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A neuroanatomical study of connections between cardiovascular control centers in the medulla and spinal cord of the rat

David Conrad Paul Baker
Iowa State University

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Baker, David Conrad Paul, Ph.D.

Iowa State University, 1988

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**A neuroanatomical study of connections
between cardiovascular control centers in
the medulla and spinal cord of the rat**

by

David Conrad Paul Baker

**A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of**

DOCTOR OF PHILOSOPHY

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Approved:

Members of the Committee:

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For the Graduate College

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SYMBOLS AND ABBREVIATIONS

- A1 - A group of monaminergic nerve cells in the ventral medulla
- A2 - A group of monaminergic nerve cells in the dorsal medulla
- ADN - Aortic depressor nerve
- C1 - A group of epinephrine containing cells in the ventral medulla
- DMV - Dorsal motor nucleus of the vagus nerve
- HRP - Horseradish peroxidase
- IML - Intermediolateral cell column of the spinal cord
- NA - Nucleus Ambiguus
- NTS - Nucleus Tractus Solitari
- PNMT - Phenylethanolamine-N-methyltransferase
- RVL - Rostral ventrolateral medulla
- VLM - Ventrolateral medulla

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It is with deep love and satisfaction that I dedicate this dissertation to my mother, Sadie. Her devotion, undying love, unselfish sacrifices, kindness and soft reproaches have molded my character and self esteem. Her insistence on far reaching educational goals can best be summarized by the great words of Longfellow:

"The heights of great men reached and kept
Were not attained by sudden flight.
But they, while their companions slept
Were toiling upwards through the night."

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"Those who have dissected many bodies,
Have at least learned to doubt,
While others who are ignorant (of Anatomy)
And do not take the trouble to learn
Are in no doubt at all."

I would also like to thank Martha Morgan for her long hours of lab work and histological processing. Also, I take great pleasure in acknowledging the expert electron microscopic and photographic assistance of Mr. Randall L. DenAdel and Ms. Cathy Martens. It is virtually impossible for me to express in words my thanks to the nice people in the Veterinary Anatomy Department especially Ms. Josie Niemand and Ms. Lori Vandermyde who tolerated my many questions during the printing of this dissertation.

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INTRODUCTION

By far the best known of the mechanisms for arterial pressure control is the baroreceptor reflex. The reflex is initiated by pressure receptors, called either baroreceptors or pressoreceptors, located in the walls of most of the large systemic arteries. A rise in pressure causes the baroreceptors to transmit afferent signals to the central nervous system. Efferent signals are, in turn, sent to the heart and the autonomic nervous system, which causes the cardiovascular system to reduce net arterial pressure back towards normal levels.

Baroreceptor activation leads to inhibition of the sympathetic nervous system vasomotor centers of the medulla and excitation of the parasympathetic nervous system vagal center. The net effects are (1) vasodilation of the peripheral circulatory system and (2) decreased cardiac rate and strength of contraction. Therefore, excitation of the baroreceptors by elevated pressure in the arteries reflexly causes the arterial pressure to decrease. Conversely, low arterial pressure has the opposite effects to those described, reflexly causing the pressure to rise back toward average levels.

There is a phylogenetic progression in cardiac control systems, starting with the aneural heart of the hagfish, which is regulated by Starling's law and accelerated by chromaffin cells. In elasmobranchs, reflex regulation is more similar to that of mammalian systems. Increased pressure in the branchial arteries leads to respiratory and cardiac inhibition. Vagal afferents from baroreceptors are the input

link of a central nervous system reflex pathway, leading to activation of medullary vagal efferents for cardiac inhibition. This is a typical parasympathetic cardiac regulatory system, but the elasmobranchs have no cardiac sympathetic efferents. Instead, elasmobranchs use circulating catecholamines to invoke their fight or flight responses, including cardiac acceleration, strengthened cardiac contraction, increased systemic blood pressure, and constricted branchial blood vessels.

The fact that the central nervous system controls blood pressure in mammals via efferent sympathetic fibers was shown more than 100 years ago by Dittmar (1873) in the rat. He studied the effects of successive transections of the brain stem, and discovered that blood pressure was affected when the medulla was transected below the level of the facial colliculus. At and below that level, transection caused a marked decrease in blood pressure from the normal 120mm Hg down to about 50mm Hg.

Neuroanatomical connections constitute one of the many operative factors which determine the function of a neuronal system. A knowledge of connections of the initial segments in the baroreceptor pathway will, in combination with other morphological and physiological data, lead toward a fundamental understanding of the process of cardiovascular regulation. The purpose of this research is to identify two synaptically associated components of a reflex arc that alter heart rate and blood pressure at the medullary level.

LITERATURE REVIEW

Resting blood pressure is reflexly regulated. Major elements of such control mechanisms are the arterial baroreceptors, stretch receptors that are localized to the major vessels of the chest and neck and are innervated by branches of the Xth and XIth cranial nerves (Andrew, 1954 and Heymans and Neil, 1958). Stimulation of baroreceptors by elevation of arterial pressure lowers blood pressure by inhibition of sympathetic discharge. Baroreceptor stimulation also slows heart rate, primarily by exciting the parasympathetic cardiac vagus nerves. In contrast, withdrawing the input from the baroreceptors by transection of the nerves or by occlusion of the vessels proximal to the receptors so that local arterial pressure falls, leads to increases in sympathetic discharge, blood pressure, and heart rate.

Receptors

Two sets of nerves, the carotid sinus nerve and the aortic depressor nerve (ADN), carry similar information from baroreceptors to the central nervous system. However, the cardiovascular reflexes originating from stimulation of receptors in these two regions are somewhat different. For example, stimulation of baroreceptors in either the carotid sinus or the aortic arch alters peripheral resistance and heart rate (Glick and Covell, 1968), but compared to the carotid baroreflex, the aortic baroreflex is more sensitive to static than dynamic stimulation (Angell-

James and De Burgh-Daly, 1970). Furthermore, in contrast to the carotid reflex the aortic reflex alters heart rate more than vascular resistance and seems to act more in hypertensive than hypotensive states (Oberg, 1981, Aars et al., 1978). Additional evidence for different physiological roles for the two buffer nerves in rats is that bilateral transection of the ADN produces a sustained increase in arterial pressure, while only a transient elevation in arterial pressure follows carotid sinus denervation (Ciriello et al., 1980)

Arterial baroreceptors do not constitute a homogeneous group; both myelinated and nonmyelinated afferent fibers are present in the baroreceptor nerves (Anyukhovskii and Beloshapko, 1978). Histologically the majority of the afferent fibers in the baroreceptor nerves are seen to be nonmyelinated fibers (Thoren et al., 1977).

Myelinated and nonmyelinated fibers have different firing characteristics and subserve different reflex pathways. The receptors associated with nonmyelinated fibers have a higher threshold than those associated with myelinated aortic baroreceptor afferents, and they have an irregular discharge rate (Thoren et al., 1977). The reflex effects elicited from baroreceptor nonmyelinated fibers include powerful effects on the vagal outflow to the heart and on sympathetic outflow to the kidney microvasculature (Abboud, 1982), but relatively small effects on the sympathetic outflow to blood vessels in the skeletal muscles (Oberg, 1981). Nonmyelinated fibers are markedly activated during acute

elevation of blood pressure, when they can induce powerful depressor reflexes (Douglas and Ritchie, 1956).

Reflex effects invoked via nonmyelinated fibers differ from those via myelinated fibers in the following ways. Increased recruitment of nonmyelinated baroreceptors at elevated arterial blood pressures contribute mainly to the vagally mediated control of the heart (Kardon et al., 1973). Normal tonic cardiovascular control mediated by the myelinated baroreceptors may, in contrast, be accomplished more via reflex changes in the skeletal muscle vascular bed and by simultaneous reflex changes in sympathetic and vagal activity to the heart (Douglas and Ritchie, 1956). The myelinated fibers are able to provide the cardiovascular regulatory centers with information about beat to beat changes in aortic pressure, while the nonmyelinated fibers provide information about mean aortic pressure over a period of time (Thoren and Lundin, 1983). The nonmyelinated fibers thus give more information about average pressure levels than about instantaneous pressure levels.

The left aortic nerve is larger and more apparent than the right aortic nerve. Von Schumacher (1902) described the left ADN as principally innervating the ventral region of the aorta; he believed the right ADN innervated the dorsal aorta and brachiocephalic artery. Embryological studies have revealed unequal development of the left and right ADN. The ADN arises in relation to the fourth primitive arterial arch, which develops asymmetrically in mammals. Lassila (1928) performed a series of developmental experiments and found that the

circular plexus surrounding the aorta is formed by the left ADN, whereas the right ADN innervates the origin of the subclavian and the brachiocephalic artery.

Medulla

This section provides an overview of the medullary centers, their interrelations, and their efferent connections that may play roles in the baroreflex. Individual nuclei will be treated in more detail in separate sections.

Impulses originating in the receptors of the ADN and carotid sinus nerve travel with vagal afferent fibers to the central nervous system, and most seem to terminate in and around the nucleus tractus solitarius (NTS) (Crill and Reis, 1968). The afferent fibers of the vagus nerve enter the dorsolateral medulla as a single bundle about 6 mm anterior to the obex. As the fiber bundle travels dorsomedially in the medulla it separates into a number of fascicles that reconverge and travel caudally in the solitary tract. The fibers exit from the solitary tract throughout its rostral-caudal extent and are distributed within the area postrema, the ipsilateral NTS, dorsal motor nucleus of the vagus (DMV), and nucleus intercalatus (Ingram and Dawkins, 1945, and (Spyer, 1981). The area postrema and ipsilateral medial NTS receive the heaviest projections of vagal afferents (Chernicky et al., 1983).

The carotid sinus nerve has a wider area of distribution in the NTS than does the ADN (Ciriello and Calaresu, 1981), so although the ADN and

carotid sinus nerve exhibit some convergence of inputs in this nucleus, they probably also affect the activity of separate neuronal pools that respond primarily to one or the other buffer nerve. This hypothesis has been verified in the cat, in which the ADN projects primarily to the ventrolateral, lateral and commissural solitary nuclei, all of which are subnuclei of the NTS, and to the DMV, whereas the carotid sinus nerve terminates in the medial, lateral, and ventrolateral solitary nuclei (Ciriello and Calaresu, 1981). In the rat, the ADN terminates in the region of the interstitial nucleus, another subnucleus of the NTS, and in the dorsolateral region of the solitary complex just above the solitary tract. Caudally the ADN terminates in the ventrolateral region of the solitary nucleus and in the commissural nucleus, another subnucleus of the NTS. These terminations are primarily ipsilateral but some contralateral projections exist as well (Ciriello, 1983).

The area of the NTS has long been identified as a site where the first synapse of the baroreflex is located (Humphrey, 1976). It is possible that afferent information from peripheral receptors and descending information from supramedullary centers is integrated within this region. Secondary neurons of the baroreflex arc project from the NTS to preganglionic vagal cardiomotor neurons in the DMV and nucleus ambiguus (NA) and to cells associated with descending fiber systems innervating the preganglionic sympathetic neurons of the spinal cord (Cottle and Calaresu, 1975). Physiological evidence reveals that both pressor and depressor areas lie in the brain stem. The pressor area

extends more rostrally and is more laterally situated than the depressor area in most species (Alexander, 1946). Until recently thinking about central vasomotor control in the medulla has been dominated by the concept of reciprocally organized "half centers". According to this concept a medially located depressor area interacts with a more laterally placed pressor area, and activation of the baroreceptors results in stimulation of the depressor area and inhibition of the pressor area (Alexander, 1946). However, there is now convincing evidence indicating that this simplified concept is inadequate in the rat. In this species specific neuron pools in the bulbopontine region can affect the sympathetic centers in the intermediolateral cell column of the spinal cord via both inhibitory and excitatory descending pathways (Loewy and Neil, 1981). Sympathetic outflow to the heart, blood vessels and visceral organs originates as preganglionic fibers from the intermediolateral cell column (IML), intermediomedial cell column, and intercalated nucleus of the spinal cord (Chung et al., 1975) that project to postganglionic neurons that, in turn, innervate the end organs, including visceral organs, blood vessels, and the heart. The preganglionic neurons are, then, the final central sites upon which neuronal information initiated within multiple centers of the central nervous system converges to maintain or alter sympathetic functions. Autonomic innervation of the heart involves integration of sympathetic and parasympathetic outflow. The latter reaches postganglionic neurons

in the heart via vagal fibers originating from the NA and DMV in the medulla (Spyer, 1981).

Both sympathoexcitatory and sympathoinhibitory pathways descend from brain stem areas and project directly or indirectly to the cells of the IML, intermediomedial, and intracalated nuclei. These pathways have been demonstrated by anterograde and retrograde tracing techniques (Calaresu et al., 1975). They originate at medullary, pontomedullary, hypothalamic, and limbic (primarily from the amygdala) levels (Hopkins et al., 1981).

Dahlstrom and Fuxe (1964) obtained evidence for a direct projection from the monoaminergic cell group in the ventrolateral medulla (VLM) to the IML. This finding has been questioned by recent observations, which suggest that only the rostral portion of the monoaminergic cell group projects directly to the IML (Dembowsky et al., 1980). With new tract tracing techniques and receptor immunocytochemistry the monoaminergic cell column in the VLM has been subdivided further into noradrenergic and adrenergic cell groups, as will be discussed later, and Ross et al., (1981) state that the noradrenergic A1 cell group does not project directly to the IML. Recently, however, more sensitive immunohistochemical techniques have provided evidence that the A1 cell group in the VLM does project to the spinal cord (Kalia et al., 1986). Electrolytic lesions in the VLM interrupt sympathetic efferent activity via a direct pathway (Granata et al., 1983). This pathway includes projections from the C1 group of adrenergic neurons located in the VLM,

and Granata et al. (1983) claim that this pathway is involved in baroreceptor mediated inhibition of sympathetic activity at the spinal level.

At present there is little evidence for a direct projection from the NTS in the dorsomedial medulla to the preganglionic sympathetic neurons in the spinal cord. However, lesions to the NTS in rats have profound effects on sympathetic outflow, resulting in long lasting increases in blood pressure and, to a lesser extent, increases in heart rate (Reis et al., 1977).

The following sections provide more detailed evidence regarding each of the above medullary centers.

Nucleus Tractus Solitarius

The NTS is a site of termination of visceral afferent fibers of the Vth, VIIth, IXth, and Xth cranial nerves (Torvik, 1956). Although the NTS extends throughout most of the length of the medulla, which is about 3 mm in the rat, it is relatively compact and well delineated against neighboring medullary structures. Within this nucleus there is anatomical and functional segregation. It has been subdivided and the subdivisions named nearly as many times as it has been studied by neuroanatomists. Consequently, there are several different and often conflicting classification and nomenclature systems for the subdivisions of the NTS. The reasons for the inconsistent nomenclature and subdivisions have been discussed in detail (Kalia and Sullivan, 1982);

the foremost is that in most species, cytoarchitectonic boundaries within the NTS are not distinct in Nissl stained material.

The NTS is the termination of visceral afferent fibers of the trigeminal, facial, glossopharyngeal, and vagus nerves. It is concerned mainly with the transmission of visceral afferent information from the lungs, heart, mouth, pharynx, esophagus, and upper part of the digestive system with its associated glands. The rostral portion of the NTS receives fibers primarily from the VIIth and IXth cranial nerves, and it mediates autonomic responses to taste sensations (Torvik, 1956). The caudal and intermediate portions of the NTS receive projections of the IXth and Xth cranial nerves, including the ADN and carotid sinus nerve (Wallach and Loewy, 1980, Czachurski et al., 1982). The intermediate region of the NTS is critical for control of the blood circulation; it integrates a number of important cardiovascular reflexes, including the baroreflexes (Miura and Reis, 1969). Within this region afferent information from peripheral receptors and descending information from supramedullary centers involved in cardiovascular regulation are integrated. The resultant activity of the secondary neurons of the baroreflex arc is then transmitted to preganglionic vagal cardiomotor neurons in the DMV and NA (Loewy and McKeller, 1980), and to centers giving rise to descending fiber systems innervating the preganglionic sympathetic neurons of the spinal cord (Ross et al., 1984a). Functionally, therefore, the intermediate region of the NTS can be designated as the cardiovascular NTS. The use of tracer technology has

now demonstrated that in addition to its visceral afferent input, the NTS is innervated by a number of areas of the brain, from cerebral cortex down through brain stem, and by the spinal cord (Sawchenko and Swanson, 1982, Ross et al., 1981 and Kostreva, 1984). Projections reach the NTS from subcortical nuclei such as the amygdala and bed nucleus of the stria terminalis in the limbic system. Specific projections arise from the hypothalamus, particularly from the paraventricular nucleus, the zona incerta, and the lateral hypothalamic area. The parabrachial nucleus of the pons, an important integration area for a variety of visceral functions, also projects to the NTS. Other inputs to the NTS are from the fastigial nucleus of the cerebellum and from neurochemically specific systems of the brain stem, particularly those synthesizing and releasing catecholamines. For a review of these connections see Brody (1986).

Outputs of the NTS have also been traced (Ricardo and Koh, 1978, Loewy and McKeller, 1980 and Sawchenko, 1983). In general, regions projecting to the NTS receive reciprocal projections, which are highly collateralized, from the NTS. Of particular interest with respect to the physiology of the baroreflex response is a recently discovered projection from this nucleus to the VLM (Dampney et al., 1982, and Ruggiero et al., 1982). In summary, the recent neuroanatomic findings described above have established the probability that information from baroreceptors is relayed via projections from the NTS to widespread areas of the nervous system, that the projection pathways have a

particular topography and are not widely generalized, and that the terminal fields are often areas of the brain with autonomic and behavioral functions other than simply cardiovascular regulation. Examples of such multipotent centers that receive input from the NTS include paraventricular nucleus, which controls the release of vasopressin, oxytocin, ACTH and endorphins (Swanson and Sawchenko, 1983), and regions of the lateral hypothalamus that have a critical role in regulating emotional behavior (Smint and DeVito, 1984).

The NTS is known to contain significant quantities of the peptides substance P and enkephalins, both in fibers and neuronal cell bodies (Maley and Elde, 1982). Both of these putative neurotransmitters are differentially localized to specific subdivisions of the NTS in the rat (Kalia et al., 1984). The medial and commissural subdivisions of the NTS show immunoreactivity to both substance P and enkephalin, which are localized within synaptic terminals, unmyelinated axons, and neuronal cell bodies (Maley, 1985).

The transmitter of the primary baroreceptor afferent input is uncertain, but recent research points to the excitatory amino acid L-glutamate as probably being responsible for the baroreceptor response. L-glutamate injected directly into the NTS results in a dose dependent bradycardia, and lowering of blood pressure (Reis et al., 1984). This response pattern is identical to the effects of stimulation of baroreceptor afferents (Reis et al., 1980). Other evidence supporting a role for L-glutamate includes the facts that the NTS contains large

amounts of L-glutamate, and numerous receptors for L-glutamate are present in the NTS (Reis et al., 1981). Microinjection of antagonists of L-glutamate into the NTS blocks baroreceptor responses and produces hypertension (Reis et al., 1981).

On the other hand, there is histochemical and immunochemical evidence that substance P also is contained in baroreceptor afferent fibers that innervate the NTS (Helke et al., 1980, and Gillis et al., 1980). The possibility that substance P plays a role is supported by observations that substance P applied to the NTS at the level of the obex elicits hypotension and bradycardia in both rats and cats (Haeusler and Osterwalder, 1980). However other peptides also have been shown to elicit hypotension when injected into the NTS.

Opioids may also have a role in the baroreflex. High concentrations of the neuroactive opioids, leu-enkephalin and meth-enkephalin-Arg-Gly-Leu have been found in the NTS and DMV, and, as a group, the nuclei of the reticular formation exhibit moderate levels of both opioid peptides (Zamir et al., 1985). Injections of enkephalins or their stable analogs into the NTS of normotensive rats caused dose dependent increases in blood pressure, and increases are maintained over long periods of time (Maley, 1985).

Ventrolateral Medulla

It has only been in recent years that neurons in the VLM have become an important target of inquiry for researchers studying the role of the

brain in controlling the circulation. Increasing evidence reveals that the region is physiologically complex, and is subdivided into functionally distinct rostral and caudal portions. It is important, before proceeding with an analysis of the baroreceptor reflex pathway, to consider some of the newer information.

The demonstration, first made in the mid-nineteenth century, that transection of the brain stem above the pons failed to alter resting levels of blood pressure, while transection of the brain stem at its junction with the spinal cord resulted in a fall of blood pressure to its lowest levels, has been confirmed repeatedly over the past century (Alexander, 1946 and Bard, 1960). The finding provides evidence that there are neurons in the medulla that act tonically to maintain blood pressure within the normal range, and hence function as tonic vasomotor neurons. The hypothesis that a nucleus of neurons participates in tonic control of blood pressure led to a search for the tonic vasomotor center.

However, failure over the years to identify this vasomotor center by production of discrete lesions raised the prospect that such neurons were distributed diffusely, and not centralized or restricted to a single group. Therefore the concept that a single area is responsible for tonic vasomotor control was often strongly challenged. Finally, within the past few years, an area of the brain has been shown to be critical in subserving vasomotor function. This center has been identified within the ventrolateral portions of the medulla oblongata.

The region that appears to be of particular importance in establishing normal control of blood pressure, as well as in relaying and integrating the baroreflex projections from the NTS, lies within the VLM (Dampney, 1981, Dampney et al., 1982, Caverson et al., and Reis et al., 1984). Anatomical studies of the VLM have revealed the presence of as many as seven morphological cell types (Andrezik et al., 1981). Furthermore, many known and putative neurotransmitters have been found in cells in the general vicinity. These include epinephrine (presumed, due to the presence of the synthesizing enzyme phenylethanolamine-N-methyltransferase [PNMT], serotonin, substance P, (Ruggiero et al., 1984 and Ruggiero et al., 1985) and neuropeptide Y (Harfstrand et al., 1984, Hokfelt et al., 1974, 1978, 1983 and Steinbusch et al., 1981). In rats, neurons containing some of these substances have been demonstrated to project to the spinal cord, specifically, to the IML and central autonomic area where preganglionic sympathetic vasomotor neurons lie (Bowker et al., 1981, and Ross et al., 1984a). Thus, it is possible that the cardiovascular cell group of the ventrolateral medulla comprises a variety of cell types, with cells producing different transmitters subserving different functions.

Functionally, anatomically and neurochemically, the VLM can be subdivided into a rostral portion, the rostral VLM and a caudal portion, the caudal VLM. The rostral VLM appears to function as the tonic vasomotor center. Inactivation of neurons in this region by cooling, electrolytic lesions, use of the neurotoxin tetrodotoxin or local

application of barbiturate drugs reduces blood pressure, sometimes to a level comparable with that produced by transection of the spinal cord (Ross et al., 1983, and Granata et al., 1983). Moreover, topical application of the antihypertensive agent, clonidine, to this region has a similar hypotensive or depressor effect (Bousquet and Schwartz, 1975). Destruction of the region also prevents the fall in blood pressure that is normally elicited by stimulation of arterial baroreceptors (Granata et al., 1983). In contrast, electrical or chemical stimulation of the area elevates blood pressure (Blessing et al., 1982). Anatomical techniques have been used to demonstrate that neurons in the rostral ventrolateral medulla project to the region of the preganglionic sympathetic neurons in thoracic segments of the spinal cord (Blessing et al., 1981). This pathway could be the anatomic substrate for medullary regulation of preganglionic vasomotor neurons via direct action on local neurons of the sympathetic nervous system.

In contrast, the actions of the caudal VLM are almost opposite to those of the rostral VLM. Bilateral electrolytic lesions, injection of neurotoxins, or application of inhibitory amino acids to this region result in elevation of blood pressure (Blessing et al., 1981). Conversely, electrical or chemical stimulation of the caudal VLM leads to lowering of blood pressure (Blessing and Reis, 1982). It has now been established that neurons in the caudal VLM do not project to the spinal cord, but rather appear to have heavy projections rostrally,

particularly to the paraventricular nuclei of the hypothalamus (Swanson and Sawchenko, 1983).

C1 region

The biochemical characters of the neurons within the rostral VLM and caudal VLM that exert control over circulation have been revealed in the past few years by application of immunocytochemical techniques combined with physiological and biochemical assays. The ventrolateral medulla has long been known to contain a sheet of cells that contain catecholamine neurotransmitters; this group runs from the rostral to the caudal portions of the medulla (Hokfelt et al., 1974). Recent studies have clearly demonstrated that functions attributed to the rostral VLM can be assigned to an action of the C1 group of epinephrine containing neurons (Ross et al., 1984b), and those associated with the caudal VLM appear attributable to actions of the A1 group of noradrenergic neurons (Blessing et al., 1981 and Blessing et al., 1982). These findings have lead to the hypothesis that: 1) the tonic vasomotor center of the brain is contained in the group of epinephrine neurons in the rostral VLM and 2) cells in the caudal ventrolateral medulla form a tonic vasodepressor center of the brain stem, acting partly via inhibition of the sympathetic nervous system. Immunocytochemical, anatomical, and physiological techniques have recently been used to demonstrate that the region within the rostral VLM controlling arterial pressure corresponds almost precisely to the distribution of neurons containing epinephrine

synthesizing enzymes (Reis et al., 1984, Ross et al., 1983, and Ross et al., 1984). These neurons are part of the C1 group, as described by Hokfelt et al. (1974), and can be precisely defined only immunocytochemically, with antibodies to the epinephrine synthesizing enzyme, PNMT. The epinephrine producing neurons of the C1 group project to the spinal cord, where they selectively terminate among autonomic neurons of the IML and intermediomedial cell columns (Ross et al., 1983 and Ross et al., 1984a). Therefore, the cardiovascular responsive area of the rostral VLM is now believed to be the C1 area, which is furthermore considered to be the source of tonic background drive to the preganglionic sympathetic neurons. This tonic stimulation is believed to be responsible for maintaining resting levels of arterial pressure (Granata et al., 1985).

Not all of the C1 adrenergic group is contained in the rostral ventrolateral medulla. Portions continue as a caudal cell column where they are mixed with noradrenergic neurons of the A1 group (Granata et al., 1985). The caudal cells, however, probably project rostrally, in part to the hypothalamus with the A1 norepinephrine group, and not to the spinal cord (Ross et al., 1984b).

It is not clear whether C1 adrenergic neurons are responsible for sympathetic functions other than cardiovascular tone, or whether their cardiovascular effects are mediated by the release of epinephrine onto preganglionic sympathetic neurons in the IML. Evidence from various pharmacological studies and physiological studies is often

contradictory. For example, microiontophoresis of epinephrine into the IML reduces, rather than enhances, the discharge of preganglionic sympathetics (Guyenet and Stornetta, 1982) as does administration of either adrenergic agonists or adrenergic antagonists (Sangdee and Franz, 1983). Such studies do not rule out the possibility that epinephrine, when released by discharge of C1 cells, may be excitatory. Epinephrine released in the spinal cord by natural stimulation might reach receptors at different sites from those activated by exogenously administered transmitter. Another possibility is that the physiologically active substance released from the C1 neurons is not epinephrine. Neuropeptide Y, for example has been demonstrated to coexist in adrenergic neurons of the ventrolateral medulla (Hokfelt et al., 1983), and could therefore be the substance released by the terminals of the C1 cells in the IML.

Opioid peptides such as leucine-enkephalin and methionine-enkephalin have been reported to briefly reduce systemic blood pressure after either intravenous administration or injection into the central nervous system (Moore and Dowling, 1980). Opioid receptors are distributed in many areas of the brain, including regions in the vagal system and NTS (Atweh and Kuhar, 1977), that participate in the baroreflex. Opioid receptors and also adrenoceptors are located within the brain stem circuitry that regulates blood pressure (Hokfelt et al., 1974). Similarities in cardiovascular actions of centrally acting alpha-adrenergic and opiate receptor stimulating drugs suggest that similar control pathways could be involved in these actions (Sander et

al., 1982). Stimulation of alpha-adrenoceptors in the brain stem produces both hypotension and bradycardia. These effects are mediated by central presynaptic alpha2-adrenoceptors (Hausler, 1974). The heart rate response to opioid peptides is not correlated with changes in blood pressure (Koyama et al., 1984), which suggests that the interaction between alpha2-adrenoceptors and opiate receptors does not occur in the heart rate modulating pathways. In other studies, bradycardia due to opioid peptides microinjected into the NA, was shown to result from the facilitation of vagal efferent pathways (Laubie et al., 1979). On the other hand the hypotensive effect of opioid injections into the NA resulted from inhibition of sympathetic efferent pathways (Bolme et al., 1978).

A1 area

Electrical stimulation of the caudal ventrolateral medulla inhibits vasomotor activity (Blessing and Reis, 1982). Neurogenic hypertension results when neural activity in this caudal ventrolateral depressor area is abolished by electrolytic lesions or inhibited by stimulation of GABA receptors (Blessing et al., 1981, and Willette et al., 1983). The pathway involved arises from noradrenergic sympathetic neurons of the A1 group. Coote et al. (1981), claim that this pathway is involved in baroreceptor mediated inhibition of sympathetic activity at the spinal level, but the exact pathway through which the cells in the caudal ventrolateral medulla alter arterial pressure is not clear. Axons from

neurons in the A1 area ascend within the ventrolateral medulla, innervating neurons in the C1 area (Willette et al., 1984). This short pathway to the C1 area may be responsible for the inhibitory cardiovascular effects elicited from the A1 area. Direct noradrenergic projections to the IML are rare (Blessing et al., 1981), however the cardiovascular effects elicited from the caudal ventrolateral medulla may be mediated by inhibitory non-noradrenergic neurons that do project to the IML (Blessing et al., 1981).

A1 neurons have been shown to ascend to the hypothalamus, where they probably innervate the paraventricular and supraoptic nuclei (Swanson and Sawchenko, 1983) and participate in baroreflex regulation of the release of vasopressin (Blessing et al., 1982).

Spinal Cord

Both sympathoexcitatory and sympathoinhibitory pathways descend from brain stem areas and project directly or indirectly to the cells of the IML, intracalated nucleus, and intermediomedial nucleus (Loewy and Neil, 1981). These pathways have been demonstrated by anterograde and retrograde tracing techniques.

Information from the central nervous system is transmitted to the cardiovascular system via sympathetic nerves. Cell bodies of preganglionic sympathetic neurons are located predominantly in the thoracolumbar level of the spinal cord in the IML. Some preganglionic neurons may extend from this area into the adjacent white matter of the

cord and into the intercalated area (Chung et al., 1975, and Wurster, 1977). Axons of preganglionic neurons are myelinated, and they leave the spinal cord via the ventral root of the segment in which their cell bodies of origin lie. The preganglionic supply to the heart originates from the first through fifth thoracic segments, with the greatest density of neurons in the IML being in segments T1-2 (Henry and Calaresu, 1972). After leaving the spinal cord the preganglionic fibers separate from the ventral root to form the white ramus to the sympathetic trunk, where they synapse on cells located in ganglia of the sympathetic trunk and peripheral ganglia. The postganglionic neurons give rise to unmyelinated axons that project to various neuroeffector organs, including the heart and blood vessels. A more detailed discussion of this anatomical arrangement, with particular reference to cardiac control, is given in a review by Wurster (1977).

MATERIALS AND METHODS

Experiment 1: Terminations of the Aortic Depressor Nerve

Seventeen male Wistar rats 300-350 gm in body weight were used in this experiment. They were housed 2 per cage, permitted free access to food and water, and remained on a 12 hr light-dark cycle for at least 1 week before experimentation.

The animals were anesthetized by means of intramuscular injections of Ketamine (Ketaset, Bristol Laboratories), 50 mg/kg. The left aortic depressor nerve and the left vagus nerve were dissected in the neck at the level of the superior cervical ganglion, and the ADN was transected. Care was taken to avoid applying tension to the nerve. To verify the identification of the ADN, the proximal stump of the ADN was electrically stimulated using a bipolar silver electrode, with pulses that were from 1-12 volts, 2-12 milliseconds in duration and lasting for 0.1 to 1.0 seconds while blood pressure and heart rate was monitored. When the proximal stump was stimulated a decrease in blood pressure and heart rate was observed, indicating that the ADN had been properly dissected and identified. Only animals that showed this response were used.

In 10 of the animals the proximal stump of the ADN was placed in a capsule containing 20% HRP in 2% dimethylsulfoxide, in distilled water, sealed with silicon grease and sutured to the surrounding tissues. In 5 animals the proximal stump of the ADN was isolated from surrounding

tissue by placing it on a piece of parafilm, and then exposing it to HRP for 20 minutes, after which it was carefully rinsed to remove all traces of HRP from the external sheath. The proximal stump was then placed into a capsule made of polyethylene tubing, which was sealed on both ends with silicon grease and sutured to surrounding tissues. In two control cases the same procedures were followed except that the vagus was cut proximal to the nodose ganglion before placement of the ADN into the capsule or onto parafilm.

The wound was closed in layers, and the animal was allowed to recover from anesthesia. After 36-48 hours the animal was again anesthetized and perfused under pressure transcardially with 100 ml of normal saline. This was followed by 500 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3, at room temperature, followed by 500 ml of 5% sucrose in the same buffer at 40 C. The brain and cervical spinal cord were removed and stored in 20% sucrose in buffer at 40 C for 24 hours. Brains were then blocked in the Horsley-Clarke plane with the aid of the device described by Papaioannau (1970). Transverse serial 40 um thick frozen sections of the brain stem and nodose ganglion were cut in the coronal plane on an American Optical sledge microtome with a Blue M freezing stage. The tissue was treated with tetramethyl benzidine (TMB) as the chromogen for the demonstration of HRP reaction product (Mesulam and Rosene, 1978).

All sections were mounted on gelatin coated slides and initially examined under darkfield illumination for HRP reaction product. Every

other section was counterstained with neutral red. A drawing tube was used to facilitate marking of labeled terminals and retrogradely labeled cell bodies on outlines of the brain stem. Cell bodies were counted in sections that were spaced 100 microns apart.

Results

No noticeable differences of labeling in cell bodies or terminals were observed between the two methods used to expose the ADN to HRP. Isolating the ADN on parafilm and exposing it to HRP for 20 minutes resulted in the same amount of labeling as did sealing the ADN in a capsule containing HRP. Moreover, the pattern of distribution of HRP was the same in both groups. HRP-labeled cell bodies of the primary afferent neurons giving rise to the ADN were identified in the nodose ganglion. They were localized in the margins of the ganglion.

Labeled axons enter the ipsilateral medulla by multiple rootlets approximately 1.5-2.0 mm rostral to the obex. The labeled fibers pass through the ventral part of the spinal tegmental nucleus and tract, and then curve dorsomedially to join the solitary tract (Fig. 1). At this level some labeled axons terminate within the dorsolateral and medial aspects of the solitary complex (Figs. 2 and 3). The most dense terminal field labeling is ipsilateral near the level of the obex in and adjacent to the interstitial nucleus and in the dorsolateral aspects of the solitary complex just dorsal the solitary tract. There is no evidence of contralateral labeling.

HRP reaction product is also present in the dorsal motor nucleus of the vagus, primarily in the nucleus ambiguus (Fig. 2). Approximately 10% of the motor neurons in the rostral pole of that nucleus are labeled. Labeled motor neurons are seen in the dorsolateral margin of DMV and in the rostral pole of the NA from 1.5 mm to 2.0 mm rostral to the obex.

In the two control animals no labeling was detected in either the terminal field in the NTS or in motor neurons of the DMV and NA. The nodose ganglion, however, in the control animals showed heavy neuronal and fiber labeling. HRP-labeled cells filled the entire ganglion and were not confined to the periphery as in the experimental animals.

Figure 1. Drawings of typical transverse sections of the dorsal medulla at 0.5 mm intervals from 2.0 mm rostral to the obex (top) to 1.0 mm caudal to the obex (bottom). The distribution of HRP reaction product after application of HRP to the cut end of the left ADN is shown. Labeled fibers are indicated by stippling, and the location of HRP-filled cell bodies is indicated by filled circles. AP, area postrema; CC, central canal; CU, cuneate nucleus; GR, nucleus gracilis; IX, dorsal motor nucleus of the vagus

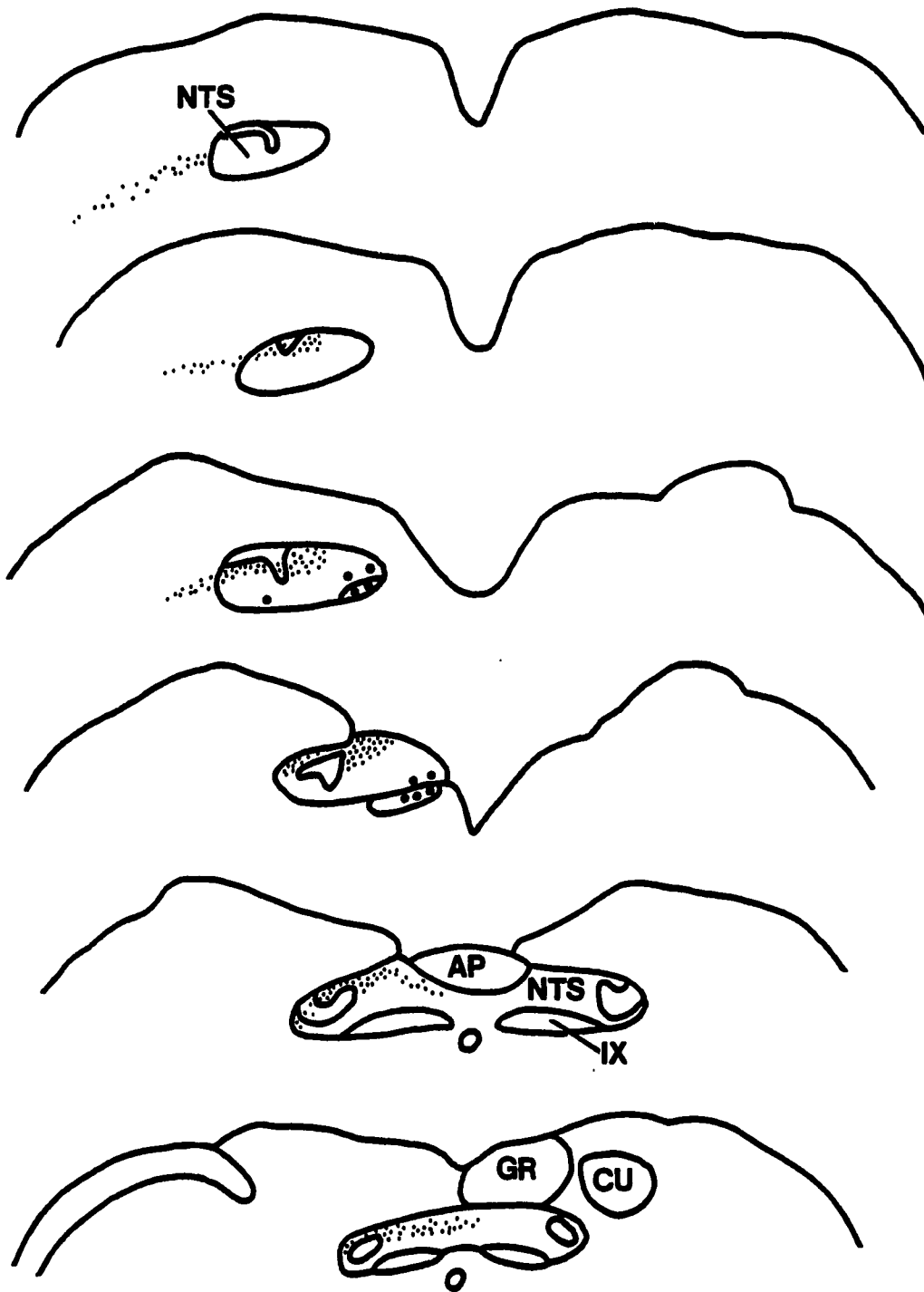
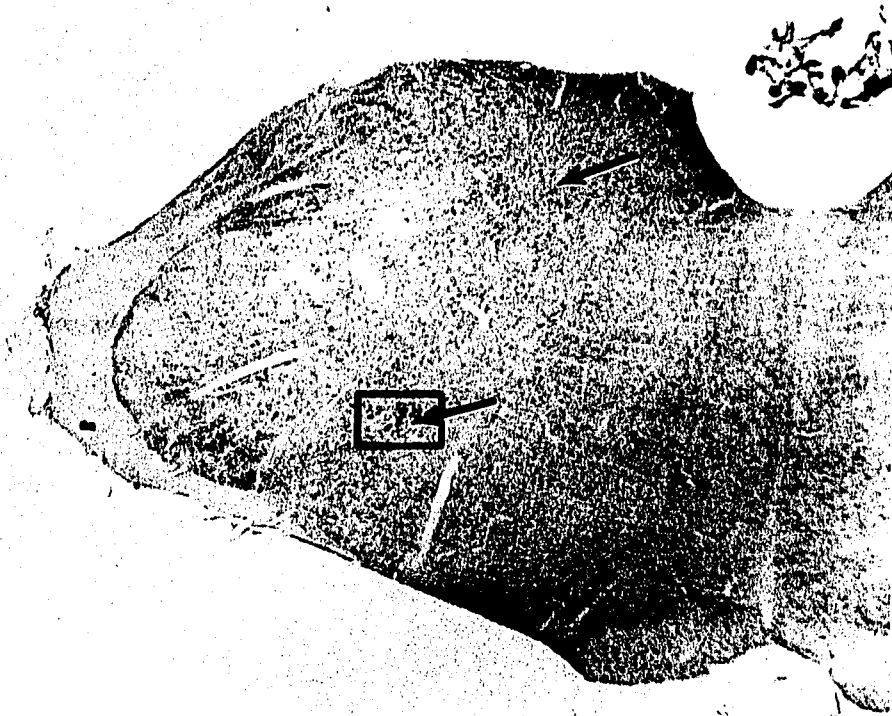


Figure 2a. Brightfield micrograph of the brain stem 2.0 mm rostral to the level of the obex. These sections have been counterstained with neutral red. Low power (25 X) showing region of labeled cell bodies and terminals after exposing the ADN to HRP (arrows)

Figure 2b. High magnification (100 X) brightfield micrograph of labeled cell bodies in the nucleus ambiguus. This pattern of labeling of cell bodies in the rostral NA was typical of the pattern seen in the experimental group

a



b

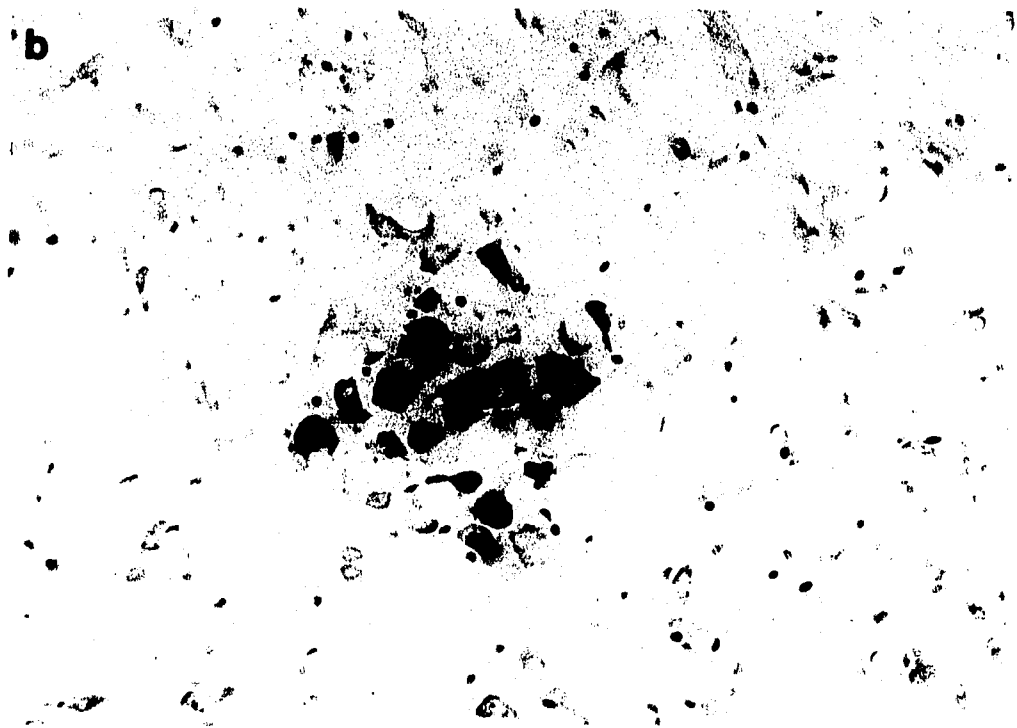
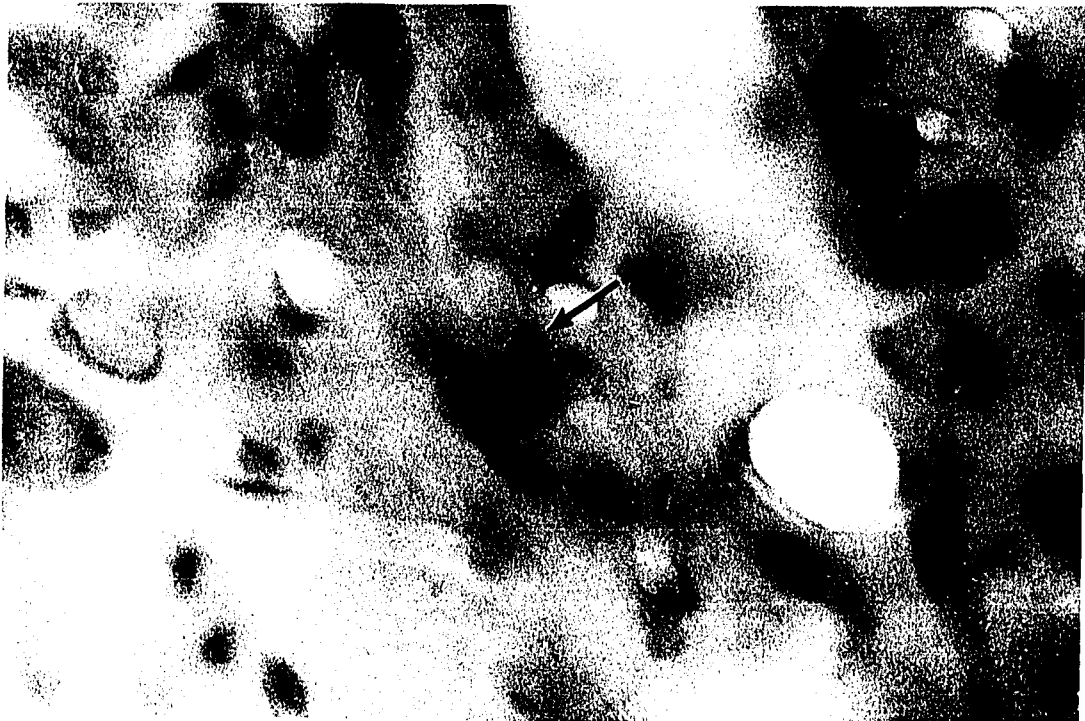


Figure 3. Brightfield micrographs of terminals filled with HRP (arrows) by transganglionic transport from the ADN. These terminals lie in close proximity with neuronal cell bodies in the NTS 1.0 mm rostral to the obex. These sections have been counterstained with neutral red (260 X)



Experiment 2: Projections of the NTS to Motor Nuclei of the Vagus

Experiments were performed on 5 male Wistar and 10 male Sprague Dawley rats 275-395 gm in body weight. All animals were housed 2 per cage, permitted free access to food and water, and remained on a 12 hr light-dark cycle for at least 1 week before experimentation.

Retrograde axonal transport of the fluorescent tracers, Fluoro-gold (Fluorochrome Inc) and True Blue (Sigma Chemical Co.), was employed to label the motor nuclei that give rise to the vagus nerve. Rats were anesthetized by means of intramuscular injections of Ketamine (Ketaset, Bristol Laboratories), 50 mg/kg, followed by Sodium Pentobarbital (Nembutal, Abbott Laboratories), 25 mg/kg, injected intraperitoneally. The left vagus nerve was dissected from the carotid sheath at the level of the thoracic inlet, and transected. The proximal stump of the vagus was isolated from surrounding tissue by placing it on a piece of parafilm, and 0.5 ul of 1% Fluoro-Gold, or 0.5 ul of 1% True Blue was then injected into the vagal sheath by means of a Hamilton microsyringe. After the injection was completed the nerve was carefully rinsed to remove all traces of Fluoro-Gold from its external sheath.

Anterograde transport of HRP was used to label projections from the NTS to the motor nuclei of the vagus. Immediately following the above procedure, rats were placed in a stereotaxic apparatus (David Kopf Inc.), and a midline incision was made extending from the level of spinal vertebra C4 to 1 cm rostral to the occipito-parietal suture. The

musculature was dissected and the occipital bone and tela were removed to expose the floor of the fourth ventricle. HRP (20-40 nl of a 2% HRP solution containing 2% dimethylsulfoxide in distilled water) was injected, by means of a Hamilton microsyringe fitted with a glass micropipette with a tip diameter of 0.5 μ m, into the NTS. The injection was placed just lateral to the border of the area postrema, 1 mm rostral to the level of the obex, to a depth of 0.4-0.6 mm below the surface of the medulla.

Incisions were closed in layers, and the animals were allowed to recover from anesthesia. After 24-48 hours the animals were again anesthetized and perfused transcardially with 100 ml of normal saline. This was followed by transcardial perfusion of 4% formaldehyde buffered with 0.1M phosphate buffer, at pH 7.3. The brains were then removed and blocked in the Horsley-Clarke plane with the aid of the device described by Papaioannau (1970). Transverse serial sections, 50 μ m in thickness, were cut on a vibratome. The tissue was treated with tetramethyl benzidine (TMB) as the chromogen for the demonstration of HRP reaction product (Mesulam and Rosene, 1978).

All sections were mounted on gelatin coated slides and initially examined under darkfield illumination for HRP reaction product. Every other section was counterstained with neutral red for identification of the HRP-labeled cell groups.

Slides were examined with a Leitz epifluorescence microscope. Filters that transmit light of 360 nm wavelength (filter cube A) were

used to examine the True Blue or Fluoro-Gold containing cells. Labeled terminals and retrogradely labeled cell bodies were traced on outlines of the brain stem by means of a drawing tube attached to the microscope. Cells were counted and measured using an image analysis program (R&M Biometrics, Nashville, Tn.) on sections that were spaced 100 microns apart.

Results

Examination of the medullary HRP injection sites in all animals confirmed that they were centered in the rostral one third of the NTS. Several regions surrounding the NTS, including the hypoglossal nucleus, dorsal reticular formation, DMV, caudal portions of the medial and lateral vestibular nuclei, and the dorsal column nuclei also react positively for HRP indicating that there was spread of the injection. However, these areas contain labeled fibers and terminals and few cell bodies (Figs. 4b and 5a).

Both retrograde Fluoro-gold labeling of neuronal cell bodies from the vagus nerve and anterograde HRP-labeling of terminals from injections in the NTS are present in the NA and RVL just ventral to the NA. Fluoro-Gold labeled cells in the vagal complex are all ipsilateral to the injection, and they are distributed throughout the NA from the level of the obex to 2.0 mm rostral to the obex. They are brightly fluorescent. The heaviest concentration of labeled cells is 1.0 to 1.5 mm rostral to the obex. The cells in this region range in diameter from 40 to 60 μm . Approximately 40% of the Fluoro-Gold labeled cells in the

NA and VLM just ventral to the NA appear to be associated with HRP-labeled terminals arising from the region of the NTS (Figs. 5a and 5b). Ninety percent of the HRP-labeled terminals in this region appear to be associated with vagal motor neurons.

The ipsilateral DMV was well delineated by Fluoro-gold labeling throughout its entire extent. Terminals labeled with HRP were also observed in association with fluorescent cells in the DMV (Fig. 5b). However, the significance of this observation is limited by the fact that the injection site is in such close proximity to the DMV that spread of the injection was observed.

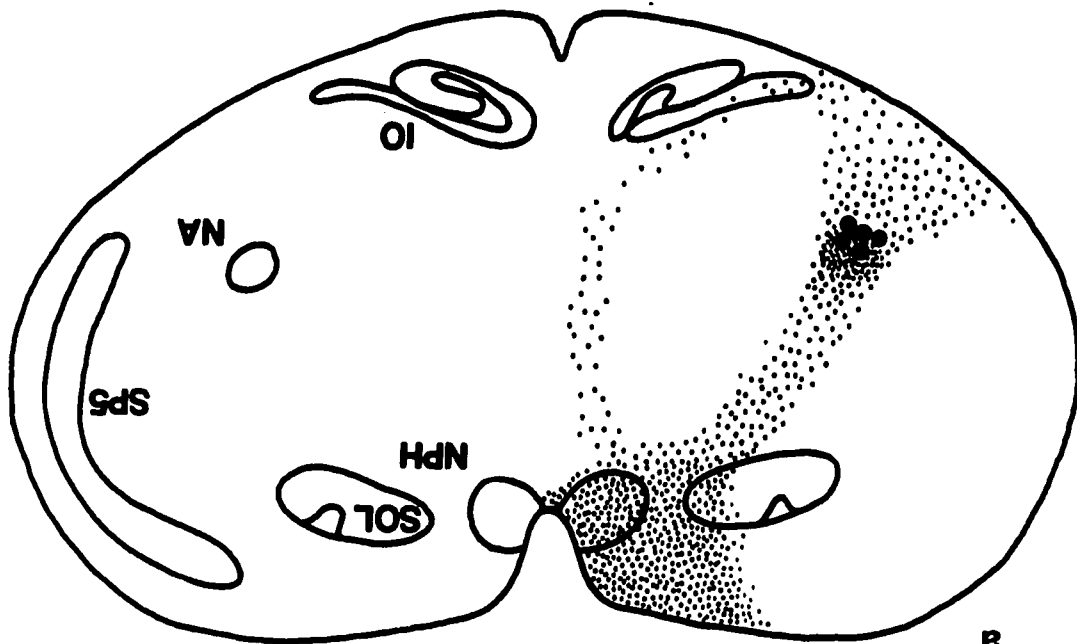
In addition to cells in the dorsal vagal complex, labeled neurons are seen in the cardiovascular portion of the VLM after exposing the cervical vagus nerve to Fluoro-gold. In the NA and adjacent RVL extending from the pyramidal decussation to 2.0 mm rostral to the obex there are numerous large (40-60 μm in diameter) and medium sized (20-30 μm in diameter) labeled neurons (Fig. 5b). Labeled neurons were ipsilaterally distributed. Retrograde transport of Fluoro-gold from the vagus also labeled the DMV group of neurons lying dorsolateral to the floor of the fourth ventricle. There, brightly fluorescent neurons are large, ranging from 40 to 60 μm in diameter.

Figure 4a. Drawing of a typical cross section of the medulla 1.5 mm rostral to the level of the obex showing the distribution of HRP (Stippling), after injecting HRP into the NTS and the distribution of Fluoro-gold after exposing the cervical vagus to the fluorochrome. The injection site was centered in the NTS approximately 0.5 mm rostral to the level of the obex. Filled circles represent cell bodies filled with Fluoro-gold (see results). IO, inferior olive; NA, nucleus ambiguus; NPH, prepositus hypoglossal nucleus; NTS, nucleus of the solitary tract, SP5, spinal tract of the trigeminal nerve

Figure 4b. Low power bright field micrograph (25 X) illustrating a typical injection site in the NTS. This micrograph show a section 0.5 mm caudal to the level shown in Figure 4a. Note spread of injection



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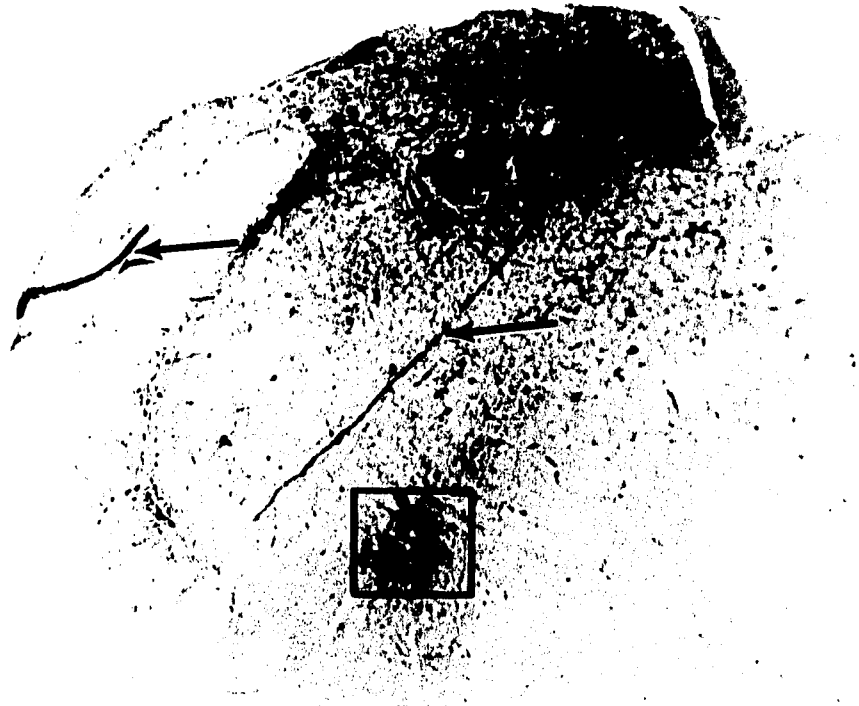


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Figure 5a. Low power bright field micrograph (25 X) 1.0 mm rostral to the injection site. HRP-labeled terminals can be seen surrounding cells in the NA (outlined). Labeled fiber tracts were observed that correspond to the sensory and motor tracts of the DMV (arrows)

Figure 5b. Combination brightfield-fluorescence micrograph of the NA and adjacent RVL showing fluorescent cells labeled by injection of Fluoro-gold into the vagus and HRP-filled terminals from an injection in the NTS. Note the close association of the HRP-filled terminals with the fluorescent cell bodies (arrows) (25 X)

a



Experiment 3: Pathways from the NTS to Sympathetic Centers

Experiments were performed on 10 male Sprague Dawley rats 275-395 gm in body weight. All animals were housed 2 per cage, permitted free access to food and water, and remained on a 12 hr light-dark cycle for at least 1 week before experimentation.

Anterograde and retrograde transport of HRP were used to map projections between the NTS and VLM. Rats were anesthetized by means of intramuscular injections of Ketamine (Ketaset, Bristol Laboratories), 50 mg/kg, followed by Sodium Pentobarbital (Nembutal, Abbott Laboratories), 25 mg/kg, injected intraperitoneally. The rat was placed in a stereotaxic apparatus (David Kopf Inc.) and a midline incision was made extending from the level of spinal vertebra T5 to 1 cm rostral to the occipito-parietal suture. The musculature was dissected and the occipital bone and tela were removed to expose the floor of the fourth ventricle.

HRP (20-40 nl of a 2% HRP with 2% dimethylsulfoxide in distilled water) was injected, by means of a Hamilton microsyringe fitted with a glass micropipette with a tip diameter of 0.5 μ m, into the NTS. The injection was placed just lateral to the border of the area postrema, 1.0 mm rostral to the level of the obex, to a depth 0.4-0.6 mm ventral to the surface of the medulla.

Retrograde axonal transport of the fluorescent tracers, Fluoro-gold (Fluorochrome, Inc.) and True Blue (Sigma Chemical Co.), was employed to

map projections from the VLM to the spinal cord. The dorsal spine and laminae of the spinal vertebrae were removed to expose the spinal cord at the level of the second thoracic vertebra, permitting injections of Fluoro-gold or True Blue into the IML to be made under visual control. In 6 rats a 2% suspension of Fluoro-Gold in distilled water was used. In the remaining 4 rats True Blue (Bentivoglio et al., 1979), was injected into the spinal cord. A 5% solution was used with distilled water being the vehicle. In both cases the volume of the injection was between 50 and 100 nl.

Incisions were closed in layers, and the animals were allowed to recover from anesthesia. After 24-48 hours the animals were again anesthetized and perfused transcardially with 100 ml of normal saline. This was followed by transcardial perfusion of 4% formaldehyde buffered with 0.1M phosphate buffer at pH 7.3. The brains and spinal cords were then removed and brains were blocked in the Horsley-Clarke plane with the aid of the device described by Papaioannau (1970). Transverse serial sections, 50 um in thickness, were cut on a vibratome. The tissue was treated with tetramethyl benzidine (TMB) as the chromogen for the demonstration of HRP reaction product (Mesulam and Rosene, 1978).

All sections were mounted on gelatin coated slides and initially examined under darkfield illumination for HRP reaction product. Alternate sections were counterstained with neutral red for the identification of HRP-labeled nuclear groups.

Slides were then examined with a Leitz epifluorescence microscope. Filters that transmit light of 360 nm wavelength (filter cube A) were used to examine the True Blue or Fluoro-Gold containing cells. Labeled terminal fields and retrogradely labeled neurons were drawn on outlines of the brain stem by means of a drawing tube attached to the microscope.

Results

Microscopic examination of the injection site in the spinal cord indicated that the injection was centered in the IML in all cases. The labels had diffused from the site, and labeled cell bodies and fiber tracts were seen in the MM, the ipsilateral lateral and dorsal funiculi, and the contralateral IML. Because of technical considerations, low power micrographs are not possible. The injection sites are typical of those seen in HRP injections (Fig. 10)a.

After injection of either retrograde tracer into the spinal cord at the level of the second thoracic segment, labeled cell bodies are present throughout the medulla. Fluoro-gold fills the neuronal cell bodies and their processes and does not appear to leak out and fill adjacent glial cells, which can be identified by their smaller size and lack of specific processes. True Blue fills only the cell bodies and not their processes and does invade surrounding glial cells. Fluoro-gold also appears brighter than True Blue when viewed with a 50 W mercury lamp. Because of these differences this section describes the results of the Fluoro-gold injections.

The cardiovascular centers in the rostral medulla containing neurons labeled by spinal injection of Fluoro-gold are the RVL, including the region containing the C1 adrenergic cell group, the NA, and the dorsal medial medulla around the NTS. The neuronal labeling is predominantly ipsilateral with some bilateral labeling seen. The heaviest labeling is in the RVL and NA 1.0 to 1.5 mm rostral to the obex. The contralateral labeling follows the same pattern (Figs. 7a and 7b).

At caudal medullary levels neurons are labeled throughout the VLM, including the region known to contain the caudal A1 noradrenergic neuron group. Cell bodies are also labeled in the NTS and area postrema. At the level just rostral to the obex labeled neurons are present in the NA. Bilateral labeling does occur, but compared to the rostral VLM fewer neurons are labeled on the contralateral side.

Examination of the medullary injection sites in all animals confirmed that they are centered in the rostral one third of the NTS. Several regions surrounding the NTS, including the hypoglossal nucleus, dorsal reticular formation, DMV, caudal portions of the medial and lateral vestibular nuclei, and the dorsal column nuclei also react positively for HRP (Fig. 6). However, these areas contain labeled fibers and terminals and few cell bodies; this indicates that HRP was not taken up and transported by these neurons.

Injection of HRP into the rostral one-third of the NTS results in retrograde and anterograde transport of the label to the VLM. HRP transported anterogradely from the NTS occupies two very distinct

regions in the VLM. One is a circular area of very dense labeling overlying the region of the NA. The other is a larger and less dense area ventral to the NA (Figs. 4a and 7a). The latter area overlies the region known to contain the C1 group of neurons. In the VLM just ventral to the NA, 0.5 mm to 1.5 mm rostral to the obex, HRP-labeled terminals are seen in apparent juxtaposition with Fluoro-Gold labeled cell bodies. HRP-filled fibers could be seen coursing from the NTS towards this region, however, these fibers could not be seen in their entirety (Fig. 7a).

In addition to labeled fibers, which are seen as bundles or thin lines filled with HRP, and terminals, which are identified in close association with neuronal cell bodies, the VLM contains neuronal cell bodies labeled by HRP from the NTS. Such retrogradely labeled neurons are present in the region thought to contain the C1 adrenergic cell group, the NA, and the RVL just ventral to the NA. Retrogradely labeled neurons are not observed in other cardiovascular regions.

Figure 6. Injection site in the medulla 1.5 mm rostral to the level of the obex. The section shown in this micrograph is typical of those seen in this series of experiments (25 X). Note spread of injection

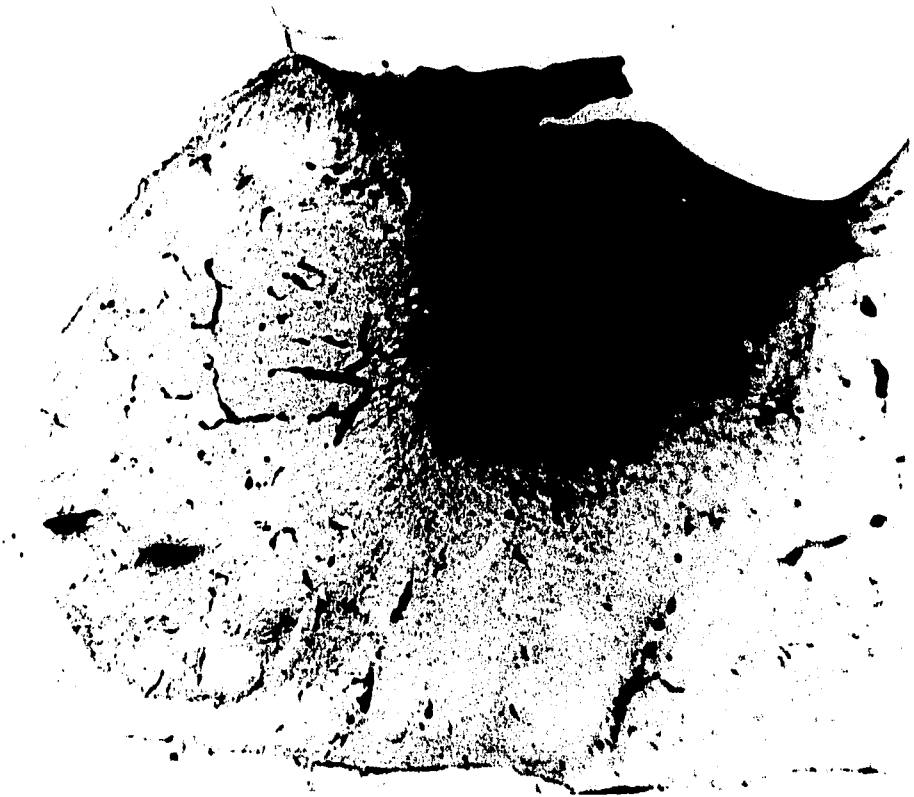


Figure 7a Low power bright field micrograph of the VLM 0.2 mm caudal to the injection site (25 X). Arrow indicates a region just ventral to the NA corresponding to the region shown in figure 7b where, at higher magnification HRP, filled axon swellings could be seen in association with cells that had been retrogradely labeled from injections at the level of the 2nd thoracic vertebra

Figure 7b Combination bright field-fluorescence micrograph of a cell filled with Fluoro-gold in the region indicated by the arrow in figure 7a (400 X). This cell was retrogradely labeled by an injection in the spinal cord at the level of the 2nd thoracic vertebra. A large bouton anterogradely filled with HRP from an injection in the NTS can be seen in close association with this fluorescent cell

a



Experiment 4: C1 Cells in the RVL Project to the Spinal Cord

Experiments were performed on 10 male Sprague Dawley rats 275-395 gm in body weight. All animals were housed 2 per cage, permitted free access to food and water, and remained on a 12 hr light-dark cycle for at least 1 week before experimentation.

Retrograde axonal transport of the fluorescent tracer, Fluoro-gold (Fluorochrome, Inc.) was employed to map projections from the VLM to the IML. Rats were anesthetized by means of intramuscular injections of Ketamine (Ketaset, Bristol Laboratories), 50 mg/kg, followed by Sodium Pentobarbital (Nembutal, Abbott Laboratories), 25 mg/kg, injected intraperitoneally. The dorsal spine and laminae of the spinal vertebrae were removed to expose the spinal cord at the level of the second thoracic vertebra, permitting injections of Fluoro-gold into the IML to be made under visual control. A 2% suspension of Fluoro-Gold in distilled water was used, and the volume of the injection was between 50 and 100 μ l.

After the incision was closed in layers, the animal was allowed to recover from anesthesia. In 24 hours the animals were again anesthetized and perfused transcardially with 100 ml of normal saline followed by transcardial perfusion of 4% formaldehyde buffered with 0.1M phosphate buffer at pH 7.3. The brains and spinal cords were then removed and brains were blocked in the Horsley-Clarke plane with the aid

of the device described by Papaioannau (1970). Transverse serial sections, 75 μ m in thickness, were cut on a vibratome.

Immunocytochemistry for localization of PNMT immunoreactivity was used to identify epinephrinergic cells of the C1 group in the RVL. The brain sections were washed two times in phosphate buffered saline for 5 minutes each, then incubated in goat serum diluted 1/30 in buffered saline for 30 minutes and again given two 5 minute washes in buffered saline. PNMT antibody (Eugene Tech Int.) was reconstituted in phosphate buffered saline at pH 7.4 to a final volume of 3 ml and dilution of 1:3000. The sections were then placed in antibody with 0.1% Triton X-100 added, for 16 hours at room temperature, again washed two times for 5 minutes each in buffered saline containing 1% goat serum, and incubated with goat antirabbit antiperoxidase for 30 minutes. After another wash in buffered saline, the sections were placed in a solution of 3,3'-diaminobenzadine for visualization of the reaction product. For control purposes, alternate sections were treated in the same way except no antibody was used in the staining procedure.

All sections were mounted on gelatin coated slides and examined with a Leitz epifluorescence microscope. Filters that transmit light of 360 nm wavelength (filter cube A) were used to examine the True Blue or Fluoro-Gold containing cells. Sections were subsequently counterstained with neutral red for identification of the Fluoro-gold labeled nuclear groups.

Slides were examined, and labeled terminal fields and retrogradely labeled neurons were drawn on outlines of the brain stem by means of a drawing tube attached to the microscope.

Results

Microscopic examination of the injection site in the spinal cord indicated that the injection was centered in the IML in all cases. Fluoro-Gold had diffused from the site, and labeled cell bodies and fiber tracts were seen in the MM, the ipsilateral lateral and dorsal funiculi, and the contralateral IML. Due to technical limitations a low power micrograph of the injection site can not be photographed. The injection site appears similar to that seen in HRP injections (Fig. 10a). After injection of a retrograde tracer into the spinal cord at the level of the second thoracic segment labeled cell bodies are present throughout the medulla, as described in detail in the results section of experiment 3. Briefly, the cardiovascular centers containing labeled cells are the RVL, including the region known to contain the C1 group of neurons, the NA, and the dorsal medial medulla around the NTS (Fig. 8). At caudal medullary levels neurons were labeled throughout the VLM, including the region known to contain the caudal A1 noradrenergic neuron group. Cell bodies are also labeled in the NTS and area postrema. No cells were labeled in the control sections.

The C1 group of neurons lies in the RVL from a level 0.5 mm rostral to the obex to 2.0 mm rostral to the obex. Results of immunocytochemical localization of PNMT immunoreactivity revealed that

RVL cells retrogradely labeled by Fluoro-gold from the spinal cord do include cells in the C1 group of neurons (Figs. 9a and 9b). In this region approximately 45% of the neurons that showed PNMT immunoreactivity were also labeled with Fluoro-gold.

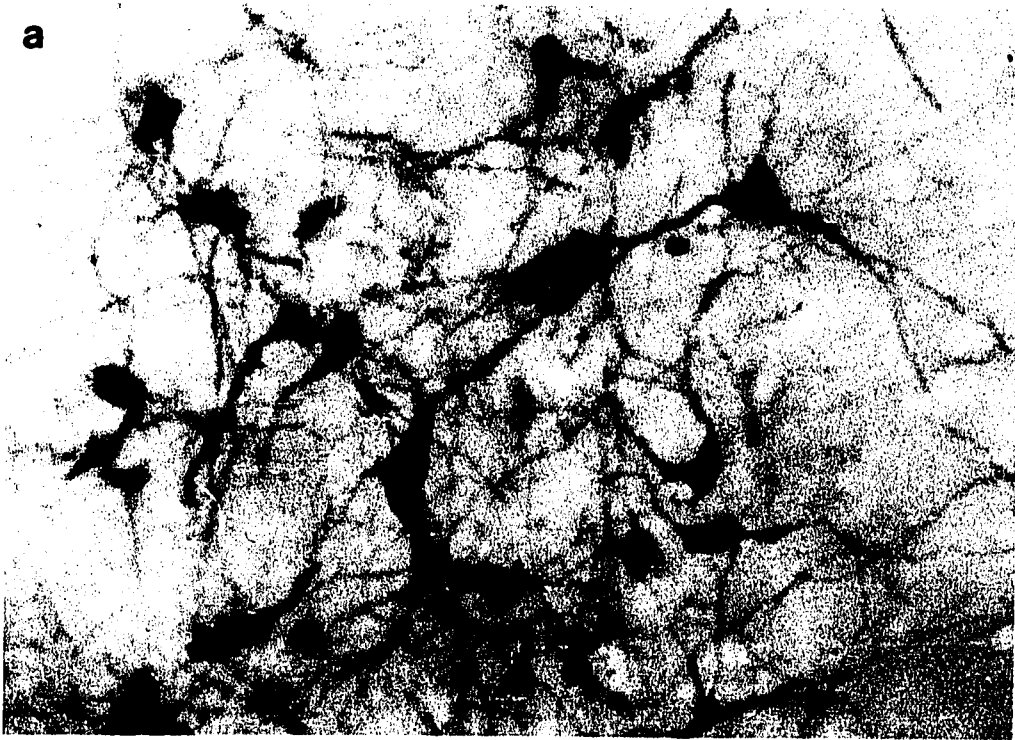
Figure 8. Survey micrograph of the medulla showing the VLM 1.5 mm rostral to the level of the obex (100 X). Arrow indicates cells that are positive for the enzyme PNMT. Many of these cells were also labeled by injections of Fluoro-gold at the level of the 2nd thoracic vertebra



Figure 9a. PNMT positive cells in the C1 area of the RVL. These cells were labeled by retrograde transport of Fluoro-gold injected into the second thoracic segment of the spinal cord. This group of labeled neurons is just ventral to the NA in the periambiguual region of the RVL. Note the high density and compactness of the cell group, and the filling of cell processes (260 X)

Figure 9b. Combination bright field-fluorescence micrograph of PNMT positive cells with nucleoli that are fluorescent due to retrograde transport of Fluoro-gold from the spinal cord (400 X)

a



Experiment 5: Spinal and Vagal Components of the Nucleus Ambiguus

Experiments were performed on 10 male Sprague Dawley rats 275-395 gm in body weight. All animals were housed 2 per cage, permitted free access to food and water, and remained on a 12 hr light-dark cycle for at least 1 week before experimentation.

Retrograde axonal transport of the fluorescent tracer, Fluoro-gold (Fluorochrome Inc) was employed to identify cells that give rise to fibers that travel in the vagus nerve. Rats were anesthetized by means of intramuscular injections of Ketamine (Ketaset, Bristol Laboratories), 50 mg/kg, followed by Sodium Pentobarbital (Nembutal, Abbott Laboratories), 25 mg/kg, injected intraperitoneally. The left vagus nerve was dissected from the carotid sheath at the level of the thoracic inlet and transected. The vagus was isolated from surrounding tissue by placing it on a piece of parafilm, and 0.5 ul of 1% Fluoro-Gold was then injected into the vagal sheath by means of a Hamilton microsyringe. After the injection was completed the nerve was carefully rinsed to remove all traces of Fluoro-Gold from its external sheath.

The retrograde axonal transport of HRP was employed to map projections from the medulla to the spinal cord. Immediately following the above procedure the rats were placed in a stereotaxic apparatus (David Kopf Inc.), and a midline incision was made extending from the fifth thoracic to the second cervical vertebra. The dorsal spine and laminae of the spinal vertebrae were removed to expose the spinal cord

at the level of the second thoracic vertebra; this permitted injections of Fluoro-gold into the IML to be made under visual control. A 4% solution of HRP with 2% dimethylsulfoxide in distilled water was used, and the volume of the injection was between 50 and 100 μ l.

The wound was closed in layers, and the animal was allowed to recover from anesthesia. After 36-48 hours the animal was again anesthetized and perfused transcardially with 100 ml of normal saline. This was followed by 500 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3, at room temperature, followed by 500 ml of 5% sucrose in the same buffer at 40 C. The brain and cervical spinal cord were removed and stored in 20% sucrose in buffer at 40 C for 24 hours. Brains were then blocked in the Horsley-Clarke plane with the aid of the device described by Papaioannau (1970). Transverse serial 40 μ m thick frozen sections of the brain stem and nodose ganglion were cut on an American Optical sledge microtome with a Blue M freezing stage. The tissue was treated with tetramethyl benzidine (TMB) as the chromogen for the demonstration of HRP reaction product (Mesulam and Rosene, 1978).

All sections were mounted on gelatin coated slides and initially examined under darkfield illumination for HRP reaction product. Every other section was counterstained with neutral red for the identification of nuclear cell groups. Cells in the region of interest were counted in sections that were spaced 100 microns apart.

Slides were then examined with a Leitz epifluorescence microscope. Filters that transmit light of 360 nm wavelength (filter cube A) were

used to examine the True Blue or Fluoro-Gold containing cells.

Retrogradely labeled neurons were traced on outlines of the brain stem by means of a drawing tube attached to the microscope.

Results

Microscopic examination of the HRP injection site in the spinal cord indicated that the injection was centered in the IML in all cases. The label had diffused from the site, and labeled cell bodies and fiber tracts were seen in the MM, the ipsilateral lateral and dorsal funiculi, and the contralateral IML.

Cell bodies filled with HRP are present in the NA, the RVL, and throughout the entire extent of the DMV after injection of HRP into the second thoracic segment of the spinal cord (Fig. 11). After application of Fluoro-Gold to the cut end of the left vagus nerve cell bodies throughout the ventrolateral medulla, including the region that contains the C1 group of neurons, are filled with Fluoro-gold, as detailed in the results section of experiment 3. Approximately 40% of all the neurons in the NA and the VLM just ventral to the NA were double labeled with Fluoro-Gold and HRP. The remainder of the neurons in this area are labeled with either HRP, (approximately 10%) or Fluoro-Gold (approximately 50%).

Figure 10a. This low power micrograph is typical of the injection sites seen after HRP or Fluoro-gold injections at the level of the 2nd thoracic vertebra (25 X). The heaviest labeling can be seen in the region of the IML. Anterograde transport has contributed to concentration of HRP in the ventral horn. HRP in the dorsal horn (arrow) is due to leakage as the pipette is withdrawn

Figure 10b. Micrograph showing the pattern of labeling seen in the NA and surrounding RVL (160 X) after injecting HRP into the IML at the level of the 2nd thoracic vertebra. This group of cells also contain fluorescent Fluoro-gold transported retrogradely in the vagus nerve, as can be seen in figure 11

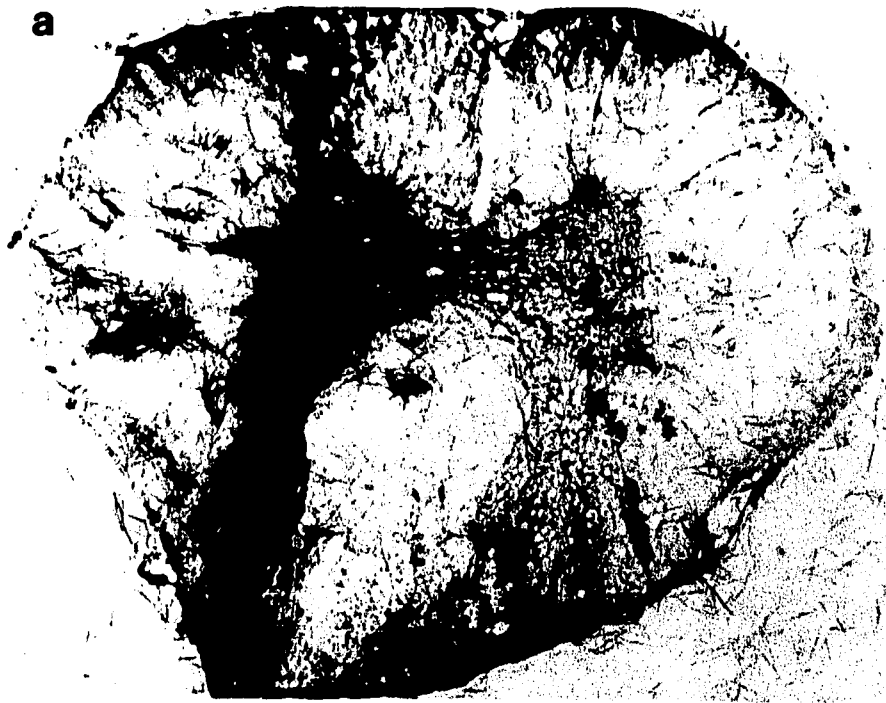
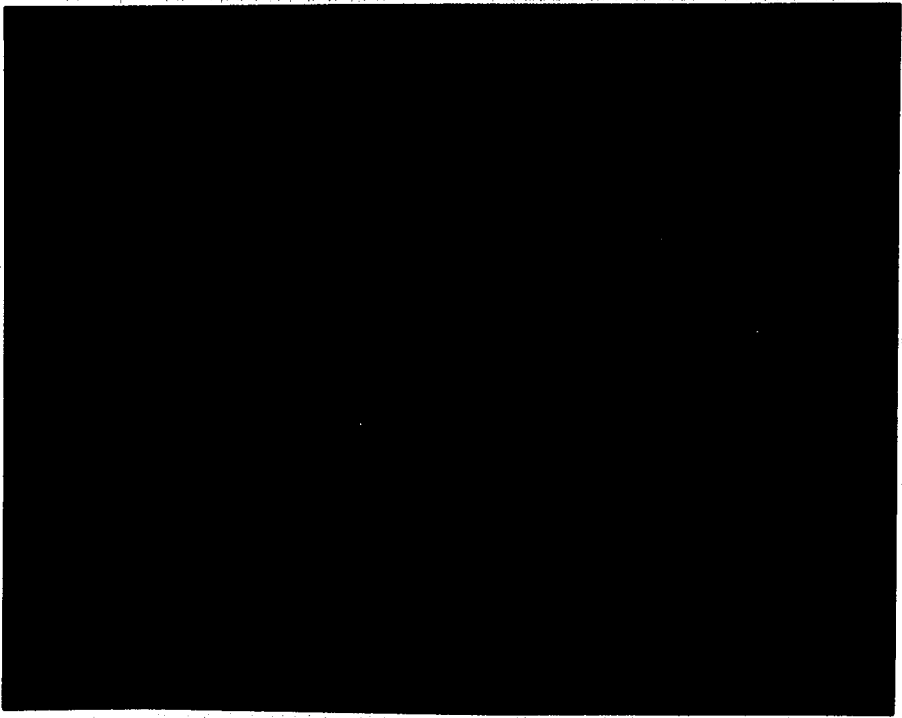
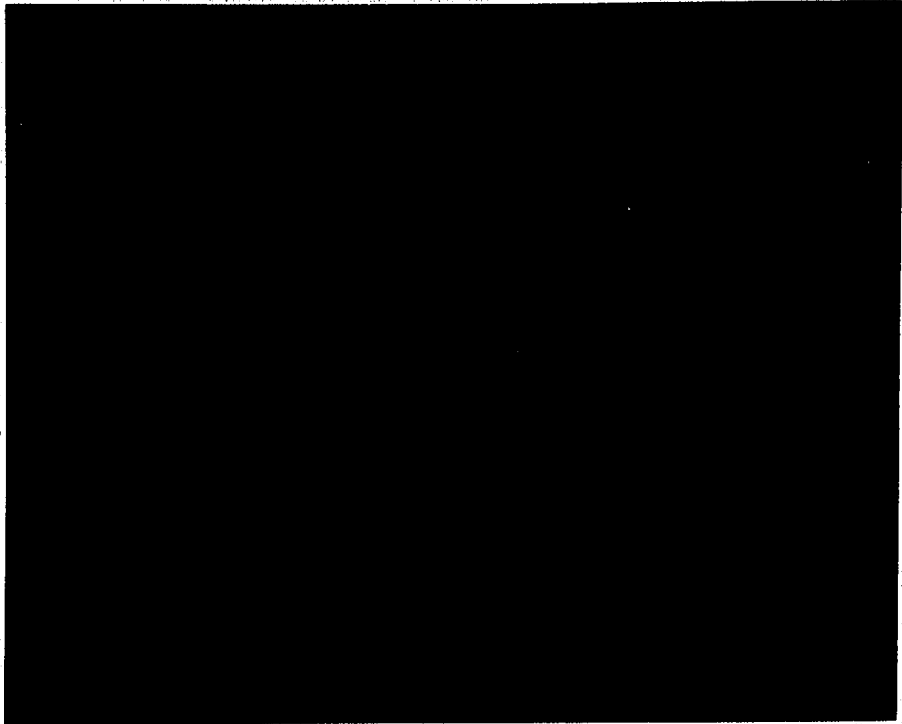


Figure 11. Combination brightfield fluorescence and fluorescence micrographs showing the double labeling of cells shown in figure 10b (400 X). These cells were double labeled by injections of HRP into the spinal cord at the level of the 2nd thoracic vertebra and injection of Fluoro-gold into the vagus nerve at the thoracic inlet. Arrows in the upper micrograph indicate cells that are filled with HRP and are faintly fluorescent. Arrows in the lower micrograph indicate cells that are brightly fluorescent and also contain HRP



Experiment 6: Identification of NTS Terminals on C1 Neurons

Experiments were performed on 10 male Sprague Dawley rats 275-395 gm in body weight. All animals were housed 2 per cage, permitted free access to food and water, and remained on a 12 hr light-dark cycle for at least 1 week before experimentation.

Anterograde and retrograde transport of HRP were used to map projections between the NTS and VLM. Rats were anesthetized by means of intramuscular injections of Ketamine (Ketaset, Bristol Laboratories), 50 mg/kg, followed by Sodium Pentobarbital (Nembutal, Abbott Laboratories), 25 mg/kg, injected intraperitoneally. They were then placed in a stereotaxic apparatus (David Kopf Inc.), and a midline incision was made extending from the level of C4 to 1 cm rostral to the occipito-parietal suture. The musculature was dissected and the occipital bone and tela were removed to expose the floor of the fourth ventricle. HRP (20-40 nl of a 2% aqueous solution) was injected, by means of a Hamilton microsyringe fitted with a glass micropipette with a tip diameter of 0.5 μ m, into the NTS. The injection was placed just lateral to the border of the area postrema, 1.0 mm rostral to the level of the obex, to a depth 0.4-0.6 mm ventral to the surface of the medulla.

Incisions were closed in layers, and the animals were allowed to recover from anesthesia. After 24-48 hours the animals were again anesthetized and perfused transcardially with 4% formaldehyde buffered with 0.1 M phosphate buffer, pH 7.3

Transverse serial sections of the brain stem 75 μm in thickness were cut on a vibratome and transferred to cold (4 C) 5% sucrose in 0.1 M phosphate buffer, pH 7.3. Sections were stored in compartmentalized plastic boxes overnight.

The tissue was treated with 3,3'-diaminobenzidine for visualization of the HRP reaction product. Sections were washed three times, for 5 minutes each, in pH 5.5, 0.1 M phosphate buffer. The sections were then incubated in 0.5% 3,3'-diaminobenzidine and 1% hydrogen peroxide in distilled water for 45 minutes. Sections were then washed in three 5 minute changes of the pH 5.5 buffer, mounted on gelatin coated slides and initially examined under darkfield illumination for HRP reaction product.

Sections with positive reactions for HRP in cell bodies, terminals, and fibers in the region known to contain the C1 cell group were examined under a stereomicroscope, and areas of about 2 mm^2 that were of interest were excised. These microsections were then post fixed for 1 hour in 1% OsO_4 in 0.1 M phosphate buffer at pH 7.3, dehydrated through a series of graded alcohols for 15 minutes each and transferred through 3 changes of propylene oxide for 15 minutes each. Tissues were then embedded in an epon-araldite resin and cured for 24 hours at 35 $^{\circ}$ C, 24 hours at 45 $^{\circ}$ C, and 48 hours at 60 $^{\circ}$ C. Blocks were trimmed on a pyramitome, and semithin sections (0.5-1.0 μm) were collected, stained with 1% toluidine blue, and examined under a light microscope. Thin sections, 900-600 Angstroms as judged by interference colors of pale

gold through silver to grey, were prepared and mounted on uncoated 300 mesh nickel grids.

The following procedure was used for immunocytochemical labeling of the PNMT antibody: Grids were rinsed on drops of water for ten minutes. Next, some of the grids were etched using a solution of saturated sodium metaperiodate under various conditions to determine the optimum time and dilution. Ten minutes in a 25% solution resulted in the least amount of tissue destruction while providing the optimum amount of deplastinated tissue for a good reaction. After etching, the grids were washed on drops of water for 5 minutes, transferred to drops of primary antibody diluted to 1:1500 for 1 hour and then washed on drops of 0.1% BSA for 5 minutes. Grids were then transferred to drops containing a 1/200 dilution of colloidal gold (AuroProbe EM, Jensen Scientific) in 0.1% BSA for 1 hour, washed twice for 5 minutes each on drops of 0.1% buffered BSA, washed with a stream of distilled water and contrasted with uranyl acetate and lead citrate. Sections were examined on a Hitachi HU-12A electron microscope.

Results

After injection of HRP into the rostral one-third of the NTS several regions surrounding the NTS including the hypoglossal nucleus, dorsal reticular formation, DMV, caudal portions of the medial and lateral vestibular nuclei, and the dorsal column nuclei also react positively for HRP. These areas contain labeled fibers and terminals but few labeled cell bodies.

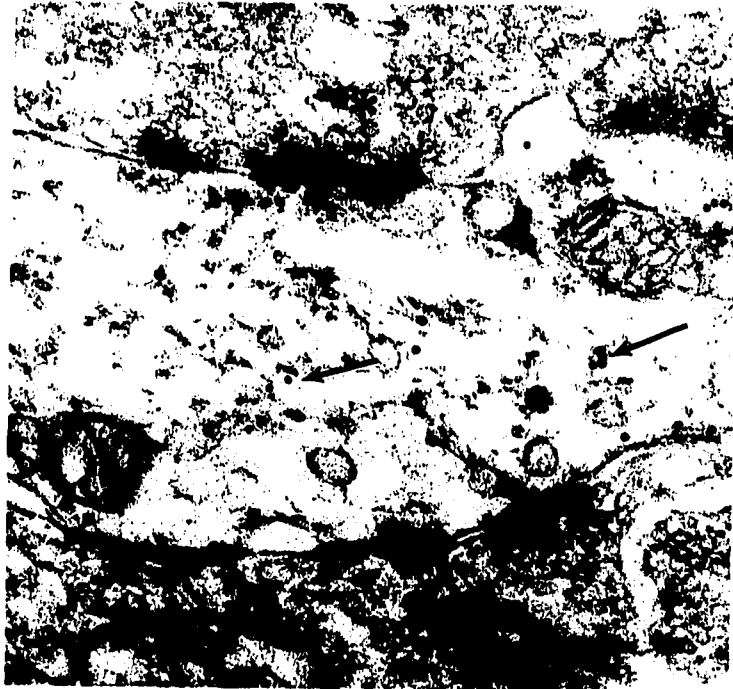
In the VLM anterogradely transported HRP occupies two very distinct regions. One is a circular area of very dense labeling overlying the NA, and the other is a larger and less dense area ventral to the NA. The latter field overlies the region containing the C1 group of neurons (Figs. 4a and 5a). In addition to anterogradely labeled fibers and terminals, some neuronal somas retrogradely labeled with HRP are present in the VLM after HRP injections into the NTS. All HRP-filled cells in these microsections are epinephrinergic neurons of the C1 group, as demonstrated by their positive reaction to the PNMT antibody. Electron micrographs of the RVL reveal many fiber tracts. These are known to be orientated mainly in a rostro-caudal direction. The neurons that exhibit PNMT immunoreactivity after the use of immunocytochemical techniques are interspersed among these fiber tracts (Fig. 12a). Colloidal gold is present free in the cytoplasmic matrix in processes of these cells. HRP containing terminals from the NTS are observed in synaptic relationships with colloidal gold labeled processes (Figs. 12b and 13).

Figure 12a. Brightfield micrograph of the VLM showing cells that are immunocytochemically positive for the enzyme PNMT (100 X). This region corresponds to the location of the C1 group of cells in the RVL. This area was micro-dissected and processed for electron microscopy (arrow)

Figure 12b. Electron micrograph from the region shown in figure 12a (76,000 X). This micrograph demonstrates that cells from the NTS filled with HRP are in synaptic contact with PNMT cells of the C1 group. HRP, as indicated by asterisks, is seen on the presynaptic side and the immunocytochemical marker colloidal gold (arrows) is present in the postsynaptic process of an adrenergic cell of the C1 group.



Figure 13. These electron micrographs show processes of adrenergic cells of the C1 group, as demonstrated by the presence of the immunocytochemical marker PNMT, colloidal gold (arrow). They are contacted by presynaptic processes that contain HRP (asterisks) that has been anterogradely transported from an injection into the NTS. This indicates that C1 cells of the RVL, cells that are known to project to the IML, receive direct input from the NTS



DISCUSSION

This investigation focused on the organization of the baroreflex arc within the brain stem of the rat. A series of experiments was designed to elucidate the simplest pathway by which the baroreflex could stimulate the parasympathetic centers, and to identify connections through which it might inhibit cardiovascular sympathetic centers. The first set of experiments was designed to identify the terminal field of the ADN. A second series of experiments was designed to verify the expected direct connections from the NTS to the NA. Additional experiments were designed to reveal anatomical connections that might exist from the NTS to sympathetic centers in the medulla.

Termination of the Aortic Depressor Nerve

The ADN carries afferent information from baroreceptors in the aortic arch to the NTS, which serves as a terminus for baroreceptor input and for virtually all other visceral afferent information reaching the central nervous system, whether relayed directly or indirectly (reviewed by Brody 1986). Localization reported here of cell bodies of the ADN in the periphery of the nodose ganglion, and of terminals of the ADN in the NTS, particularly in its medial and dorsolateral subnuclei, are in agreement with the observations on the termination of the ADN in the rat by Calaresu and Ciriello (1980), Ciriello and Calaresu (1981), and Ciriello (1983).

Retrogradely labeled neuronal cell bodies in the DMV and NA, as observed in the present study after exposure of the ADN to HRP have also been reported previously, but only in rabbits (Wallach and Loewy, 1980). Researchers using similar techniques in rats did not find any evidence of retrogradely labeled cell bodies in the brain stem (Jordan and Spyer, 1979, and Kidd and McWilliams, 1979). Therefore we attempted to determine whether this unexpected retrograde labeling occurred from uptake by efferent fibers within the ADN itself or if it resulted from leakage of HRP into the neck, where it could subsequently be taken up by fibers innervating the pharynx or other visceral structures. Several techniques were employed to prevent leakage of HRP. In some animals the ADN was placed in a piece of polyethylene tubing containing HRP, and both ends were sealed with dental wax and sutured to the surrounding tissue. In other animals the cut stump of the ADN was placed on a piece of parafilm that completely isolated it from other structures during the exposure period. The HRP was carefully flushed from the surface of the ADN, and in addition, the nerve was placed in a piece of polyethylene tubing that was sealed on both ends with dental wax before removing the parafilm shield. In both of the above cases, the pattern of neuronal cell labeling in the brain stem was the same. Therefore, retrograde labeling did not appear because of leakage at the exposure site. This did not, however, eliminate the possibility that the label was being transferred proximally to motor fibers in the vagus nerve.

To control for the possibility the label was transferred between axons in the vagal trunk, the distribution of cells retrogradely labeled from the ADN was compared with that of cells labeled by exposing the cut end of the vagus nerve to HRP distal to the separation of the ADN from the vagus. In the latter group, labeled neurons were distributed throughout the extent of the DMV, which was very well delineated due to the many retrogradely labeled neurons. Moreover, the rostral pole of the NA was only lightly labeled when the vagus was exposed to HRP. In contrast, most of the neurons labeled from the ADN are concentrated in the rostral pole of the NA, and others are confined to a small area overlying the DMV and ventromedial NTS. Clearly the distribution of neurons retrogradely labeled from the ADN is different from that of those labeled by application of HRP to the vagus nerve. These results indicate that the ADN in the rat is not purely afferent as is the case in the cat (Kalia and Welles, 1980, and Ciriello et al., 1981), and in contradiction to the findings of Ciriello (1983), who found no labeled neurons in the brain stem of the rat. We conclude that in the rat some vagal motor fibers are distributed through the ADN.

The possibility that the efferent neurons that project through the ADN in the rat might have a specialized function is very interesting. Because ADN afferents arise from the aortic sinus and are associated with cardiovascular function, it seems likely that these efferent neurons project to corresponding cardiovascular structures. If so then it would be possible to mimic the effects of stimulating these cells by

electrically stimulating their distal stumps, and a cardiovascular effect could be detected by simultaneously monitoring heart rate and blood pressure.

An attempt to determine if these labeled motor neurons were involved in cardiovascular regulatory mechanisms was made in this investigation. The distal stump of the transected ADN was electrically stimulated while blood pressure and heart rate were monitored. No changes in blood pressure or heart rate were seen. However, this does not rule out the possibility that efferent neurons that course in the ADN do participate in vagal modulation of cardiovascular activity. One possibility is that these are cardioinhibitory cells, but that they are too few in numbers to produce such obvious effects as could be observed under our experimental conditions. Another possibility is that these efferent fibers terminate in the aortic sinus. This pathway might participate in the resetting of baroreflex sensitivity such as occurs after prolonged elevation of the mean arterial pressure. Afferents in the ADN arise from receptors in the aortic sinus; efferents in the same nerve may well terminate in close proximity to those receptors. This efferent pathway could have a modulating effect on the baroreceptors themselves. Alternatively, an indirect mechanism analogous to that occurring in muscle spindles could be involved.

It would be illuminating to inject HRP directly into the tunica muscularis of the aortic arch at the origin of the ADN. This approach should avoid labeling motor fibers from the vagus, and might elucidate

the site of termination of efferent motor fibers that course in the ADN of the rat.

Projections of the NTS to Motor Nuclei of the Vagus

Functionally, the cells of the NA and neighboring RVL seem to be responsible for heart rate rather than myocardial contractility. Electrical stimulation of the VLM reduces the heart rate (Calaresu and Pearce, 1965, Geis and Wurster, 1980, and McAllen and Spyer, 1976), while stimulation of the DMV has been reported to influence contractility (Geis and Wurster, 1981). Further evidence that the NA is directly responsible for bradycardia is given by Thomas and Calaresu (1974), who have shown that bradycardia elicited from stimulation of the NA is abolished by ipsilateral vagotomy in the cat, while bradycardia elicited by stimulation of the NTS and DMV is only partially reduced by ipsilateral vagotomy.

Two separate studies (Thomas and Calaresu, 1974), and Nosaka et al., 1979) have demonstrated that the region encompassing the NA and the neighboring RVL from 2.0 mm caudal to the obex to 1.5 mm rostral to the obex is the site of origin of the vagal efferent fibers that innervate the myocardium, and the major myocardial projection originates from the rostral pole of the NA. In the present investigation it has been shown that cells in these regions that contain the cardiac preganglionic parasympathetic motor neurons receive projections from cells in the region of the NTS. The parasympathetic efferent cell bodies were

labeled by exposing the whole left vagus nerve to the retrograde tracer Fluoro-Gold; consequently all fibers, including those destined for the heart, were labeled in this experiment. The neuronal cell bodies that were labeled in this study lay in the same regions of the brain stem as the labeled cells that were observed after exposing the vagus to HRP in experiment 1. These results are also in agreement with other studies (Geis and Wurster, 1980 and Kalia and Mesulam, 1980), on the distribution of the motor components of the vagus nerve.

HRP-filled terminals from cells in the region of the NTS were identified in synaptic association with these vagal neurons in the present investigation. These results are the first demonstration that the NTS projects directly to cardioinhibitory preganglionic neurons of the parasympathetic nervous system. This connection completes the simplest known reflex pathway by which stimulation of baroreceptors can induce a reduction in heart rate.

Connections between the NTS and C1

The finding that neurons in the NTS send efferent axons to the general region of the VLM is in agreement with results of earlier autoradiographic studies. Labeled fibers and terminals have been demonstrated in the VLM after injections of tritiated amino acids in the NTS in the rat (Loewy and Burton, 1978, Loewy and McKeller, 1980, and Sawchenko and Swanson, 1982). However, those previous investigators concentrated on the more caudal portions of the VLM and not on the

region of the C1 group as in the present study. This is of particular significance because, as explained in the following paragraphs, the more caudal VLM has been shown to be vasodepressive in function, while, in contrast, the C1 group is responsible for vasomotor tone. Also the previous studies did not use immunocytochemistry to identify the cells in the terminal field, but relied on physiological responses to identify this region.

Granata et al. (1985), bilaterally lesioned the RVL in the area containing the C1 group. He found that such lesions caused drops in arterial pressures comparable to those seen after transection of the spinal cord. Unilateral lesions produced a partial reduction of arterial pressure. By carefully mapping the distribution of the lesion with respect to the localization of the C1 cell group, as defined immunocytochemically using antibodies to PNMT, Granata was able to demonstrate close concordance of the lesion site, the distribution of C1 neurons and the fall of arterial pressure. Lesions placed outside of the C1 area did not reduce arterial pressure. In contrast, lesions placed more caudally, in the region of the caudoventrolateral medulla containing the A1 noradrenergic cell group, caused elevations in arterial pressure (Blessing and Reis, 1982). This was interpreted as indicating that the caudoventrolateral medulla has an inhibitory role in the control of blood pressure (Blessing et al., 1981, Blessing and Reis, 1982). Thus, it appears that the caudoventrolateral medulla and RVL

together contain a column of aminergic neurons that are involved with autonomic regulation of blood pressure.

C1 cells by definition contain the neurotransmitter epinephrine, and, as previously stated, they project to the IML. However, the evidence that epinephrine is the excitatory neurotransmitter for preganglionic sympathetic activation is unclear. Microiontophoresis of epinephrine onto the cell bodies of preganglionic sympathetic neurons in the IML inhibits rather than stimulates the activity of these neurons (Coote et al., 1981, and Guyenet and Cabot, 1981). Other putative neurotransmitters are colocalized in the C1 cells and may be released by these cells to stimulate spinal preganglionic sympathetic neurons. These include substance P, enkephalins, and GABA (Willette et al., 1983, Ruggiero et al., 1984 and Ruggiero et al., 1985), and neuropeptide Y (Hokfelt et al., 1983 and Everitt et al., 1984). Although the neurotransmitter utilized remains to be determined, it is very likely that neurons in the RVL play a critical role in the maintenance of arterial pressure (Brody et al., 1986).

Labeled terminals were seen in this investigation in the C1 area after HRP injections in the region of the NTS. To determine if these labeled terminals were associated with neurons that project to the spinal cord a double label was employed. This set of experiments showed that after injections of HRP into the NTS and Fluoro-Gold into the spinal cord, terminals anterogradely labeled with HRP from the NTS lay in close association with fluorescent cells in the C1 area. Alternate

adjoining sections from experiment 3 were reacted for the presence of the enzyme PNMT, and it was found that the fluorescent cells in the RVL showed a positive reaction for that enzyme. Thus, confirmation was obtained that cells in the NTS project to the immediate proximity of adrenergic cells of the C1 group.

Further examination of this area using electron microscopy and immunocytochemistry revealed that presynaptic terminals containing HRP from injections in the region of the NTS terminate upon PNMT-containing processes in the C1 region of the RVL. This result indicates that fibers from cells in the region of the NTS actually synapse upon C1 cells in the RVL. Therefore, an anatomical substrate by which cells in the NTS may influence the medullary cardiac sympathetic outflow has been demonstrated in this investigation. It may be this connection that is responsible for the fact that vagotomy does not completely prevent bradycardia elicited by stimulation of the NTS (Thomas and Calaresu, 1974).

Projections of the NA to the Spinal Cord

The cell bodies of cardiac vagal preganglionic neurons have been shown to lie in the DMV, NA, and the intermediate zone between the DMV and NA in cats (Geis and Wurster, 1980), and rats (Nosaka et al., 1979). The NA contained 72% of the total number of cell bodies, while 19% were found in the DMV. Geis et al. (1981), found that electrical stimulation of the NA decreased heart rate, but had no direct effect on the force of

the ventricular contraction. In contrast, stimulation of the DMV led to a decrease in the force of the ventricular contraction but did not change the heart rate.

Other physiological studies have shown that cells in the retroambiguus discharge in phase with the respiratory cycle, suggesting a role for this region in respiratory control (Merrill, 1970, and Kalia, 1981). The NA and nucleus retroambiguus are unusual in that they have both spinal and vagal efferents. This has been demonstrated indirectly by Leong et al. (1984), who injected HRP into the spinal cord in rats and found labeled cell bodies in the vagal complex. However, the present experiment is the first to use a double labeling technique and thereby demonstrate the spinal and vagal efferents directly.

After HRP was injected into the second thoracic segment, there were efferent neurons in the NA and DMV that were filled with retrogradely transported label. Similar results have been briefly mentioned in single label studies by Tohyama et al. (1979) in the cat, and by Leong et al. (1984) in the rat. In the present investigation such retrograde labeling revealed that cells at all levels of the NA and surrounding VLM project to the thoracic spinal cord. The most concentrated region of neurons labeled from the spinal cord in the NA is between 1.0 and 2.0 mm rostral to the obex. Cells at this level are, in fact, preganglionic parasympathetic motor neurons, as demonstrated by the fact that they are double labeled when the vagus nerve is exposed to a second retrograde marker. These results reveal that the neurons in the NA have a dual

projection; 1) to the spinal cord and 2) to an end organ that is innervated by fibers of the vagus nerve. Results of anatomical and physiological studies discussed earlier in this section, (Merrill, 1970, Geis and Wurster, 1980, Kalia, 1981, and Leong et al., 1984), indicate that collateral projections from preganglionic parasympathetic vagal neurons in the NA and retroambiguus may have an influence on respiratory preganglionic sympathetic neurons at the spinal cord level. However, it seems unlikely that only respiratory function is subserved by these connections, as a large percentage of these neurons project to the heart (Nosaka et al., 1979), and affect heart rate (Calaresu and Pearse, 1965, Geis and Wurster, 1980). These nuclei have been shown to receive inputs from the NTS in this study. Therefore it is tenable that vagal neurons of the NA that project to the myocardium in the region of the sinoatrial and atrioventricular nodes, could also influence sympathetic outflow to the heart via collateral projections to the preganglionic sympathetic neurons in the IML.

The significance of the spinal collaterals from vagal motor neurons will remain uncertain until further studies are carried out. For example it should be possible to inject a retrograde label such as HRP into the myocardium around the sinoatrial or atrioventricular nodes and wait for retrograde transport to fill all axon collaterals. From this experiment it might be possible to label spinal terminals of vagal collaterals that project to cells in the IML between thoracic segments T1 and T5.

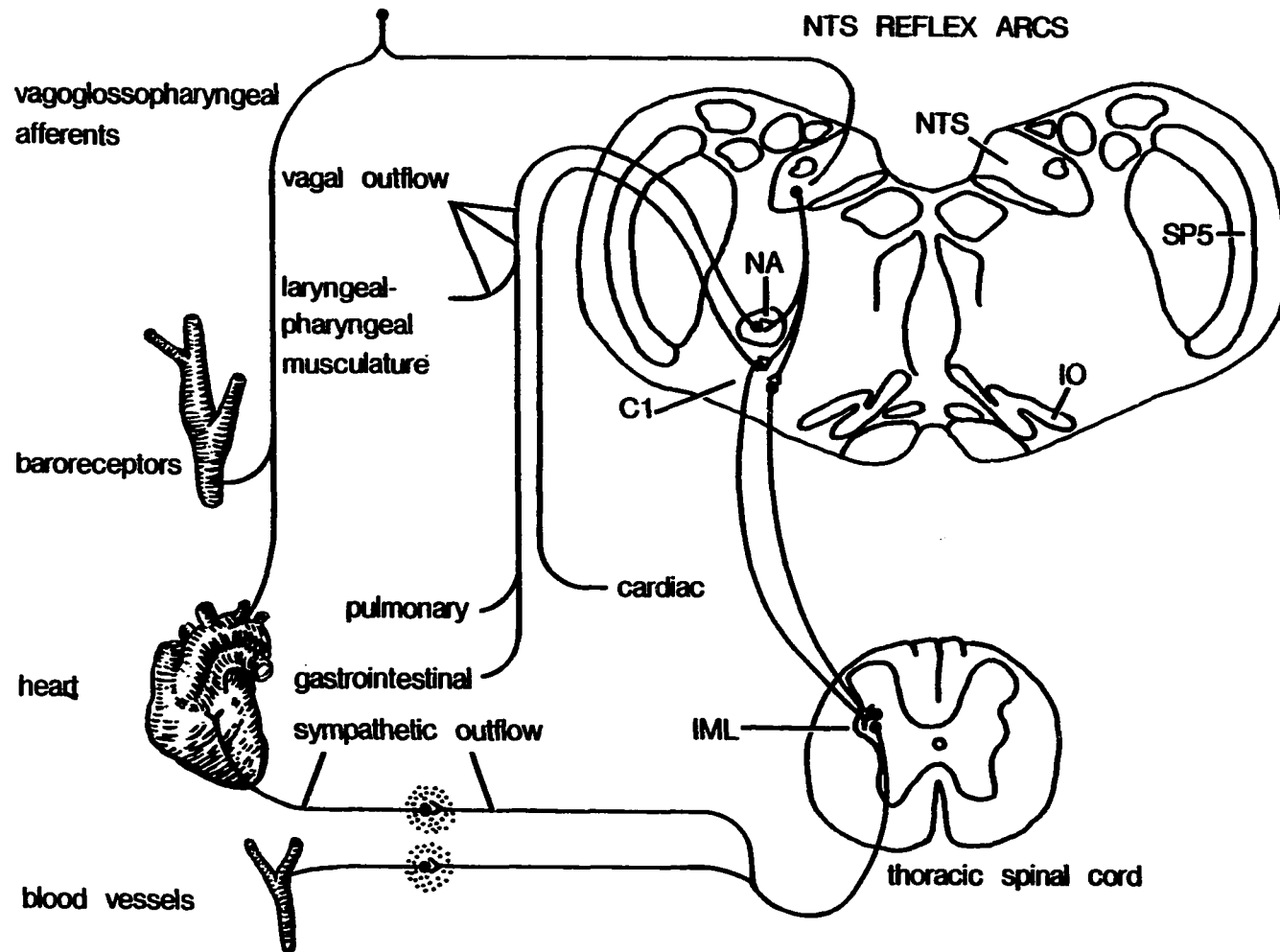
Conclusions

Baroafferent fibers in the ADN terminate in the NTS, which in turn projects to parasympathetic and sympathetic medullary centers. The parasympathetic center, the vagal motor complex, sends cardioinhibitory fibers through the vagus nerve. The sympathetic center, comprising adrenergic neurons of the C1 group in the VLM, projects to the spinal cord, where preganglionic cells give rise to the final pathway of the sympathetic vasomotor pathway. These pathways appear to represent the smallest number of neurons that could constitute reflex arcs that effect slowing of the heart rate and decreasing vascular resistance in response to baroreceptor stimulation (see Fig. 9).

The NTS affects cardiovascular function in response to baroreceptor stimulation by stimulating the parasympathetic nervous system and by inhibition of the sympathetic nervous system. Experiments reported here have shown projections from the NTS to the NA and surrounding VLM, a region known to be the location of the preganglionic vagal efferent neurons that play a role in regulation of cardiovascular function. This completes a neuroanatomical pathway through which barostimulation can activate parasympathetic cardioinhibition. Other experiments reported here have demonstrated that the NTS projects to the RVL, and specifically to the immunocytochemically identified adrenergic neurons of the C1 group, which in turn project to the spinal cord. The termination of the ADN in the NTS, and the projections of the NTS to the

ventrolateral medulla, demonstrate a simple pathway by which the baroreflex can influence sympathetic output to the heart and blood vessels. In addition, these experiments have revealed that in the region of the NA and surrounding VLM that receives projections from the NTS, there are cells that project not only through the vagus but also to the spinal cord. Therefore an anatomical substrate has been demonstrated that could subserve baroreflex inhibition of the sympathetic nervous system via a second pathway arising from the dorsal vagal complex.

Figure 14. Proposed pathway of the baroreflex arc. Afferent fibers from baroreceptors in the aortic arch travel first via the ADN and then with the vagus nerve, and they terminate in the NTS. The NTS projects to C1 neurons in the RVL. C1 neurons project to the IML to preganglionic sympathetic neurons that control sympathetic outflow. The NTS also projects to the NA, which controls parasympathetic outflow. The NA sends efferent fibers to the heart, which decreases heart rate, and to the spinal cord, which may modulate or inhibit sympathetic outflow. (adapted from Reis et al., Hypertension, volume 6: 1984)



LITERATURE CITED

- Aars, H., L. Myhre, and B. A. Haswell. 1978. The function of baroreceptor C fibers in the rabbit's aortic nerve. *Acta. Physiol. Scand.* 102: 84-93.
- Abboud, F. M. 1982. The sympathetic system in hypertension. State-of-the-art review. *Hypertension* 4 Suppl. 2: 208-225.
- Alexander, R. S. 1946. Tonic and reflex functions of medullary sympathetic cardiovascular centers. *J. Neurophysiol.* 9: 205-217.
- Andrew, B. L. 1954. A laryngeal pathway for aortic baroreceptor impulses. *J. Physiol.* 125: 352-360.
- Andrezik, J. P., V. Chan-Palay and S.L. Palay. 1981. The nucleus paragigantocellularis lateralis in the rat. Demonstration of afferents by the retrograde transport of horseradish peroxidase. *Anat. Embryol. (Berl.)* 161: 373-390.
- Angell-James, J. E., and M. De Burgh-Daly. 1970. Comparison of the reflex vasomotor response to separate and combined stimulation of the carotid sinus and aortic arch baroreceptors by pulsatile and non-pulsatile pressures in the dog. *J. Physiol.* 209: 257-293.
- Anyukhovskii, P. E., and G. G. Beloshapko. 1978. Electrophysiological characteristics of aortic baroreceptors. *Byulleten' Eksperimental'noi Biologii i Meditsiny* 85(1): 6-8.
- Atweh, S. F., and M. J. Kuhar. 1977. Autoradiographic localization of opiate receptors in rat brain. *Brain Res.* 124: 53-67.
- Bard, P. 1960. Anatomical organization of the central nervous system in relationship to the control of heart and blood vessels. *Physiol. Rev.* 40: 3-24.
- Bentivoglio, M., H. G. J. M. Kuypers, C. E. Catsman-Berrevoets, and O. Dann. 1979. Fluorescent retrograde neuronal labeling in rat by means of substances binding specifically to adenine-thymine rich in DNA. *Neurosci. Lett.* 12: 235-240.
- Blessing, W. W., A. K. Goodchild, R. A. L. Dampney, and J. P. Chalmers. 1981. Cell groups in the lower brain stem of the rabbit projecting to the spinal cord, with special reference to catecholamine-containing neurons. *Brain Res.* 221: 35-55.

- Blessing, W. W., and D. J. Reis. 1982. Inhibitory cardiovascular function of neurons in the caudal ventrolateral medulla of the rabbit: Relationship to the area containing A1 noradrenergic cells. *Brain Res* 253: 161-167.
- Blessing, W. W., A. F. Sved, and D. J. Reis. 1982. Destruction of noradrenergic neurons in rabbit brain stem elevates plasma vasopressin, causing hypertension. *Science* 217: 661-663.
- Blessing, W. W., M. J. West, and J. P. Chalmers. 1981. Hypertension, bradycardia and pulmonary edema in the conscious rabbit after brain stem lesions coinciding with the A1 group of catecholamine neurons. *Circ. Res.* 49: 949-958.
- Bolme, P., K. Fuxe, L. F. Agnati, R. B. Bradley, and J. Smythies. 1978. Cardiovascular effects of morphine and opioid peptides following intracisternal administration in chloralose anesthetized rats. *Eur. J. Pharmacol.* 48: 319-324.
- Bousquet, P., and J. Schwartz. 1975. Alpha adrenergic drugs: Pharmacological tools for the study of the central vasomotor control. *Biochem. Pharmacol.* 34: 151.
- Bowker, R.M., K.N. Westlund and J.D. Coulter. 1981. Serotonergic projections to the spinal cord from the midbrain in the rat: an immunocytochemical and retrograde study. *Neurosci. Lett.* 24: 221-226.
- Brody, M.J.. 1986. Central nervous system control of arterial pressure regulation. *Fed Proc.* 45: 2700-2706.
- Calaresu, F. R., and J. Ciriello. 1980. Projections of buffer nerves to the medulla and hypothalamus. Baroreceptors and hypertension. pp. 253-260. In P. Sleight (ed.). *Arterial Baroreceptors and Hypertension.* Oxford Medical Publications, New York.
- Calaresu, F. R., A. A. Faiers, and G. J. Mogenson. 1975. Central neural regulation of heart and blood vessels in mammals. *Prog. Neurobiol.* 5: 1-35.
- Calaresu, F.R. and J.W. Pearce. 1965. Effects on heart rate of electrical stimulation of medullary vagal structures in the cat. *J. Physiol. Lond.* 176: 241-251.
- Caverson, M. M., J. Ciriello, and F. R. Calaresu. 1984. Chemoreceptor and baroreceptor inputs to ventrolateral medullary neurons. *Am. J. Physiol.* 247(5)2: R761-R767.

- Chernicky, C. L., K. L. Barnes, C. M. Ferrario, and J. P. Conomy. 1983. A morphological characterization of the canine area postrema. *Brain Res. Bull.* 10: 345-351.
- Chung, J. M., K. Chung, and R. D. Wurster. 1975. Sympathetic Preganglionic Neurons of the Cat Spinal Cord: Horseradish Peroxidase Study. *Brain Res.* 91: 126-131.
- Ciriello, J. 1983. Brain stem projections of aortic baroreceptor afferent fibers in the rat. *Neurosci. Lett.* 36: 37-42.
- Ciriello, J., and F. R. Calaresu. 1981. Projections from buffer nerves the nucleus of the solitary tract: An anatomical and electrophysiological study in the cat. *J. Auton. Nerv. Sys.* 3: 299-310.
- Ciriello, J., and M. M. Caverson. 1984. Ventrolateral medullary neurons relay cardiovascular inputs to the paraventricular nucleus. *Am. J. Physiol.* 246(6): R968-R978.
- Ciriello, J., A. W. Hryciyshyn, and F. R. Calaresu. 1981. Horseradish peroxidase study of brain stem projections of carotid sinus and aortic depressor nerves in the cat. *J. Auton. Nerv. Sys.* 4: 43-61.
- Ciriello, J., E. M. J. Palmer, and F. R. Calaresu. 1980. Arterial pressure and heart rate in the rat after section of the aortic depressor or the carotid sinus nerve. *Proc. Can. Fed. Biol. Soc.* 23: 103.
- Coote, J. H., V. H. Macleod, S. M. Fleetwood-Walker, and M. P. Gilbey. 1981. Baroreceptor Inhibition of Sympathetic Activity at a Spinal Site. *Brain Res.* 220: 81-93.
- Cottle, M. K. W., and F. R. Calaresu. 1975. Projections from the nucleus and tractus solitarius in the cat. *J. Comp. Neurol.* 161: 143-158.
- Crill, W. E., and D. J. Reis. 1968. Distribution of carotid sinus and depressor nerves in the cat brain stem. *Amer. J. Physiol.* 214: 269-276.
- Czachurski, J., K. J. Lackner, D. Ockert, and H. Seller. 1982. Localization of neurones with baroreceptor input in the medial solitary nucleus by means of intracellular application of horseradish peroxidase in the cat. *Neurosci. Lett.* 28: 133-137.

- Dahlstrom, A., and K. Fuxe. 1964. Evidence for the existence of monamine containing neurons in the central nervous system. I. Demonstration of monamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.* 62,S232: 1-55.
- Dampney, R. A. L. 1981. Brain stem mechanisms in the control of arterial pressure. *Clin. Exp. Hyperten.* 3: 379-391.
- Dampney, R. A. L., A. K. Goodchild, L. G. Robertson, and W. Montgomery. 1982. Role of ventrolateral medulla in vasomotor regulation: a correlative anatomical and physiological study. *Brain Res.* 249: 223.
- Dembowsky, K., J. Czachurski, K. Amendt, and K. Horst. 1980. Tonic descending inhibition of the spinal somatosympathetic reflex from the lower brain stem. *J. Auton. Nerv. Sys.* 2:157-182
- Dittmar, C. 1873. Ein neuer beweis fur die reizbarkeit der centripetalen endfasern des ruckenmarks. *Akad. Wiss. Leipzig, Math. Phys. Kl.* 22: 18-45.
- Douglas, W. W., and J. M. Ritchie. 1956. Cardiovascular reflexes produced by electrical excitation of non-medullated afferents in the vagus, carotid sinus, and aortic nerve. *J. Physiol. Lond.* 134: 167-178.
- Everitt, B. J., T. Hokfelt, L. Terenius, K. Tatemoto, V. Mutt, and M. Goldstein. 1984. Differential co-existence of neuropeptide Y (NPY) like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11: 443-462.
- Geis, G. S., J. W. Kozelka, and R. D. Wurster. 1981. Organization and reflex control of vagal cardio-motor neurons. *J. Auton. Nerv. Sys.* 3: 437-450.
- Geis, G. S., and R. D. Wurster. 1980. Horseradish peroxidase localization of cardiac vagal preganglionic somata. *Brain Res* 182: 19-30.
- Gillis, R. A., C. J. Helke, B. L. Hamilton, W. P. Norman, and D. M. Jacobowitz. 1980. Evidence that substance P is a neurotransmitter of baro- and chemoreceptor afferents in nucleus tractus solitarius. *Brain Res.* 181: 476-481.
- Glick, G. and J. W. Covell. 1968. Relative importance of the carotid and aortic baroreceptors in reflex control of heart rate. *Amer. J. Physiol.* 209: 257-293.

- Granata, A. R., D. A. Ruggiero, D. H. Park, T. H. Joh, and D. J. Reis. 1983. Lesions of epinephrine neurons in the rostral ventrolateral medulla abolish the vasodepressor components of baroreflex and cardiopulmonary reflex. *Suppl. V. Hypertension* 5(6): V80-V84.
- Granata, A. R., D. A. Ruggiero, D. H. Park, T. H. Joh, and D. J. Reis. 1985. Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor responses. *Am. J. Physiol.* 248: H547-H567.
- Guyenet, P. G., and J. B. Cabot. 1981. Inhibition of sympathetic preganglionic neurons by catecholamines and clonidine: Mediation by an alpha adrenergic receptor. *J. Neurosci.* 1: 908-927.
- Guyenet, P. G., and R. L. Stornetta. 1982. Inhibition of sympathetic preganglionic discharges by epinephrine and L-methylepinephrine. *Brain Res.* 235: 271-283.
- Harfstrand, A., K. Fuxe, L. F. Agnati, D. Ganten, P. Eneroth, K. Tatemoto, and V. Mutt. 1984. Studies on neuropeptide-Y catecholamine interactions in central cardiovascular regulation in the alpha-chloralose anaesthetized rat. Evidence for a possible new way of activating the alpha-2 adrenergic transmission line. *Clin. Exp. Theroy Practice* A6: 1947-1950.
- Haeusler, G. and R. Osterwalder. 1980. Is substance P the transmitter at the first synapse of the baroreflex in rats and cats. *Clin. Sci.* 59: 2955-2967.
- Hausler, G. 1974. Clonidine induced inhibition of sympathetic nerve activity: No indication for a central presynaptic or an direct sympathomimetic mode of action. *Naunyn Schmiedeberg's Arch. Pharmacol.* 286: 97-111.
- Helke, C. J., T. L. O'Donohue, and D. M. Jacobowitz. 1980. Substance P as a baro- and chemoreceptor afferent neurotransmitter: Immunocytochemical and neurochemical evidence in the rat. *Peptides* 1: 1-9.
- Henry, J. L., and F. R. Calaresu. 1972. Topography and numerical distribution of neurons of the thoaco-lumbar intermediolateral nucleus in the cat. *J. Comp. Neurol.* 144: 205-214.
- Heymans, C., and E. Neil. 1958. Reflexogenic areas of the cardiovascular system. Churchill, London.
- Hokfelt, T., K. Fuxe, M. Goldstein, and O. Johanssen. 1974. Immunohistochemical evidence of adrenaline neurons in the rat brain. *Brain Res.* 66: 235-251.

- Hokfelt, T., A. Ljungdahl, H. Steinbusch, A. Verhofstad, G. Nilsson, E. Brodin, B. Pernow and M. Goldstein. 1978. Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine-containing neurons in the rat central nervous system. *Neuroscience* 3: 517-538.
- Hokfelt, T., J. M. Lundberg, K. Tatemoto, V. Mutt, L. Terenius, J. Ploak, S. Bloom, and C. Sasek. 1983. Neuropeptide Y (NPY)- and FMRFamide neuropeptide-like immunoreactivities in catecholamine neurons of the rat medulla oblongata. *Acta Physiol. Scand.* 117: 315-318.
- Hopkins, D. A., J. H. Mc Lean, and Y. Takeuchi. 1981. Amygdalotegmental projections: Light and electron microscope studies utilizing anterograde degeneration and the anterograde and retrograde transport of horse radish peroxidase. pp. 133-147. In Y. Ben-Ari (ed.) *The amygdaloid complex*. Elsevier, Amsterdam.
- Humphrey, D.R. 1976. Neuronal activity in medulla oblongata of the cat evoked by stimulation of the carotid sinus nerve. pp. 131-167. In Kezdi, P. (ed.). *Baroreceptors and hypertension*. Pergamon Press, New York
- Ingram, W. R., and E. A. Dawkins. 1945. The intramedullary course of afferent fibers of the vagus in the cat. *J. Comp. Neurol.* 82: 157-168.
- Jordan, D. and K.M. Spyer. 1979. Studies on the excitability of sinus nerve afferent terminals. *J. Physiol. Lond.* 297: 123-134.
- Kalia, M. 1981. Anatomical organization of central respiratory neurons. *Annu. Rev. Physiol.* 43: 105-120.
- Kalia, M. and M.M. Mesulam. 1980. Brain stem projections and motor components of the vagus complex in the cat: 1. The cervical vagus and nodose ganglion. *J. Comp. Neurol.* 193: 435-453.
- Kalia, M., and M. Sullivan. 1982. Brain stem projections of sensory and motor components of the vagus in the rat. *J. Comp. Neurol.* 211: 248-264.
- Kalia, M., J. J. Viola, M. E. Hudson, K. Fuxe, A. Harfstrand, and M. Goldstein. 1986. Brain stem mechanisms involved in cardiovascular regulation. In IUPS satellite symposium on the organization of the autonomic nervous system: Central and peripheral mechanisms, Montreal.

- Kalia, M., and R. V. Welles. 1980. Brain stem projections of the aortic nerve in the cat: A study using tetramethyl benzidine as the substrate for horseradish peroxidase. *Brain Res.* 188: 23-32.
- Kalia, M., K. Fuxe, T. Hokfelt, D. Johansson, R. Lang, D. Ganten, C. Cuello, and L. Terenius. 1984. Distribution of neuropeptides immunoreactive nerve terminals within the subnuclei of the nucleus tractus solitarius of the rat. *J. Comp. Neurol.* 222: 409-444.
- Kardon, M. B., D. F. Peterson, and V. S. Bishop. 1973. Reflex bradycardia due to aortic nerve stimulation in the rabbit. *Amer. J. Physiol.* 225: 7-11.
- Kidd, C. and P.N. McWilliams. 1979. Cell bodies of aortic nerve efferent fibers in the rabbit and cat. *J. Physiol.* 293: 55-56.
- Kostreva, D. R. 1984. Functional mapping of cardiovascular reflexes and the heart using 2-14C-deoxyglucose. *Physiologist* 26: 333.
- Koyama, S., N. Terada, Y. Shiojima, and T. Takeuchi. 1984. Pharmacological evidence that central α_2 -adrenoceptors are involved in cardiovascular responses to intravenously injected enkephalins in anesthetized dogs. *Jpn. J. Physiol.* 34: 995-1002.
- Lassila, V.. 1928. The aortic arch and its branches, their relation to sympathetic, vagus and recurrent laryngeal nerves in mammals. *Ann. Acad. Sc. Fenniae* 27: 1-269.
- Laubie, M., H. Schmitt, and M. Vincent. 1979. Vagal bradycardia produced by microinjection of morphine-like drugs into the nucleus ambiguus in anesthetized dogs. *Eur. J. Pharmacol.* 59: 287-291.
- Leong, S. K., J. Y. Shieh, and W. C. Wong. 1984. Localizing spinal cord projecting neurons in adult albino rats. *J. Comp. Neurol.* 228: 1-17.
- Loewy, A. D., and H. Burton. 1978. Nuclei of the Solitary Tract: Efferent Projections to the Lower Brain Stem and Spinal Cord of the Cat. *J. Comp. Neurol.* 181: 421-450.
- Loewy, A. D., and S. McKeller. 1980. The neuroanatomical basis of central cardiovascular control. *Fed. Proc.* 39: 2495.
- Loewy, A. D., and J. J. Neil. 1981. The role of descending monoaminergic systems in the central control of blood pressure. *Fed. Proc.* 40: 2778-2785.

- Maley, B., and R. Elde. 1982. Immunohistochemical localization of putative neurotransmitters within the feline nucleus tractus solitarii. *Neuroscience*. 7: 2469-2490.
- Maley, B. E. 1985. The ultrastructural localization of enkephalin and substance P immunoreactivities in the nucleus tractus solitarii of the cat. *J. Comp. Neurol.* 233: 490-496.
- McAllen, R. M. and K. M. Spyer. 1976. The location of cardiac vagal preganglionic motoneurons in the medulla of the cat. *J. Physiol. Lond.* 258: 187-204.
- Merrill, E. G. 1970. The lateral respiratory neurons of the medulla: Their associations with nucleus ambiguus, nucleus retroambiguus, the spinal accessory nucleus and the spinal cord. *Brain Res.* 24: 11-28.
- Mesulam, M. M., and D. L. Rosene. 1978. Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *J. Histochem. Cytochem.* 26: 106-117.
- Miura, M., and D. J. Reis. 1969. Termination and secondary projections of carotid sinus nerve in the cat brain stem. *Am. J. Physiol.* 217(1): 142-153.
- Moore, R. H., and D. A. Dowling. 1980. Effects of intravenously administered leu- or met-enkephalin on arterial blood pressure. *Regul. Peptides* 1: 77-87.
- Nosaka, S., T. Yamamoto, and K. Yasunaga. 1979. Localization of vagal cardioinhibitory preganglionic neurons within rat brain stem. *J. Comp. Neur.* 186: 79-92.
- Oberg, B. 1981. Reflex cardiovascular responses to graded stimulations of medullated and non-medullated afferents in the carotid sinus and aortic nerves in the cat. *Acta Physiol. Scand.* 113: 129-137.
- Papaioannau, J. 1970. A simple device for blocking brains in the Horsley-Clarke coronal plane. *J. Physiol.* 210: 77-79.
- Reis, D. J., N. Boda, D. W. Snyder, and M. A. Nathan. 1977. Brain lesions and hypertension; chronic lability and elevation of arterial pressure produced by electrolytic lesions and 6-hydroxy-dopamine treatment of nucleus tractus solitarii (NTS) in rat and cat. *Prog. Brain Res.* 47: 169-188.

- Reis, D. J., A. R. Granata, H. J. Tong, C. A. Ross, D. A. Ruggiero, and D. H. Park. 1984. Brain stem catecholamine mechanisms in tonic and reflex control of blood pressure. *Hypertension* 6: 7-15.
- Reis, D. J., M. H. Perrone, and W. T. Talman. 1981. Glutamic acid is the neurotransmitter of baroreceptor afferents in the nucleus tractus solitarii (NTS): Possible relationship to neurogenic hypertension. pp. 37-48. In J. P. Buckley and C. M. Ferrario (eds.). *Central nervous system mechanisms in hypertension*. Raven Press, New York.
- Reis, D. J., W. T. Talman, M. Perrone, N. Doba, and M. Kumada. 1980. L-glutamate as the neurotransmitter of baroreceptor afferents. pp. 221-227. In P. Sleight (ed.). *Arterial Baroreceptors and Hypertension*. Oxford Medical Publications, New York.
- Ricardo, J. A., and E. J. Koh. 1978. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala and other forebrain structures in the rat. *Brain Res.* 153: 1026.
- Ross, C. A., D. A. Ruggiero, and D. J. Ries. 1981. Afferent projections to cardiovascular portions of the nucleus of the tractus solitarius in the rat. *Brain Res.* 223: 402-408.
- Ross, C. A., D. A. Ruggiero, T. H. Joh, D. H. Park, and D. J. Reis. 1983. Adrenaline synthesizing neurons in the rostral ventrolateral medulla: A possible role in tonic vasomotor control. *Brain Res.* 273: 356-361.
- Ross, C. A., D. A. Ruggiero, T. H. Joh, D. H. Park, and D. J. Reis. 1984a. Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. *J. Comp. Neurol.* 228: 168-184.
- Ross, C. A., D. A. Ruggiero, D. H. Park, T. H. Joh, A. F. Sved, J. Fernandez-Pardal, J. M. Saavedra, and D. J. Reis. 1984b. Tonic vasomotor control by rostral ventrolateral medulla: effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. *J. Neurosci.* 4: 474-494.
- Ruggiero, D. A., M. Meeley, M. Anwar, and D. J. Reis. 1985. Newly identified GABAergic neurons in regions of the ventrolateral medulla which regulate blood pressure. *Brain Res.* 339: 171-177.

- Ruggiero, D. A., C. A. Ross, M. Anwar, and D. J. Reis. 1984. The rostral ventrolateral medulla: Immunocytochemistry of intrinsic neurons and afferent connections. *Soc. Neurosci. Abstr.* 10: 299.
- Ruggiero, D. A., C. A. Ross, and D. J. Reis. 1982. Medullary viscreal reflex arcs demonstrated by a combined retrograde and anterograde transport technique. *Soc. Neurosci. Abstr.* 8: 723.
- Sander, G., T. Giles, A. Kasten, A. Kaneish, and D. Coy. 1982. Leucine-enkephalin: Reversal of intrinsic cardiovascular stimulation by pentobarbital. *Eur. J. Pharmacol.* 78: 467-470.
- Sangdee, C. and D.N. Franz. 1983. Evidence for inhibition of sympathetic preganglionic neurons by bulbospinal epinephrine pathways. *Neurosci. Lett.* 37: 167-173.
- Sawchenko, P. E. 1983. Central connections of the sensory and motor nuclei of the vagus nerve. *J. Auton. Nerv. Sys.* 9: 13.
- Sawchenko, P. E., and L. W. Swanson. 1982. The organization of noradrenergic pathways from the brain stem to the paraventricular and supraoptic nuclei in the rat. *Brain Res. Rev.* 4: 275-325.
- Smint, O. A., and J. L. DeVito. 1984. Central neural integration for the control of autonomic responses associated with emotion. *Annu. Rev. Neurosci.* 7: 43.
- Spyer, K. M. 1981. Neural organization and control of the baroreceptor reflex. *Rev. Physiol. Biochem. Pharmacol.* 88: 24.
- Steinbusch, H.W., A.A. Verhofstad, B. Penke, J. Varga and H.W. Joosten. 1981. Immunohistochemical characterization of monoamine-containing neurons in the central nervous system by antibodies to serotonin and noradrenalin. A study in the rat and lamprey (*Lampetra fluviatilis*). *Acta Histochem.* 24: 107-122.
- Swanson, L. W., and P. E. Sawchenko. 1983. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Ann. Rev. Neurosci.* 6: 269-283.
- Thomas, M. R., and F. R. Calaresu. 1974. Localization and function of medullary site mediating vagus bradycardia. *Am. J. Physiol.* 226: 201-208.
- Thoren, P., and S. Lundin. 1983. Autonomic nervous system and blood pressure control. pp. 31-61. In D. Ganten and D. Pfaff (eds.). *Central cardiovascular control.* Springer-Verlag, New York.

- Thoren, P., W. R. Saum, and A. M. Brown. 1977. Characteristics of aortic baroreceptors with non medullated vagal afferents in the cat. *Circ. Res.* 40: 321-337.
- Tohyama, M. K., M. Sakai, D. Touret, D. Salvert, and M. Jouvet. 1979. Spinal projections from the lower brain stem in the cat as demonstrated by the horseradish peroxidase technique. II. Projections from the dorsolateral pontine tegmentum and raphe nuclei. *Brain Res.* 176: 215-231.
- Torvik, A.. 1956. Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures. *J. Comp. Neurol.* 106: 51-141.
- von Schumacher, S. 1902. Zur frage der herzzinnervation bei der saugetieren. *Anatomischer Anzeiger* 21: 1-7.
- Wallach, J. H., and A. D. Loewy. 1980. Projections of the aortic nerve to the nucleus tractus solitarius in the rabbit. *Brain Res.* 188: 247-251.
- Willette, R. N., A. J. Krieger, P. P. Barcas, and H. N. Sapru. 1983. Medullary γ -aminobutyric acid (GABA) receptors and the regulation of blood pressure in the rat. *J. Pharmacol. Exp. Ther.* 226: 893-899.
- Willette, R. N., S. Punnen, A. J. Krieger, and H. N. Sapru. 1984. Interdependence of rostral and caudal ventrolateral medullary areas in the control of blood pressure. *Brain Res.* 321: 169-174.
- Wurster, J. M. 1977. Spinal sympathetic control of the heart. pp. 211-246. In W. C. Randal (ed.). *Neural regulation of the heart.* Oxford University Press, New York.
- Zamir, N., M. Palkovits, and M. Brownstein. 1985. Distribution of immunoreactive Met-Enkephalin-Arg6-Gly7-Leu8 and Leu-Enkephalin in discrete regions of the rat brain. *Brain Res.* 326: 1-8.