F RATIO 27DIVIDE25	.30741 n.s.

THIS INDICATES THAT THE SLOPES OF THE REGRESSIONS DO NOT
DIFFER BETWEEN GROUPS.

CALCULATE SUM OF SQUARES FOR TOTAL AND GROUPS

29.	TOTAL SUM OF PRODUCTS	75787.000
30.	GROUPS SUM OF PRODUCTS	-7471.150
31.	UNEXPLAINED SUM OF SQUARES-TOTAL	93780.283

TABLE 2.1

```
32.          UNEXPLAINED SUM OF SQUARES-GROUPS          0.113
33.            ADJUSTED SUM OF SQUARES 31-20         46130.639
34.              ADJUSTED MEAN SQUARE               46130.639
35.                F RATIO 34DIVIDE21                  10.649
```

FINAL ANALYSIS OF COVARIANCE TABLE

SOURCE OF VARIATION	DF	SS	MS	F	P
ADJUSTED MEANS	1	46130.64	46130.64	10.65	<0.01
ERROR	11	47649.64	4331.78		

THIS SHOWS THAT WHEN THE DIFFERENT BASELINES ARE TAKEN
INTO ACCOUNT, THERE IS A SIGNIFICANT DIFFERENCE BETWEEN
THE TWO GROUPS.

Chapter 3: Table of Contents

<u>Cell groups in the ventrolateral medulla involved</u>
<u>in the control of sympathetic outflow.</u>

1 Introduction

Since the pioneering work of Dittmar (1870) and Owsjannikow
(1871) over one hundred years ago, the importance of the
medulla oblongata in the tonic and reflex control of
sympathetic outflow has been well established. As discussed
in chapter 1, particular importance has been placed on the
role of neurons whose cell bodies lie in the ventrolateral
part of the medulla oblongata in a region extending from the
level of the calamus scriptorius to the caudal pole of the
facial nucleus. This region has been further subdivided
into a portion rostral to the level of the obex,
corresponding to the location of the C1 group of adrenaline
synthesizing neurons (rostral ventrolateral medulla), and a
portion caudal to the obex, corresponding to the location of
the A1 group of noradrenaline synthesizing neurons (caudal
ventrolateral medulla) (figures 1.1, 1.3, 2.10).

In 1981, Blessing et al reported that electrolytic or
chemical lesions of the caudal ventrolateral medulla in the
rabbit, coinciding with the A1 group of
noradrenaline-containing neurons, elicited a marked rise in
blood pressure, and a fall in heart rate which lasted for
one to two hours. On the basis of these experiments, it was

postulated that the hypertensive component of this response
was due to an increase in the activity of a bulbospinal
sympathoexcitatory pathway, possibly originating in the
rostral ventrolateral medulla (Blessing et   al   1981).
However, in 1982, Blessing et al reported that during the
period of hypertension that followed the destruction of this
region, there was a massive elevation in plasma vasopressin
concentrations. These authors suggested that this increase
in the level of a potent vasoconstrictor hormone, was
responsible for the   elevation in arterial pressure, since
the increase in pressure persisted after   spinal   cord
transection, and   was   attenuated   by   intravenous
administration of the vasopressin antagonist.

In subsequent experiments performed by   Minson   et   al
(1984,1986) in the rat, and by Elliott et al (1985) in the
rabbit the relative importance of vasopressin and   the
sympathetic   nervous   system   was   examined   using
pharmacological techniques. Briefly, these workers found
that the hypertensive response was still present following
electrolytic lesions in Brattleboro rats, which do not
synthesize vasopressin;   and that the hypertension could be
blocked by prior chemical sympathectomy with intravenous
6-hydroxydopamine, or by the intravenous administration of
the alpha   receptor   blocking   agent   phentolamine.
Furthermore, by the use of a vasopressin antagonist, it was
found that the heart rate component of the response was due
partly to a baroreflex-mediated increase in vagal tone, and

partly as an effect of vasopressin. These results supported the original idea that the increase in arterial pressure was consequent upon an increase in peripheral sympathetic nerve activity.

In this chapter the effect of electrolytic lesions of the caudal ventrolateral medulla on renal nerve activity is described. In addition, the effect of chemical stimulation and inhibition of the rostral and caudal ventrolateral medulla, on arterial pressure and renal nerve activity is examined.

2 Methods

In the rabbits in which electrolytic lesions of the caudal ventrolateral medulla were performed, renal nerve electrodes were implanted under halothane anaesthesia (see chapter 2) either 1-2 days (5 animals), or 1-2 hours (5 animals), prior to the commencement of the experiments. In these experiments, arterial pressure was monitored from a central ear artery (see chapter 2); a marginal ear vein was cannulated for the administration of drugs.

After the preliminary procedures had been completed, the animals were allowed to rest for at least thirty minutes. Renal nerve activity, blood pressure and heart rate were

then recorded at five minute intervals for thirty minutes.
At the end of this period, electrolytic or sham lesions of
the caudal ventrolateral medulla were carried out (see
chapter 2), and renal nerve activity, blood pressure and
heart rate, were then monitored for a further forty-five
minutes.

In the second series of experiments, rabbits were
anaesthetized with urethane (see chapter 2) and an electrode
was implanted for the recording of renal nerve activity.
Blood pressure and heart rate were recorded from a catheter
in a femoral artery.   The animals were then placed in a
stereotaxic frame and the dorsal surface of the medulla
oblongata was exposed to enable the microinjection of
glutamate or GABA (see chapter 2).

Histological verification was performed following lesions or
microinjections (see chapter 2).

3 Results

3.1 Electrolytic lesions

Electrolytic lesions of the caudal ventrolateral medulla were found to destroy most of the catecholamine fluorescent neurons in this area. No formal cell counts were performed, since the object of these experiments was to examine the efferent mechanisms of the well documented hypertensive response, rather than the role of the A1 catecholamine neurons. The effect of electrolytic lesions in the caudal ventrolateral medulla on blood pressure, heart rate and renal nerve activity, is shown in figure 3.1. Immediately following the cessation of anaesthesia and the recommencement of recordings, blood pressure and renal nerve activity were similar, whilst heart rate had already fallen in the lesioned group. Over the next forty-five minutes, blood pressure and renal nerve activity rose progressively, with a peak increase in blood pressure of 41.0 mmHg and a peak increase in renal nerve activity of 440% when compared with the sham group.

The increase in blood pressure was statistically significant when compared with the sham group ($F=15.6$,
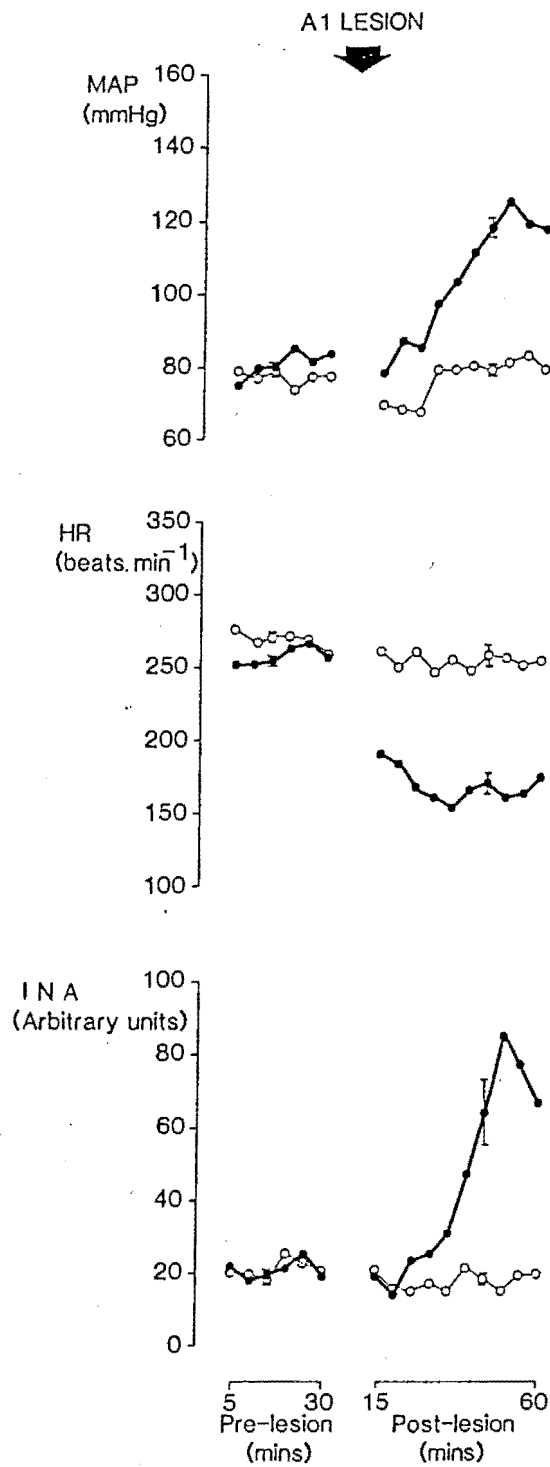
Figure 3.1 Effect of electrolytic lesions in the region of the caudal ventrolateral medulla corresponding to the A1 cell group on integrated nerve activity (INA), blood pressure (MAP), and heart rate (HR).

p=0.006)', as was the increase in renal nerve activity
(F=8.3, p=0.021), and the fall in heart rate (F=7.2,
p=0.028). Furthermore, the increase in renal nerve
activity corresponded temporally with the increase in
blood pressure (r=0.946 p<0.001), rather than the fall in
heart rate (r=-0.423 p>0.1).

3.2 Microinjections

The effect of microinjections of GABA or glutamate on
blood pressure and renal nerve activity was assessed in
four rabbits. Figure 3.2 shows the typical effect of
these microinjections. In each case there was a change
in mean arterial pressure of about 20 mmHg. In each case
the change was accompanied by a directionally equivalent
change in renal nerve activity. Injections of the
excitatory amino acid glutamate into the caudal
ventrolateral medulla elicited falls in arterial pressure
and renal nerve activity, whilst injections of GABA had
the opposite effect. In contrast, glutamate elicited
increases in renal nerve activity and arterial pressure
from the rostral medulla whilst GABA had the opposite
effect (figure 3.2). The changes observed in renal nerve
activity did not always parallel the changes in arterial
pressure.
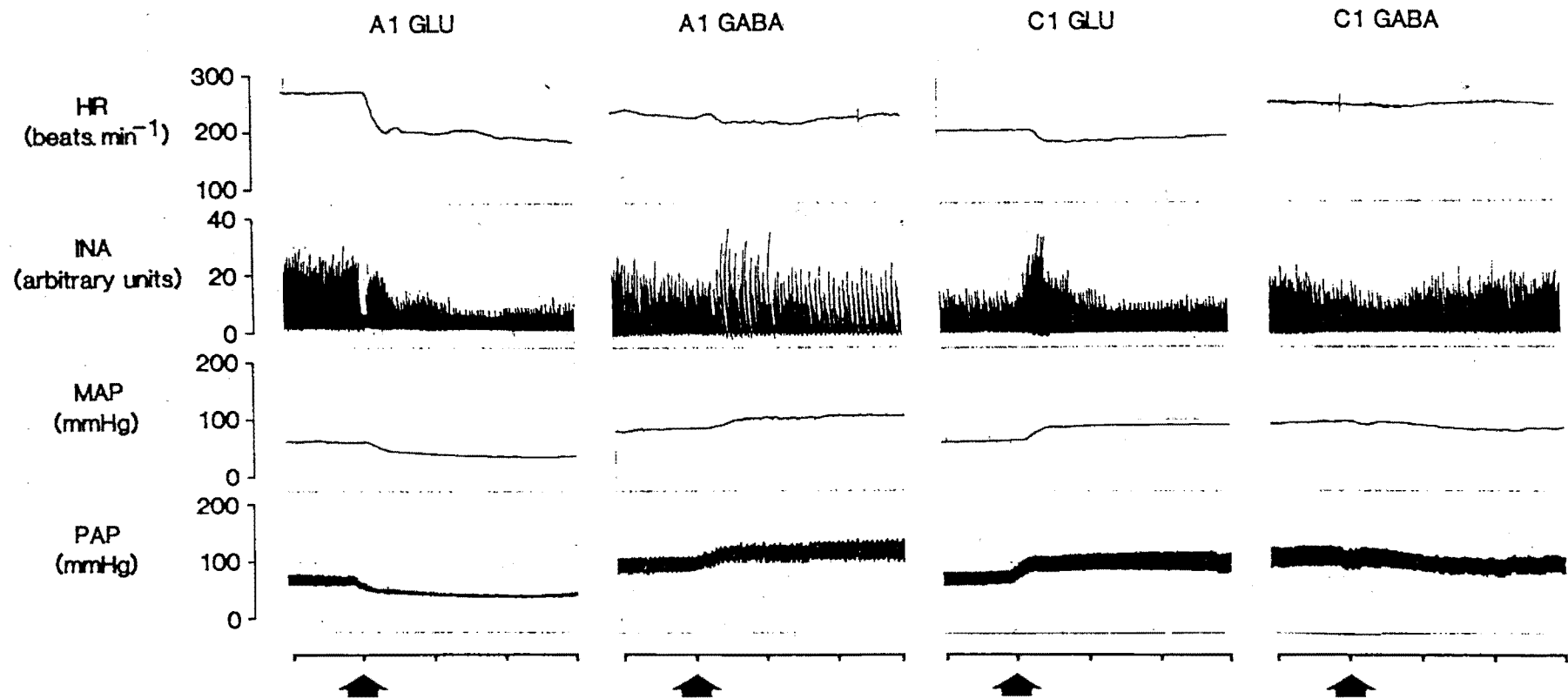
Figure 3.2 Effect of microinjection of l-glutamate (GLU), and gamma- aminobutyric acid (GABA) in the caudal (A1) and rostral (C1) regions of the ventrolateral medulla on heart rate (HR), integrated nerve activity (INA) and mean (MAP) and phasic (PAP) arterial pressure.

4 Discussion

In agreement with previous studies, anodal lesions of the caudal ventrolateral medulla in the region of the A1 neurons elicited increases in blood pressure and falls in heart rate. Anodal lesions rather than cathodal or chemical lesions, were used rather than cathodal electrolytic lesions, since the cardiovascular response to anodal lesions has been well characterized. The finding that renal nerve activity is increased following lesions, and that the increase corresponds temporally to the increase in blood pressure but not the fall in heart rate, supports the findings of previous workers (Minson and Chalmers 1984; Elliott et al 1985), who have suggested that the hypertensive response is predominantly sympathetically mediated without ruling out a role for vasopressin.

These findings are at variance with the original conclusions of Blessing et al (1982), who reported that the electrolytic destruction of this area in the same way as described above leads to a vasopressin-mediated hypertension. This conclusion was based mainly on three findings. Firstly, there was a significant increase in plasma vasopressin levels after the lesions. Secondly, administration of a specific vasopressin vascular antagonist to rabbits that had been lesioned produced a significant fall in arterial pressure, although without restoring pressure to the same

level as that seen in the sham-treated animals. Thirdly, animals that had been spinally transected, and whose blood pressure had been restored with an infusion of noradrenaline, responded to lesions of the A1 region, produced with the neurotoxic agent kainic acid, with an increase in arterial pressure. Furthermore, the increase in arterial pressure seen in this group was reversed by the administration of the vasopressin antagonist.

The finding that vasopressin levels are markedly increased following A1 lesions has been confirmed in a number of studies in both the rat (Chalmers et al 1984; Minson and Chalmers 1984; Minson et al 1986), and the rabbit (West et al 1984; Elliott et al 1985). Furthermore, studies by these authors have shown that rats that do not synthesize vasopressin (Brattleboro rats) respond to A1 lesions in the same way as those that do. In contrast, Imaizumi et al (1985), have reported in an apparently identical series of experiments, that the degree of hypertension present in these non-vasopressin synthesizing rats is much less than in control animals. The reason for this discrepancy is not clear. In the studies in rabbits (West et al 1984; Elliott et al 1985), the finding that administration of a vasopressin antagonist produced a fall in arterial pressure following lesions was replicated, however the magnitude was quite small. Finally, the evidence obtained from spinally transected rabbits must be treated with caution since this preparation differs so markedly both in the way in which the

'lesion' was performed, the conscious state of the animal, and the effect of spinal transection. In fact, other workers have demonstrated a pressor response to chemical stimulation of the caudal ventrolateral medulla in spinally transected rats (Kubo et al 1985). In addition to demonstrating a relatively minor role for vasopressin in the genesis of 'A1 hypertension', these authors have also provided pharmacological evidence that the sympathetic nervous system is involved. In the rabbit, the hypertensive response was prevented or abolished with the alpha adrenergic antagonist phentolamine, whilst in the rat, the response was abolished by prior destruction of the sympathetic nervous system with the neurotoxin 6-hydroxydopamine and adrenalectomy. In the experiments reported by Imaizumi et al (1985), a similarly hypotensive effect of phentolamine was found, although these authors also found a hypotensive effect of the vasopressin antagonist. Despite the considerable controversy surrounding the relative roles of the different effector mechanisms in this form of experimental hypertension, the results provide a strong indication that the sympathetic nervous system participates in mediating the increase in blood pressure, a suggestion that is supported by the direct recordings of renal nerve activity reported here.

In an attempt to define the site of this sympathoexcitatory pathway more precisely (figure 3.2) excitatory (glutamate) and inhibitory (GABA) amino acids were injected into the

ventrolateral medulla.   Injections of glutamate into the
caudal ventrolateral medulla elicited falls in blood
pressure demonstrating that this is a pressor centre, whilst
injections of GABA elicited increases, suggesting that it is
tonically active.   These findings confirm those of previous
workers (Blessing and Reis 1982, Sved et al 1985a), and have
been extended to show that the changes are accompanied by
directionally similar changes in renal nerve activity. This
result suggests that the increases and decreases in arterial
pressure are mediated by sympathoexcitatory and
sympathoinhibitory pathways, since mediation by another path
would result in a baroreflex mediated change in renal nerve
activity that is directionally opposite to the change in
blood pressure, such as occurs during the intravenous
administration of hyper- or hypo- tensive drugs, or during
occlusion of the aorta or vena cava (figure 2.8).

Injections of glutamate and GABA into the rostral
ventrolateral medulla produced effects on blood pressure
opposite to those seen in the caudal ventrolateral medulla,
in agreement with the findings of many other workers
(Dampney et al 1982,1985; Goodchild et al 1982,1984; Ross
et al 1984;   Willette et al 1984a,b). As with the caudal
ventrolateral medulla, these changes were accompanied by
directionally equivalent changes in renal nerve activity,
supporting the idea that this is a tonically active
vasopressor centre.

These findings confirm the existence of sympathoexcitatory and sympathoinhibitory centres in the rabbit, and show that the effects of stimulating, inhibiting or destroying these regions is related to effects on cell bodies rather than axons of passage.

The unanswered question concerns the nature of the neurons responsible for mediating these effects. In the remainder of this thesis I will describe experiments that were designed to address this problem. Three approaches were used. Firstly, the effect of inducing a mass release of neurotransmitter from catecholamine- or indoleamine-containing neurons on renal nerve activity, blood pressure, heart rate, and plasma adrenaline, noradrenaline and vasopressin was studied. Secondly, the release of putative neurotransmitters from the spinal cord in response to a variety of hypertensive stimuli was examined. Thirdly, immunohistochemical and fluorescence techniques were used in an attempt to define the different sub-populations of neurons present in the ventrolateral medulla.

Chapter 4: Table of Contents

Are catecholamine and indoleamine neurons
sympathoexcitatory?

1 Introduction

In the   previous   chapter,   work   was   presented   that
demonstrated the   presence   of   a sympathoexcitatory pathway
originating in the medulla.   In this and subsequent chapters
I will describe experiments in which   I   have   attempted   to
identify the   neurons responsible for mediating this effect.
Several approaches are available to achieve this.   Firstly,
one can   apply   compounds   that   are   known to be present in
neurons, and   see   if   they   have   effects   on ᛫ sympathetic
outflow.   Secondly, one can apply agonists or antagonists of
various compounds and see if these have an effect.   Thirdly,
one can   stimulate   a   pathway and see if compounds that are
known to be present in nerves are released, and fourthly one
can examine a   pathway   electrophysiologically ᛫at   cellular
level so as it determine its inputs and projections and then
examine   the   neuron   morphologically.   Finally,   various
combinations of these techniques may be used.

In this chapter the results of investigations into the ᛫ role
of serotonin-   and   catecholamine-   containing   neurons   are
described.

As discussed in chapter 1, serotonin-containing neurons   are

found throughout the medulla, pons and midbrain of many species, and their role in the central control of blood pressure has been a subject of much controversy (see chapter 1). Similarly, the widespread distribution of catecholamine-containing neurons throughout the neuraxis has led to many studies on their possible role in cardiovascular control (see chapter 1).

In this chapter I will describe experiments where the effects of intracisternally administered 5,7-dihydroxytryptamine (57DHT) and 6-hydroxydopamine (6OHDA) on blood pressure, heart rate, renal nerve activity and plasma vasopressin, adrenaline and noradrenaline in the unanaesthetized rabbit were assessed. These neurotoxins have been used extensively in this fashion by a number of workers and have been demonstrated to elicit an acute release of neurotransmitter lasting for two to three hours (Lewis et al 1974; Korner et al 1978; Head and Korner 1980,1982), followed by a destruction of these neurons (Hedreen and Chalmers 1972; Chalmers and Reid 1972; Lewis et al 1974; Wing and Chalmers 1974; Korner et al 1978; Korner and Head 1981; Head and Korner 1982; Berge et al 1985; Elliott et al 1985) over one to two weeks. The fact that these neurotoxins exert an acute effect of release has enabled a number of workers to use this model as a way of assessing the role of substances released from these neurons (Lewis et al 1974; Finch 1975; Korner et al 1978; Head and Korner 1980, 1982; Korner and Head 1981; Elliott et al

1985; Reidel 1985). The cardiovascular changes that occur include a rise in blood pressure, a fall in heart rate and well documented changes in peripheral blood flow (Korner et al 1978; Korner and Head 1981). In addition, an increase in renal nerve activity has been reported in animals treated with 6-hydroxydopamine, an increase that was significant at an ambient temperature of 22 degrees C, but not at a temperature of 28 degrees C (Reidel 1985). The purpose of the present experiments was to examine the extent to which administration of these neurotoxins would increase renal sympathetic nerve activity. In addition, plasma adrenaline levels (an index of adrenal nerve activity) were measured. In this way, it was possible to assess directly the role of central serotonin- and catecholamine- neurons on sympathoexcitatory pathways. Finally, plasma vasopressin levels were also measured, since these have been shown to increase in several models of neurogenic hypertension (Blessing et al 1982; Sved et al 1985; Sved 1986; Alexander and Morris 1986), and during activation of central serotonin pathways (Steardo and Iovino 1986) and central noradrenaline pathways (Day and Renaud 1984; Day et al 1985a,b; Benetos et al 1986; Randle et al 1986a,b).

2 Specific methods and experimental protocol

The drugs injected into the previously implanted cisternal catheters (see chapter 2) were 6OHDA hydrobromide (0.6mg/kg, n=5), 57DHT creatinine sulphate (0.633mg/kg, n=7), and creatinine sulphate (0.333mg/kg, n=5). All of these compounds were dissolved in 0.15ml of vehicle (0.9% NaCl and 1% Ascorbic acid) immediately prior to injection. Following injection, the catheter was flushed with 0.3ml of vehicle.

The surgical procedures and methods for recording physiological variables, as well as the biochemical assays are described in chapter 2. Statistical analysis was performed as described in chapter 2, using analysis of variance and covariance. The vasopressin, catecholamine and renal nerve activity data were subjected to a logarithmic transformation for the reasons discussed in chapter 2. The symbols in the graphs of these results therefore represent 95% confidence intervals (figures 4.4,4.5). For the purposes of the analysis, the responses were divided into four phases, control, initial, intermediate and late. The time points used were 10 and 20 minutes prior to the injections (control), 5 and 15 (initial), 30 and 60 (intermediate), and 120 and 180 (late), minutes after the injections. These times correspond to the times at which blood was sampled for the biochemical assays.

3 Results

3.1 Effects on blood pressure and heart rate

Injection of creatinine sulphate produced a small rise in
blood pressure during the initial phase when compared
with the control phase (table 4.1, figure 4.1), but
returned to control levels within 30 minutes and remained
stable for the remainder of the experiment. Heart rate
rose gradually throughout the experiment, and was
significantly greater than control levels in the late
phase (table 4.1, figure 4.1).

Treatment with 57DHT elicited a prompt rise in blood
pressure that was maximal in the initial phase, but
persisted throughout the post- injection period (tables
4.1,4.2, figure 4.1,4.2). The initial rise in blood
pressure was associated with a pronounced bradycardia
(tables 4.1,4.2, figure 4.1,4.2) that returned to control
levels during the intermediate phase. During the late
phase, there was a significant tachycardia when compared
with the control period, but when compared with the
vehicle treated animals in the late phase, heart rate was
decreased (tables 4.1,4.2, figure 4.1).

TABLE 4.1

DIFFERENCES BETWEEN POST-INJECTION AND PRE-INJECTION VALUES FOR SHAM ANIMALS AND ANIMALS TREATED WITH 5,7DHT OR 6OHDA

(The errors for these data are shown in Figures 4.1, 4.4, 4.5. Each value represents the difference between the average of 2 control phase values and 2 test phase values, corresponding to the time points at which blood samples were taken for hormone assays)

	n	INITIAL PHASE-CONTROL PHASE						INTERMEDIATE PHASE-CONTROL PHASE						LATE PHASE-CONTROL PHASE					
		BP mm Hg	HR bmin^{-1}	%INA	AVP pgml^{-1}	NA pgml^{-1}	A pgml^{-1}	BP mm Hg	HR bmin^{-1}	%INA	AVP pgml^{-1}	NA pgml^{-1}	A pgml^{-1}	BP mm Hg	HR bmin^{-1}	%INA	AVP pgml^{-1}	NA pgml^{-1}	A pgml^{-1}
SHAM	5	13*	1	10	3	130	149	4	23	8	-1	123	101	1	42*	4	9	110	66
5,7-DHT	7	32*	-84*	54	100*	698*	1685*	24*	6	36	47*	431*	1388*	20*	42*	59	59*	409*	630*
6-OHDA	5	29*	-54*	16	9	251	615*	17*	17	17	-4	161	97	24*	16	16	30*	256*	231*

* P < 0.05

TABLE 4.2

DIFFERENCES BETWEEN VALUES OBTAINED IN SHAM ANIMALS, ANIMALS TREATED WITH 5,7DHT AND ANIMALS TREATED WITH 6OHDA

(The errors for these data are shown in Figures 4.1, 4.4, 4.5. Each value represents the difference between 2 values obtained in one group of animals with 2 values obtained in another group of animals corresponding to the time points at which blood samples were taken for hormone assays).

	INITIAL PHASE						INTERMEDIATE PHASE						LATE PHASE					
	BP mm Hg	HR bmin^{-1}	%INA	AVP pgml^{-1}	NA pgml^{-1}	A pgml^{-1}	BP mm Hg	HR bmin^{-1}	%INA	AVP pgml^{-1}	NA pgml^{-1}	A pgml^{-1}	BP mm Hg	HR bmin^{-1}	%INA	AVP pgml^{-1}	NA pgml^{-1}	A pgml^{-1}
5,7DHT-SHAM	19*	-108*	43*	96*	495	1550*	20*	-41	26	47*	234	1301	19*	-24	54*	49*	225	57*
6-OHDA-SHAM	16*	-65*	11	-8	89	485	13	-16	14	-17	6	15	23*	-36	17*	7	114	184
6-OHDA-5,7DHT	-4	44	-32	-105*	-406	-1065*	8	25	-13	-63*	-228	-1285*	-4	-12	-37	-42	-111	-394

* p < 0.05
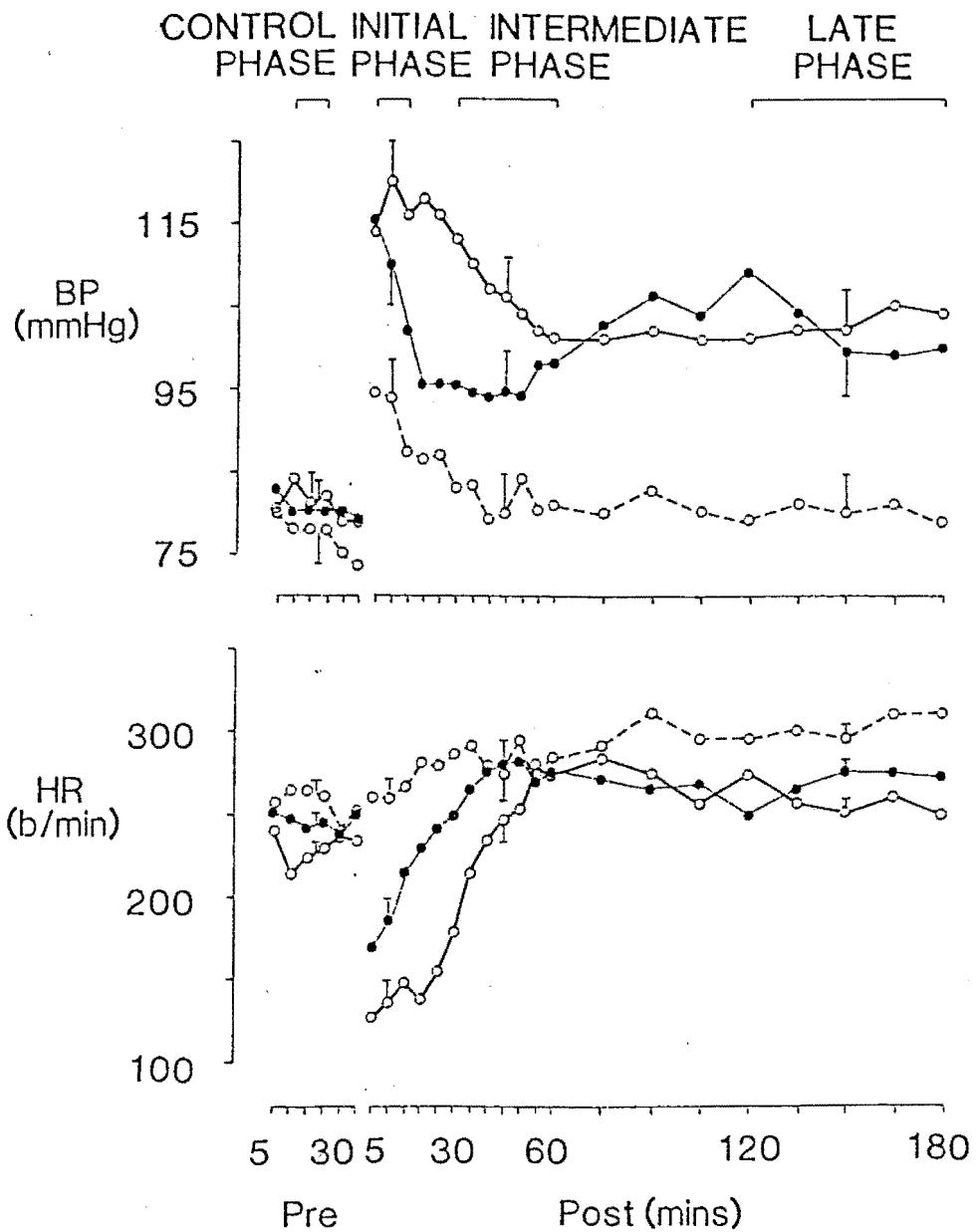
Figure 4.1 Effect of vehicle (open circles, dotted lines), 6OHDA (closed circles, continuous lines) and 57DHT (open circles, continuous lines), on mean arterial pressure (BP) and heart rate (HR).
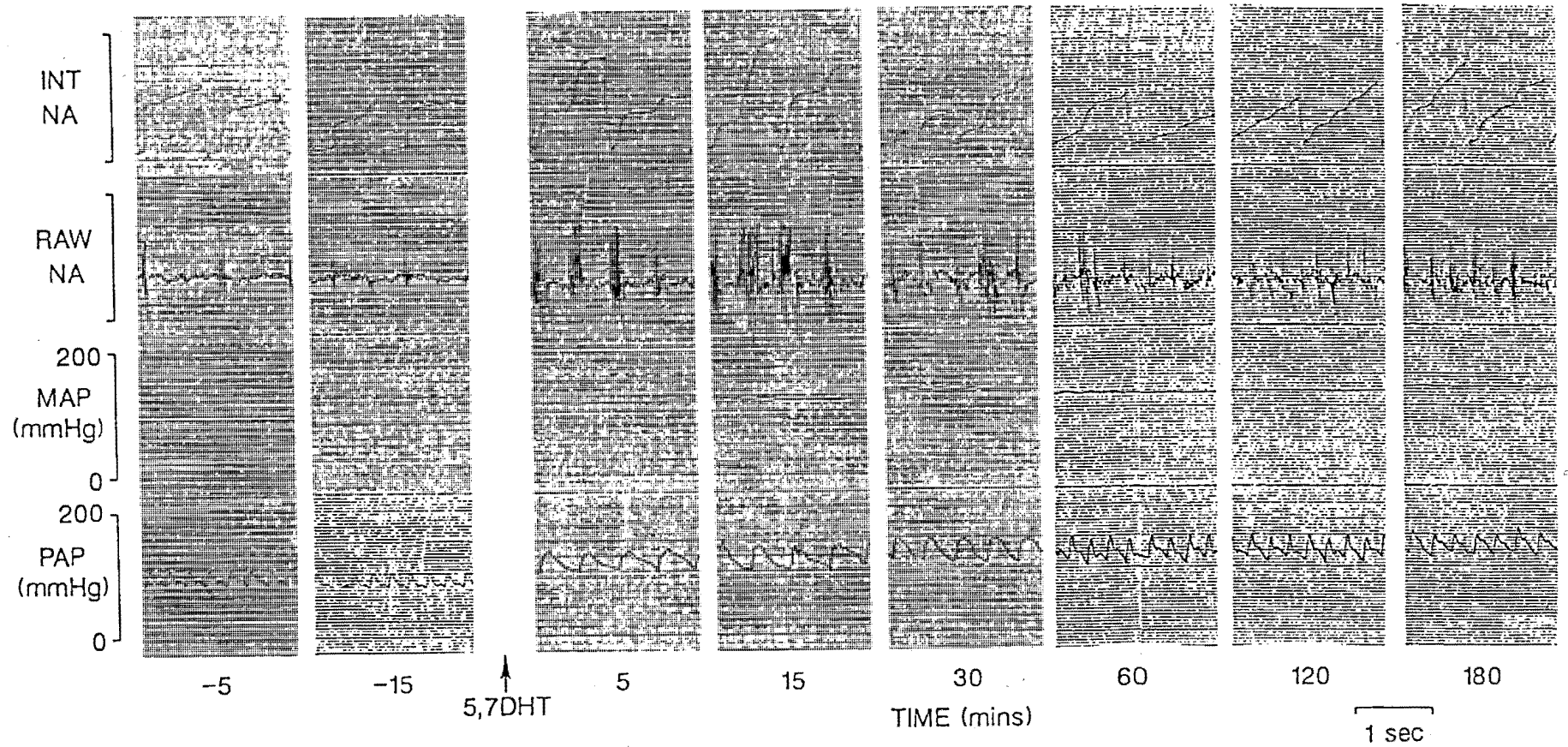
Figure 4.2 An example of the effect of 57DHT on nerve
activity and blood pressure. The sensitivity of the
integrator was decreased five-fold after the injection.

Injection of 6OHDA produced a hypertensive response that was clearly biphasic. There was a prompt increase in blood pressure immediately following the injection. Over the next 15 minutes, blood pressure returned towards control levels, and was not significantly greater than in the vehicle treated animals during the intermediate phase. In the late phase, there was a second rise in blood pressure, and during this period, blood pressure remained significantly greater than in the vehicle treated animals (tables 4.1,4.2, figure 4.1,4.3).

3.2 Effects on renal nerve activity

Renal nerve activity was unaffected by injection of vehicle (figure 4.4, table 4.1), but was increased both by treatment with 57DHT (in the initial and late phase) and 6OHDA (in the late phase), when compared with injection of vehicle (figure 4.4, tables 4.1,4.2).
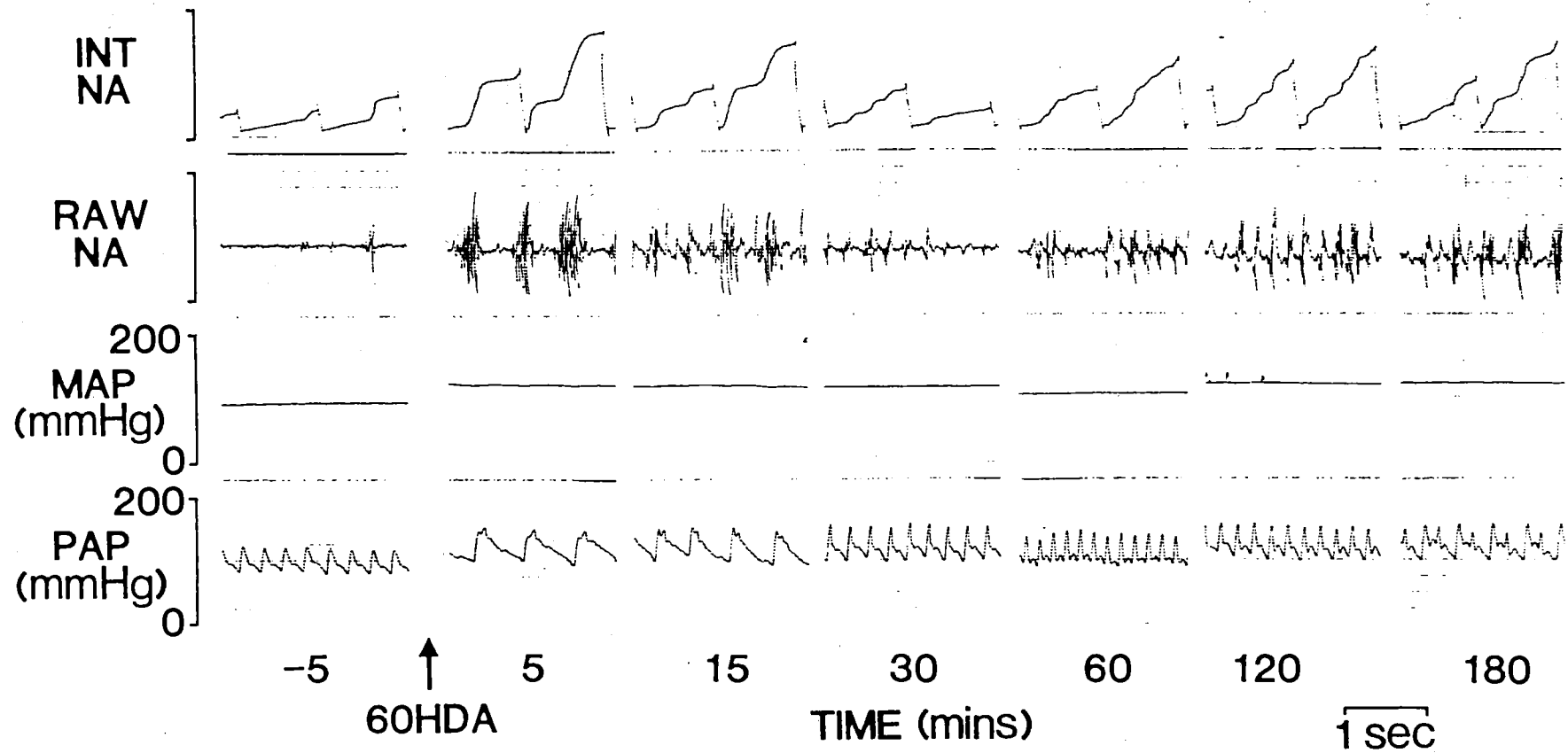
Figure 4.3 An example of the effect of 6OHDA on nerve
activity and blood pressure.
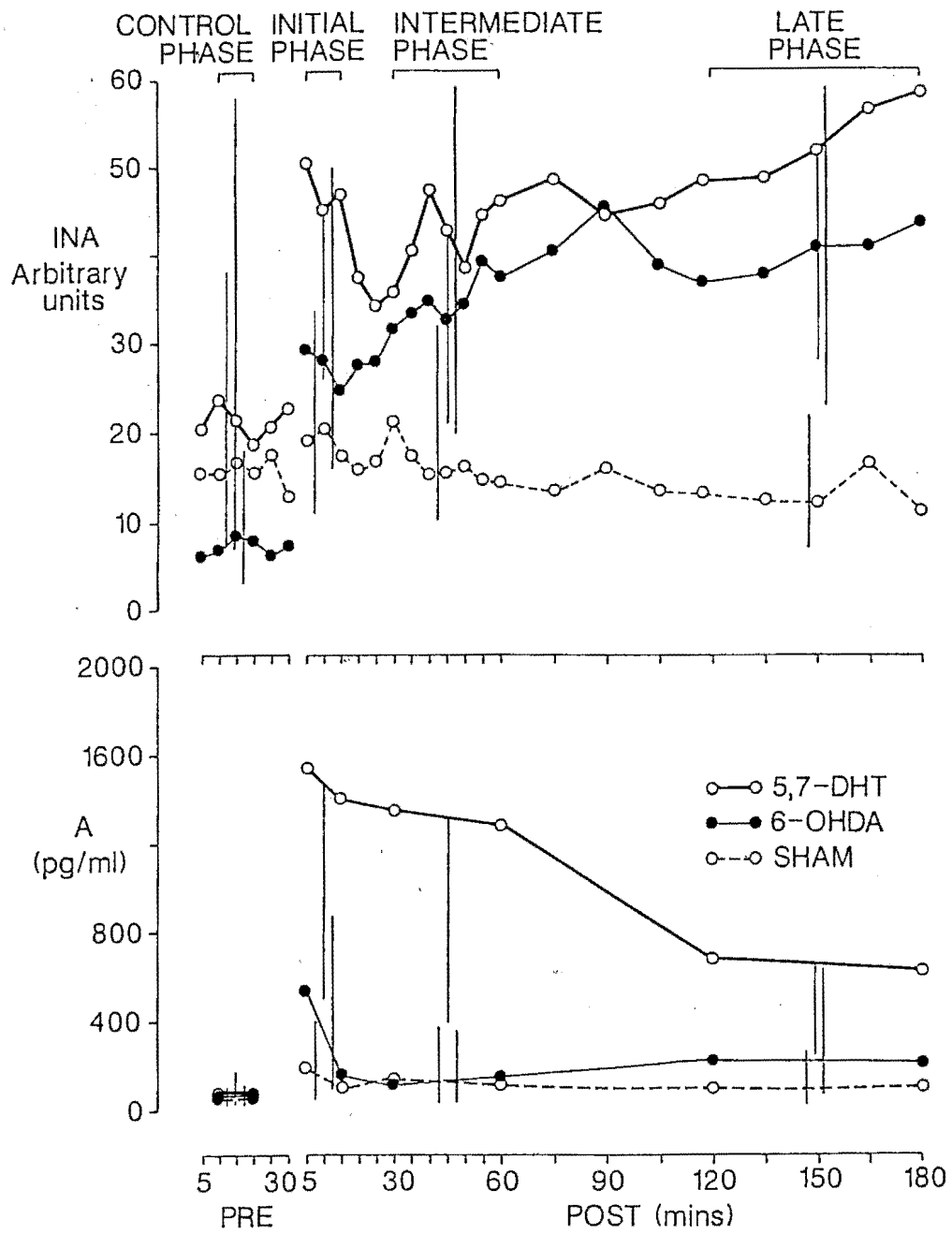
Figure 4.4 Effect of vehicle, 6OHDA and 57DHT, on integrated nerve activity and plasma adrenaline levels. Notation as in Figure 4.1.

3.3 Effects on plasma adrenaline levels

The changes observed in plasma adrenaline levels are shown in figure 4.4. Plasma adrenaline levels were not changed by injection of vehicle (table 4.1), but were significantly elevated by the injection of 57DHT throughout the three post-injection phases (table 4.2, figure 4.4). Injection of 6OHDA produced much smaller changes in plasma adrenaline levels, that were significantly different from those seen in the vehicle treated animals, but only in the initial phase (table 4.2, figure 4.4).

3.4 Effects on plasma noradrenaline levels

The effects of the three interventions on plasma noradrenaline levels are shown in figure 4.5. When compared with the pre-injection levels, the increase seen in the 57DHT group was significant during all three phases, as was the increase seen in the 6OHDA group during the initial and late phases (table 4.1). When compared with the levels seen in the sham group, there was no significant change in noradrenaline levels in either group in any phase.
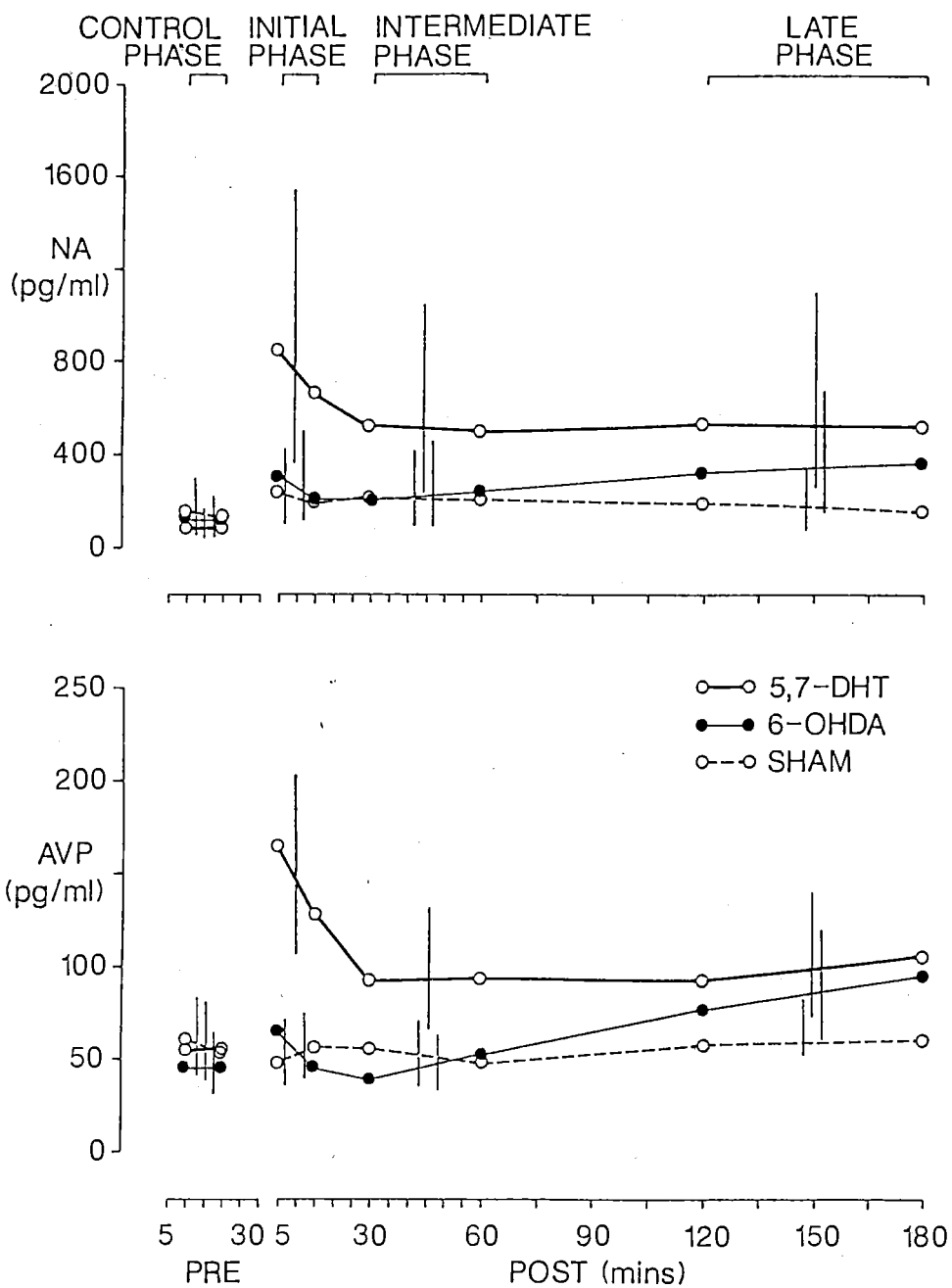
Figure 4.5 Effect of vehicle, 6OHDA and 57DHT, on plasma noradrenaline and vasopressin concentrations. Notation as in Figure 4.1.

3.5 Effects on plasma vasopressin levels

The pre-injection levels of vasopressin seen in all of the animals was higher than that seen in experiments by other workers (Elliott et al 1985), possibly reflecting the surgical procedures undertaken two days earlier. Plasma vasopressin levels were unaffected by injections of either vehicle or 6OHDA (tables 4.1, 4.2, figure 4.5). In the animals treated with 57DHT, there was a three-fold increase in plasma vasopressin levels initially; thereafter the levels decreased, but remained significantly elevated for the duration of the experiments (tables 4.1, 4.2 figure 4.5).

4 Discussion

The hypertensive response to intracisternal 57DHT described here is associated with an increase in renal nerve activity, plasma adrenaline and plasma vasopressin levels throughout most of the response (tables 4.1, 4.2, figures 4.1, 4.4, 4.5). In contrast, the response to intracisternal 6OHDA is associated with an increase in renal nerve activity, without a significant effect on plasma hormone levels. Interestingly, despite a 300-400% increase in renal nerve activity, there was no consistent corresponding increase in

plasma noradrenaline levels, which have been suggested by several groups as a useful index of sympathetic nerve activity (Esler et al 1984; West et al 1984; Elliott et al 1985; Hubbard et al 1986).

The changes in blood pressure and heart rate seen after injection of 57DHT, 6OHDA, or vehicle, were similar to those reported previously by Korner and Head (1981) and Elliott et al (1985), with the exception that I did not observe a tachycardia during the late phase of the response to 57DHT when compared with vehicle treated animals at the same time. Several reasons could account for this discrepancy. First, in the report by Korner and Head (1981), the results were analysed as a 'within animals' experiment. If by this, it is meant that the significance of the difference between times before and after the injections was assessed, a result equivalent to that shown in table 4.1 would be obtained, that is a tachycardia during the late phase of the response to 57DHT. In this thesis, I have chosen to use a 'between groups' analysis as outlined in chapter 2, and since the heart rate of the vehicle treated group increased over time, no tachycardia was seen in the 57DHT treated animals. The possibility exists that a different group of vehicle treated animals would not have responded in this fashion, although similar results were obtained by Elliott et al (1985). Secondly, the animals used in these experiments received a halothane anaesthetic two days earlier (an unavoidable consequence of the short lifespan of the renal nerve

electrodes)', whereas the experiments performed by Korner and

Head (1981) were on rabbits that had been operated on two

weeks earlier. Finally, I used 57DHT, whereas Korner and

Head (1981) used 56DHT, although it should be noted that

Elliott et al (1985) reported that these compounds exert

similar effects on blood pressure and heart rate when given

intracisternally.

4.1 Contributions of the different effectors to the response

The initial phase of the response to both 6OHDA and 57DHT

was similar, with increases in blood pressure and falls

in heart rate.   In addition, the animals treated with

57DHT showed marked increases in renal nerve activity and

plasma hormone concentrations, indicating a generalized

activation of central nervous system pressor mechanisms.

In the intermediate phase, most variables were moving

towards their respective control levels, although in the

animals treated with 6OHDA, renal nerve activity was

increasing.

Finally, during the late phase (2-3 hours post-injection)

of the response, heart rate returned to control levels,

whilst blood pressure was maintained at elevated levels

in the 57DHT treated animals, and increased in the
rabbits treated with 6OHDA. The finding that adrenaline
levels were not increased during this phase of the
response in the 6OHDA treated animals, suggests that the
increase in blood pressure is mediated largely by
non-adrenal sympathetic nerves. This finding is
consistent with the idea of a non-uniform activation of
the sympathetic nervous system as has been reported in
other situations (Ninomiya et al 1971; Iriki et al 1972;
Weaver et al 1984; Young et al 1984). These results are
also in accord with the findings of Korner et al (1978),
who reported an increase in resistance of many vascular
beds following treatment with 6OHDA, but not in muscle.
The lack of effect on muscle vascular resistance is
particularly relevant in view of the report that in man,
plasma noradrenaline levels correlate well with muscle
sympathetic nerve activity (Wallin et al 1981), although
it should be noted that these authors sampled plasma from
a forearm vein, where one might expect a correlation with
muscle sympathetic activity. The latter result may be
the reason for the absence of a systematic relationship
between noradrenaline levels and sympathetic nerve
activity, nevertheless, these findings indicate a need
for caution in studies where plasma noradrenaline is used
as a measure of sympathetic nerve activity. In none of
the studies noted above where this was done (Esler et al
1984; West et al 1984; Elliott et al 1985; Hubbard et
al 1986), were sympathetic nerve activity and plasma

noradrenaline recorded simultaneously. Furthermore, in a study by Chen et al (1984), where this problem was examined by graded electrical stimulation of the renal nerve and measurement of renal venous noradrenaline overflow, no change was seen in noradrenaline release at levels of stimulation that resulted in decreases in renal blood flow. In the present study, where nerve activity and noradrenaline were measured simultaneously, no significant increases in plasma noradrenaline levels were found in the animals treated with 6OHDA, despite large increases in renal nerve activity. In the animals treated with 57DHT, nerve activity rose during the course of the experiment whilst plasma noradrenaline levels fell. Although the possibility exists that other sympathetic beds may respond differently (Weaver et al 1984; Meckler and Weaver 1985), the finding in this study that renal nerve activity tended to change in the same way as the blood pressure (figure 4.1), suggests that renal nerve activity is a good index of sympathetic vasoconstrictor activity.

4.2 Role of vasopressin

Administration of 6OHDA had no effect on the levels of plasma vasopressin, suggesting that this hormone does not contribute to the hypertensive response to this compound. This finding is surprising in view of the results of other workers who have shown a pathway from the A1 noradrenaline neurons to the vasopressin containing neurons of the hypothalamus in the rabbit (Blessing et al 1982a), and the findings of other workers who have demonstrated in the rat, that noradrenaline depolarizes vasopressin neurons (Day et al 1985a,b; Randle et al 1986) and stimulates the release of vasopressin into the circulation (Benetos et al 1986). Furthermore stimulation of the caudal ventrolateral medulla in the region of the A1 cells facilitates the activity of vasopressin neurons, an effect that is not seen after ablation of hypothalamic noradrenaline terminals by earlier microinjection of 6OHDA (Day and Renaud 1984). Since these latter experiments were conducted in the rat, a species difference in the sensitivity of vasopressin neurons to noradrenaline is possible. Another possible explanation for this apparent discrepancy may be related to the route of administration of the drugs: it is conceivable that the drug does not gain access to the hypothalamic noradrenergic terminals after intracisternal injection. Finally, 6OHDA may be

exerting effects on dopamine systems, as well as noradrenergic systems (Jacks et al 1972; Sievers et al 1980; Buuse et al 1984; Edwards et al 1985) that may affect vasopressin neurons through indirect connections, these other systems may exert opposing effects on the secretion of vasopressin.

Administration of 57DHT on the other hand, elicited a large and sustained increase in plasma vasopressin levels, suggesting that this hormone may play a role in the cardiovascular changes seen. A role for serotonin in the release of vasopressin is supported by anatomical studies that have demonstrated a serotonergic input to the hypothalamus (Sawchenko et al 1983; Steinbusch 1984; Bosler and Beaudet 1985; Kawakami et al 1985), and physiological studies that have suggested that serotonergic pathways may enhance the release of vasopressin (Steardo and Iovino 1986).

Chapter 5: Table of Contents

Are spinally-projecting serotonin-containing
neurons involved in a pressor pathway?

1 Introduction

As outlined earlier, one of the putative
neurotransmitters that has been implicated in the medullary
control of sympathetic outflow is serotonin. In chapter 4,
experiments were described which confirmed that following
the mass release of serotonin from central neurons which
follows the intracisternal administration of
5,7-dihydroxytryptamine (57DHT) there is an increase in mean
arterial pressure, that lasts for at least three hours. The
finding that renal nerve activity was also increased
suggested that spinally-projecting sympathoexcitatory
neurons were involved in this response. It is not clear
which of the many spinally-projecting neurons discussed in
chapter 1 are responsible for mediating this effect. One
approach that can be used to examine this problem is to
stimulate these neurons and see if the appropriate compounds
are released at the sites of axonal termination. If this is
possible, then one can use this system in order to see if
these neurons are activated by stimuli delivered at other
sites. In this chapter, experiments are described in which
the role of spinally-projecting serotonin-containing neurons
in the medulla oblongata of the rat in the central control
of blood pressure was examined, using the technique of in

vivo tissue dialysis. This technique is similar to the method of push-pull perfusion (for example, Granata and Reis 1983), in which fluid is introduced through one limb of the system into the brain region of interest, and then removed from another limb. The technique was revolutionized by the apparently simple expedient of enclosing the fluid within a dialysis membrane across which compounds of interest could pass (Delgado et al 1972). This approach had a number of important advantages; first, probes could be placed in areas that for technical reasons would be inaccessible to a push-pull cannula. Secondly, because the perfusing fluid is enclosed, only the trauma of the initial insertion damages the tissue, instead of the continuing damage that accompanies push-pull systems. Thirdly, large molecular weight substances are unable to cross the membrane and interfere with subsequent biochemical analyses. This method has been applied in studies of the striatum (Ungerstedt et al 1982; Zetterstrom et al 1982,1983,1984,1986; Brodin et al 1983; Johnson and Justice 1983; Tossman et al 1983; Blakely et al 1984; Herrara-Marschitz et al 1984; Imperato and Dichiari 1984,1985; Marsden and Routledge 1984; Zetterstrom and Ungerstedt 1984; Tossman et al 1985), thalamus (Hernandez et al 1983), frontal cortex (Marsden and Routledge 1984), nucleus tractus solitarius (Lindefors et al 1986) and lateral geniculate nucleus (Sandberg and Lindstrom 1983). A large variety of substances have been measured in these studies, including amino acids, substance P, catecholamines and their metabolites, indoleamines and their

metabolites, ascorbic acid and purines. Since this technique has not previously been used for the quantitation of neurotransmitter release in the spinal cord, it was necessary to perform a number of in vitro experiments in order to ensure that serotonin could cross the dialysis membrane, and that the recovery of serotonin in the dialysate samples accurately reflected changes in the extracellular environment.

2 Methods

2.1 In vivo dialysis

The manufacture of the dialysis probes used in these experiments is described in chapter 2. In the in vitro experiments, probes were placed with their tips in a beaker containing Ringers solution (as described in chapter 2). The recovery of indoleamines in the dialysate was then measured at varying flow rates and at varying concentrations in the beaker. The arrangement used in these experiments is shown in figure 5.1.
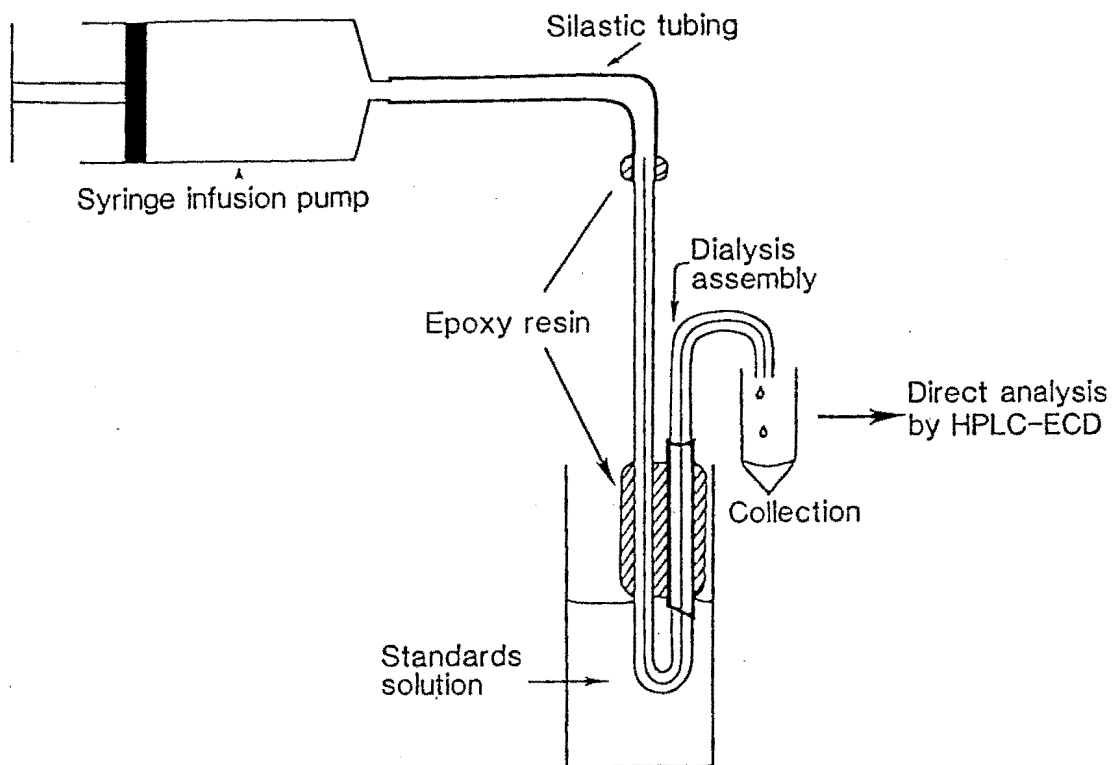
Figure 5.1 Arrangement used for the in vitro testing of dialysis probes.

2.2 Operative procedures

The in vivo experiments outlined below were performed in urethane anaesthetized Wistar-Kyoto rats (see chapter 2). A femoral artery was cannulated for recording blood pressure (see chapter 2) and the trachea was intubated to allow assisted ventilation where necessary.

The rats were then positioned in a stereotaxic frame (David Kopf) and burr holes were drilled bilaterally to allow microinjections to be made into the medial rostral ventrolateral medulla in the region of the B3 cell group. A dialysis probe was then inserted as described in chapter 2. In each rat the dialysis tubule was perfused at a constant rate. This rate varied from 2.5-5.0 ul/min. Each collection period lasted for twenty minutes, since this was the minimum time in which measurable basal levels could be reproducibly obtained given the sensitivity of the assay. After a control period lasting eighty minutes (four samples), microinjections of either kainic acid (5nmol in 100nl of vehicle: 10mM phosphate-buffered saline pH 7.4, containing 0.01% pontamine sky blue: 5 rats), or vehicle (5 rats), were made into the rostral ventrolateral medulla at sites 1.5mm rostral to the obex, and 1.3mm lateral to the midline (figure 5.10). A further five

rats that had been treated with an intracerebroventricular injection of 57DHT (0.2mg in 0.01ml of normal saline containing 1mg/ml ascorbic acid) were also injected with kainic acid. In order to confirm that this procedure had caused a reduction in spinal cord serotonin levels, samples of spinal cord tissue were taken at the end of the experiment, and the serotonin concentration estimated and compared with spinal cord taken from vehicle injected controls.

In a separate group of animals that did not have microinjections into the medulla a dialysis probe alone was inserted into the spinal cord, in order to examine the effect of potassium ions on release.

In all of the in vivo studies zimelidine HCl (0.1g/l) was included in the perfusate. This compound blocks the reuptake of released serotonin, preventing its removal from the extracellular space and degradation by intraneuronal monoamine oxidase. In preliminary experiments performed without zimelidine, there was no observable basal release of serotonin.

3 Results

3.1 In vitro studies

The effect of varying the rate of flow of fluid through the dialysis tubule on the recovery of serotonin and 5-hydroxyindoleacetic acid (5HIAA) is shown in figure 5.2. As shown in this figure, the percentage recovery of indoleamines decreased as the flow rate increased. This apparent loss of recovery was offset by the fact that the total amount of indoleamine in a sample remained constant. Of critical importance was that the recovery not change with changes in the concentration of indoleamines in the fluid surrounding the probe; the effect of varying this concentration on recovery at a set flow rate is shown in figure 5.3. It can be seen that over a wide range of concentrations, the recovery was unchanged. Finally, it is essential that the recovery remain stable over time. The recovery over a three hour period is shown in figure 5.4, and is constant.
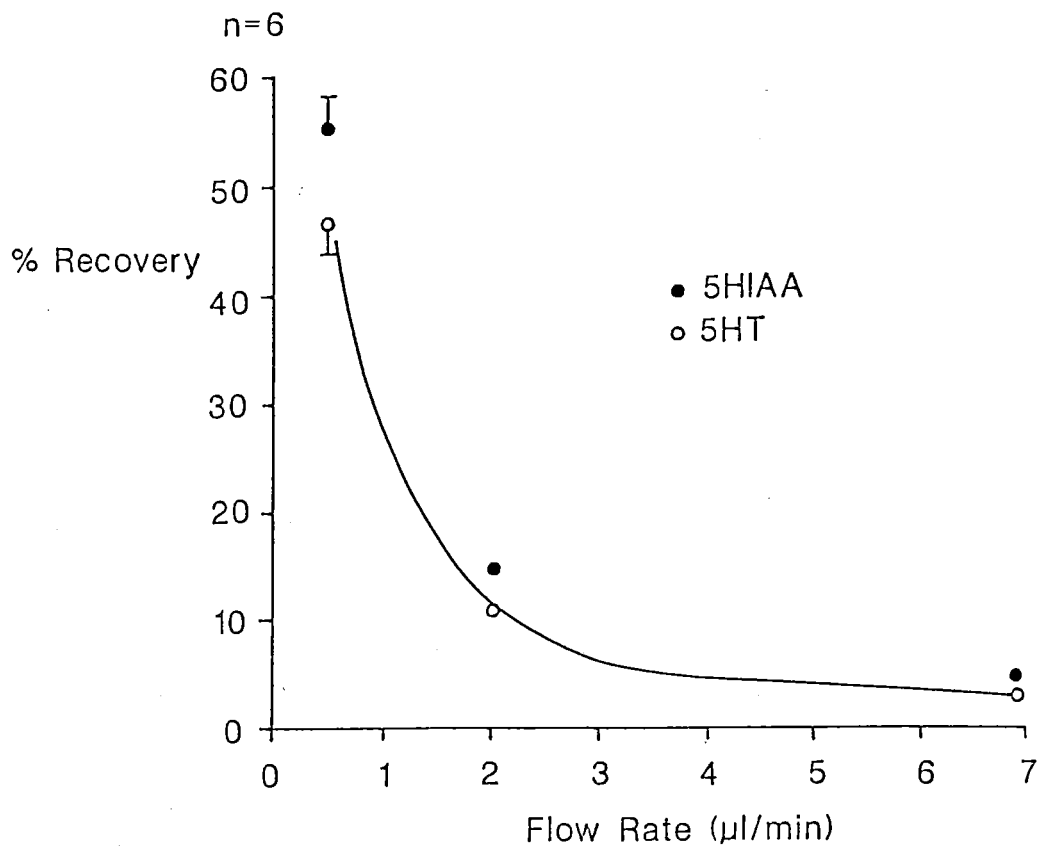
Figure 5.2 Effect of flow rate on the recovery of indoleamines in vitro.
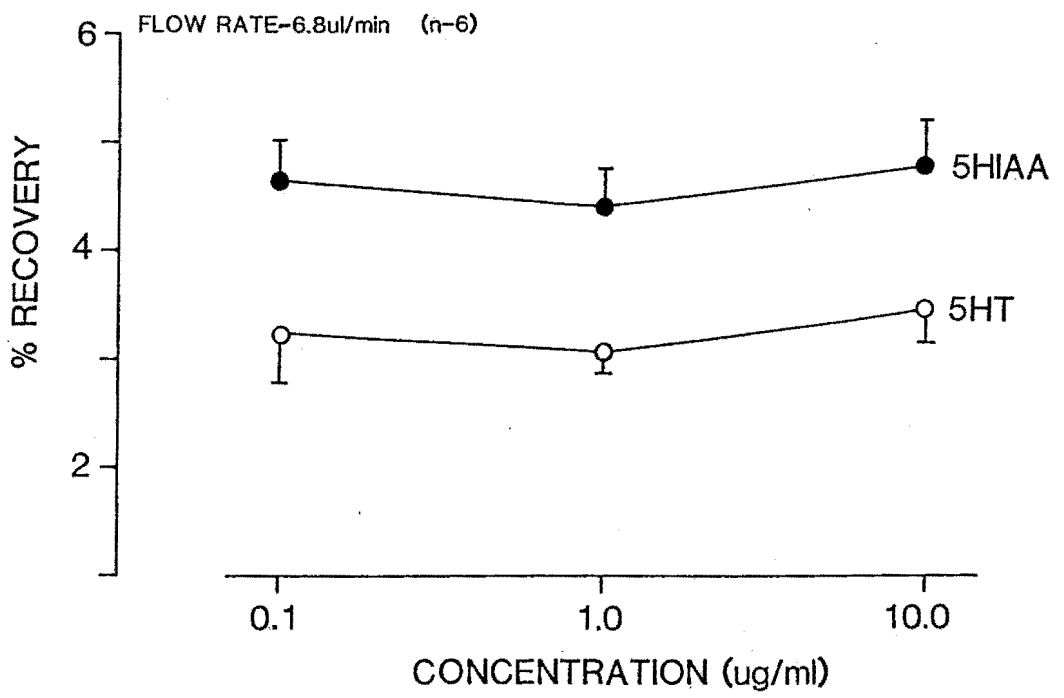
Figure 5.3 Effect of the concentration in the beaker on the recovery of indoleamines in vitro.
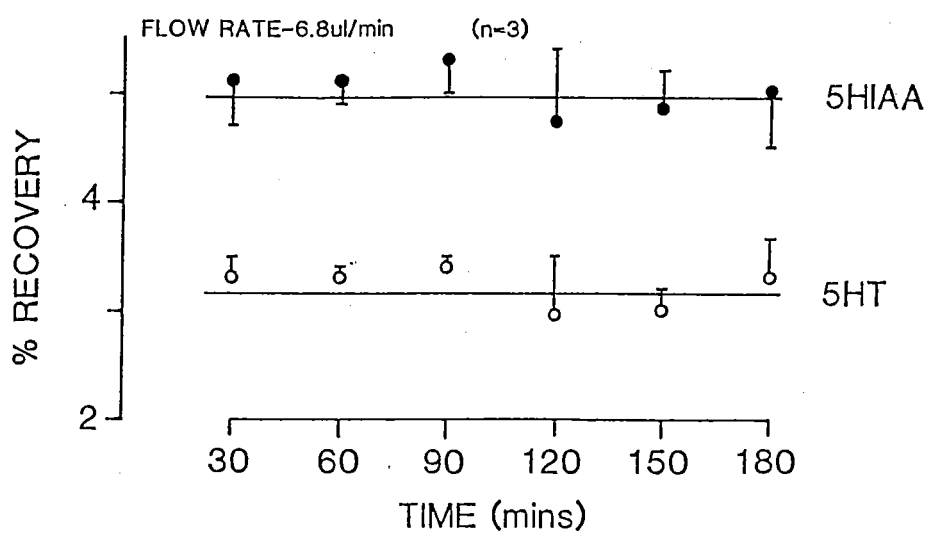
Figure 5.4 Effect of time on the recovery of indoleamines in vitro.

3.2 Effect of potassium

The effect of including 100mM potassium chloride in the dialysing solution is shown in figure 5.5. The amount of serotonin in the dialysate increased from 76.2 ± 45.3 to 210.4 ± 90.0 ($n=5$, $p<0.05$, paired students t test). In contrast, levels of 5HIAA were unaffected (figure 5.5).

3.3 Effect of chemical stimulation in the brainstem

In the vehicle treated animals there was a gradual decline in the levels of serotonin during the control phase. Vehicle injection had no effect on the release of serotonin into the dialysate (figure 5.6). Blood pressure levels in this group were stable prior to the vehicle injection, and were increased slightly, but not significantly, by the vehicle injection. Blood pressure levels in the kainic acid injected animals were similar to those in the vehicle group during the pre-injection period, but increased markedly after kainic acid injection (figure 5.6,5.7). Similarly, serotonin levels in this group were not significantly different from those seen in the vehicle treated animals prior to injection, but increased after injection of kainic acid (figure

EFFECT OF POTASSIUM
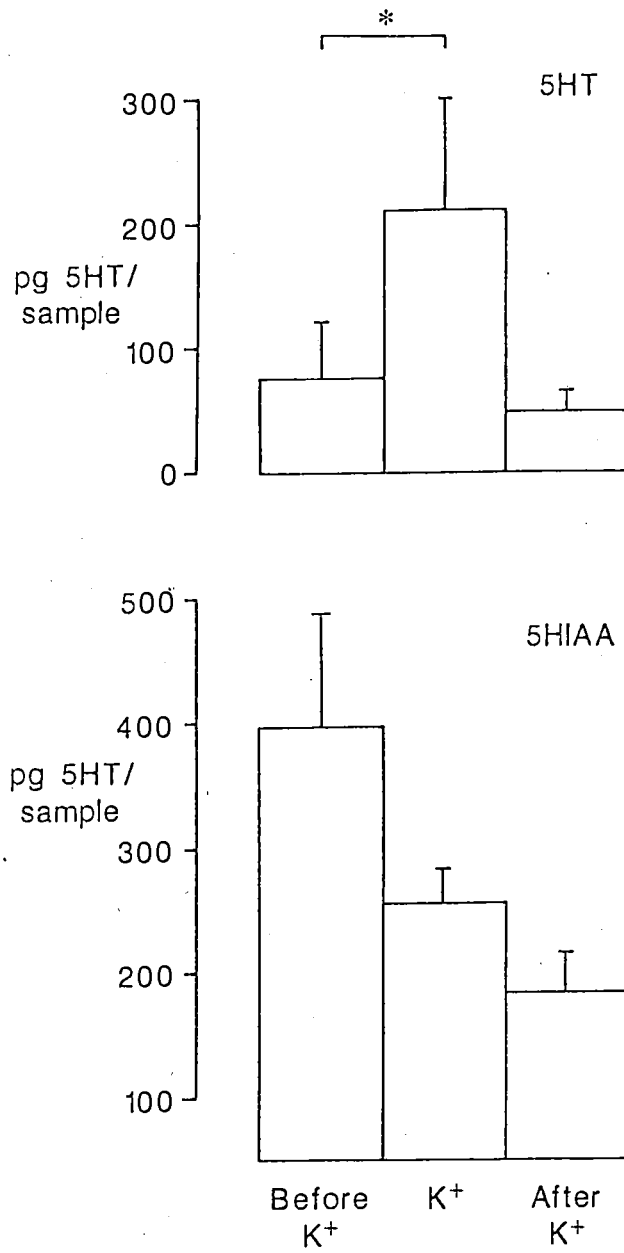
Figure 5.5 Effect of potassium ions on the release of indoleamines from the spinal cord. (*,P<0.05).
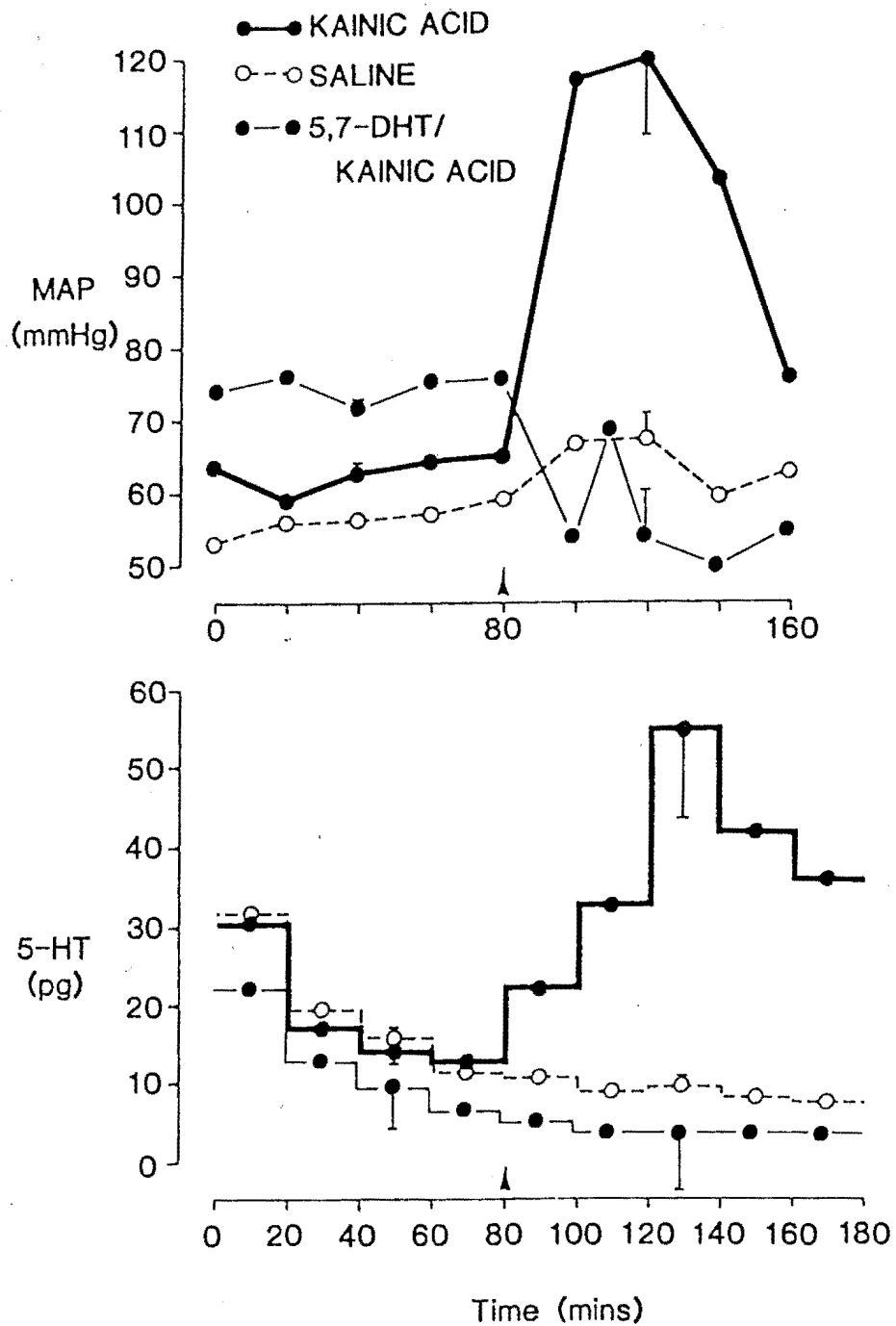
<u>Figure 5.6</u> Effect of microinjections of kainic acid, or vehicle in normal rats, and rats pretreated 10-14 days earlier with 57DHT, on blood pressure and serotonin (5-HT) release into spinal cord dialysates.
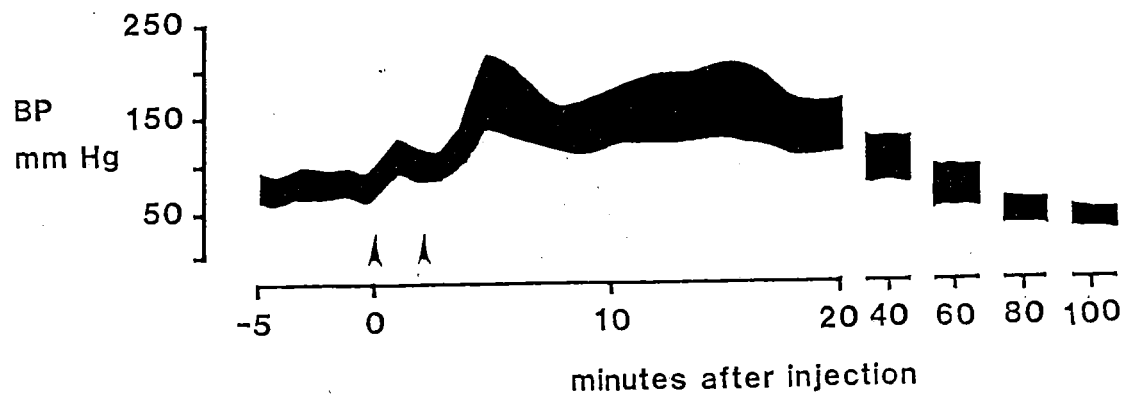
KA into ventral medulla

Figure 5.7 An example of the blood pressure response to kainic acid microinjected onto the B3 lateral cell group in a normal rat.

5.6,5.9). Both the serotonin levels and the blood
pressure remained elevated for the duration of the
experiment. In the rats that had been pre-treated with
57DHT, the serotonin content in the spinal cord, measured
at the end of the experiment was, dramatically reduced
compared with the levels seen in the spinal cord of
vehicle treated rats (figure 5.8). In these animals, the
basal levels of serotonin were less than in the other two
groups, although this was not statistically significant,
since the lower limit of detectability was used as the
value in samples where the serotonin levels were below
this point. As a result, there appears to be a basal
efflux of serotonin throughout the experiment in this
group (figure 5.6), even though in most animals there was
no detectable serotonin present after 80 minutes of
collection. Injection of kainic acid in these animals
had no effect on the efflux of serotonin. In addition,
blood pressure no longer rose after kainic acid injection
in this group.

In all of the groups, 5HIAA levels showed a similar
pattern. The levels were high initially, and gradually
declined during the course of the experiment. There was
no apparent effect of kainic acid administration on
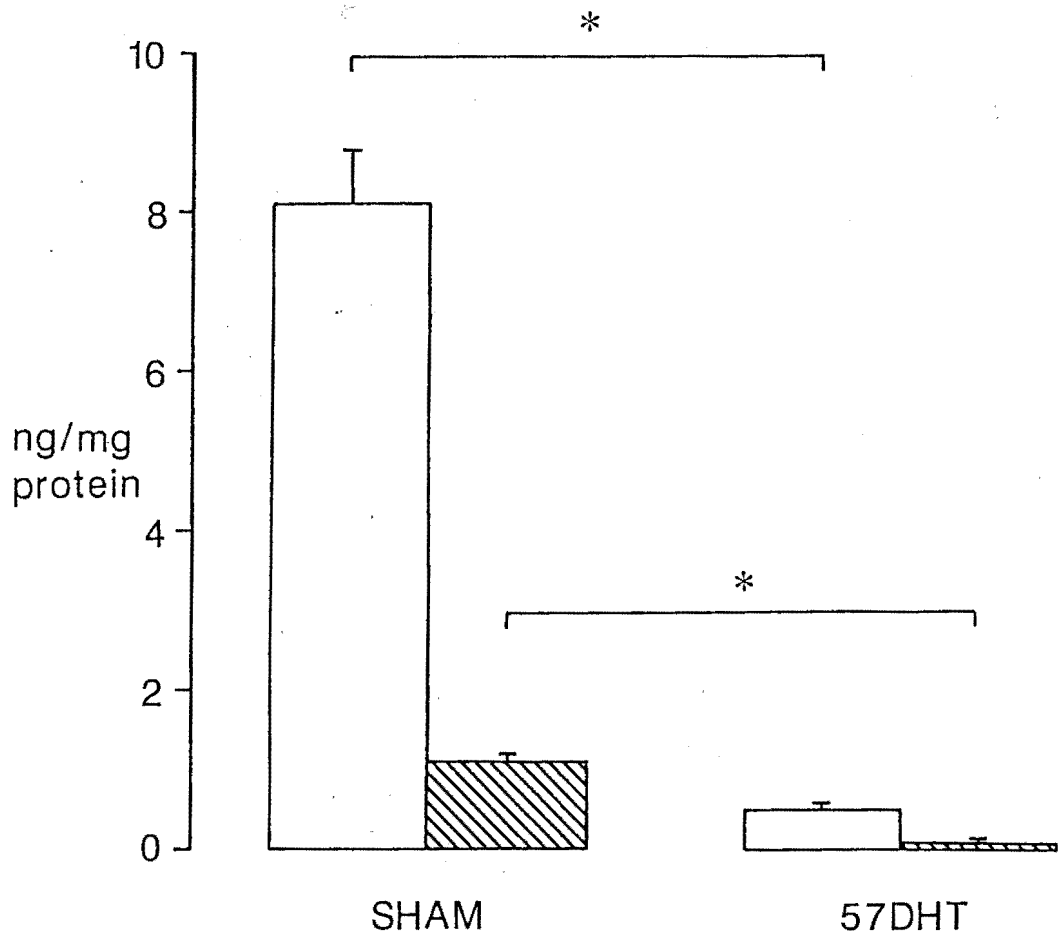5HIAA.

EFFECT OF 57DHT ON SPINAL CORD INDOLEAMINE LEVELS

Figure 5.8 Effect of pretreatment with 57DHT on tissue levels of 5HIAA (open) and serotonin (shaded) in the spinal cord of rats 10-14 days after teatment. (*,P<0.001).

5HIAA

5-HT
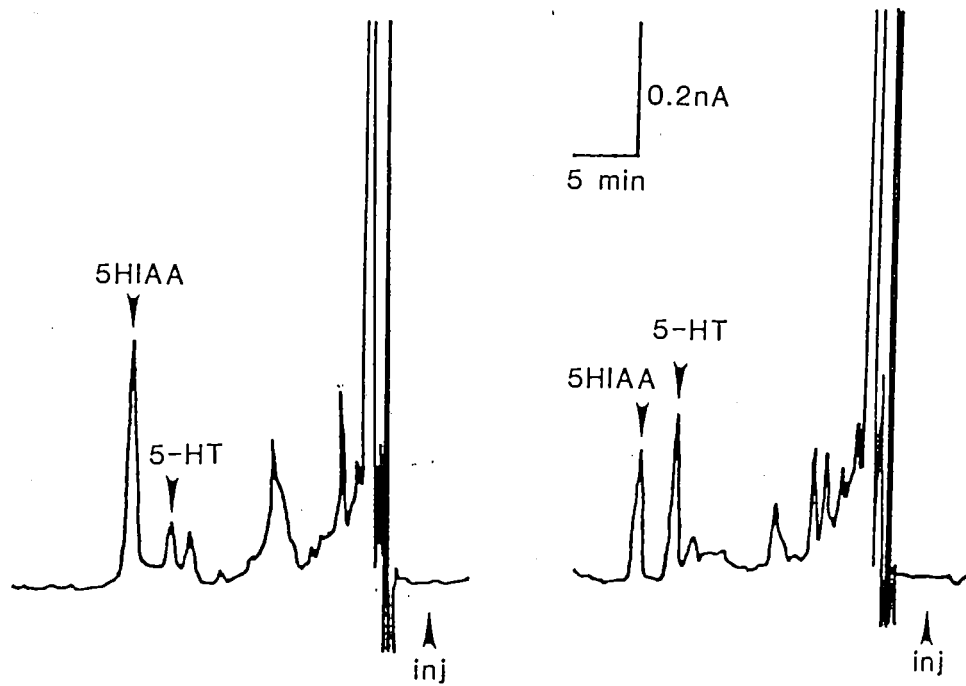
0.2nA

5 min

5-HT

5HIAA

inj

inj

Figure 5.9 Chromatograms showing the detection of serotonin (5-HT) and 5HIAA in spinal cord dialysates: before (left side), and after (right side) microinjection of kainic acid onto the B3 lateral cell group of the rat.
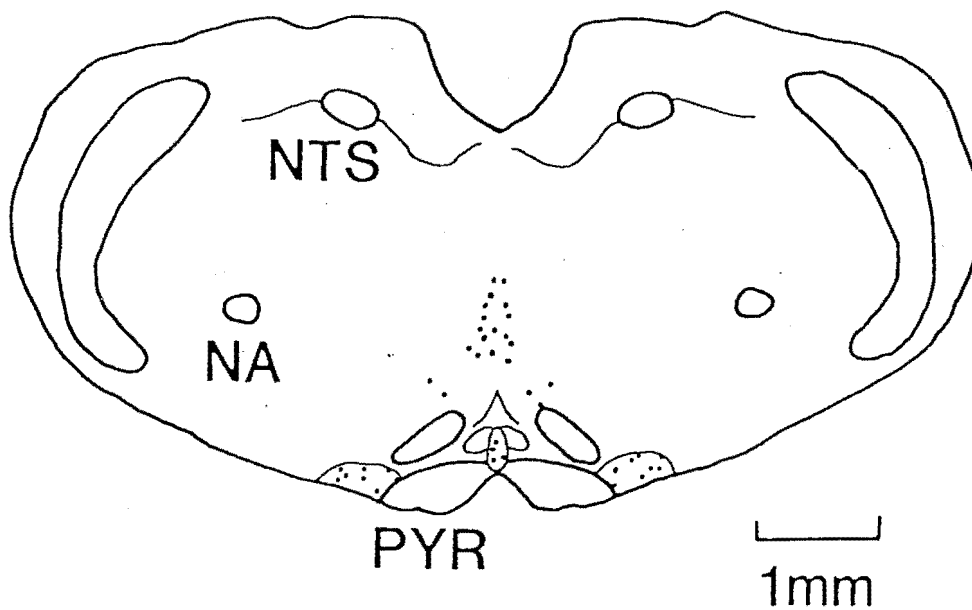
Figure 5.10 Location of the B3 lateral cell group in the
rostral medulla of the rat. This cell group consists of the
dots adjacent to the pyramidal tracts. NTS: nucleus
tractus solitarius, NA: nucleus ambiguus, PYR: pyramidal
tracts.

4 Discussion

4.1 Assessment of the model

The experiments described above demonstrate that the recovery of indoleamines using the technique of in vivo dialysis is constant over a range of different concentrations in the surrounding medium. The fact that the recovery varied with flow rate was not a problem, since in the in vivo experiments the flow rate was held constant. One factor that was of concern was that serotonin from non-neuronal sources would interfere with the ability of the method to accurately reflect the release of serotonin from neurons. One possible source of interference might be a release of serotonin from platelets as a result of tissue damage that occurs during insertion of the probe. It seems likely that this occurs to a limited extent, since serotonin was measured in the first few samples immediately after insertion of the probe in animals that had been pretreated with 57DHT, however the rapid falloff to levels below the limit of detectability in this group suggests that it is not a significant problem. Finally, the fact that increases in serotonin efflux occurred when potassium ions were included in the medium suggests that the technique is

sensitive enough to reflect changes in neuronal activity.

The lack of effect of any of the procedures on the
efflux of 5HIAA may be attributable to the presence of
zimelidine in the perfusing solution, since this compound
would prevent the usual reuptake of serotonin and
enzymatic formation of 5HIAA.

4.2 Role of the B3 serotonin neurons in the control of blood pressure

As mentioned in the introduction, and discussed in
chapter 1, considerable controversy surrounds the role of
brainstem serotonin neurons in the control of blood
pressure, with as many papers suggesting that they are
inhibitory as there are suggesting them to be excitatory.
One of the reasons for this wide range of views is that
central serotonin neurons may   not   be   functionally
homogeneous. Anatomical studies have demonstrated that
the distribution of serotonin-containing neurons   is
extensive,   and that virtually every region of the
brainstem is innervated by serotonin fibres (e.g.
Steinbusch 1984). It is therefore not surprising that in
experiments in which the entire serotonergic system is
activated, as with systemic or intrathecal administration
of pharmacological agents, diverse effects are seen.   In

addition, the use of anaesthetic agents may markedly
affect a response. Correa et al (1985) were able to show
that intracerebroventricularly administered noradrenaline
elicited a dose dependent pressor effect in awake rats,
but a dose dependent depressor effect in anaesthetized
rats. In the experiments described above, I have
examined one component of the central serotonergic
system, namely the spinally projecting neurons of the
medial rostral ventrolateral medulla. Previous workers
in this laboratory have reported that electrical
stimulation of this region elicits a pressor response
that is blocked or even reversed by pretreatment with
57DHT (Howe et al 1983). One of the disadvantages of
this technique is that electrical stimulation may affect
fibres of passage as well as cell bodies; in the present
study, kainic acid was used, since this rigid analogue of
the excitatory amino acid glutamate appears to affect
only cell bodies. In addition, kainic acid produced an
increase in blood pressure lasting for over an hour, a
time span suitable for the measurement of serotonin in
dialysate samples given the sensitivity of the assay
available. Furthermore, in vivo dialysis allows a clear
demonstration that the neuronal system under examination
has been activated. In the present study, microinjection
of kainic acid onto the B3 lateral group of serotonin
neurons increased blood pressure and the release of
serotonin. This effect was abolished by pretreatment
with 57DHT, suggesting that it is specific to these

neurons. In fact, after destruction of central serotonin neurons with 57DHT, there was a decrease in blood pressure after the microinjection of kainic acid into this region, suggesting the presence of a second population of neurons in this region that may subserve a sympathoinhibitory function.

Another possible application of studies of this type where the release of a putative neurotransmitter is assessed, is in determining whether or not a particular neuronal cell type is activated during a given response. In the next chapter, I will discuss the results of experiments in which this was done for neuropeptide Y, and in chapter 7 I will discuss the results of experiments in which serotonin and neuropeptide Y release from the spinal cord were measured before and after acute sinoaortic denervation in the anaesthetized rabbit.

Chapter 6: Table of Contents

Are spinally-projecting neuropeptide Y-containing
neurons involved in a pressor pathway?

1 Introduction

In chapter 1, the chemical content of spinally-projecting neurons with cell bodies in the rostral ventrolateral medulla was discussed. As noted there, the major substances so far identified in this region were substance P, serotonin, neuropeptide Y and adrenaline. In chapter 5, the role of the serotonin-containing neurons was examined by measuring the release of serotonin in the spinal cord after stimulation of the rostral ventrolateral medulla. In a complementary series of experiments, described here, the role of the neuropeptide Y containing neurons was examined by measuring the release of neuropeptide Y-like immunoreactivity into the spinal sub-arachnoid space. Measurement of neuropeptide Y rather than adrenaline was performed because the quantities of adrenaline that are present in the spinal cord are minute (Zivin et al 1975; Van Der Gugten et al 1976; Fleetwood-Walker and Coote 1981a,b; Coote 1985). In preliminary experiments, it proved impossible to detect adrenaline in spinal cord dialysates. Initially I planned to collect neuropeptide Y by in vivo dialysis. Unfortunately, although dialysis tubules were obtained with a pore size large enough to allow the passage of neuropeptide Y, preliminary studies

demonstrated that these tubules had an apparently limitless

capacity to bind this peptide, rendering them useless for

this purpose.   It was therefore decided to attempt to

measure neuropeptide Y release in perfusates of the spinal

sub-arachnoid space.

These experiments were performed in rabbits for a number of

reasons; firstly, the experiments described in chapters 3

and 4 had been performed in this species.   Secondly,

implantation of the spinal perfusing catheters proved to be

technically easier in rabbits.   Thirdly, rabbit

cerebrospinal fluid and spinal cord contain large quantities

of neuropeptide Y-like immunoreactivity, and fourthly, I

intended to examine the effects of alterations in

baroreceptor input on, neuropeptide Y release, experiments

that are more feasible in the rabbit, since the baroreceptor

afferent nerves are separate and readily identifiable.

In this chapter, two experiments are described.   In the

first group, neuropeptide Y-like immunoreactivity was

measured in perfusates before and after unilateral

microinjections of kainic acid or vehicle into the rostral

ventrolateral medulla.   In the second series of experiments

neuropeptide Y-like immunoreactivity was measured before and

after microinjection of the GABA receptor agonist muscimol,

or vehicle, into the sympathoinhibitory region of the caudal

ventrolateral medulla delineated in chapter 3, in order to

see if the neuropeptide Y- containing neurons of the rostral

medulla are involved in the sympathoexcitatory response to inhibition of the caudal medulla.

2 Methods

The operative procedures for the cannulation of the cisterna magna and the spinal sub-arachnoid space, cannulation of a femoral artery, and exposure of the dorsal surface of the medulla oblongata, are outlined in chapter 2. In the first group of experiments the cisternal catheter lay just within the cisterna magna; the fluid lying between the two catheters was collected at ten minute intervals by infusing 0.5ml of a modified Ringers solution (8.6g NaCl, 0.3g KCl, 0.32g $CaCl_2$, 5mg bacitracin and 200mg bovine serum albumin in 1000ml H_2O, pH 6.3) into the spinal catheter and collecting the same volume from the cisternal catheter (shown diagrammatically in fig. 6.1). Each collection took about one minute. After a ninety minute equilibration period, either kainic acid (5 nmol) in 100 nl of vehicle (100mM phosphate buffered saline, pH 7.4, containing 0.01% pontamine sky blue, n=6), or 100 nl of vehicle alone (n=7), was microinjected into the left rostral ventrolateral medulla (3mm rostral to obex, 3mm lateral to the midline, and 4.5mm ventral to the dorsal surface), in the region of the C1 adrenaline/neuropeptide Y-containing neurons. The microinjections were performed immediately after the ninth
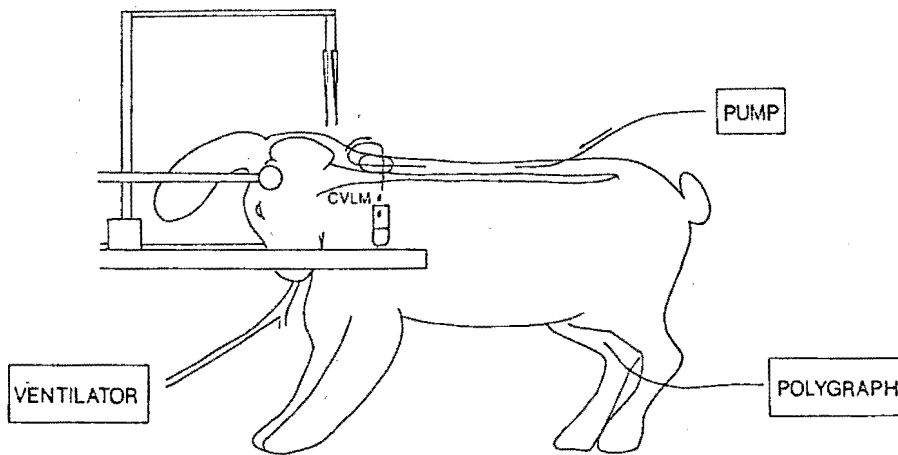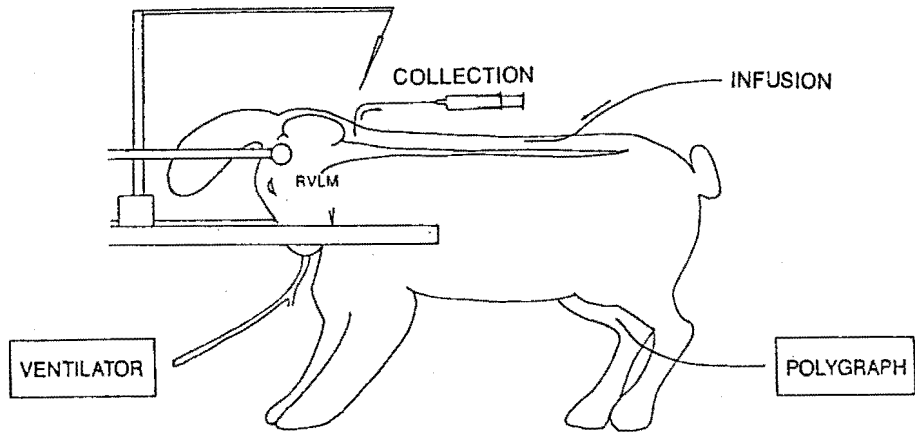
Figure 6.1 Diagrams of the arrangements used for the collection of spinal subarachnoid space perfusates in the experiment in which kainic acid was injected (upper panel) and in subsequent experiments (lower panel).

sample was collected, and collections were continued for a further sixty minutes. Microinjections were accomplished with glass micropipettes as described in chapter 2. At the end of the experiments, the rabbits were fixed by perfusion through the abdominal aorta as described in chapter 2, and the brain removed in order to confirm   the   site   of microinjection.

In the second series of experiments, the cisternal catheter was advanced to the junction of the cervical and thoracic spinal cord, and sealed with a solution of 5% agar.   The space between the two catheters was   then   perfused continuously with the modified Ringers solution described above, using an infusion pump at a rate of 0.15ml/min (shown diagrammatically in fig 6.1).   Collections were made over five minute periods into tubes containing 0.5ml of 1M acetic acid.   Samples were collected for thirty minutes before, and thirty minutes after bilateral microinjection of either 1nmol of muscimol in 200nl of Ringers solution (n=7) or Ringers alone (n=7), into the caudal ventrolateral medulla in the region of the A1 noradrenaline synthesizing neurons (1mm caudal to obex, 3mm lateral to the midline, and 3mm ventral from the dorsal surface of the brain) (figure 6.2). In this experiment, plasma levels of neuropeptide Y-like immunoreactivity were measured five minutes before, and one and ten minutes after the microinjections.

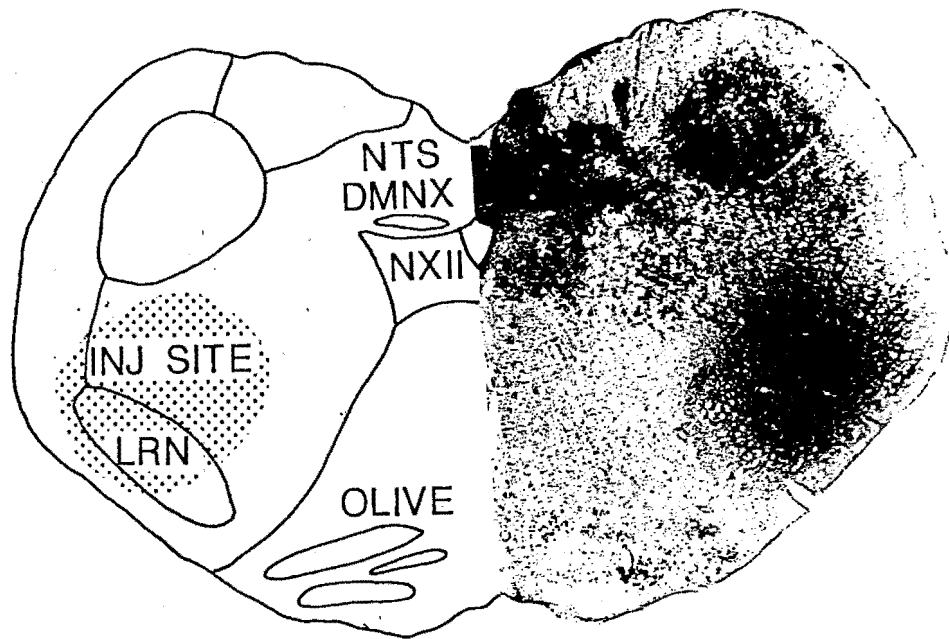The neuropeptide Y radioimmunoassay is described in chapter

Figure 6.2 A coronal section through the brainstem of the
rabbit, 1mm caudal to the obex, showing the extent of the
injection site in the experiments where muscimol was
injected. NTS: nucleus tractus solitarius, DMNX: dorsal
motor nucleus of the vagus, INJ SITE: injection site, NX11:
hypoglossal nucleus, LRN: lateral reticular nucleus.

2. Statistical analysis was performed as described in chapter 2.

3 Results

The effect of a unilateral microinjection of kainic acid on arterial pressure in one animal is shown in figure 6.3. This treatment elicited a rapid increase in mean arterial pressure that was sustained for the duration of the experiment (fig. 6.4). Associated with the increase in arterial pressure was an increase in neuropeptide Y release into the sub-arachnoid space (fig 6.4) that was also sustained for the duration of the experiment. The peak increase in blood pressure occurred immediately after the injection of kainic acid, whereas the levels of neuropeptide Y appeared to peak towards the end of the experiment. Microinjections of vehicle had no effect on arterial pressure or neuropeptide Y levels.

In the second group of rabbits, microinjection of muscimol elicited increases in arterial pressure that were slower developing, with a peak increase seen after fifteen to twenty minutes (figs 6.5, 6.7). In these rabbits plasma neuropeptide Y levels were measured before and after the microinjections of muscimol. This treatment did not cause any change in plasma neuropeptide Y levels (fig 6.8). In
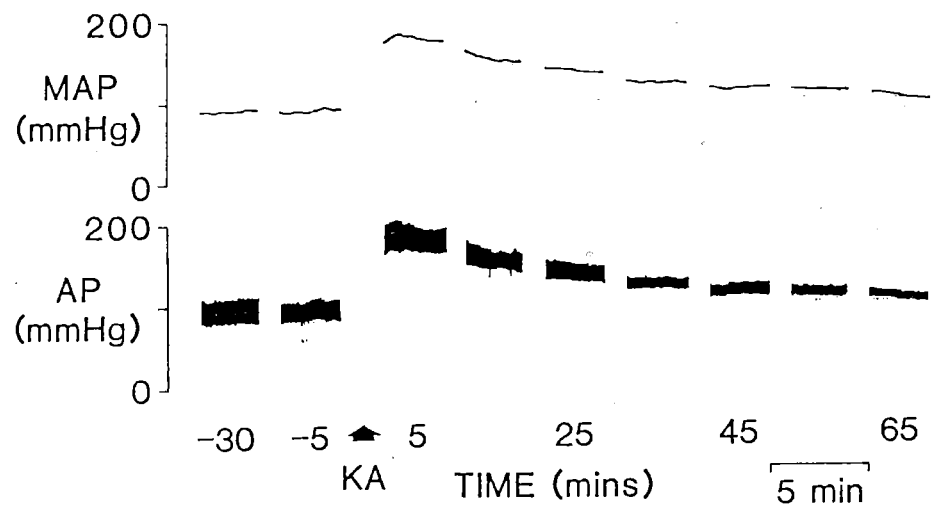
Figure 6.3 Effect of microinjection of kainic acid into the right rostral ventrolateral medulla on blood pressure.
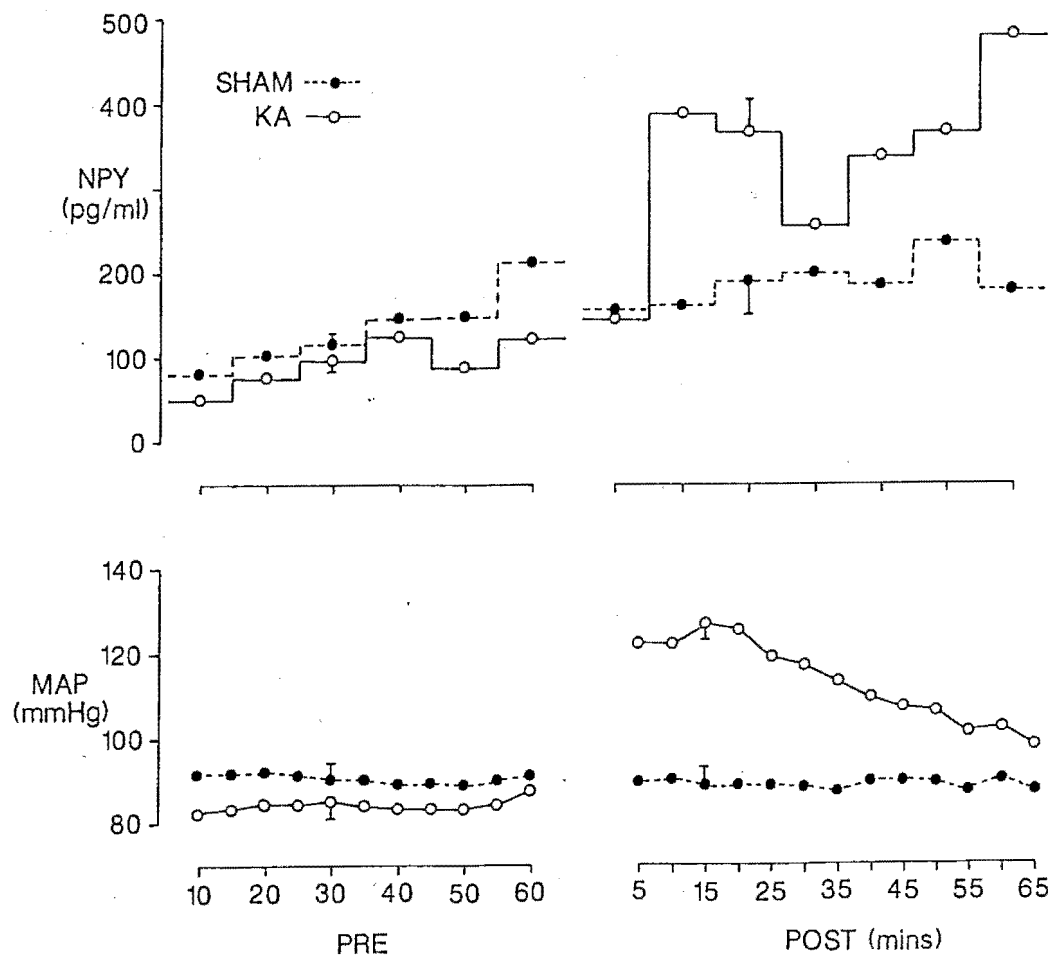
Figure 6.4 Effect of microinjection of kainic acid (open circles, continuous lines) or vehicle (closed circles, dashed lines) into the right rostral ventrolateral medulla. Upper panel is neuropeptide Y - like immunoreactivity (NPY-LI), lower panel is mean arterial pressure (MAP).
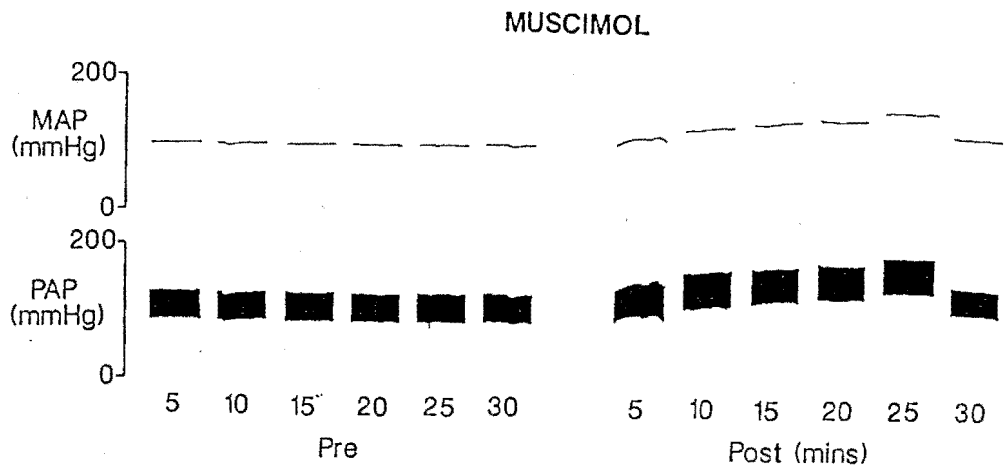
MUSCIMOL

Figure 6.5 Effect of bilateral microinjections of muscimol into the caudal ventrolateral medulla on mean (MAP) and phasic (PAP) arterial pressure in one rabbit.

SHAM

MAP (mmHg) 200 — 0

PAP (mmHg) 200 — 0

5   10   15   20   25   30   5   10   15   20   25   30
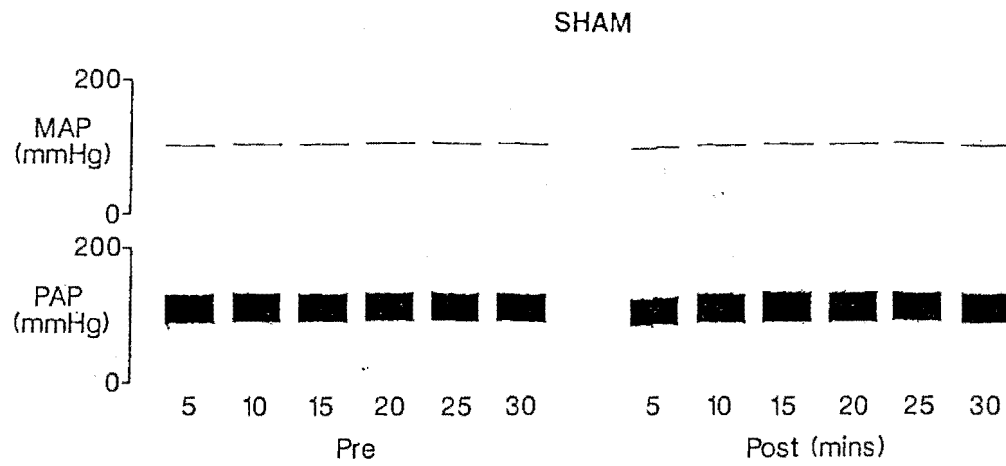Pre   Post (mins)

Figure 6.6 Effect of bilateral microinjections of Ringers solution into the caudal ventrolateral medulla on blood pressure. Notation is as in Figure 6.5.
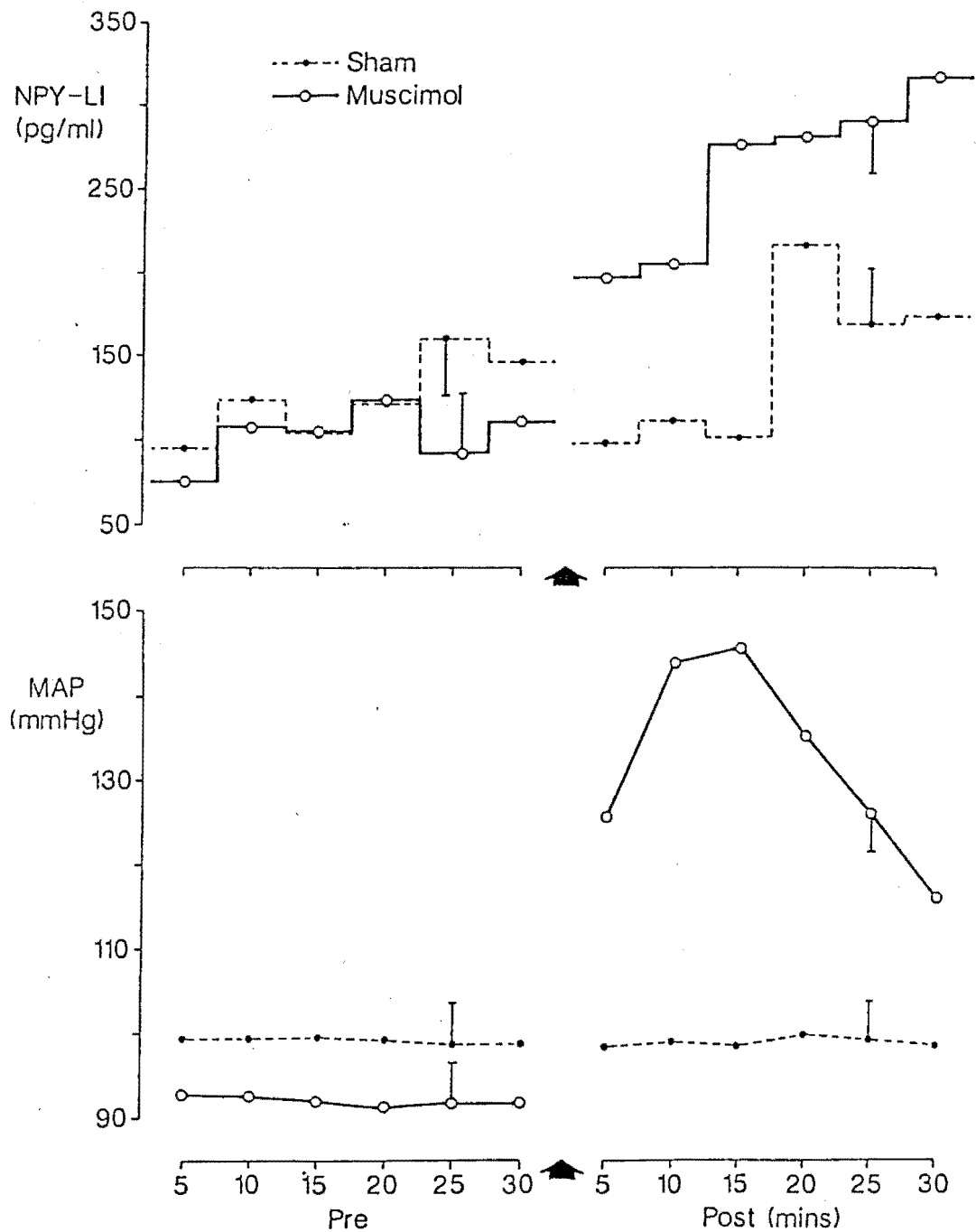
Figure 6.7 Effect of microinjections of muscimol (open circles, continuous lines) or Ringers solution (closed circles, dashed lines) on the release of neuropeptide Y - like immunoreactivity into the spinal sub-arachnoid space (upper panel) and blood pressure (lower panel).
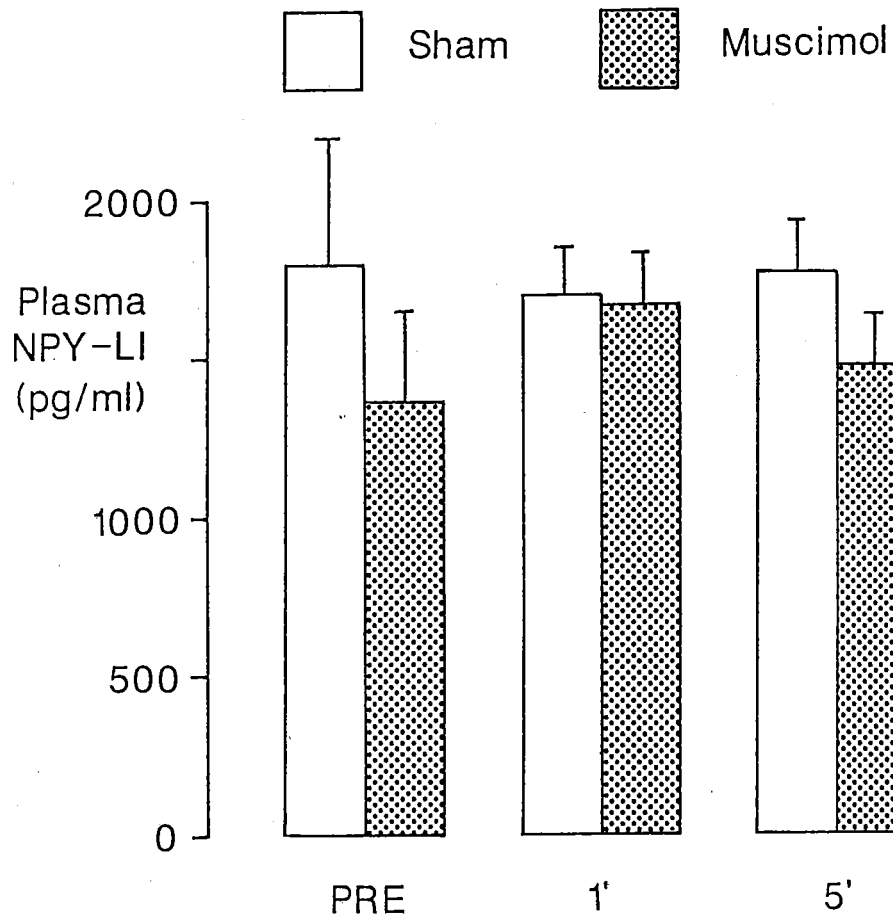
Figure 6.8 Effect of microinjections of muscimol or Ringers into the caudal ventrolateral medulla on the concentration of neuropeptide Y - like immunoreactivity in the plasma. Before the injections (PRE), and one and five minutes after.

contrast, the concentration of neuropeptide Y in the perfusates increased significantly over the period examined. Microinjection of vehicle did not elicit a change in blood pressure (figure 6.6).

An example of an injection site in this latter group of animals is shown in fig 6.2.

4 Discussion

The results of the first group of experiments demonstrate that stimulation of cell bodies in the rostral ventrolateral medulla elicits a release of neuropeptide Y-like immunoreactivity in the spinal sub-arachnoid space. Associated with this increase was an increase in arterial pressure, that preceded the increase in neuropeptide Y release, unlike the situation with serotonin release described in chapter 5, where there was a close correspondence between the increase in blood pressure and the increase in serotonin release. The explanation for this temporal discrepancy is not clear. One possibility is that since in the serotonin experiments the collection system was located adjacent to the intermediolateral cell column, the serotonin would have to diffuse over a shorter distance to reach the dialysis probe. In contrast, in these experiments the neuropeptide Y released from terminals in the

intermediolateral cell column, would have to diffuse through the spinal cord before being collected and measured. Since neuropeptide Y is a 36 amino acid peptide, this diffusion time may be quite substantial. A second possibility is that since kainic acid was applied unilaterally, baroreceptor inhibition of sympathetic tone mediated through the contralateral side of the medulla may have been operating to reduce blood pressure at a time when the cells on the ipsilateral side were still being stimulated by kainic acid. These results provide evidence that the neuropeptide Y-containing neurons of the ventrolateral medulla are spinally-projecting, and that stimulation of their cell bodies elicits a release of neuropeptide Y from their terminals. These findings are essential if a neurotransmitter or neuromodulatory role for this peptide is to be considered. Furthermore, the results demonstrate the feasibility of measuring neuropeptide Y release as an index of the activity of these neurons. Since the stimulus in these experiments was applied at the same site as the cell bodies, and since it is not possible at the present time to selectively lesion these neurons, as was done with the serotonin experiments with the selective serotonergic neurotoxin 57DHT, as described in the previous chapter, it is not possible to conclude from this experiment that these neurons are involved in the sympathoexcitation that occurs during stimulation of this area.

In the second group of experiments, this question was

addressed by inhibiting the caudal ventrolateral medulla, a
procedure that, as shown in chapter 3, results in an
increase in sympathetic nerve activity. Since inhibition of
this area also resulted in an increase in neuropeptide Y
release, it seems likely that the neuropeptide Y-containing
neurons of the rostral ventrolateral medulla subserve a
sympathoexcitatory function, although the possibility that
they are inhibitory, and were activated as a result of an
increase in baroreceptor activity, cannot be ruled out. In
the next chapter experiments are described in which an
attempt was made to assess the role of both neuropeptide Y-
and serotonin- containing neurons in baroreflex pathways.
As noted in chapter 1, anatomical studies have provided
evidence that adrenaline and neuropeptide Y-containing
neurons project to the spinal cord, whilst
electrophysiological studies (Henry and Calaresu 1974;
Caverson et al 1983; Barman and Gebber 1985) have shown
that neurons in this area are sympathoexcitatory. The
results presented here demonstrate that neuropeptide
Y-containing neurons are activated during stimulation of the
rostral ventrolateral medulla, as well as during the
sympathoexcitation that occurs during inhibition of the
caudal ventrolateral medulla. Taken together, these results
provide strong evidence that these neuropeptide Y-containing
neurons are indeed sympathoexcitatory. The results do not,
however, shed light on the question of which chemical
mediator released from the terminals of these neurons is
responsible for excitation of the sympathetic preganglionic

neurons.

Chapter 7: Table of Contents

Effect of acute sinoaortic denervation
on the release of serotonin and neuropeptide Y
from the spinal cord

1 Introduction

In chapter 1, the role of the arterial baroreceptor neurons
in cardiovascular control was discussed. Increases in the
activity of these neurons results in a decrease in the
activity of sympathetic nerves, and a decrease in heart
rate and blood pressure. Conversely, decreases in their
activity increases sympathetic nerve activity, and produces
tachycardia and a rise in blood pressure. As noted in
chapter 6, a role for neurons in the ventrolateral medulla
in mediating baroreceptor impulses has been suggested by
other studies (McAllen et al 1982; Granata et al 1983,
1985) in the rat and the cat, without specifying their
chemical content. In the rabbit an essential role for
serotonin-containing neurons has been suggested in some
studies (Wing and Chalmers 1974a,b; Chalmers and Wing
1975), whilst a significant but not crucial role has been
suggested by others (Korner and Head 1981; Head and Korner
1982).

In the previous chapter, a possible role for
spinally-projecting neuropeptide Y-containing neurons in
mediating increases in sympathetic outflow in the rabbit was

suggested, whilst in chapter 5 a similar role for serotonin
in the rat was reported. In this chapter I will present the
results of experiments in which acute baroreceptor
denervation was used as a method of determining whether or
not these bulbospinal neurons are involved in baroreceptor
pathways. These experiments were performed in the rabbit,
since apart from the other technical reasons outlined in
chapter 6, it is a fortuitous anatomical quirk of the rabbit
that all four baroreceptor nerves are separately
identifiable, as opposed to the situation in most other
species, where the aortic depressor nerve and the vagus run
together. In these experiments, the four baroreceptor
nerves were isolated on threads, enabling a complete
denervation to be performed in one to two minutes. As in
the experiments described in chapter 4, plasma vasopressin
levels were measured in some of these animals, in order to
see if there were any changes in the levels of this hormone
accompanying the increase in arterial blood pressure that
follows acute denervation of the baroreceptor input.

2 Methods

The experiments were performed in urethane anaesthetized
rabbits. The four baroreceptor nerves were exposed, and
confirmed as baroreceptor nerves by their characteristic
pulse-related electrical activity. The nerves were then

isolated on loops of 5/0 thread, and the wounds closed with Michell clips. The thoracic spinal cord was exposed and a dialysis probe inserted into the rabbits in which serotonin was measured. In the rabbits where the spinal sub-arachnoid space was perfused, the arrangement was the same as that in the experiments where muscimol was injected into the caudal ventrolateral medulla. In some of the rabbits where the spinal cord was dialysed for serotonin, blood samples were taken for the estimation of plasma vasopressin by radioimmunoassay. The methods used in the estimation of neuropeptide Y and serotonin are described in chapter 2.

After a control period, the wounds in the neck were reopened. In the denervated animals the carotid sinus nerves were sectioned by pulling on the silk loops until the nerve broke; the aortic depressor nerves were cut with scissors. In the sham-treated animals the threads were not handled. Baroreceptor function was assessed before and after denervation or sham-denervation by observing the change in heart rate after intravenous injections of 10ug of either phenylephrine hydrochloride or noradrenaline bitartrate.

3 Results

Removal of the four baroreceptor nerves simultaneously, resulted in a rapid increase in arterial pressure of about 25-30mmHg (figures 7.1, 7.2, 7.3), that was sustained for about forty minutes. In these animals, the fall in heart rate that normally accompanies acute rises in arterial pressure was absent or markedly attenuated (less than 10 bpm for a 20mmHg rise in pressure). In the animals where serotonin release was measured, there were high levels initially, reaching a plateau after sixty to ninety minutes. There was no significant difference between the release of serotonin in the denervated animals when compared with the sham-treated animals. In the rabbits where neuropeptide Y release was measured, there was little variation in the levels of neuropeptide Y prior to denervation, and there was no significant difference between the two groups after the denervation.

In the rabbits (in which) plasma vasopressin levels were measured, the basal levels were about 50 pg/ml, similar to those seen in the experiments described in chapter 4. In the rabbits that were denervated, there was a prompt rise in plasma vasopressin levels (figure 7.4). This increase was significant at five minutes, but not afterwards, although the levels remained elevated throughout the experiment.
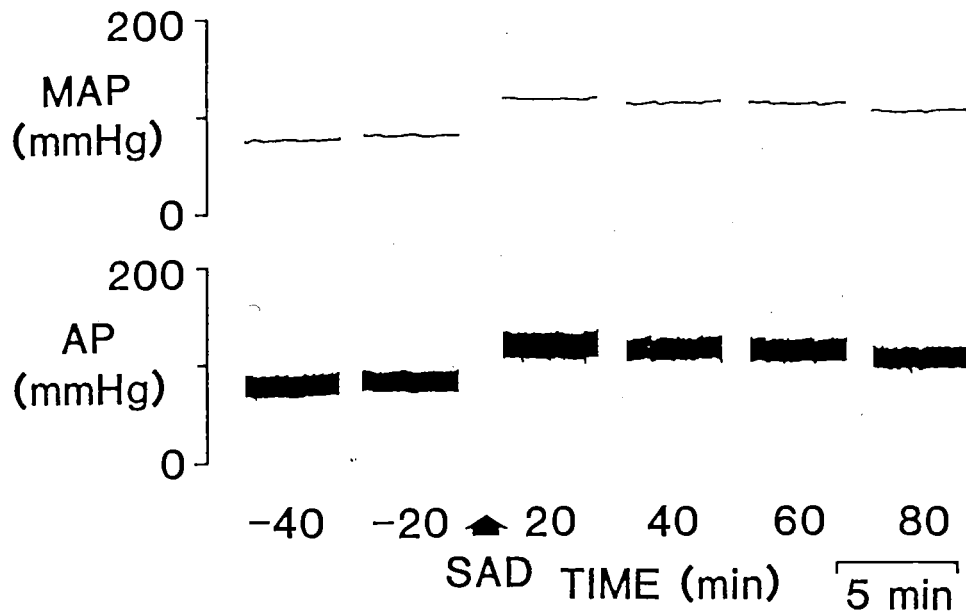
Figure 7.1 An example of the effect of an acute sinoaortic denervation on blood pressure in one rabbit.
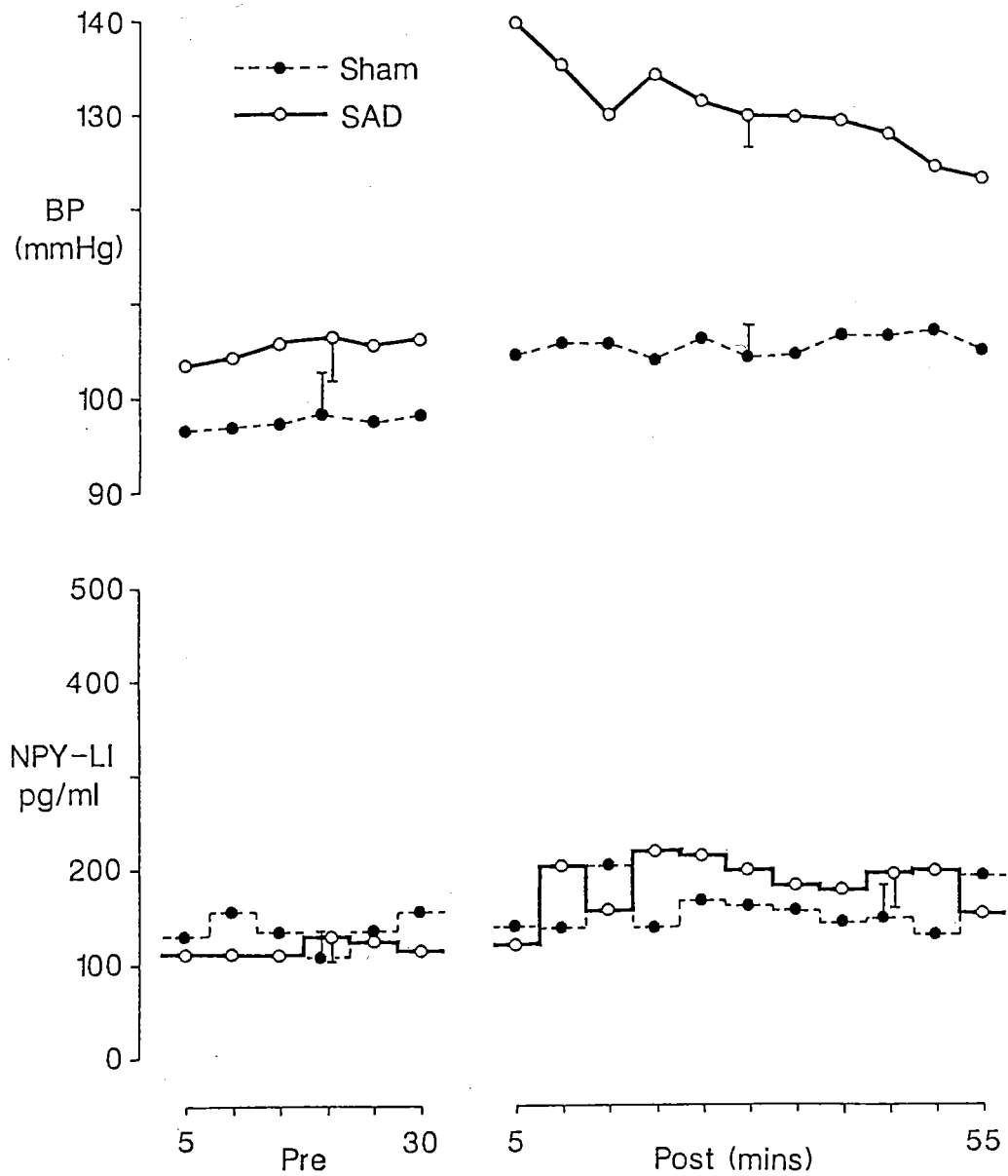
Figure 7.2 Effect of acute sinoaortic denervation (SAD) or sham denervation on blood pressure (BP, upper panel) and the concentration of neuropeptide Y – like immunoreactivity in the spinal subarachnoid space (NPY-LI, lower panel).
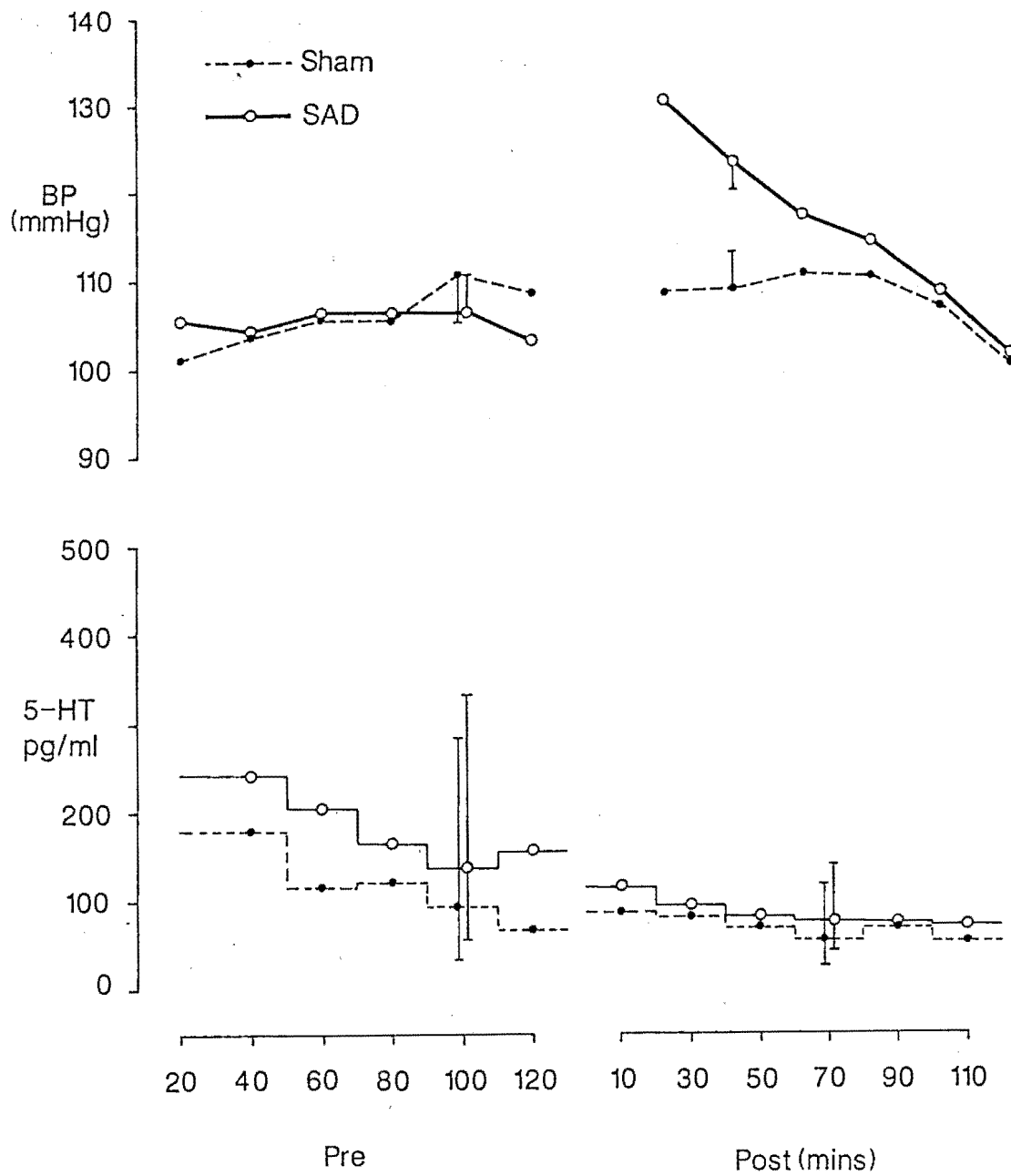
Figure 7.3 Effect of acute sinoaortic denervation (SAD) or sham denervation on blood pressure (BP, upper panel) and the concentration of serotonin in thoracic spinal cord dialysates (5-HT, lower panel).

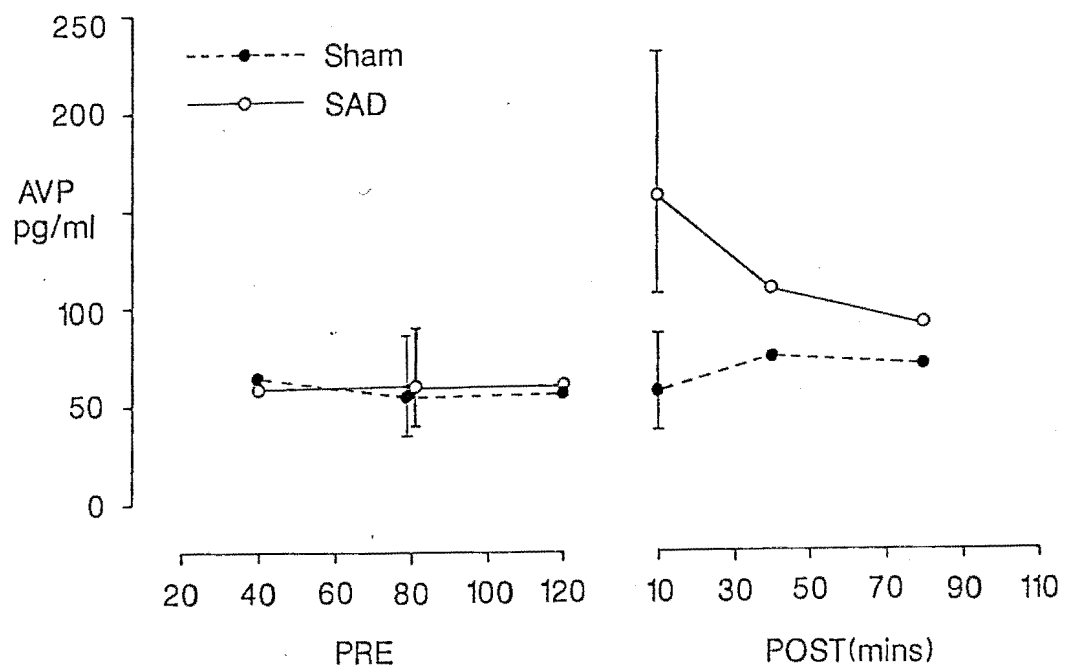Figure 7.4 Effect of acute sinoaortic denervation on the concentration of plasma vasopressin immunoreactivity. A logarithmic transformation of the data was necessary prior to the analysis, and the error bars represent 95% confidence intervals.

4 Discussion

The results of these experiments demonstrate that in the urethane-anaesthetized rabbit the increase in arterial pressure that accompanies acute sinoaortic denervation is not associated with an increase in the release of serotonin or neuropeptide Y in the spinal cord, but is associated with an increase in the levels of plasma vasopressin. There are several possible explanations for this result. Firstly, it is possible that neither serotonin- nor neuropeptide Y- containing neurons are involved in baroreceptor-mediated changes in sympathetic tone. Although possible, this conclusion seems unlikely given the weight of evidence already outlined in favour of such an involvement. Secondly, the stimulus used may not have resulted in a sustained increase in the activity of bulbospinal serotonin- or neuropeptide Y- containing neurons. On an initial examination of the data this explanation appears unlikely, since the change in blood pressure seen in these experiments is of a similar magnitude and duration to that seen in the experiments described in chapter 6. A major difference between those experiments, and the studies described here is that the animals were sino-aortically denervated: consequently, the increase in plasma vasopressin seen in these experiments may in itself have been sufficient to account for the increases in arterial pressure. This idea is supported by the fact that the time course of the change

in vasopressin and the change in arterial pressure are almost identical. This is in contrast to the findings of Blessing et al (1982), who observed increases in plasma vasopressin levels after electrolytic lesions of the caudal ventrolateral medulla. In that study, the increase in plasma vasopressin levels preceded the increase in arterial pressure. Support for this idea also comes from experiments performed by Cowley et al (1974), who found that the pressor responsiveness to vasopressin was markedly increased by sinoaortic denervation. Although their experiments were performed in conscious dogs, making comparisons difficult, inspection of their data (figure 7.5) suggests that the levels of vasopressin seen in these experiments could wholly account for the increase in arterial pressure. A number of experiments are necessary to test this idea further, including the direct measurement of sympathetic nerve activity during the period after sinoaortic denervation.

These results do not necessarily invalidate the original contention of Wing and Chalmers (1974a,b), or DeQuattro et al (1969), that the increase in blood pressure seen after sinoaortic denervation is secondary to an increase in sympathetic tone mediated by bulbospinal serotonin neurons, since in the experiments described here the animals were anaesthetised with urethane, paralyzed and ventilated. In contrast, the experiments of Wing and Chalmers (1974a,b) were performed chronically in conscious rabbits. These major differences in the preparations used could easily
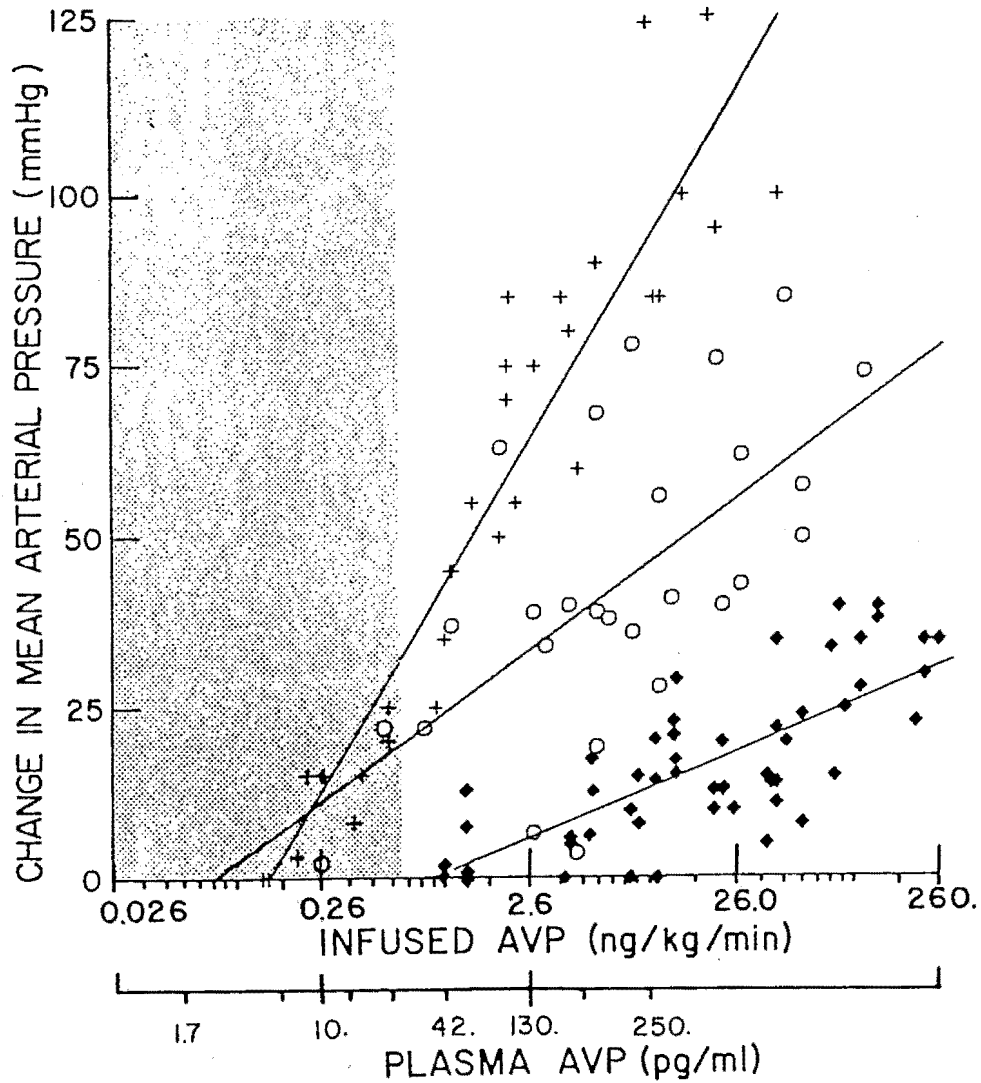
Figure 7.5 A diagram from Cowley et al (1974) showing the relationship between blood pressure and plasma vasopressin concentrations during infusions of vasopressin in normal (closed diamonds) and sinoaortically denervated dogs (open circles).

account for the differences in the observations.   Results
similar to those reported here have been made by Alexander
and Morris (1986) in the rat.   These authors also found that
the increase in blood pressure found after   sinoaortic
denervation   was associated with an increase   in   the
concentration of plasma vasopressin.   In these conscious
rats, administration of a vascular vasopressin antagonist
only attenuated the increase in blood pressure, suggesting a
role for the sympathetic nervous system.

Although these results do not provide support for the
hypothesis that bulbospinal serotonin or neuropeptide Y
containing neurons participate in the baroreflex control of
blood pressure, they do not rule out this possibility.

Chapter 8: Table of Contents

Immunoh'istochemistry of substance P and adrenaline
neurons in the ventrolateral medulla

1 Introduction

In previous chapters, the results of experiments in which
neurotransmitter release was used as an index of the
activity of bulbospinal pathways were described. It is an
essential prerequisite for these studies that the
neurotransmitter being studied is confined in its
distribution to a particular area. As outlined in chapter
5, activation of serotonin-containing neurons in the
ventrolateral medulla results in an increase in arterial
pressure associated with an increase in serotonin release.
Both of these changes were abolished by prior destruction of
serotonin neurons with 57DHT. In an attempt to further
examine these responses, and to contrast them with the
changes in arterial pressure seen during activation of the
more laterally-lying adrenaline-synthesizing neurons of the
C1 group, it was planned to measure the release of substance
P from the spinal cord, since this peptide has been
localized within neurons of the medial part of the rostral
ventrolateral medulla (Ljungdahl et al 1978; Loewy and
Marson 1985), and has been shown to coexist with serotonin
in some of these neurons (Johansson et al 1981). In order
for this peptide to be a useful index of neuronal activation
in this context, it is necessary for it to be absent from

the adrenaline-synthesizing neurons. It seemed likely that this would be the case, since in an elution-restaining study by Ljungdahl et al (1978), where the location of substance P- and tyrosine hydroxylase- immunoreactivity had been examined, no evidence for colocalization had been reported. In 1985, however, a report appeared suggesting that there was extensive colocalization of substance P with phenylethanolamine -N- methyltransferase in spinally-projecting neurons of the ventrolateral medulla (Lorenz et al 1985). Because of the clear discrepancy between these two reports, I decided to determine the extent of colocalization of substance P- and phenylethanolamine-N-methyltransferase- like immunoreactivity (SPLI and PNMTLI) within neurons of this region.

2 Methods

The experiments were performed in rabbits and rats. Three male Wistar-Kyoto (WKY) and three male Sprague-Dawley (SD) rats were treated with intracerebroventricular (ICV) colchicine under chloral hydrate (100mg/kg)/ sodium pentobarbitone (35mg/kg) intraperitoneal anaesthesia. Fixation, sectioning and treatment of sections was performed as described in chapter 2. The protocol is outlined in figure 2.11.

In two rats that had not been treated with colchicine, but fixed in the same way as those that had, longitudinal sections of the thoracic spinal cord were examined.

Three New Zealand White rabbits were treated with a microinjection of 100nl of colchicine (10mg/ml) into the rostral ventrolateral medulla at the site described in chapter 6. Twenty-four hours later the rabbits were perfused and the brains removed, sectioned and treated as described in chapter 2. Preliminary experiments revealed that the antisera to PNMT used in these experiments was ineffective in staining cells in the rabbit medulla, thus in these experiments, FAGLU-induced catecholamine fluorescence was used as a marker of C1 neurons. Previous studies have shown that colchicine pretreatment causes an increase in FAGLU-induced fluorescence in these neurons (Howe et al 1984).

3 Results

3.1 Control experiments

The interpretation of double-labelling experiments such
as those described here is critically dependent on
carefully carried out control experiments, especially
when the reagents are applied simultaneously.   In
particular, it is important to avoid the use of secondary
antisera that are likely to interact with each other,
such as sheep anti-rabbit and rabbit anti-rat. In the
studies described below, the second antibodies were
raised in the same species (sheep), to reduce the chances
of such interactions. When tissues reacted with rat
anti-substance P were reacted with fluorescein
isothiocyanate (FITC)-conjugated affinity purified sheep
anti- rabbit antisera, no labelling was seen. Similarly,
when rabbit anti-PNMT was followed by
tetramethylrhodamine isothiocyanate (TRITC)-sheep
anti-mouse, no labelled cells were seen. This shows that
the two second antibodies do not react with the
inappropriate primary antibodies. To confirm this, both
second antibodies were applied after each of the primary
antibodies had been applied alone. FITC fluorescence was
seen only after incubation in the rabbit anti-PNMT
antiserum; conversely, TRITC fluorescence was seen only
after prior incubation in the rat anti-substance P
antiserum. The latter set of controls indicate that the
two secondary antibodies did not interact. The secondary

antibodies alone were applied to sections exposed to a mixture of the two primary antibodies. Two distinct populations of neurons were seen. After application of the FITC-labelled sheep anti-rabbit, fluorescence was seen in cell bodies in the ventrolateral and dorsomedial medulla, corresponding to the location of the C1 and C2 cell groups. Conversely, TRITC-labelled sheep anti-mouse revealed fluorescence within cell bodies in the midline and in the ventrolateral medulla. Finally, omission of both primary or both secondary antisera resulted in no fluorescent labelling. Since only one primary and one secondary antibody were used in the experiments in the rabbit, the control experiments were less complicated; omission of either the primary or secondary antisera resulted in no fluorescent labelling.

3.2 Immunohistochemical labelling

In WKY and SD rats, neurons with PNMTLI and SPLI were counted in 50 micron sections. Alternate sections were used for counting. PNMTLI was seen in cells extending from 0.6mm caudal to the obex, to 2.3mm rostral to the obex (figure 8.1) in the ventrolateral medulla, forming the C1 adrenaline cell group. The FITC-fluorescence observed in these neurons was typically homogenous, and included the nucleus but not the nucleolus (figure 8.2).
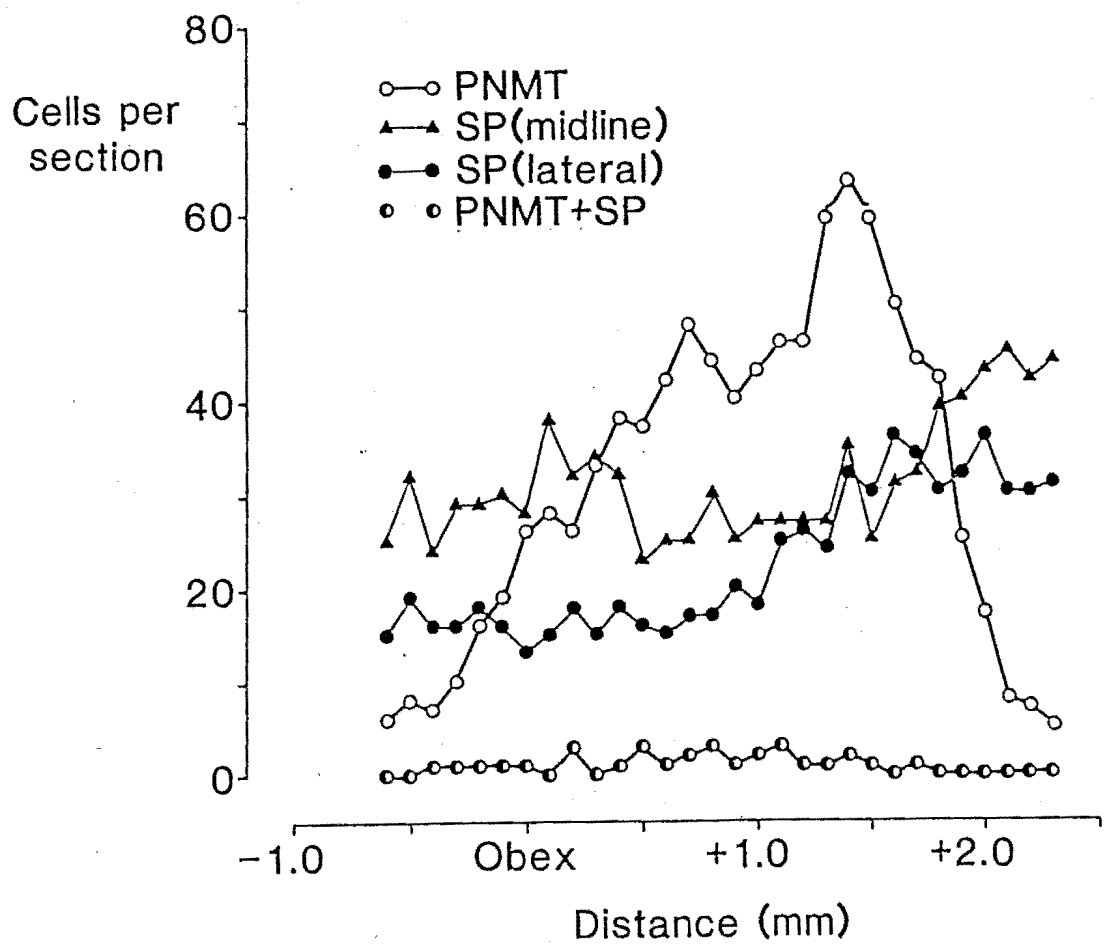
Figure 8.1 Cell counts in the ventral medulla (circles) and raphe (triangles) showing the rostrocaudal extent of cells containing phenylethanolamine -N- methyltransferase (PNMT) or substance P (SP) like immunoreactivity or both antigens.
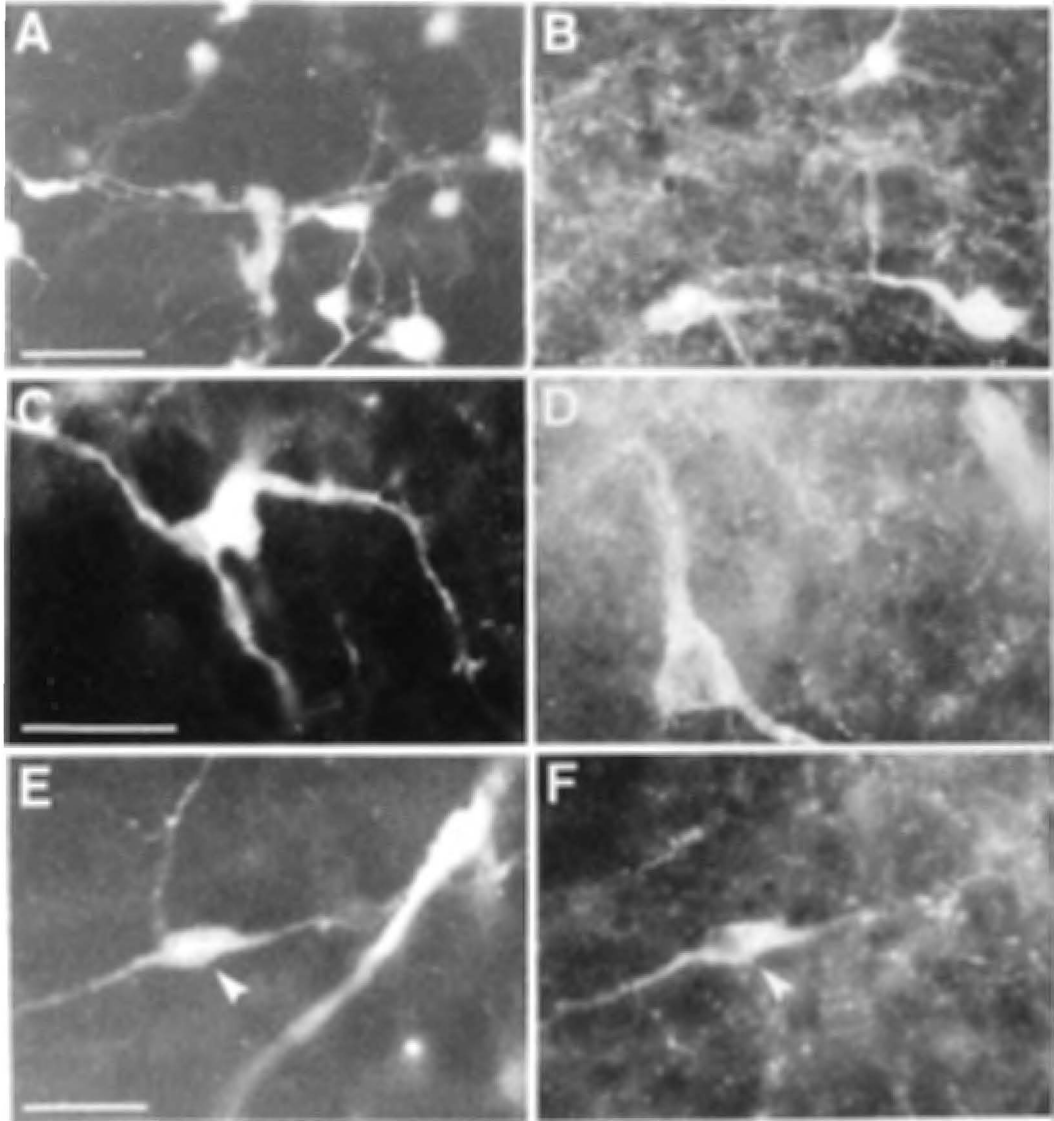
Figure 8.2 Cells containing phenylethanolamine -N-methyltransferase (A,C,E) and substance P (B,D,F) like immunoreactivity in the rat ventral medulla. Each pair of photographs were identical except for the filter used to discriminate the two fluorophores. One cell contains both antigens (arrow). Bar = 50 microns.

SPLI in neurons was counted in the same sections that contained cells with PNMTLI. These cells were found in two regions of the medulla: in the midline, and laterally adjacent to the borders of the pyramidal tracts and extending into the area of the nucleus paragigantocellularis lateralis (Andrezik et al 1981). In contrast to the fluorescence seen in PNMTLI neurons, the SPLI was clearly granular, and the nucleus was unstained (figure 8.2). In the WKY rats a small number of neurons in the ventrolateral medulla containing PNMTLI were also found to display SPLI ($3.2\pm0.6\%$, mean$\pm$sem, n=3; figure 8.1). The same proportion of dually-labelled neurons was also found in the SD rats ($3.2\pm0.9\%$, n=2).

Since, in the experiments described by Lorenz et al (1985), rats were used that had been treated with colchicine for forty-eight hours, the possibility existed that the longer time used might increase the amount of SPLI within cells, and hence increase the number of dual-labelled neurons. This seems unlikely, since in the one SD rat examined that had been treated for 48 hours with colchicine, only 0.7% of PNMTLI cells also contained SPLI.

In the experiments described above I have examined the extent of colocalization of PNMTLI and SPLI within cell bodies. In addition, the number of fibres in the medulla and spinal cord that were dually-immunoreactive was

assessed qualitatively. Apart from fibres extending from the cell bodies of dually-labelled neurons, very few doubly labelled fibres were seen. In the spinal cord, despite the presence of large numbers of fibres containing SPLI or PNMTLI, very few dually-labelled fibres were seen, in one thoracic spinal cord for example only two such fibres were seen. Since the presence of SPLI in fibres is not dependent on pretreatment with colchicine, this is unlikely to be a false negative result.

In New Zealand White rabbits numerous catecholamine fluorescent neurons were seen in the rostral ventrolateral medulla on the side of the colchicine injection. Small numbers of cells containing SPLI were also seen in the ventrolateral medulla; these cells tended to lie medially to the catecholamine fluorescent neurons. Since the numbers of cells seen appeared to depend on the colchicine injections, no formal cell counts were undertaken. No examples of dually-labelled neurons were seen in these animals (figure 8.3). In one animal, staining was attempted with the antiserum to PNMT at dilutions as low as 1 in 10. No labelled cells were seen.
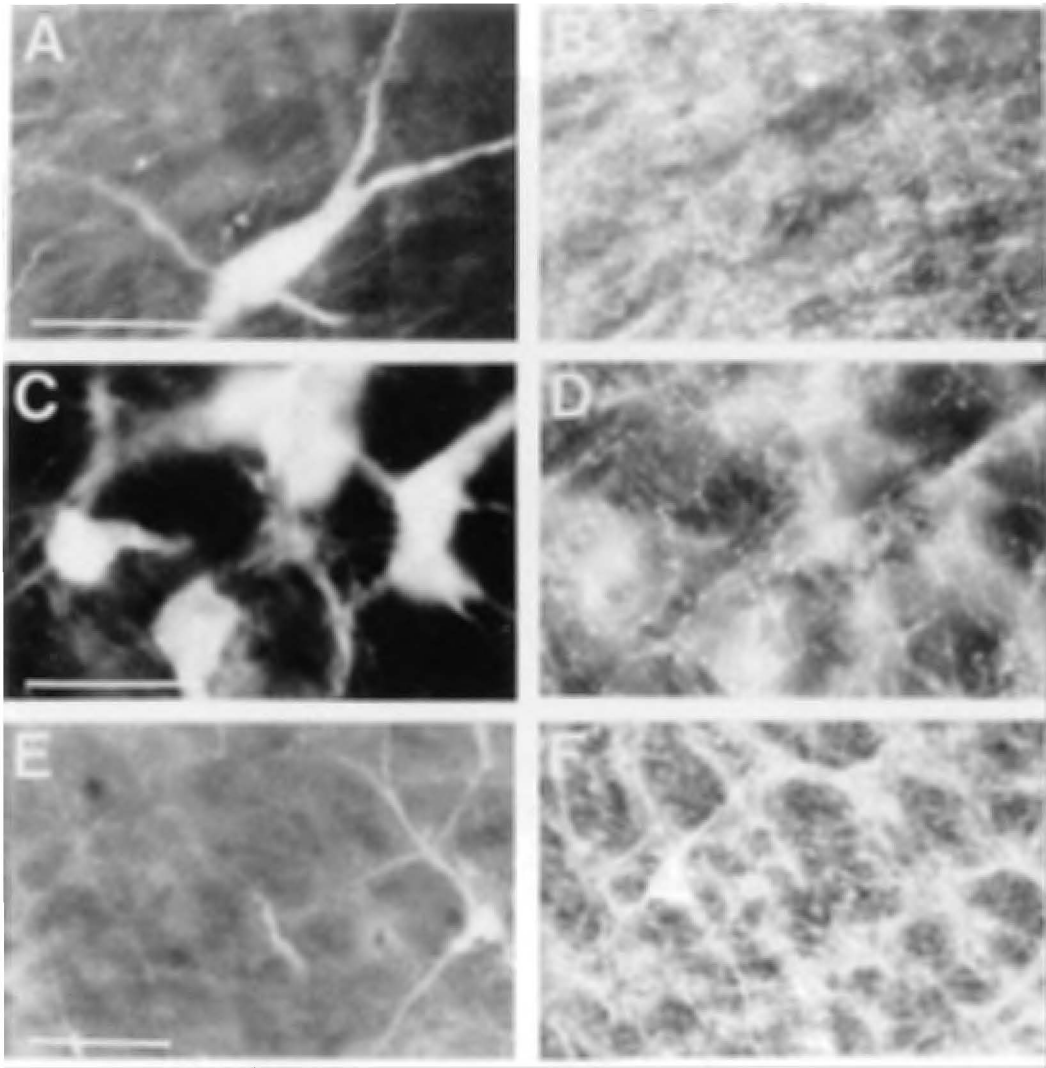
Figure 8.3 Cells containing FAGLU fluorescence (A,C,E) or substance P like immunoreactivity (B,D,F) in the rabbit ventral medulla. Bar = 50 microns.

4 Discussion

4.1 Immunofluorescence in the rabbit

The finding of no immunofluorescent staining in the
medulla with the antiserum to PNMT in the rabbit is in
accord with the literature: despite the presence of many
antisera to this enzyme, there have as yet been no
reports on its immunohistochemical localization in rabbit
brain. There are two explanations for this: either the
rabbit brain does not contain PNMT, or the antisera
available (generally produced against bovine adrenal
PNMT) do not interact with rabbit PNMT. The former
possibility seems unlikely, since one can induce
catecholamine fluorescence in neurons of the rostral
ventrolateral medulla by pretreatment with an inhibitor
of PNMT, presumably resulting in an increase in
intracellular noradrenaline levels. It should be noted
that this is indirect evidence, and it remains for
someone to demonstrate that the antisera available do not
identify rabbit adrenal PNMT, and to report on the levels
of adrenaline and PNMT activity in rabbit brain. In this
context it is worth noting that in the only report
available so far on the concentrations of adrenaline in
rabbit spinal cord, the levels were so low that they

could not be estimated directly, and were estimated as the difference between total and noradrenaline catecholamine (Zivin et al 1975). On the other hand, in a recent study on the guinea pig, it was reported that although this species contains the same amount of adrenaline in its adrenal medulla as the rat, there was no detectable adrenaline present in the medulla oblongata or hypothalamus, despite the presence of cells in the adrenal medulla that were immunoreactive for PNMT (Cumming et al 1986).

Immunoreactivity for substance P was present in fibres in the medulla, and within cell bodies after colchicine treatment. The findings here do not support the idea that this peptide is present within catecholamine neurons in the rabbit.

4.2 Immunofluorescence in the rat

The control experiments described above indicate that the second antibodies used were species specific. Furthermore, there was no binding of the second antibodies to each other, nor was there binding of the primary antibodies to each other. Under these circumstances the double-labelling procedure appears likely to be specific, in that the presence of both

labels within the one structure is due to colocalisation
of the two antigens, and the possibility of false
positive double-labelling is absent. The results
presented here differ markedly from those of Lorenz et al
(1985), who reported that the majority of
spinally-projecting neurons containing PNMTLI were also
immunoreactive for substance P. The reasons for this
difference is unclear; it seems unlikely to be due to a
sampling error, in that Lorenz et al (1985) examined
neurons that were retrogradely labelled from the spinal
cord with the fluorescent dye fast blue whereas I
examined all PNMTLI neurons, since I have also examined
the extent of colocalisation of these antigens within
fibres in the spinal cord, and found very few to be
doubly-labelled. There are two other methodological
factors that may account for the differences. Firstly,
in the report by Lorenz et al (1985), the filter
combinations used in the microscope in their study were
not specified, it is possible that bleed-through of
fluorescence could produce false positive staining. A
further possibility is that unwanted interactions may
have occurred between the second antibodies used by
Lorenz et al (1985). These authors applied their primary
antisera together as we did, in fact their substance P
antibody was the same monoclonal that we used (Cuello et
al 1979); however they then applied an FITC-labelled
sheep anti-rabbit antibody followed after a wash in
buffer by a TRITC-labelled rabbit anti-rat antibody. It

is therefore possible that free antibody binding sites on the FITC-labelled sheep anti-rabbit antibody would be able to bind the TRITC labelled rabbit anti-rat antibody. This is precisely the method used in the peroxidase anti-peroxidase bridge technique. Such an interaction, if it occurred, would be expected to produce large numbers of spuriously dual-labelled neurons.

These findings confirm the presence of neurons in the rostral ventrolateral medulla that contain both PNMTLI and SPLI, but demonstrate that they are very few in number, and their contribution to the total population of spinally-projecting PNMT-containing neurons is quite small. It is conceivable that this sub-population forms a larger proportion of the PNMT-containing neuronal input to another region.

Chapter 9: Table of Contents

General discussion and conclusions

1 Summary of major findings

In what way has our knowledge of the medullary control of sympathetic tone advanced since the landmark discoveries of Dittmar (1870), Owsjannikow (1871) and Alexander (1946)? At that time, the focus of these investigations was on changes in the activity of sites in the brainstem where changes in autonomic function could be elicited. With the advent of pharmacological agents, and the ability to localize substances within neurons, there was a change in focus to an examination of specific populations of neurons, defined by their chemical content. In one sense, these studies provided a clearer picture of the neurons involved in autonomic function, but generally speaking these were studies in which the functions of a particular area was associated with the presence of certain populations of neurons, without providing direct evidence for their involvement. These experiments, although providing a more accurate picture of the areas of the brainstem that are important in cardiovascular control, do not greatly improve on the findings of the early workers in the field. In this thesis I have started with an examination of the effects of altering neuronal function in different parts of the medulla on blood pressure and sympathetic nerve activity (chapter

3). The results of these experiments conform to the findings of many previous workers, in that there is a tonically active depressor area located in the caudal ventrolateral medulla, and a tonically active pressor area in the rostral ventrolateral medulla. The results presented in chapter 3 also demonstrate that the changes in blood pressure are accompanied by changes in sympathetic nerve activity that are in the same direction. This suggests that the effects are due to alterations in the activity of sympathetic nerves, rather than changes in the activity of an alternative pressor system. Unfortunately, as in previous studies, it is not possible to say which of the many different cell types in the area are involved in mediating these effects. The role of catecholamine- and serotonin- containing neurons in the control of sympathetic nerve activity was investigated by the instillation of specific neurotoxins into the cisterna magna (chapter 4). By measuring renal nerve activity, plasma catecholamine and plasma vasopressin levels, in addition to blood pressure and heart rate, it was possible to dissect the involvement of the different central efferent pathways that mediate the hypertensive response to these neurotoxins. It was found that in both cases there was a large increase in renal sympathetic nerve activity. These results provide evidence that specific groups of neurons (in this case serotonin- and catecholamine- containing) are involved in a sympathoexcitatory pathway. An advantage of these experiments is that they were performed in conscious

rabbits, and the possible complications of anaesthesia were avoided. A major disadvantage is that it is not clear which of the many serotonergic or catecholaminergic cell groups are involved in these responses, or even if the sympathoexcitatory responses are mediated by a bulbospinal pathway directly, or through a polysynaptic route. In order to approach these two problems, techniques were developed in the laboratory to allow the measurement of substances released in the target area (in this case the spinal cord) after stimulation of the cell bodies either directly or indirectly. With this method the problem of specificity is overcome, since the activity of the neuronal cell population of interest is being measured at the same time as the changes in autonomic function. Furthermore, it now becomes possible to examine the activity of specific cell groups innervating a specific target.

In chapter 5, experiments are described in which this approach was used to examine the role of spinally-projecting serotonin-containing neurons whose cell bodies are located in the medial part of the rostral ventrolateral medulla of the rat. It was found that in association with the increase in arterial pressure seen after stimulation of these neurons, there was a release of serotonin in the spinal cord;   presumably from the terminals of bulbospinal serotonin-containing neurons.   Since   the   increase   in arterial pressure was prevented by prior destruction of these serotonin - containing neurons, it seems likely that

these are the neurons responsible for mediating the pressor effects that can be elicited from this area.

In chapter 6, using a slightly different approach, a release of neuropeptide Y - like immunoreactivity into the spinal subarachnoid space of the rabbit was demonstrated during stimulation of the rostral ventrolateral medulla. Furthermore, a similar increase in arterial pressure and neuropeptide Y - like immunoreactivity was seen during inhibition of the caudal ventrolateral medulla. These direct findings support the view of other workers who have used correlative approaches (Dampney et al 1982; McAllen et al 1982; Ross et al 1984) that neuropeptide Y /adrenaline neurons of the rostral ventrolateral medulla mediate sympathoexcitatory information to the sympathetic preganglionic neurons of the spinal cord.

In an attempt to determine whether or not these bulbospinal serotonin or neuropeptide Y neurons participate in the baroreflex control of blood pressure, the release of these substances in the spinal cord was measured before and after acute sinoaortic denervation. In both cases no change was seen. On the other hand, the elevation in plasma vasopressin that was observed could have accounted for the increase in blood pressure, suggesting that at least under the conditions used here, acute sinoaortic denervation may not be a particularly good model for the activation of bulbospinal sympathoexcitatory pathways.

The findings of chapter 8, although preliminary, suggest that it is possible to analyse these bulbospinal neurons in terms of their chemical content. In the longer term, this chemical coding may prove to be functionally significant.

2 Implications of the present results

The work of the pioneers in this field (Dittmar 1870; Owsjannikow 1871; Bard 1930; Alexander 1946) enables us to draw a simple circuit outlining the central control of the cardiovascular system (figure 9.1). It is clear from this figure that there is an afferent limb and three efferent limbs; one excitatory to sympathetic outflow, and one inhibitory, as well as the cardioinhibitory output from the vagus. Absent from this design is an input to the vasopressin secreting cells of the hypothalamus. Furthermore, there is no understanding of how impulses are integrated within the brainstem prior to changes in efferent outflow, nor has there been a delineation of the cells in the medulla responsible for affecting sympathetic outflow. The situation recently is outlined in figure 9.2. The major features that are different include the pathway for the release of vasopressin, and the fact that it is affected by baroreceptor afferents which relay information through the A1 noradrenaline neurons of the caudal medulla. The descending inhibitory input proposed by Alexander (1946) has
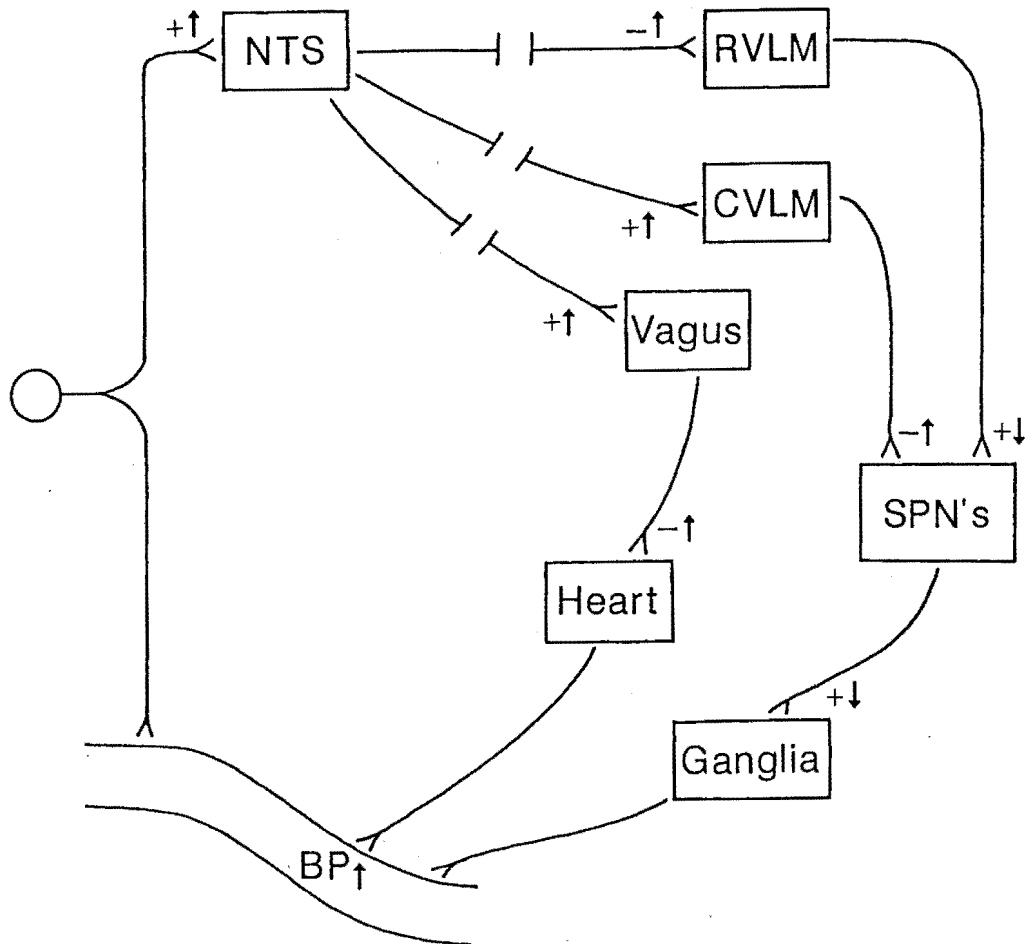
Figure 9.1 A schematic drawing showing the neuronal
circuitry of the medullary control of the cardiovascular
system based on information available prior to 1940. The
importance of afferent pathways, vagal pathways, and the
ventrolateral medulla had been established, however the
relationship between the caudal and rostral ventrolateral
medulla had not been elucidated. nucleus tractus solitarius
(NTS), rostral (RVLM) and caudal (CVLM) ventrolateral
medulla, sympathetic preganglionic neurons (SPN's), blood
pressure (BP). Arrows indicate the change in activity of
the neuron that occurs during an increase in blood pressure.
+ and - indicate whether or not the neuron is excitatory or
inhibitory.

Figure 9.2 A schematic drawing showing the current state of knowledge concerning the neuronal circuitry within the medulla underlying cardiovascular control. The pathway from the caudal ventrolateral medulla to the vasopressin secreting cells of the hypothalamus has been established, however little additional knowledge has been gained concerning the relationship between the caudal and rostral ventrolateral medulla and the spinal cord. Acetylcholine (Ach), noradrenaline (NA).

to date been neither proved nor disproved. Neurotransmitter
candidates have only been included in this schema where
there is clear evidence for their involvement. Of
particular interest is the apparent lack of progress that
has been made in the area of integration within the medulla.
Part of figure 9.2 is expanded in figure 9.3; it is this
part of the circuit that I have examined in this thesis.
There are two main cell groups that have been implicated as
having a role in mediating excitatory bulbospinal impulses;
the serotonin/substance P containing cells and the
neuropeptide Y/adrenaline containing cells. It should be
noted that since formal cell counts of this region have not
been carried out, there may be other as yet unidentified
cells in the region.

As noted above, the experiments in chapters 3 and 4
confirmed the general outline of this schema, without
providing additional evidence about which cells in the area
are involved. The experiments in chapter 5 however provided
new evidence in favour of a role for serotonin-containing
neurons in mediating sympathoexcitatory responses at least
in the rat. The experiments described in chapter 6 enable
one to conclude that in the rabbit, spinally-projecting
neuropeptide Y-containing neurons are pressor, and are
involved in mediating the pressor effects of inhibiting the
caudal medulla. This information is included in figure 9.4.
It should be noted that the possibility that descending
serotonin neurons may be involved in the caudal depressor
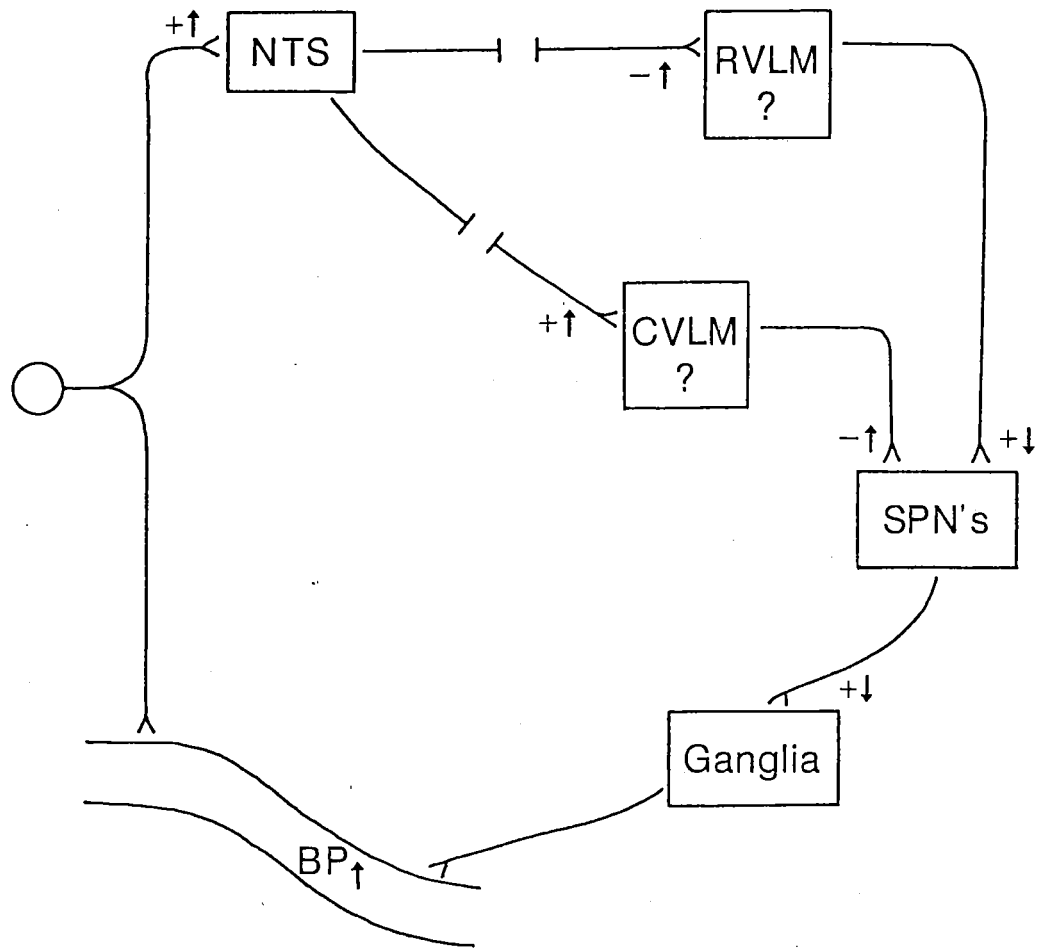
Figure 9.3 An extract from Figure 9.2, showing the connections between the afferent neurons, NTS, RVLM and CVLM. The neurotransmitters mediating the responses from the RVLM and CVLM has not been identified (?).
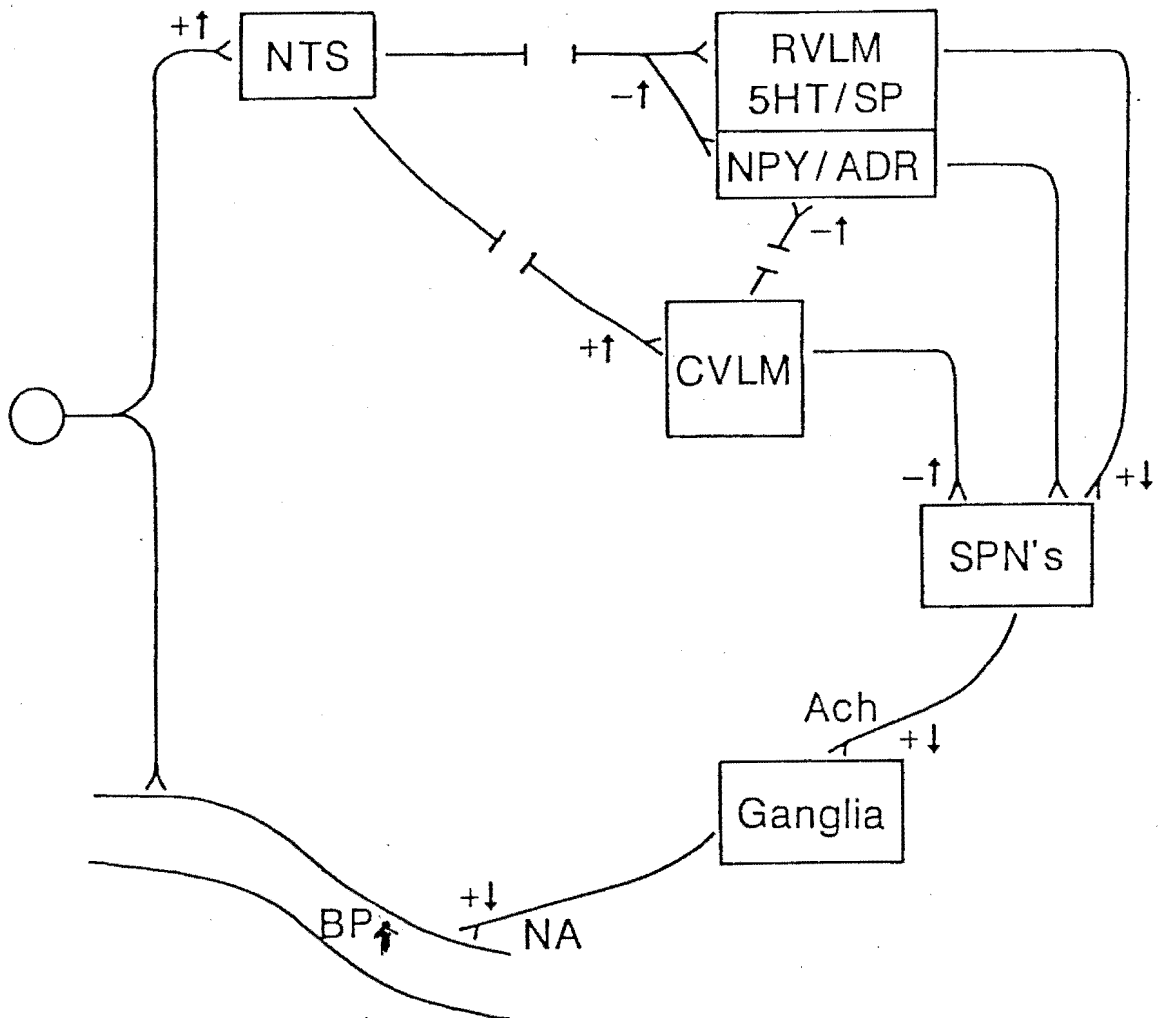
Figure 9.4 The same diagram as Figure 9.3, but including a pathway from the CVLM to the RVLM, and two pathways from the RVLM to the spinal cord. On the basis of experiments described in this thesis, it is proposed that serotonin and substance P (5HT/SP) are present in one population of neurons, whilst neuropeptide Y and adrenaline (NPY/ADR) are present in a separate population. The neurotransmitters and number of neurons involved in the pathway from the CVLM to the RVLM is not known.

response has not been formally investigated.

I had intended to see if these same neurons in the rostral ventrolateral medulla were involved in mediating baroreceptor information to neurons in the spinal cord, but unfortunately, it appears that the model chosen for this purpose was inadequate (chapter 7). Nevertheless, the negative findings of chapter 7 confirm that the findings obtained in the previous chapters are not an artefact secondary to the rise in blood pressure.

Finally, the findings of chapter 8, open the way for a more detailed histochemical and physiological analysis of neurons in this region using the methods outlined above.

Many challenges remain: the sympathoexcitatory neuron in the rostral ventrolateral medulla that mediates baroreceptor information is still unidentified, the neurotransmitter of the second order baroreceptor neuron is also unidentified, as are the number of interneurons between the primary afferent neuron and the sympathetic preganglionic neuron. Although some of the chemicals released by these neurons have been identified, the neurotransmitter used has only been identified unequivocally for the preganglionic neurons.

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