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THE AORTIC DEPRESSOR NERVES OF GUINEA PIGS

By

HONG-SHUO SUN

A THESIS

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As the water shapes itself to the vessel that contains it, so a wise man adapts himself to circumstances.

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To my parents

ABSTRACT

In guinea pigs, aortic baroreceptor afferents were found in anatomically separate fine aortic depressor nerves (ADN) that ran cephalad to join the ipsilateral superior laryngeal nerves in the mid-cervical region. Histological and electrophysiological studies revealed both myelinated and unmyelinated fibers in the ADN. Orthodromic stimulation of the ADN induced depressor responses and bradycardia. The responses to left, right or bilateral ADN stimulation were similar, and were voltage, frequency and pulse-width dependent. Efferent arcs of the aortic baroreflexes involved autonomic ganglia, central alpha$_2$, peripheral alpha$_1$ and beta$_1$ adrenoceptors. Aortic baroreflexes were similar in animals under pentobarbital, urethane or chloralose anesthesia, and were unchanged after acute bilateral adrenalectomy. The depressor responses and bradycardia induced by ADN stimulation resulted solely from a reflex reduction in sympathetic tone, and involved only the myelinated afferents in the ADN. The depressor effects were confined to the vasculature. The aortic baroreflexes did not influence heart rate via vagal efferents. Cooling, and anodal block of conduction in aortic baroreceptor afferents and of ADN stimulation, confirmed that the myelinated fibers alone conducted aortic baroreceptor afferent activity. Intra-aortic injection of chemoreceptor

stimulants did not alter the ADN's discharge pattern, and no pressor responses to ADN stimulation were detected, indicating the absence of chemoreceptor afferents in the ADN. Functional studies of the unmyelinated fibers in the ADN revealed that some were sympathetic efferents that travelled in the cervical sympathetic trunks, synapsed in the superior cervical ganglia, and then passed via the superior laryngeal nerves to the ADN; others were visceral nociceptive afferent fibers. Recordings of efferent activity from the ADN showed three types of activity: Type A associated with cardiac events, and Type B associated with respiratory events, that were both of sympathetic origin, and Type C of parasympathetic origin, that had scattered, random firing patterns. Stimulating sympathetic efferents in the ADN enhanced aortic baroreceptor activity. Carotid occlusion induced pressor responses that were abolished by sectioning the carotid sinus nerves.

In summary, a unique guinea-pig model of the ADN has been developed. ADN-mediated reflexes were studied and it was found that: 1) aortic baroreceptor afferents comprised solely myelinated fibers; 2) unmyelinated fibers were either sympathetic efferents or visceral nociceptive afferents; 3) chemoreceptor afferents were absent; and 4) carotid occlusion induced pressor responses.

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ABBREVIATIONS

ABR: aortic baroreceptor activity

ADN: aortic depressor nerve

CSN: carotid sinus nerve

CST: cervical sympathetic trunk

HR: heart rate

MABP: mean arterial blood pressure

MF: myelinated fiber

NG: nodose ganglion

P_{art}: arterial pressure

P_{sinus}: carotid sinus pressure

RLN: recurrent laryngeal nerve

SCG: superior cervical ganglion

SLN: superior laryngeal nerve

TEM: transmission electron microscope

UF: unmyelinated fiber

XN: vagus nerve

CHAPTER I. INTRODUCTION

1.1. BACKGROUND

Baroreceptors (mechanoreceptors/pressoreceptors) are sensory receptors that form part of the cardiovascular, homeostatic, control mechanisms. The baroreflex control of the cardiovascular system has been well reviewed (Bagshaw, 1985; Brown, 1980; Catton, 1970; Kirchheim, 1976; Kunze, 1985; Paintal, 1973; Pelletier et al., 1972; Sagawa, 1983).

Cardiovascular reflexes play a major part in circulatory homeostasis in health and disease. In diseases like hypertension, heart failure, and myocardial infarction, changes in these neurally mediated mechanisms may strikingly alter the resultant integrated physiologic responses. Increasing our understanding of these changes would improve the treatment of many cardiovascular diseases associated with high morbidity and mortality, and serious socio-economic consequences.

1.1.1. Anatomy of Arterial Baroreceptors

Baroreflex interactions are altered in cardiovascular disease (Bowman and Rand, 1980; Smith and Kampine, 1984). These changes are mediated via 3 groups of afferent sensory receptors: 1) arterial baroreceptors; 2) cardiopulmonary mechanoreceptors; and 3) renal afferents. Arterial baroreceptors are slowly-adapting mechanoreceptors, that respond to mechanical strain in the arterial wall secondary

to changes in blood pressure (Kirchheim, 1976; Brown, 1980; Sagawa, 1983; Bagshaw, 1985). Baroreceptors are not stimulated if the wall of artery is prevented from stretching in response to an increase in arterial blood pressure (Brown, 1980). Also, baroreceptors can be activated if the shape of the sinus wall is distorted, e.g., by a large decrease in arterial blood pressure (Franz, 1967).

There are two groups of baroreceptors which respond to acute changes in the arterial blood pressure: 1) aortic baroreceptors - located in the aortic arch and in brachiocephalic artery where it joins the right subclavian and right common carotid arteries, and 2) carotid sinus baroreceptors - located in the carotid sinuses of the bifurcation of the common carotid arteries (Kirchheim, 1976; Bagshaw, 1985).

Nonidez (1935) reviewed the early literature on the anatomy and physiology of aortic baroreceptors. The aortic (depressor) nerve was described first in rabbits, by Cyon and Ludwig in 1866. In mice, cats, dogs, swine and humans, after a variable course, the aortic nerve afferents pass cephalad in the vagi (Hasimoto and Hirohata, 1936). In rats, aortic baroreceptor afferents either pass centrally only via the vagi, or they pass via the cervical sympathetic nerves to the nodose ganglia and then via the vagi, or they pass via the recurrent laryngeal nerves to

the superior laryngeal nerves and then via the vagi (Smith and Kampine, 1984; Kirchheim, 1976; Bowman and Rand, 1980; Faber and Brody, 1983; Howell and Huber, 1891; Andrew, 1954; Krieger and Marseillan, 1963; McCubbin et al., 1958). However, in rabbits, aortic afferents pass cephalad in well-defined, separate aortic nerves that join the vagi via the superior laryngeal nerves (Alexander and Cuir, 1963; Angell, 1971a,b,c, 1973; Douglas and Ritchie, 1956; Douglas et al., 1956; Kardon et al., 1973; Nonidez, 1935; Numao et al., 1983; Yao and Thoren, 1983). In all species, the aortic nerves are composed of myelinated and unmyelinated fibres, many of which subserve baroreceptors, that pass cephalad to the cardiovascular centers of the medulla. In some species, other aortic-nerve fibers subserve chemoreceptors in the aortic glomi and aortic body (paraganglion aorticum supracardiale [Nonidez, 1935]).

The carotid sinuses are an anatomical enlargement formed at the base of the internal carotid arteries in humans and most other species (Bagshaw, 1985). However, in guinea pigs, the internal carotid arteries are absent and the carotid sinuses are located at the origin of occipital arteries (Heymans and Neil, 1958; Rees, 1967; Bagshaw, 1985). The carotid sinuses are highly elastic and contain less vascular smooth muscle than adjacent portions of the common carotid, external carotid, internal carotid and occipital arteries. They are well innervated by myelinated

and unmyelinated afferent fibers, and by sympathetic efferent fibers originating from the superior cervical ganglia (Bagshaw, 1985; Bock and Gorgas, 1976; Reis and Fuxe, 1968; Shin et al., 1987).

The aortic depressor nerves and the carotid sinus nerves are readily identified in the intact animal by their electrophysiological characteristics - bursts of electrical activity synchronized with cardiac systoles.

1.1.2. Physiology of the Arterial Chemoreceptors

The arterial chemoreceptors are located in: 1) the aortic body, found in the ascending aorta and aortic arch; and 2) the carotid body found at the bifurcation of the common carotid arteries. Afferent fibers from aortic body chemoreceptors run in the vagus nerves in most species. Afferents from carotid body chemoreceptors travel in the carotid sinus nerves. The aortic and carotid bodies are highly vascularized organs, and consist of islands of cells and capillaries termed "glomeruli" which are surrounded by capsules of connective tissue. In the carotid body, each glomerulus or glomus is a miniature carotid body. The carotid body is innervated by myelinated and unmyelinated afferents from the carotid sinus nerves. Also, the carotid body receives sympathetic efferent nerve fibers originating from the superior cervical ganglion (Ezyaguirre and Zapata, 1984; Fidone and Gonzalez, 1986).

Chemoreceptors respond to changes in arterial PO_2, PCO_2, and pH (Eyzaguirre and Zapata, 1984). Stimulation of chemoreceptors induces significant increases in ventilation, respiratory rate and tidal volume (Fizgerald and Lahiri, 1986). Circulatory responses to chemoreceptor stimulation comprise pressor responses resulting from increased sympathetic outflow accompanied by tachycardia or bradycardia (Ezyaguirre et al., 1983). In the resting animal, chemoreceptors discharge in synchrony with respiration as they respond to minor fluctuations in arterial PO_2 (Biscoe and Purves, 1967). Chemoreceptor activity is easily distinguished from baroreceptor activity by its non-pulse-related discharge in hypoxic, hypercapnic, and acidotic animals (Fidone and Gonzalez, 1986).

A number of substances act as chemoreceptor stimulants and increase their activity. Chemoreceptor stimulants include acetylcholine, serotonin, lobeline, neuropeptides, phenyldiguanide, capsaicin, and sodium cyanide (Gernandt, 1946; Paintal, 1977; McQueen, 1977; Ezyaguirre and Zapata, 1984; Ezyaguirre et al., 1983; Fidone and Gonzales, 1986).

1.1.3. Physiology of the Arterial Baroreflexes

Aortic and carotid sinus baroreceptors play a major role in regulating arterial blood pressure. Denervation of the aortic depressor nerves and the carotid sinus nerves induces acute hypertension. Conversely, stimulation of

baroreceptors induces reflex decreases in sympathetic outflow that reduces peripheral vascular resistance and induces bradycardia (Sagawa, 1983; Daly, 1986).

Bilateral occlusion of the common carotid arteries is widely used to investigate reflexes involving carotid sinus baroreceptors. In most species, carotid baroreflexes appear to dominate over aortic baroreflexes (Bagshaw, 1985). Thus, decreasing intra-sinus pressure and increasing aortic pressure by bilateral occlusion of the common carotid arteries induces pressor responses even if the aortic depressor nerves are intact (Kirchheim, 1976). In cats, dogs, rabbits and rats, carotid occlusion induces reflex hypertension and tachycardia (Kirchheim, 1976). However, in guinea pigs, occlusion of the common carotid arteries induces paradoxical depressor responses without significant changes in heart rate (Biggs et al., 1984).

1.1.4. Physiology of the Aortic Baroreceptors

In cats, dogs, rats, and swine, the aortic nerves contain afferent fibers from baroreceptors in the aortic arch and chemoreceptors from the aortic body (Brown et al., 1976; Brown et al., 1978; Daly and Evans, 1953; Douglas and Ritchie, 1956; Douglas et al., 1956; Douglas and Schaumann, 1956; Edis and Shepherd, 1971; Oberg and Thoren, 1973; Pelletier et al., 1972; Schmidt, 1968; Thoren et al., 1977). However, in rabbits, afferents in the aortic

depressor nerves subserve aortic baroreceptors and visceral nociceptive receptors (Douglas et al., 1956; Kardon et al., 1973; Numao et al., 1983). In all species examined, the aortic depressor nerves are comprised of myelinated and unmyelinated nerve fibers (Brown et al., 1976; Brown et al., 1978; Douglas et al., 1956; Oberg and Thoren, 1973; Thoren et al., 1977). Many of these fibers are baroreceptor afferents that pass centrally and eventually enter the cardiovascular centers of the medulla (Daly and Evans, 1953; Smith and Kampine, 1984); some are chemoreceptor afferents that follow a similar path. Orthodromic electrical stimulation of baroreceptor and chemoreceptor afferents induces reflex depressor and pressor responses, respectively. Reflex responses to selective electrical stimulation of myelinated (A- and B-) and unmyelinated (C-) fibers in the aortic nerves have been compared in several species. In rabbits, stimulation induces reflex hypotension and bradycardia mediated by both myelinated and unmyelinated fibers (Douglas and Ritchie, 1956; Douglas et al., 1956; Kardon et al., 1973; Pelletier et al., 1972). In swine, A-fibers subserving baroreceptors mediate depressor responses, and C-fibers mediate pressor responses (Schmidt, 1968). In cats, A- and C-fibers mediate depressor responses, and B-fibers mediate only pressor effects (Douglas and Schaumann, 1956). In dogs, aortic nerve stimulation results in both depressor and

pressor responses. However, selective stimulation of unmyelinated afferents induced pressor responses and bradycardia (Pelletier et al., 1972).

Numao et al. (1983) reported three subsystems in the aortic depressor nerves of rabbits: 1. the aortic baroreceptor A-fiber afferents, 2. C-fiber afferents, both with sympatho-inhibitory function, and 3. the nociceptive C-fiber afferents with sympatho-excitatory function. They showed that the initial pressor component of the aortic baroreflex was evoked by activation of aortic C-fibers, and that it was eliminated selectively by pretreating animals with capsaicin. They confirmed that the aortic depressor nerves of rabbits contained afferent fibers of non-barosensory origin by acute intracisternal injections of opioid peptides (Numao et al., 1983).

It is noteworthy that many researchers have neglected - deliberately or otherwise - to denervate the carotid sinuses in studies of the aortic baroreflexes. There is little direct evidence of how aortic baroreceptor reflexes work in humans, largely because there are no techniques for studying them. Mancia et al. (1977; 1978) and Mancia and Mark (1983) studied the actions of aortic afferents in relation to heart rate, and Guz et al. (1964; 1966) investigated their reflex actions on blood pressure. These workers concluded that these receptors have minimal reflex effects; however, they did not eliminate the effects of

carotid baroreceptors, and it is known that the cardiovascular effects of vagal or aortic-nerve blocks are reduced unless the influence of the carotid-sinuses is eliminated (Guazzi et al., 1962; Oberg and White, 1970; Edis, 1971; Mancia et al., 1973).

There are aortic afferents in the sympathetic innervation - the so-called "sympathetic afferents" (Bishop et al., 1983). Uchida (1975), Pagani (1975), and Malliani and Pagani (1976), who studied aortic receptors with sympathetic afferents, reported that both myelinated and unmyelinated fibers showed rhythmic activity. In Uchida's experiments, myelinated fibers were often silent, but in Pagani and Malliani's experiments, all could be induced to fire in phase with the systolic pulse (at 1 impulse/fiber/beat). Unmyelinated afferents were either silent or discharged in synchrony with respiration and were excited by asphyxia (Uchida, 1975); they appeared functionally similar to myelinated fibers apart from their higher (pressure) threshold (Malliani and Pagani, 1976).

1.1.5. Efferent Innervation of Baroreceptors

In addition to an afferent innervation, the aortic and carotid-sinus pressure-sensing regions receive a rich efferent innervation. Most of this efferent innervation derived from the sympathetic branch of the autonomic nervous system (Nonidez, 1935), although some may be of

parasympathetic origin (Eyzaguirre et al., 1983; Majcherczyk et al., 1980). Little is known of the physiology of the efferent innervation of the baroreceptor regions (Sagawa, 1983). Thus, how impulse traffic changes in response to different pressure inputs, whether the effects of sympathetic efferent discharge result in a net gain or fal[illegible]eceptor sensitivity, and whether the discharges c[illegible]tting without affecting transduction gain is unknown. Differences between baroreceptors subserved by myelinated or unmyelinated afferents further complicate matters.

In 1944, Palme reported that carotid baroreceptor function could be altered by stimulating the efferent sympathetic nerves supplying the carotid bifurcation (Rees, 1967; Reis and Fuxe, 1968), thereby increasing the sensitivity of the carotid baroreceptors and reflexly decreasing arterial blood pressure. This increased sensitivity was assumed to result from a direct action on the baroreceptors, because of the immediacy of the change (Koizumi and Sato, 1969) and the delayed response of the vessel wall (Bagshaw and Peterson, 1972; Brattstrom, 1980, 1981). This conclusion was questioned because of the difficulty of distinguishing between direct excitatory sympathetic effects on receptors and indirect inhibitory sympathetic effects mediated via the vessel walls (Munch et al., 1987). It is now known that the reflex sympathetic

bursts that coincide with diastole are fast (Brattstrom, 1980, 1981), but that vessel-wall responses, which could alter baroreceptor sensitivity, are slow (Heymans and Van den Heuvel-Heymans, 1950; Catton, 1970; Koushanpour and Kelso, 1972; Keith et al., 1974), i.e., vessel wall responses could not alter sensitivity within a single pulse cycle. Also, experiments in vitro with a rat aortic-arch preparation modified to separate receptor from vessel wall effects showed conclusively that noradrenaline increased baroreceptor discharge via alpha-adrenoceptors (Kunze et al., 1984; Kunze, 1985). Thus, the sensitivity of baroreceptors may be controlled by a feedback burst of sympathetic activity during diastole; this would accord the pulse a major role in reflex control of the cardiovascular system.

The notion of sympathetic modulation of baroreceptor activity is not new (Nonidez, 1935). Kunze's brief review (1985) assessed conflicting views of nerve- and drug-induced modulation of baroreceptor activity. Kunze and co-workers (1984, 1985) showed that alpha-adrenoceptor agonists increased baroreceptors' sensitivity, and that beta-adrenoceptor stimulants had no effect. These findings were confirmed by Munch et al. (1987), who showed that "baroreceptor responses to vasoactive agents reflect not only changes in wall dimension but perhaps changes in wall tension and/or the coupling relation between baroreceptors

and smooth muscle structures." The adrenoceptors on these baroreceptors have been characterized pharmacologically: they are blocked by nonspecific alpha-blockers such as tolazoline, phentolamine, and phenoxybenzamine (Landgren et al., 1952; Brattstrom et al., 1980; Brattstrom, 1981; Kunze et al., 1984), and by the specific $alpha_1$-blocker prazosin (Munch et al., 1987; Munch and Brown, 1987). As responses to alpha-adrenoceptor agonists are usually cGMP-mediated and slow, lasting minutes, any rapid pulse-by-pulse modulatory effects of these receptors must occur via another, possibly channel-mediated, mechanism.

Many of the studies cited above refer to carotid baroreceptors. Only Munch & Brown (1987) have shown that orthodromic electrical stimulation of the efferent sympathetic nerves to the aortic arch causes "baroreceptor unloading via vasoconstriction and may cause direct excitation of some units...." Their experiments, in vitro, are the only reported demonstration of sympathetic efferent modulation of aortic arch baroreceptors.

Efferent activity in the carotid sinus nerve has been studied in some detail and can be used as a model of activity in the aortic nerve. However, the role of the efferent fibers to the carotid body is controversial (Eyzaguirre et al., 1983). Biscoe & Sampson (1968), who studied efferent impulses in the cut central end of feline carotid nerve, reported rhythmic activity from sympathetic

fibers that decreased when blood pressure was elevated. Also, they noted nonrhythmic activity of central origin. Similar findings have been reported in rabbits (Laurent and Jager-Barres, 1969) and cats (Neil and O'Regan, 1971); its physiologic significance is unknown (Eyzaguirre et al., 1983).

Pharmacodynamic aspects of the functioning of the sympathetic nervous system have been well reviewed by Vanhoutte et al.(1981). However, more recent observations have indicated a noncatecholaminergic component of sympathetic nerve activity (Hakanson et al, 1986; Lundberg and Hokfelt, 1986; Stjarne and Lundberg, 1986; Edvinsson et al., 1988). In particular, it has been shown that neuropeptide Y co-exists and is co-released with noradrenaline from many sympathetic neurons supplying the cardiovascular system. Three effects of neuropeptide Y have been described: 1) it activates specific neuropeptide Y receptors on the target cell, receptors that are different from those for noradrenaline; 2) it enhances the actions of noradrenaline on the target cell; and 3) neuropeptide Y suppresses the release of noradrenaline from sympathetic nerve endings. The effects of neuropeptide Y on baroreceptors, and the effects of its co-release when the sympathetic nerves to baroreceptors are stimulated, are unknown. ATP is co-released with noradrenaline from many sympathetic post-ganglionic endings (Burnstock, 1986;

Stjarne & Lundberg, 1986). Its effects on baroreceptors are unknown.

1.1.6. Pressure Transduction by Arterial Baroreceptors

Arterial baroreceptors are activated by acute distortions of receptor endings within the walls of the vessels in which they are located. In rats, most of the receptor endings are in the adventitia (Krauhs, 1979; Yates and Chen, 1980). In guinea pigs, the receptors penetrate into the media and some endings can be found adjacent to the intima (Bock and Gorgas, 1976; Shin et al., 1988). The receptors are innervated by myelinated or unmyelinated afferents in rats, rabbits, and dogs (Kirchheim, 1976). In guinea pigs, carotid sinus baroreceptors are innervated by myelinated and unmyelinated nerve fibers (Shin et al., 1988), but the innervation of the aortic baroreceptors is unknown.

Electrical activity is believed to be initiated via strain-dependent mechanotransducer ion channels: increased membrane tension increases the probability of their opening (Sachs, 1986a,b, 1987). Membrane tension is coupled to the channels by cytoskeletal strands that focus strain energy from a large area of the receptor's membrane and enhance its sensitivity. In diseases such as hypertension, the baroreceptors are chronically reset and operate at a higher pressure than in normotension. However, baroreceptors can

also reset in minutes - acute resetting. Sachs (1987) proposed that the viscoelastic properties of the cytoskeletal strands account for acute mechanoreceptor resetting. Others (Coleridge et al., 1984, 1986) have proposed that viscoelastic-wall creep is responsible. Munch and Brown (1985, 1986) felt that the evidence excluded "changes in gross wall mechanics." Although the time course of resetting has been established, its mechanisms are unknown. Chronic resetting, as in hypertension, appears to involve changes in vessel caliber (Krieger, 1987).

The effects of altered ionic composition of the fluid surrounding baroreceptors have been well reviewed by Andresen and Kunze (1987). Receptor discharge is modulated by the extracellular [Na^+]. As even small changes (<5%) depress baroreflexes (Kunze and Brown, 1978), it seems that the receptor membrane potential is highly dependent upon the Na^+ gradient across the membrane. These findings accord with those of Saum et al. (1976), who postulated that an electrogenic sodium pump helps to determine receptor sensitivity and set point. The extracellular [Na^+] indirectly affects blood pressure, through changes in blood volume. Also, cardiac mechanoreceptors reflexly influence the release of atrial natriuretic factor (ANF). Thus, it is reasonable to postulate that extracellular [Na^+] affects baroreceptor function. Interestingly,

calcium entry does not appear to have a role in baroreceptor transduction (Andresen and Kunze, 1987).

It will be clear from the above review that the anatomy, physiology and electrophysiology of the aortic baroreceptors in guinea pigs are unknown. Also, understanding of the relationship between aortic baroreceptor functioning in vivo and its sympathetic modulation is minimal.

1.2. HYPOTHESES

1. That aortic baroreceptor afferent pathways in guinea pigs differ from those of other species.
2. That the sympathetic nervous system modulates the activity of aortic baroreceptors.
3. That a better understanding of the functions and electrophysiological properties of the aortic baroreceptors is important for control and regulation of the arterial blood pressure. This knowledge can be extended for future rational design of therapy to combat or prevent cardiovascular disease.

1.3. SPECIFIC GOALS

Using the guinea pig as animal model:

1. To determine the afferent pathways of the aortic baroreceptors, anatomically and physiologically.

2. To characterize the afferent activity of the aortic baroreceptors (using whole nerve and a few fiber preparations).
3. To characterize the reflex responses to electrical orthodromic stimulation of aortic baroreceptor afferents.
4. To determine the reflex efferent arcs of any aortic baroreflexes.
5. To characterize the receptors that mediate aortic baroreflexes, centrally and peripherally.
6. To describe the histology of the aortic depressor nerves using transmission electron microscope (TEM).
7. To characterize the electrophysiological properties (e.g. stimulation thresholds, conduction velocities, etc.) of individual groups of nerve fibers in the aortic depressor nerves.
8. To compare the reflex effects of orthodromic stimulation of left- vs right- aortic depressor nerve at various stimulus voltages, frequencies and pulse durations.
9. To characterize the reflex responses of orthodromic stimulation of both aortic depressor nerves at various stimulus voltages, frequencies and pulse durations.
10. To establish the effects of different anesthetics (pentobarbital, chloralose and urethane) on the responses to aortic depressor nerve stimulation.

11. To characterize the effects of bilateral adrenalectomy on responses to aortic depressor nerve stimulation.
12. To establish the effects of selective stimulation of chemoreceptors in order to determine whether chemoreceptor afferents are present in the aortic depressor nerves.
13. To compare the effects of aortic depressor nerve stimulation to the effects of carotid sinus nerve stimulation.
14. To determine the blocking temperatures for conduction of myelinated and unmyelinated fibers in the aortic depressor nerves.
15. To establish the effects of differential blocks (anodal block and cooling block) on conduction in aortic baroreceptor afferent fibers.
16. To characterize the effects of anodal block on responses to aortic depressor nerve stimulation.
17. To characterize the effects of cooling block on responses to aortic depressor nerve stimulation.
18. To confirm that the myelinated fibers in the aortic depressor nerves are the only afferents from the aortic baroreceptors.
19. To determine the functions of the remaining unmyelinated fibers in the aortic depressor nerves.
20. To characterize efferent and afferent activities in the aortic depressor nerves.

21. To establish the effects of physiologic stimulation of sympathetic nerves on aortic baroreceptor activity.
22. To determine whether sympathetic activity modulates aortic baroreceptor function.
23. To characterize the effects of sympathetic stimulation pharmacologically using selective agonists and antagonists.
24. To confirm that some of the unmyelinated fibers in the aortic depressor nerves are sympathetic efferent fibers.
25. To confirm that some of the unmyelinated fibers in the aortic depressor nerves are visceral nociceptive afferent fibers.
26. To confirm that there are no chemoreceptor afferents in the aortic depressor nerves.
27. To study any histological changes in the aortic depressor nerves induced by treating animals with capsaicin, using TEM.
28. To determine whether the paradoxical depressor responses to occlusion are due to activation of carotid sinus baroreceptors.
29. To confirm that bilateral occlusion of the common carotid arteries induces pressor responses, and that these are eliminated by sectioning the carotid sinus nerves bilaterally.

30. To characterize the effects of capsaicin treatment on the responses to the carotid occlusion.

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CHAPTER II. GENERAL METHODS

2.1. Animals

Hartley strain guinea pigs, male or female, weighing 250 - 700 g, were purchased from Charles River Canada Ltd., St Constant, Quebec. Animals were shipped in filter-top boxes. They were housed on grids in cages suspended over trays of rock salt, in laminar-flow units (Bioclean, Hazelton System, Aberdeen, MD), at 25°C, on a 12-h dark-light cycle. They were allowed water ad lib, and fed normal chow supplemented with apples.

2.2. Anesthesia

Animals were anesthetized with pentobarbital (30 mg/kg i.p., with additional doses of 5 mg/kg, i.v., p.r.n.), urethane (1.25 -1.75 g/kg, i.p.), or alpha-chloralose (50 mg/kg, i.v., after induction with methoxyflurane). In most experiments, urethane was not used because of its alpha$_2$-adrenoceptor blocking actions (Armstrong et al., 1982; Moore et al., 1984), and its hypotensive effects in guinea pigs. In all animals, the trachea was cannulated (PE240) below the larynx via a midline longitudinal incision on the ventral surface of the cervical region. Animals breathed spontaneously, artificial respiration was applied only if needed. A catheter (PE50) was inserted into a jugular vein for administering drugs.

2.3. Recording of Arterial Blood Pressure and Heart Rate

Arterial blood pressure was monitored via a tapered cannula (PE90) inserted into a carotid artery or sometimes into a femoral artery. The cannula was connected to a Gould P50 or a Statham P23Dd pressure transducer (Hewlett-Packard 7702B physiograph, or a Grass 7D polygraph). Mean arterial blood pressure was determined as the average of the systolic and diastolic pressures. Heart rate was monitored via ECG lead II, or via the systolic pulse (Hewlett-Packard HP8812A rate computer).

2.4. Surgical Preparation

In guinea pigs, a mid-line longitudinal incision was made on ventral surface of the mid-cervical region. The underlying adventitia were separated by blind dissection and the larynx located. The larynx was carefully exposed, and the common carotid arteries as far as the carotid sinuses were carefully cleared bilaterally.

A dissecting microscope (American Optical) was used to aid dissection. As the common carotid arteries were freed from their connective tissues, the superior laryngeal nerves were identified beneath the common carotid arteries. The superior laryngeal nerves merged into the nodose ganglia of the vagi and formed internal and external branches to the larynx. The main trunks of the superior laryngeal nerves laid upon the superior cervical ganglia.

The aortic depressor nerves were identified as a pair of extremely fine nerves running close to, but separate from, the vagi and the cervical sympathetic trunks along their whole length in the neck to the aortic arch. They joined the main trunk of the superior laryngeal nerves adjacent to its bifurcation into the internal and external branches of the superior laryngeal nerves in most animals (see Fig. 3-1 in Chapter III). However, there were exceptions: sometimes the aortic depressor nerves joined the superior laryngeal nerve at a point along the main trunk of the nerve, or near the nodose ganglia, or even entered the nodose ganglia directly. After identification, the aortic depressor nerves were carefully freed from the surrounding tissues. It was confirmed that they were the aortic depressor nerves by placing them on electrodes (described later) and recording signals synchronized with cardiac systoles. This procedure was performed in every guinea pig before any studies were made of sectioning, recording and stimulating the aortic depressor nerves.

Guinea pigs have no internal carotid arteries (Rees, 1967) (see Fig. 6-1 in Chapter VI) and their common carotid arteries divide into the external carotid and the occipital arteries. The carotid sinus was isolated near the base of the occipital artery. The carotid sinus nerve was identified as it emerged from the carotid sinus/carotid body region and merged into the glossopharyngeal (IX)

nerve, and dissected free from the surrounding tissues. The carotid sinus nerve is extremely fine and its length is less than 2 cm.

The following nerves were also located and visualized: the recurrent laryngeal nerves on both sides of the trachea, the vagi, the nodose ganglia of the vagi, the cervical sympathetic trunks, and the superior cervical ganglia of the cervical sympathetic trunks. The cervical regions were flooded with mineral oil to insulate the electrodes, to reduce current spread, and to prevent drying of the tissues.

2.5. Equipment for Nerve Recording

A bio-electric amplifier system was built and used for recording whole-nerve and few-fiber activities. The amplifier system (shown in block diagram, Appendix A-1) consists of: 1) a pair of bipolar platinum recording electrodes; 2) a high-input impedance differential amplifier (gain = 20X; Appendix A-2); 3) a main instrumentation amplifier (AD522) (gain = 1X, 2X, 5X, 10X, 50X, 100X, 500X, or 1000X; Appendix A-3); 4) a 60 Hz notch filter to reduce 60 Hz noise from the AC power supply by 100X; 5) a full-wave precision rectifier (Appendix A-4), with high-input impedance, and X1 gain; 6) an integrator (Appendix A-5) with a reset timer (0.1, 0.25, 0.5, 1, 2, 5 s), which integrates the rectified signals for

quantification; and 7) several voltage followers to reduce the output resistance of the main amplifier and the integrator, and to prevent any possible loss of the amplified and integrated signals

Amplified and integrated signals were displayed on a Tektronix 5113 dual-beam storage oscilloscope (band width setting: 0.1 - 1 KHz), and permanent records were made on Polaroid film (Type 667) using a Tektronix C-5A oscilloscope camera.

2.6. Nerve Recording and Nerve Stimulation

These will be discussed in detail in the following chapters (Chapter III to VI).

The other experimental procedures, such as differential block (including cold block and anodal block), and morphological studies (gross anatomy and transmission electron microscopy, etc.) will be described in detail in the relevant chapters. Drugs used in the each group of experiments will be listed in each chapter.

2.7. Experimental Design and Statistical Analysis

A minimum of four replicates was obtained in each series of experiments. Data are expressed as mean $\pm$ SE. Student's t test, one-way analysis of variance, and Duncan's multiple comparison test were used to examine data. Significance was assumed at the 5% level.

2.8. REFERENCES

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CHAPTER III. THE ANATOMY OF THE AORTIC DEPRESSOR NERVES - THE AFFERENT PATHWAYS OF THE AORTIC BARORECEPTOR FIBERS IN GUINEA PIGS*.

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3.1. INTRODUCTION

Arterial baroreceptors mediate cardiovascular reflexes that induce acute circulatory changes (Smith and Kampine, 1984). Most of these baroreceptors are in the walls of the aortic arch and carotid sinus. Aortic baroreceptors are located at the origin of the left common carotid artery and left subclavian artery, and in the brachiocephalic artery at its bifurcations into the right subclavian and right common carotid artery (Kirchheim, 1976).

In most species (including rabbit, cat, dog and human), afferents from aortic baroreceptors travel centrally in the main trunk of the vagus nerve or in separate aortic depressor nerves adjacent to the vagi (Bowman and Rand, 1980; Faber and Brody, 1983). However, in rats, the pathways of aortic afferents can follow four routes: 1) via the vagus nerve; 2) via the cervical sympathetic trunk to the nodose ganglion, and hence centrally via the vagus; 3) via separate aortic depressor nerves; and 4) via the recurrent laryngeal nerve to the superior laryngeal nerve through the communicating branch, and then centrally via the vagus (Faber and Brody, 1983; Howell and Huber 1891; Andrew, 1954; Krieger and Marseillan, 1963; McCubbin and Masson, 1958). These pathways differ from rat to rat and between sides in an animal.

During a preliminary investigation of upper airway reflexes in guinea pigs, we observed that central stimulation of one or both superior laryngeal nerves induced reflex hypotension. Recordings of afferent activity from the superior laryngeal nerves revealed bursts of firing that were synchronous with cardiac systole. We now report experiments in which we traced the afferent pathways of aortic baroreceptor fibers in guinea pigs by three approaches: dissection, electrical stimulation and electrophysiologic studies.

3.2. METHODS

Guinea pigs of either sex, weighing 440 ± 5.6 g, were anesthetized with urethane (1.25-1.75 g/kg body weight, intraperitoneally). The trachea was cannulated and animals breathed room air spontaneously. Arterial blood pressure was monitored from a femoral artery via a tapered PE90 catheter (Statham P23Dd pressure transducer; Hewlett-Packard 7454A physiograph). Heart rate was monitored via the electrocardiogram (lead II) using a heart-rate monitor (HP8812A). Drugs were given via a PE50 catheter inserted into a jugular vein.

3.2.1. Preparation of Animals

We used a dissecting microscope to help isolate the following nerves: both superior laryngeal nerves adjacent

to the larynx, both aortic depressor nerves at the point where they joined the superior laryngeal nerves, and the recurrent laryngeal nerves on both sides of the trachea. The nerves were cut, and bipolar platinum electrodes, connected via an isolation unit to a Grass S44 stimulator, were applied to their central ends. Stimulation (10 V, 10 Hz, 1 ms) was applied for 30 s. After each period of stimulation, 10-15 min were allowed for arterial blood pressure and heart rate to stabilized and return to baseline values before further stimulation was applied. Peripheral nerve activity was recorded from the distal ends of the cut nerves with a bioamplifier built in our laboratory. Amplified and integrated signals were displayed on a Tektronix 5113 dual-beam oscilloscope; photographs were taken as permanent records. The regions around the electrodes were flooded with mineral oil to insulate the electrodes and prevent drying of the tissues.

Central electrical stimulation of the superior laryngeal nerves, aortic depressor nerves and recurrent laryngeal nerves was performed. Changes in arterial blood pressure and heart rate were recorded. In some experiments, stimulation was repeated after giving atropine, mepyramine, cimetidine, propranolol, hexamethonium, or clonidine, or after bilateral vagotomy, or extirpation of the nodose ganglia. In other experiments, activity from the recurrent and superior

laryngeal nerves was recorded. In other experiments, afferent activity from the aortic depressor nerves was recorded before and after giving phenylephrine or sodium nitroprusside. At the end of each experiment, necropsy was performed and the course of the aortic depressor nerves to the region of the aortic arch was traced.

3.2.2. Statistical Analysis

A minimum of 4 replicates was obtained in each series of experiments. All values are expressed as the mean ± S.E. Groups of data were compared using paired Student's *t* tests. Significance was assumed at the 5% level.

3.2.3. Drugs

Drugs used were atropine sulfate (Fluka AG, Buchs SG, Switzerland), cimetidine (Smith Kline and French Canada, Mississauga, Ont.), clonidine (gift from Boehringer Ingelheim (Canada), Burlington, Ont.), hexamethonium (K & K Laboratories, Plainview, N.Y., U.S.A.), mepyramine (Rhone-Poulenc Pharma Inc., Montreal, PQ.), phenylephrine (USP), propranolol hydrochloride (Ayerst Laboratories, Montreal, PQ.), and sodium nitroprusside (Hoffman-La Roche, Vaudreuil, PQ.).

3.3. RESULTS AND DISCUSSION

After preparing the animals, they were left for 30 min

to stabilize. Only animals with stable baseline values of arterial blood pressure, heart rate and respiration were used.

Bilateral central electrical stimulation of the superior laryngeal nerves induced reflex hypotension without altering heart rate significantly. Stimulation reduced mean arterial pressure from 39 ± 2 to 28 ± 2 mm Hg ($p < 0.01$, $n = 12$) and heart rate fell from 228 ± 9 to 218 ± 8 beats/min ($p > 0.05$, $n = 12$). The depressor responses were abolished by hexamethonium (1 mg/kg, i.v.) or clonidine (0.2 mg/kg, i.v.), and eliminated by extirpating both nodose ganglia, indicating that they were mediated via vagal afferents, and that autonomic ganglia and central alpha$_2$-adrenoceptors were involved in responses. However, they were unaffected by atropine (0.1 mg/kg), mepyramine (0.5 mg/kg), cimetidine (1 mg/kg), and propranolol (1 mg/kg) or by bilateral mid-cervical vagotomy. Thus, muscarinic receptors, H_1 or H_2 histaminergic receptors, and beta-adrenoceptors are not involved in the depressor responses, nor are they mediated via vagal efferents.

Results of bilateral central stimulation of the aortic depressor nerves were similar to those produced by superior laryngeal nerve stimulation. Mean arterial blood pressure fell from 53 ± 2 to 38 ± 4 mm Hg ($p < 0.01$, $n = 5$) and the heart rate fell from 274 ± 19 to 266 ± 17 beats/min ($p > 0.05$, $n = 5$). Bilateral stimulation of the recurrent

laryngeal nerves had no effect on blood pressure or heart rate.

Recordings from the intact superior laryngeal nerves revealed bursts of activity in synchrony with cardiac systole. This activity was unaltered after the internal and external branches of the superior laryngeal nerves to the larynx were cut, but was abolished by sectioning the ipsilateral aortic depressor nerve. Similar bursts of activity were recorded from the aortic depressor nerves (Fig. 3-1 and 3-2). Phenylephrine (50-100 μg/kg, i.v.) significantly increased arterial blood pressure and aortic depressor nerve activity (Fig. 3-3), and induced reflex bradycardia. Sodium nitroprusside (100 μg/kg/min, i.v.) decreased arterial blood pressure and activity in the aortic depressor nerve (Fig. 3-4). No recordings from the recurrent laryngeal nerves showed phasic activity.

Tracing the aortic depressor nerves at necropsy showed that usually they left the superior laryngeal nerves and ran caudally toward the heart as separate, fine nerves adjacent to, but distinct from, the cervical sympathetic trunks and the vagus nerves. They reached the aortic arch at the origin of the left common carotid artery on the left side, and at the bifurcation of the brachiocephalic artery into the right subclavian and common carotid arteries on the right (Fig. 3-5).

Our results show that, in guinea pigs, aortic-baroreceptor afferents form physiologically and anatomically separate aortic depressor nerves. In the mid-cervical region, the aortic depressor nerves are readily distinguishable from the recurrent laryngeal nerves, the vagi, and the sympathetic trunks, as separate fine nerves running cephalad to join the ipsilateral superior laryngeal nerves. This pattern of innervation was consistent in 11 of the 12 guinea pigs examined - unlike findings in rats, in which the aortic baroreceptor fibers can form part of the vagi, the recurrent laryngeal nerves, and the sympathetic trunks, or appear as separate aortic depressor nerves. (In the 12th guinea pig, we were unable to demonstrate or find the aortic depressor nerves).

We conclude that: 1) the aortic depressor nerves in guinea pigs are readily accessible; and 2) reflex responses to central stimulation of the aortic depressor nerves are mediated via autonomic ganglia and central alpha$_2$-adrenoceptors.

3.4. ACKNOWLEDGEMENTS

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H.S. Sun is a Research Fellow of the Alberta Heritage Foundation for Medical Research.

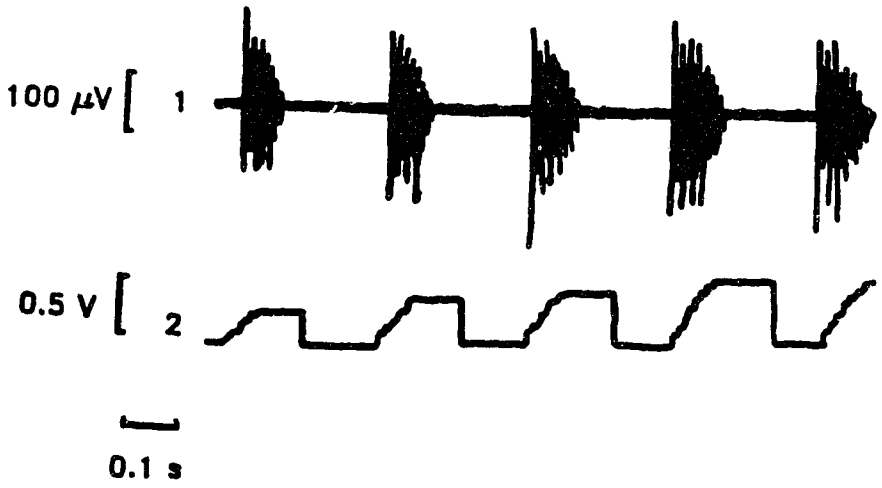

Figure 3-1. Typical bursts of electrical activity in an aortic depressor nerve (upper trace) and the integrated signals (lower trace). Mean arterial blood pressure = 50 mm Hg.

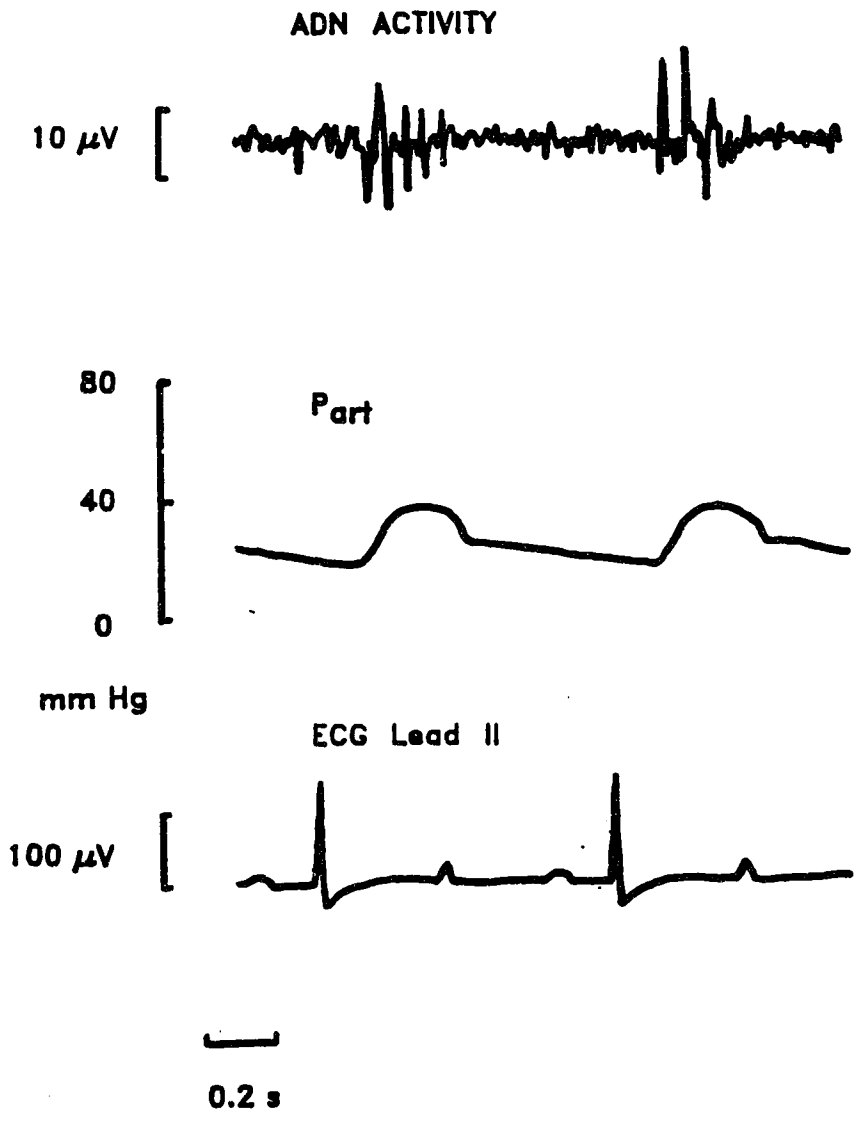

Figure 3-2. Signals showing synchrony among aortic depressor nerve activity (upper trace), arterial blood pressure (middle trace), and lead II of the electrocardiogram (lower trace).

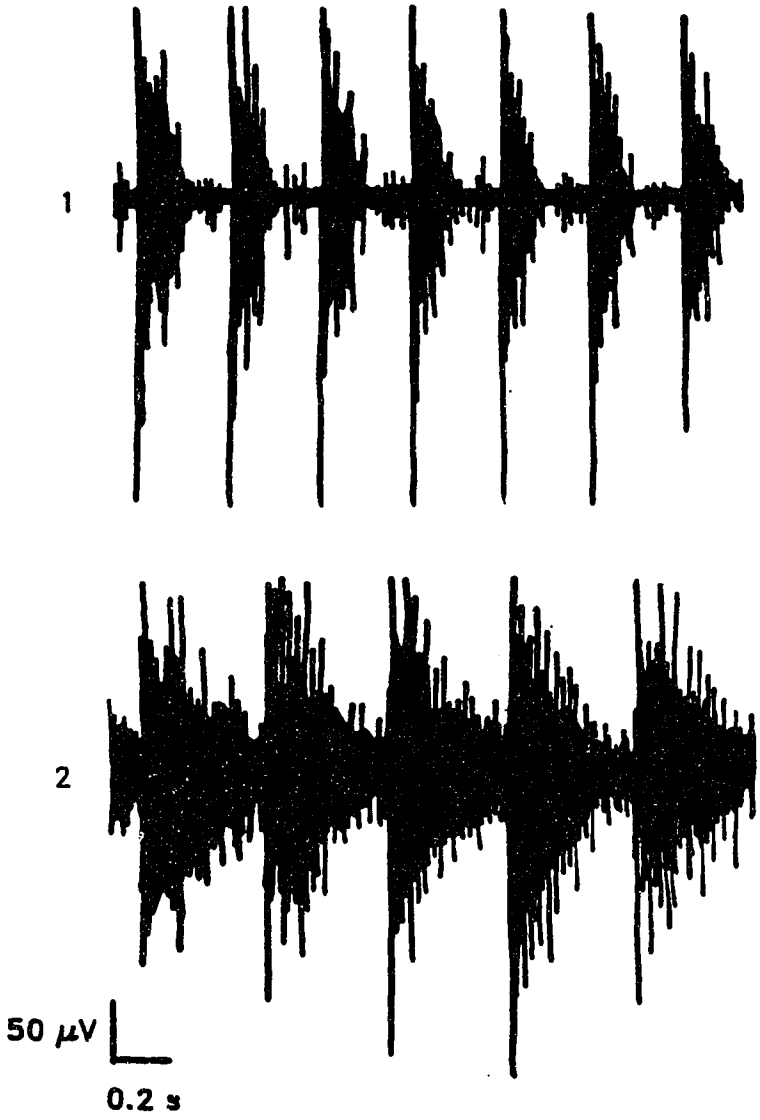

Figure 3-3. Increases in electrical activity in the aortic depressor nerve after raising arterial pressure with phenylephrine (100 μg/kg). Upper trace: control, MABP = 60 mm Hg. Lower trace: after phenylephrine, MABP = 85 mm Hg.

Figure 3-4. Decreases in electrical activity in the aortic depressor nerve after lowering arterial blood pressure with sodium nitroprusside (100 μg/kg/min). Upper trace: control; MABP = 60 mm Hg. Lower trace: after sodium nitroprusside; MABP = 30 mm Hg.

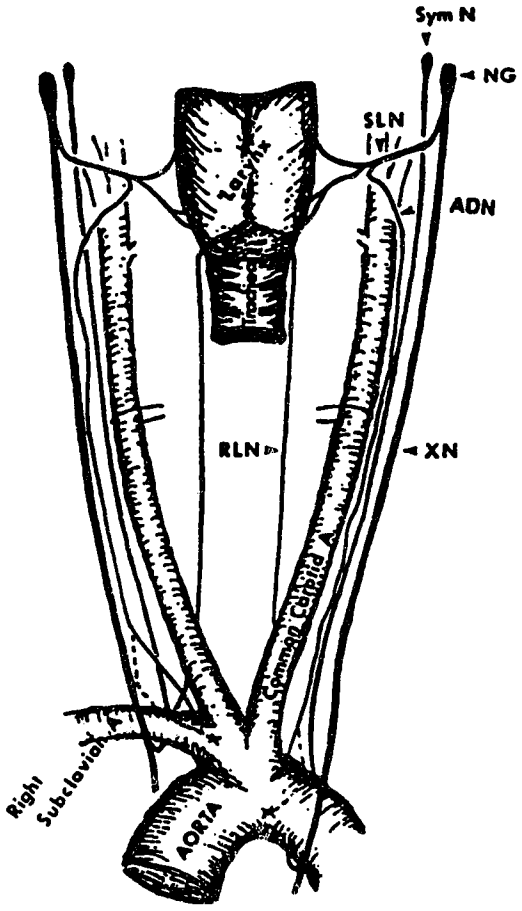

Figure 3-5. Drawing of nerves in the cervical region of the guinea pig: AN, aortic depressor nerve; NG, nodose ganglion; RLN, recurrent laryngeal nerve: SLN, superior laryngeal nerve; Sym N, sympathetic nerve; and XN, vagus nerve. Scale approximately 2 X normal.

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CHAPTER IV. AORTIC BAROREFLEXES IN GUINEA PIGS.*

* The results of this chapter have been published in abstract form (SUN, H.S., and BIGGS, D.F. 1988. "Aortic baroreflex in guinea pigs." FASEB J. 2: A384, Abstract #515.)

4.1. INTRODUCTION

The aortic depressor nerves form part of the sensory system that mediates homeostatic cardiovascular responses to acute changes in arterial blood pressure and anoxia. In cats, dogs, rats, and swine, the aortic depressor nerves comprise afferents from baroreceptors in the aortic arch and chemoreceptors from the aortic body (Brown et al., 1976; Brown et al., 1978; Daly and Evans, 1953; Douglas and Ritchie, 1956; Douglas et al., 1956; Douglas and Schaumann, 1956; Edis and Shepherd, 1971; Oberg and Thoren, 1973; Pelletier et al., 1972; Schmidt, 1968; Thoren et al., 1977). However, in rabbits, aortic depressor nerve afferents are derived mainly from baroreceptors (Douglas et al., 1956; Kardon et al., 1973). In all species examined, the aortic depressor nerves contain myelinated and unmyelinated nerve fibers (Brown et al., 1976; Brown et al., 1978; Douglas et al., 1956; Oberg and Thoren, 1973; Thoren et al., 1977). Many of these fibers are baroreceptor afferents that pass centrally and eventually enter the cardiovascular centers of the medulla (Daly and Evans, 1953; Smith and Kampine, 1984), and others are chemoreceptor afferents that follow a similar path. Investigators have compared the reflex responses to selective (orthodromic) electrical stimulation of myelinated (A- and B-fibers) and unmyelinated (C-fibers) afferents in the aortic depressor nerves. In rabbits,

stimulation induced reflex hypotension and bradycardia mediated by both myelinated and unmyelinated afferent fibers (Douglas and Ritchie, 1956; Douglas et al., 1956; Kardon et al., 1973; Pelletier et al., 1972). In swine, A-fibers from aortic baroreceptors mediated depressor, and C-fiber afferents pressor responses (Schmidt, 1968), and in cats, both A- and C-fibers mediated depressor responses, but, B-fibers mediated only pressor effects (Douglas and Schaumann, 1956). In dogs, Edis and Shepherd (1971) noted depressor and pressor responses to aortic depressor nerve stimulation. However, selective stimulation of unmyelinated afferents induced pressor responses and bradycardia (Pelletier et al., 1972).

Sun and Biggs (1986; 1987) described the anatomy and physiology of the aortic depressor nerves in guinea pigs. They reported that the afferent fibers from aortic baroreceptors emerged from the aortic arch, travelled centrally as separate fine aortic depressor nerves, distinct from the vagi, the cervical sympathetic trunks and the recurrent laryngeal nerves, and finally merged into the ipsilateral superior laryngeal nerves (Fig. 4-1). However, the types of axons present in the aortic depressor nerves and the effects of stimulating them selectively are unknown. We report here the findings from experiments in which we determined: 1) the types of nerve fibers present in the aortic depressor nerves; 2) the physiological

characteristics of the axons; and 3) the reflex effects of selectively stimulating myelinated and unmyelinated afferents in these nerves.

4.2. METHODS

4.2.1. Animals

Male or female, Hartley-strain guinea pigs (Charles River, St Constant, Quebec, Canada), weighing 350-600 g, were used. Animals were housed in laminar-flow units, (Bioclean, Hazleton, MD) on grids, in cages suspended over trays of rock salt, at 25°C on a 12-h dark/light cycle. They were allowed water ad lib and fed normal chow supplemented with apples.

4.2.2. Animal Preparation

Animals were anesthetized with pentobarbital (30 mg/kg i.p., with additional doses of 5 mg/kg, i.v., p.r.n.). Their tracheas were cannulated, and they breathed room air spontaneously. Arterial blood pressure was monitored via a tapered cannula (PE90) inserted into a carotid artery and a Statham P23Dd pressure transducer (HP8805B carrier amplifier, Hewlett-Packard 7702B physiograph), and mean arterial blood pressure was determined from the systolic and diastolic pressures. Heart rate was determined from the systolic peaks in the arterial blood pressure signal via a heart-rate meter (HP8812A rate computer). Drugs were

administered via a catheter (PE50) inserted into a jugular vein.

A dissecting microscope was used to help visualize and isolate: both superior laryngeal nerves adjacent to the larynx, both aortic depressor nerves at the point where they join the superior laryngeal nerves, both carotid sinus nerves close to the carotid sinuses, and the vagi in the mid-cervical region.

4.2.3. Experiments

4.2.3.1. Histological Studies

The characteristics of the nerve fibers in the left aortic depressor nerves of 2 guinea pigs were examined. Tissues were fixed with conventional techniques (Bancroft and Stevens, 1982). Following induction of anesthesia, 1 cm of the left aortic depressor nerve was exposed in the mid-cervical region, and 3 ml of cold (4°C) freshly prepared glutaraldehyde solution (3% in phosphate buffer, pH 7.2) was poured into the area and left in contact with the nerve for 10 min. Then, the nerve was dissected free, and fixed in more glutaraldehyde solution for 3 h. After postfixing in 1% osmium tetroxide solution for 2 h, and dehydration with graded ethanols, the tissue was embedded in LX-112 epoxy resin, and sectioned with a Reichert ultramicrotome. Ultrathin sections (60-90 nm) were cut with diamond knives, mounted on uncoated copper grids,

stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (Siemens Elmiskop 102). The cross sectional area of individual fibers was quantified with an ImagePlus^{R} System.

4.2.3.2. Electrophysiological Measurements

Measurements of stimulus thresholds and conduction velocities were used to distinguish different types of nerve fibers in the aortic depressor nerves. The left aortic depressor nerve was exposed and carefully dissected free for 2-3 cm. Single shocks (0.05 ms duration) were applied caudally via bipolar platinum electrodes. Compound action potentials were recorded from a second set of electrodes placed about 2 cm more centrally. Evoked potentials were recorded with an amplifier system (Sun and Biggs, 1986; 1987) built in our laboratory. The stimulus and nerve potentials were displayed on a Tektronix 5113 dual-beam oscilloscope, and permanent records were made on Polaroid film (Type 667) with a Tektronix C5A oscilloscope camera. Nerve fiber thresholds were determined by increasing the voltage applied in 1-V steps. The time interval between the stimulus artifact and each component of the compound action potential and the distance between the stimulating and recording electrodes were measured. Conduction velocity was calculated by dividing the distance by the time interval (Hurch, 1939).

4.2.3.3. Effects of Anesthetics and Sectioning the Barosensory Nerves on Arterial Blood Pressure and Heart Rate

Baseline values of arterial blood pressure and heart rate in guinea pigs under pentobarbital (30 mg/kg, i.p.), urethane (1.5 g/kg, i.p.) and chloralose (50 mg/kg, i.v., after induction with methoxyflurane) anesthesia were compared. The effects of sequentially sectioning the barosensory nerves on arterial blood pressure and heart rate were noted in these animals. First, the aortic depressor nerves were sectioned and then the carotid sinus nerves, and, in separate experiments, vice versa.

4.2.3.4. Orthodromic Electrical Stimulation of the Aortic Depressor Nerves

The nerves were cut distally and laid upon bipolar platinum stimulating electrodes connected via an isolation unit (SIU5) to a Grass S44 stimulator. The area around stimulating electrodes was flooded with mineral oil to insulate the electrodes and to prevent drying of the tissues. Stimuli were applied at various voltages, frequencies, and pulse durations for 30 s, in increasing order of stimulus parameters. Blood pressure and heart rate were monitored. After each period of stimulation, 10-15 min were allowed for arterial blood pressure and heart rate to return to baseline values and to stabilize

before the next period of stimulation. Most experiments were performed after bilateral aortic and carotid denervation. The responses to left, right, and bilateral aortic depressor nerve stimulation were compared.

4.2.3.5. Cold Block

This procedure can be used to selectively inhibit conduction via myelinated afferents (Franz and Iggo, 1968; Paintal, 1965; Paintal, 1967; Paintal, 1973). A double folding hook made from an 18-G needle was placed on the left aortic depressor nerve near the superior laryngeal nerve. About 5-6 mm of the aortic depressor nerve was laid on the hook, and mineral oil was applied to the area. Cooled water at 0-15°C (20 ml/min) was pumped through the cooling hook by a MasterFlex pump. The temperatures that blocked the myelinated and the unmyelinated fibers were determined by monitoring responses to orthodromic stimulation of the aortic depressor nerve. Arterial blood pressure and heart rate were monitored throughout this procedure.

4.2.3.6. Anodal Block

Conduction in myelinated fibers can be selectively blocked by polarizing electrodes (Guz and Trenchard, 1971; Kardon et al., 1975; Paintal, 1973). Three pairs of electrodes were placed along the length of the left aortic

depressor nerve. The most caudal pair were the platinum stimulating electrodes connected to a Grass SD9B stimulator. A second pair of electrodes, placed more centrally, applied a polarizing current (anode distal) via a Grass SIU5 stimulus isolation unit connected to the DC output of a Grass S44 stimulator. The third pair placed most centrally served as the recording electrodes. Nerve activity was recorded as before.

4.2.3.7. Effects of Anesthetics on Responses to Stimulation of the Aortic Depressor Nerves

The effects of aortic depressor nerve stimulation were compared in animals anesthetized with pentobarbital (30 mg/kg, i.p.), urethane (1.5 g/kg, i.p.), or chloralose (50 mg/kg, i.v., after induction with methoxyflurane), after sectioning the aortic depressor and carotid sinus nerves bilaterally. Both aortic depressor nerves were stimulated (10 Hz, 1 ms) simultaneously via bipolar platinum electrodes at various voltages for 30 s. Arterial blood pressure and heart rate were monitored throughout.

In some animals under pentobarbital anesthesia, chloralose (50 mg/kg, i.v.) was injected, and both aortic depressor nerves were stimulated (10 v, 10 Hz, 1 ms) for 30 s, while arterial blood pressure and heart rate were monitored.

4.2.3.8. Adrenalectomy

In 4 animals under pentobarbital anesthesia, bilateral adrenalectomies were performed using lateral abdominal incisions without entering the peritoneal cavity. The incisions were closed with simple continuous sutures. After sectioning both aortic depressor and carotid sinus nerves, the aortic depressor nerves were stimulated (10 Hz, 1 ms) at various voltages for 30 s, while arterial blood pressure and heart rate were monitored.

4.2.3.9. Selective Stimulation of Chemoreceptors

In 8 animals under pentobarbital anesthesia, after sectioning both the aortic depressor and carotid sinus nerves, sodium cyanide (0.1 mg/kg), phenylbiguanide (500 μg/kg), lobeline (1 mg/kg) and serotonin (50 μg/kg) were injected intra-aortically via a second cannula (PE50) inserted down the left carotid artery into the root of the ascending aorta. Electrical activity from the aortic depressor nerves was recorded before, during, and after injection of these chemoreceptor stimulants.

In some experiments, low-intensity (2-4 V), high-frequency (100-150 Hz), or high-intensity (8-15 V), low-frequency (5-15 Hz) stimuli, of 0.1 ms duration were applied for 20 s to the nerves to try and stimulate baroreceptor and chemoreceptor afferents selectively (Douglas and Schaumann, 1956; Edis and Shepherd, 1971;

Schmidt, 1968). Arterial blood pressure and heart rate were monitored as before.

4.2.3.10. Stimulation of the Carotid-Sinus Nerves

The carotid sinus nerves and aortic depressor nerves were sectioned bilaterally in pentobarbital-anesthetized animals. Bipolar platinum electrodes were applied to central ends of both carotid sinus nerves. The nerves were stimulated (10 Hz, 1 ms) orthodromically with various voltages for 30 s, and arterial blood pressure and heart rate were monitored.

4.2.4. Drugs

Drugs used were angiotensin amide (Hypertensin-CIBA^{R}, CIBA, Dorval, PQ., Canada), atenolol (Sigma, St Louis, USA), alpha-chloralose (Fisher, Fair Lawn, New Jersey, USA), sodium cyanide (General Intermediates of Canada, Edmonton, AB., Canada), clonidine (gift from Boehringer Ingelheim (Canada), Burlington, Ont.), hexamethonium bromide (K & K Laboratories, Plainview NY, USA), 5-hydroxytryptamine (serotonin) (Sigma, St Louis, USA), lobeline (Sigma, St Louis, USA), methoxyflurane (Penthrane^{R}, Abbott Laboratories, Montreal, PQ., Canada), sodium pentobarbital (Euthanyl^{R}, 3 ml in 117 ml distilled water, M.T.C. Pharmaceuticals, Mississauga, Ont., Canada), phenylbiguanide (Aldrich Chemical Company, Inc., Milwaukee,

USA), phenylephrine (USP), prazosin hydrochloride (gift of Pfizer, Kirkland, PQ., Canada), propranolol hydrochloride (Sigma, St Louis, USA), and yohimbine hydrochloride (Sigma, St Louis, USA).

4.2.5. Statistical Analyses

A minimum of four replicates was obtained in each series of experiments. Values are expressed as mean ± SE. Differences were examined with Student's t test, and significance was assumed at the 5% level. Also, one-way analysis of variance and Duncan's multiple comparison test were used to compare data in Tables 4-2 to 4-4.

4.3. RESULTS

4.3.1. Histology of the Aortic Depressor Nerves

Electron microscopy of cross sections of the aortic depressor nerves from guinea pigs revealed that both myelinated and unmyelinated nerve fibers were present (Fig. 4-2 and 4-3). Each aortic depressor nerve contained about 180-190 myelinated and 540-720 unmyelinated axons. The ratio of myelinated to unmyelinated axons was 1 : 3-4. Myelinated fibers were scattered throughout the sections; by contrast, unmyelinated fibers were grouped together in bundles sheathed by Schwann cells. The cross-sectional areas of the myelinated fibers ranged from 9 - 2175 square microns (mean = 424.6 ± 20.5, n = 206) (Fig. 4-4), and they

appeared to have a bimodal distribution. The areas of unmyelinated fibers ranged from 0.004 - 0.257 square microns (mean = 0.074 ± 0.002, n = 1006) (Fig. 4-5).

4.3.2. Measurements of Thresholds and Conduction Velocities

Measurements of conduction velocity confirmed the presence of at least two groups of nerve fibers in the aortic depressor nerves of guinea pigs. The first potentials that could be distinguished from the stimulus artifact were evoked at low thresholds (1.2-5.0 V; mean = 3.0 ± 0.7, n=18). Their conduction velocities ranged from 1.3 to 12.5 m/s (mean = 5.97 ± 0.74, n=18). These potentials appeared to arise from myelinated axons (Fig. 4-6). At higher voltages (2.0-8.0 V; mean = 5.7 ± 0.8, n=31), another group of more slowly propagated potentials were evoked. They had lower conduction velocities (0.25-0.94 m/s; mean = 0.53 ± 0.03, n=31), and appeared to represent propagation via unmyelinated afferents (Fig. 4-7). All compound action potentials observed were all-or-none in nature. In three experiments, some potentials evoked appeared to be obscured by the stimulus artifact, suggesting the presence of a group of nerve fibers with higher conduction velocities. However, the length of aortic depressor nerve that could be cleared for recording (2-3 cm) was too short to discriminate them.

4.3.3. Effects of Anesthetics and Sectioning Barosensory Nerves on the Arterial Blood Pressure and Heart Rate

Baseline values for mean arterial blood pressure and heart rate in guinea pigs under pentobarbital, urethane, or chloralose anesthesia are summarized in Table 4-1. The effects of sequential sectioning of the barosensory nerves (aortic depressor and carotid sinus nerves) on arterial blood pressure and heart rate in guinea pigs under the three anesthetics are set out in Tables 4-2 and 4-3. In the first series of experiments, both aortic depressor nerves were sectioned and then the carotid sinus nerves were cut. Changes were similar with each anesthetic: sectioning the aortic depressor nerves induced an initial rise in arterial blood pressure that fell slightly and stabilized at levels above baseline. After the carotid sinus nerves were sectioned, a further rise in arterial blood pressure occurred that also fell slightly and stabilized at levels further above baseline. Arterial blood pressure after sectioning the aortic depressor nerves increased significantly above baseline except in pentobarbital anesthetized animals; sectioning the carotid sinus nerves induced a further significant increase. No significant changes in heart rate were detected after any nerve sections.

In a second series of experiments under pentobarbital anesthesia, the carotid sinus nerves were sectioned first

and then the aortic depressor nerves were cut. Results are summarized in Table 4-4. The effects of the two series of sequential sections on arterial blood pressure are compared in Tables 4-2 and 4-3. Arterial blood pressure was significantly increased only after the carotid sinus nerves were sectioned. Heart rate was unchanged (Table 4-4).

4.3.4. Stimulation of Aortic Baroreceptor Afferents

In 14 pentobarbital anesthetized animals, the aortic depressor nerves were sectioned, but the carotid sinus nerves were left intact. After allowing 15 min for animals to stabilize, mean arterial blood pressure and heart rate were 69 $\pm$ 1 mm Hg and 267 $\pm$ 6 beats/min, respectively.

Orthodromic electrical stimulation (10 V, 10 Hz, 1 ms, 30 s) of afferents in the aortic depressor nerves induced hypotension and slight bradycardia. Mean arterial pressure fell from 69 $\pm$ 2 to 54 $\pm$ 2 mm Hg ($p<0.01$) and heart rate fell from 250 $\pm$ 5 to 239 $\pm$ 4 beats/min ($p<0.01$). The hypotensive responses were blocked by hexamethonium (1 mg/kg, i.v.), clonidine (0.2 mg/kg, i.v.) or prazosin (0.1 mg/kg, i.v.), but were unaffected by yohimbine (0.5 mg/kg, i.v.). To prevent masking by hexamethonium or prazosin's hypotensive effects, arterial pressure was restored to baseline levels by infusing angiotensin (1 μg/kg/min). Findings were similar in these experiments. The relationship between the stimulus voltage and the responses

was determined: maximal falls of arterial blood pressure occurred at 6 V (Fig. 4-8).

In 4 animals under pentobarbital anesthesia, both the aortic depressor and carotid sinus nerves were sectioned. Mean arterial pressure rose from 63 ± 3 to 73 ± 4 mm Hg ($p<0.05$) after bilateral aortic denervation. This increase lasted about 5 min before returning to baseline. Heart rate was unchanged. In these animals, sectioning both carotid sinus nerves increased mean arterial blood pressure from 65 ± 4 to 91 ± 5 mm Hg ($p<0.05$), without changing heart rate significantly. Pressure remained elevated for about 10 min, before falling slowly to stabilize at 74 ± 3 mm Hg. Maximal falls of arterial blood pressure occurred at 3 V (Fig. 4-8) in these "deafferented" animals. Responses to aortic depressor nerve stimulation at different stimulus voltages, frequencies and pulse durations are shown in Fig.4-9, 4-10 and 4-11. Responses to stimulation of the left, the right, or both aortic depressor nerve(s) were similar (Fig. 4-9, 4-10 and 4-11). Atropine (0.1 mg/kg), or bilateral vagotomy had no effect on the reflex bradycardia noted during aortic afferent stimulation. However, propranolol (1 mg/kg) and the specific $beta_1$-adrenoceptor antagonist, atenolol (0.6 mg/kg), abolished any changes in heart rate.

The effects of selective pharmacologic antagonists on the depressor and heart-rate responses to aortic depressor nerve stimulation are summarized in Table 4-5.

4.3.5. Cold Block

The effect of gradual cooling on conduction in the myelinated and unmyelinated fibers of the aortic depressor nerves was determined. The "cold block" temperature for myelinated fibers was 5.3 ± 0.3 °C (n=4) (Fig. 4-12), and for unmyelinated fibers 0.3 ± 0.3 °C (n=4) (Fig. 4-13).

The effect of gradually cooling the aortic depressor nerves on aortic baroreceptor activity was also monitored. Aortic baroreceptor activity disappeared after the nerve was cooled to 6.4 ± 0.5 °C (n=8) (Fig. 4-14).

In another series of cooling experiments, baseline arterial pressure and heart rate were 75 ± 9 mm Hg and 254 ± 12 beats/min. After placing the cooling hook, left aortic depressor nerve stimulation (10 V, 10 Hz, 1 ms, 30 s) reduced mean arterial pressure from 75 ± 9 to 61 ± 7 mm Hg ($p<0.01$, n=4), and heart rate from 254 ± 12 to 245 ± 12 beats/min ($p<0.01$). After the nerve was cooled to 8°C for 20 min, stimulation still reduced mean arterial pressure from 78 ± 8 to 74 ± 8 mm Hg ($p<0.05$, n=4), and heart rate from 235 ± 8 to 233 ± 9 beats/min. However, after the nerve was cooled to 5°C for 30 min, stimulation induced no changes in arterial pressure or heart rate. After

rewarming to 37°C for 20 min, depressor responses to nerve stimulation reappeared, and reduced mean arterial pressure from 78 ± 3 to 62 ± 2 mm Hg ($p<0.01$), and heart rate from 196 ± 10 to 190 ± 11 beats/min. The effects on mean arterial blood pressure matched the changes in nerve conduction (Fig. 4-15). Thus, the fast wave of myelinated-fiber conduction was eliminated by cooling the nerve to 5°C, as were the depressor responses to nerve stimulation; the slower wave of unmyelinated-fiber conduction remained. After cooling the nerve to 0°C, both myelinated and unmyelinated fiber conduction disappeared. Upon rewarming to 37°C, both myelinated and unmyelinated fiber conduction reappeared, as did the depressor responses.

4.3.6. Anodal Block

In 3 experiments, the effect of anodal block of the nerve was similar to that of cold block. A small blocking current (2-10 μA) selectively eliminated myelinated afferent conduction (Fig. 4-16), and the depressor responses and bradycardia to left aortic depressor nerve stimulation were greatly reduced.

4.3.7. Effects of Different Anesthetics on Responses to Aortic Depressor Nerve Stimulation

The effects of various anesthetics on baseline levels of mean arterial blood pressure and heart rate in guinea

pigs were shown in Table 4-1. The choice of anesthetic did not alter the response to aortic depressor nerve stimulation. After sectioning both aortic depressor and carotid sinus nerves, depressor and bradycardiac responses were similar under pentobarbital, urethane or chloralose anesthesia (Fig. 4-17).

In 5 animals under pentobarbital anesthesia, aortic depressor nerve stimulation reduced mean arterial pressure from 80 ± 5 to 64 ± 3 mm Hg ($p<0.01$), and heart rate from 237 ± 11 to 222 ± 9 beats/min ($p<0.05$). After injection of chloralose (50 mg/kg, i.v.), stimulation reduced mean arterial pressure from 73 ± 1 to 66 ± 2 mm Hg ($p<0.05$), and heart rate from 217 ± 9 to 205 ± 10 ($p<0.05$). Pressor responses were not detected.

4.3.8. Effects of Adrenalectomy

In 6 pentobarbital anesthetized, bilaterally adrenalectomized animals, baseline mean arterial pressure and heart rate were 55 ± 4 mm Hg and 261 ± 4 beats/min, respectively. Aortic and carotid denervation caused mean arterial pressure to rise to 71 ± 4 mm Hg ($p<0.01$), without any change in heart rate. Pressure remained elevated for about 10 min, and then fell and stabilized at 64 ± 4 mm Hg. Aortic depressor nerve stimulation yielded depressor responses (Fig. 4-18), similar to those in non-adrenalectomized controls.

4.3.9. Chemoreceptor Stimulation

In 8 animals with both carotid sinus nerves sectioned, intra-aortic injection of the chemoreceptor stimulants, sodium cyanide, phenylbiguanide, lobeline, or serotonin, did not alter the firing pattern of the aortic depressor nerves, even though these drugs induced changes in blood pressure and heart rate.

In 4 pentobarbital anesthetized animals, aortic depressor nerve stimulation with low-intensity, high-frequency stimuli (3 V, 150 Hz, 0.1 ms for 20 s) induced depressor responses. Mean arterial pressure fell from 78 ± 4 to 60 ± 2 mm Hg ($p<0.01$), and heart rate fell from 272 ± 8 to 266 ± 7 beats/min ($p<0.05$). However, high-intensity, low-frequency stimuli (15 V, 15 Hz, 0.1 ms for 20 s) also induced depressor responses: mean arterial pressure fell from 79 ± 4 to 61 ± 5 mm Hg ($p<0.01$)>, and heart rate fell from 262 ± 7 to 251 ± 6 beats/min ($p<0.05$). No pressor responses were noted to either form of stimulation.

4.3.10. Comparison with the Carotid Baroreflex

In 4 pentobarbital anesthetized animals, carotid denervation elevated mean arterial pressure from 62 ± 5 to 88 ± 7 mm Hg ($p<0.05$), and heart rate from 256 ± 10 to 258 ± 10 beats/min (NS). After 10 min, mean arterial pressure and heart rate stabilized at 75 ± 7 mm Hg and 254 ± 11

beats/min, respectively. After aortic denervation, mean arterial pressure stabilized at 78 ± 7 mm Hg (NS), and heart rate at 250 ± 12 beats/min (NS). Central (orthodromic) stimulation of the carotid sinus nerves induced depressor responses and bradycardia (Fig. 4-19). Two peaks were noted with maxima at 2 and 8 V, respectively.

4.4. DISCUSSION

In most species, after a variable course, aortic baroreceptor afferents pass cephalad in the vagi (Sun and Biggs, 1986; 1987). However, in rabbits, aortic baroreceptor afferents pass cephalad in a pair of well-defined, separate aortic depressor nerves that join the vagi via the superior laryngeal nerves and the nodose ganglia (Douglas and Ritchie, 1956; Douglas et al., 1956). We are the first to describe the pathways of the aortic depressor nerves in guinea pigs (Sun and Biggs, 1986; 1987).

4.4.1. Axons in the Aortic Depressor Nerves

Histological findings showed that the types of fiber in the aortic depressor nerves of guinea pigs were similar to those of cats, rabbits and rats, whose aortic depressor nerves contain myelinated and unmyelinated fibers. In guinea-pig aortic depressor nerves, there appeared to be 2

types of myelinated axons: we observed thick (A) and thin (B) wall myelinated axons. These findings were similar to those reported in cats (Agostoni et al. 1957). The ratio of myelinated to unmyelinated fibers in cat aortic nerves was 2.5 : 1. However, in guinea pigs, there were more unmyelinated than myelinated fibers, and the ratio of myelinated to unmyelinated fibers was 1 : 3-4. Interestingly, in rats, unmyelinated fibers predominate (Thoren et al., 1977), and the ratio of myelinated to unmyelinated fibers is 1 : 9 (personal communication, Dr M.C. Andresen). This suggests that functional organization in guinea-pig aortic depressor nerves is different from cats and rats.

The electrophysiological studies of the axons in the aortic depressor nerves indicated that one group represented the myelinated fibers and the other the unmyelinated fibers noted in the sections.

Thus, we obtained electrophysiological and histological evidence that the aortic depressor nerves of guinea pigs contained myelinated and unmyelinated fibers. Next, we sought to determine the functions of these fibers.

4.4.2. Buffering effects of the Aortic Depressor Nerves and the Carotid Sinus Nerves

The results of two series of sequential sections of the buffering nerves showed that the carotid sinus nerves

are more important and dominate baroreflex control in guinea pigs. Nevertheless, depressor responses to carotid sinus nerve stimulation were similar in magnitude to those seen in response to aortic depressor nerve stimulation. Carotid sinus nerve stimulation yielded "double-peaked" responses; by contrast to the "single-peaked" responses seen in response to the aortic depressor nerve stimulation. This suggested that the carotid sinus nerves contain both myelinated and unmyelinated axons subserving baroreceptors, whereas only myelinated fibers subserve baroreceptors in the aortic depressor nerves.

4.4.3. Aortic Baroreflexes

Superior laryngeal nerve stimulation caused reflex hypotension and bradycardia in rats (Faber and Brody, 1983). In guinea pigs, the aortic baroreceptor afferent stimulation resulted in reflex hypotension, and mild bradycardia. Responses were voltage, frequency and pulse-width dependent. In guinea pigs, afferents mediating aortic baroreflexes travelled via the aortic depressor nerves, the superior laryngeal nerves, the nodose ganglia and vagal afferents, to the central nervous system (Sun and Biggs, 1986; 1987; 1988). Experiments with selective blockers showed that the efferent arcs of aortic baroreflexes were mediated via central alpha$_2$-adrenoceptors, autonomic ganglia and peripheral alpha$_1$- and

beta$_1$-adrenoceptors; vagal efferents were not involved (Table 4-5). Thus, the depressor responses and bradycardia resulted solely from a reflex reduction in sympathetic tone. Interestingly, the aortic baroreflex in guinea pigs did not involve any effect of vagal efferents on heart rate. However, active reflex bradycardia was seen in guinea pigs after phenylephrine was injected. Presumably this was mediated by carotid sinus baroreceptors or cardiac-pulmonary receptors.

Either aortic depressor nerve was capable of controlling arterial blood pressure in guinea pigs, as the responses to stimulating the left, right or both aortic depressor nerves were similar.

Acute bilateral adrenalectomy did not alter the responses to aortic depressor nerve stimulation, indicating that the release of catecholamines from the adrenal medulla was not involved in the responses.

The anesthetic used (pentobarbital, urethane or chloralose) had no effect on responses to aortic depressor nerve stimulation. In animals under anesthetic doses of chloralose, and after injection of chloralose into guinea pigs under pentobarbital anesthesia, aortic depressor nerve stimulation caused only depressor responses, as has been reported in rabbits (Neil and Redwood, 1949). By contrast, in cats under pentobarbital anesthesia, injection of chloralose converted the depressor response to aortic

depressor nerve stimulation to a pressor response (Neil and Redwood, 1949). These workers concluded that the aortic depressor nerves in cats contained chemoreceptor fibers, but that these fibers were absent in rabbits. As depressor responses to the aortic depressor nerve stimulation under chloralose anesthesia were smaller, this might represent the summation of the usual depressor response and a small pressor response.

The depressor responses and bradycardia evoked by nerve stimulation were mediated via myelinated fibers, as maximal responses occurred at 3 V, the excitation threshold for myelinated fibers. Results from the cooling and anodal block experiments supported this. These findings indicate that aortic baroreflexes in guinea pigs differ from those of rabbits, cats, rats and dogs. In those species, the effects of stimulation are mediated via both myelinated and unmyelinated afferents (Douglas and Ritchie, 1956; Douglas et al., 1956; Oberg and Thoren, 1973; Thoren et al., 1977).

The role of the unmyelinated afferents in the aortic depressor nerves of guinea pigs is unknown. They might be chemoreceptor afferents that give pressor responses on stimulation (Pelletier et al., 1972). However, pressor responses were never observed to aortic nerve stimulation, even under chloralose anesthesia, or to high intensity and low frequency stimulation (Douglas and Schaumann, 1956; Edis and Shepherd, 1971; Schmidt, 1968). Also,

intra-aortic injection of the chemoreceptor stimulants cyanide, phenylbiguanide, lobeline or serotonin failed to alter the firing pattern in the aortic depressor nerves. Thus, there was no evidence of a chemoreceptor component in guinea-pig aortic depressor nerves. Alternatively, these unmyelinated fibers might have long-term effects on blood pressure regulating systems that involve fluid-hormonal and renal control mechanisms. These fibers could represent afferents from nociceptive, or intrathoracic stretch receptors (Covell, 1985), or sympathetic efferents.

The principal findings of this investigation in guinea pigs were: 1) aortic depressor nerves contain both myelinated and unmyelinated nerve fibers; 2) the carotid sinus nerves contribute a greater baroreflex buffering effect than the aortic depressor nerves; 3) stimulation of aortic baroreceptor afferents results in reflex hypotension and bradycardia; 4) either aortic depressor nerve is capable of controlling blood pressure; 5) the efferent arcs of the aortic baroreflexes involve central $alpha_2$-adrenoceptors, autonomic ganglia and peripheral $alpha_1$- and $beta_1$-adrenoceptors; 6) the reflex hypotension and bradycardia are mediated via myelinated afferents; 7) reflex responses result solely from a reduction in sympathetic tone; 8) neither the choice of anesthetic nor adrenalectomy alter responses to aortic depressor nerve

stimulation; and 9) there is no evidence of a chemoreceptor component in the aortic depressor nerves of guinea pigs (Sun and Biggs, 1988).

4.5. ACKNOWLEDGEMENTS

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I am grateful to Halyna Marusyk for assisting me with the transmission electron microscopy studies.

TABLE 4-1.

BASELINE VALUES OF MEAN ARTERIAL BLOOD PRESSURE (MABP) AND HEART RATE (HR) UNDER DIFFERENT ANESTHETICS.

Anesthetic	MABP (mm Hg)	n	HR (beats/min)	n
Pentobarbital (30 mg/kg, i.p.)	69 ± 1	22	267 ± 6	22
Urethane (1.5 g/kg, i.p.)	52 ± 1	8	302 ± 5	8
Chloralose * (50 mg/kg, i.v.)	72 ± 2	8	245 ± 6	8

*: Chloralose was injected after induction with methoxyflurane.

Values are mean ± S.E.M.

TABLE 4-2.

EFFECTS OF SEQUENTIALLY SECTIONING THE AORTIC DEPRESSOR NERVES (ADN) AND THE CAROTID SINUS NERVES (CSN) ON MEAN ARTERIAL BLOOD PRESSURE (MABP)

Anesthetic	MABP (mm Hg)				
	C1	ADN-X	C2	+CSN-X	C3
Pentobarbital (30 mg/kg, i.p.)	63±3 (n=4)	73±4 (NS)	65±4 (NS)	91±5 (*)	74±3 (NS) C1 (NS) C2
Urethane (1.5 g/kg, i.p.)	52±3 (n=4)	68±4 (*)	56±3 (NS)	69±4 (*)	60±1 (NS) C1 (NS) C2
Chloralose (50 mg/kg, i.p.)	71±3 (n=4)	86±5 (*)	73±3 (NS)	95±4 (*)	88±7 (*) C1 (*) C2

C1: baseline values; ADN-X: both ADN sectioned; C2: 10 min after sectioning both ADN; +CSN-X: both CSN sectioned; C3: 10 min after +CSN-X.

(*): $p<0.05$; (NS): not significant.
ADN-X tested against C1, C2 against C1, +CSN-X against C2, C3 against C1 or C2.

TABLE 4-3.

EFFECTS OF SEQUENTIALLY SECTIONING THE AORTIC DEPRESSOR NERVES (ADN) AND THE CAROTID SINUS NERVES (CSN) ON HEART RATE

Anesthetic	Heart Rate (beats/min)				
	C1	ADN-X	C2	+CSN-X	C3
Pentobarbital (30 mg/kg, i.p.)	276±6 (n=4)	276±7 (NS)	271±4 (NS)	282±6 (NS)	275±6 (NS) C1 (NS) C2
Urethane (1.5 g/kg, i.p.)	306±4 (n=4)	311±8 (NS)	305±4 (NS)	309±7 (NS)	293±6 (NS) C1 (NS) C2
Chloralose (50 mg/kg, i.p.)	244±5 (n=4)	248±8 (NS)	245±6 (NS)	241±7 (NS)	240±4 (NS) C1 (NS) C2

C1: baseline values; ADN-X: both ADN sectioned; C2: 10 min after sectioning both ADN; +CSN-X: both CSN sectioned; C3: 10 min after the +CSN-X.

(*): $p<0.05$; (NS): not significant.
ADN-X tested against C1, C2 against C1, +CSN-X against C2, C3 against C1 or C2.

TABLE 4-4.

EFFECTS OF SECTIONING THE CAROTID SINUS NERVES (CSN), THEN THE AORTIC DEPRESSOR NERVES (ADN) ON MEAN ARTERIAL BLOOD PRESSURE (MABP) AND HEART RATE, UNDER PENTOBARBITAL ANESTHESIA (30 mg/kg, i.p.)

Measurements	C1	CSN-X	C2	+ADN-X	C3
MABP (mm Hg)	61±4 (n=5)	92±7 (*)	78±6 (NS)	82±7 (NS)	79±6 (NS) C1 (NS) C2
Heart Rate (beats/min)	255±8 (n=5)	262±9 (NS)	259±10 (NS)	256±11 (NS)	240±7 (NS) C1 (NS) C2

C1: baseline values; CSN-X: both CSN sectioned; C2: 10 min after sectioning both CSN; +ADN-X: both ADN sectioned; C3: 10 min after +ADN-X.

(*): $p<0.05$; (NS): not significant.
CSN-X tested against C1, C2 against C1, +ADN-X against C2, C3 against C1 or C2.

TABLE 4-5.

EFFECTS OF PHARMACOLOGIC ANTAGONISTS ON DEPRESSOR (ABP-R) AND BRADYCARDIAC (HR-R) RESPONSES TO STIMULATION OF THE AORTIC DEPRESSOR NERVES

Antagonists	Action	Dose	ABP-R	HR-R
Atropine	M blocker	0.1	unchanged	unchanged
Mepyramine	H_1 blocker	0.5	unchanged	unchanged
Cimetidine	H_2 blocker	1.0	unchanged	unchanged
Clonidine	$alpha_2$ agonist	0.2	eliminated	eliminated
Clonidine +			eliminated	eliminated
Hexamethonium	ganglionic blocker	1.0	eliminated	eliminated
Hexamethonium+			eliminated	eliminated
Prazosin	$alpha_1$ blocker	0.1	eliminated	unchanged
Prazosin +			eliminated	unchanged
Yohimbine	$alpha_2$ blocker	0.5	unchanged	unchanged
Propranolol	beta blocker	1.0	unchanged	eliminated
Atenolol	$beta_1$ blocker	0.6	unchanged	eliminated

Dose: mg/kg, i.v.
+: MABP restored to control levels by infusing angiotensin amide (1 μg/kg/min, i.v.).

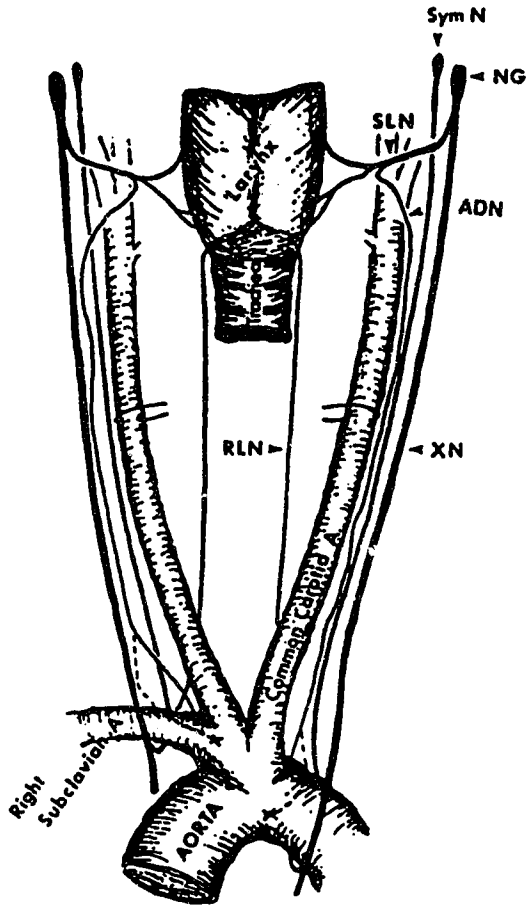


Figure 4-1. Anatomy of the cervical region in the guinea pig showing relevant nerves: ADN - aortic depressor nerve; NG - nodose ganglion; RLN - recurrent laryngeal nerve; SLN - superior laryngeal nerve; Sym N - sympathetic nerve; and XN - vagus nerve.

Figure 4-2. Electron micrograph (X2,500) of cross section of the aortic depressor nerve in guinea pigs showing both myelinated and unmyelinated fibers.

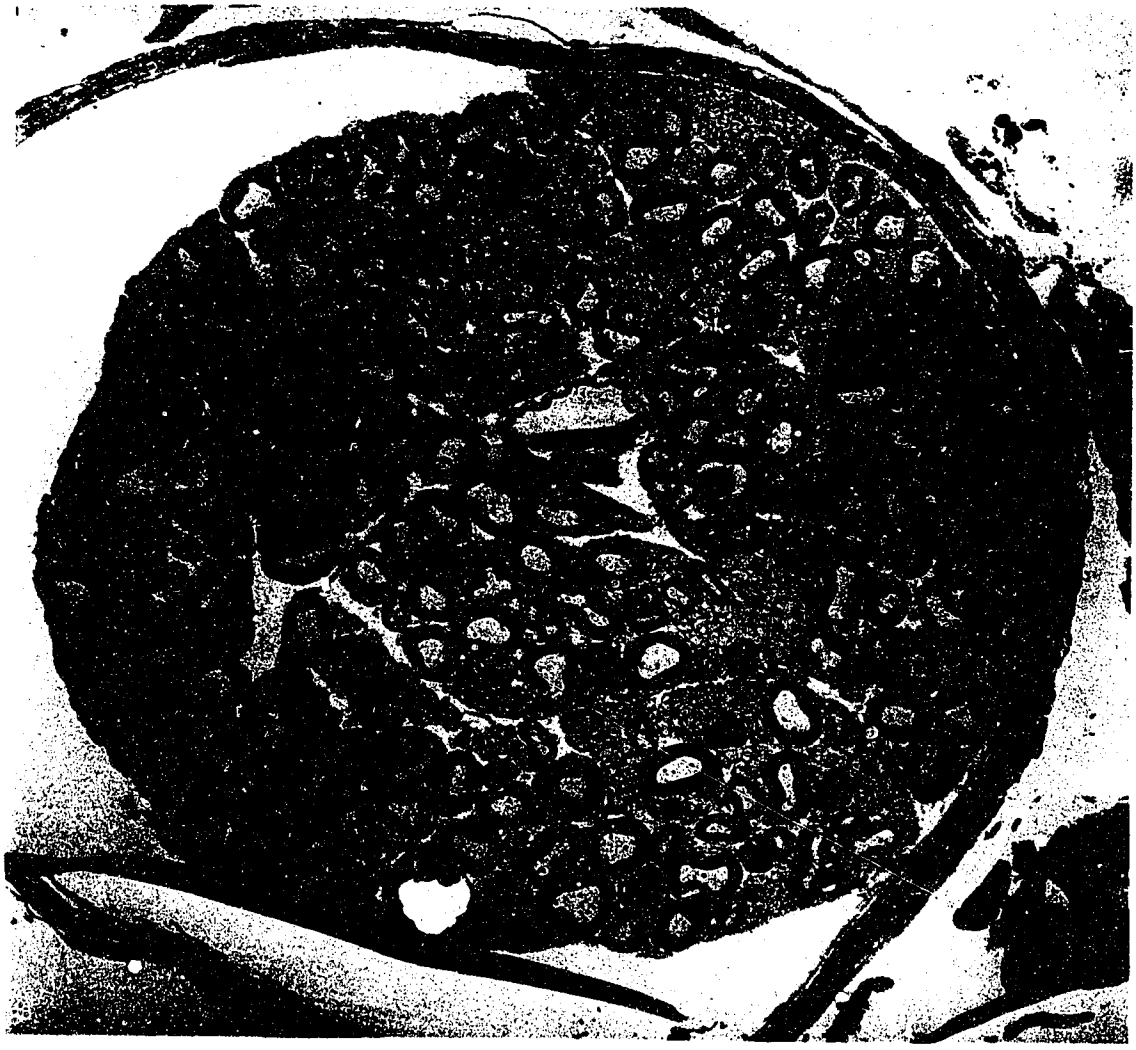
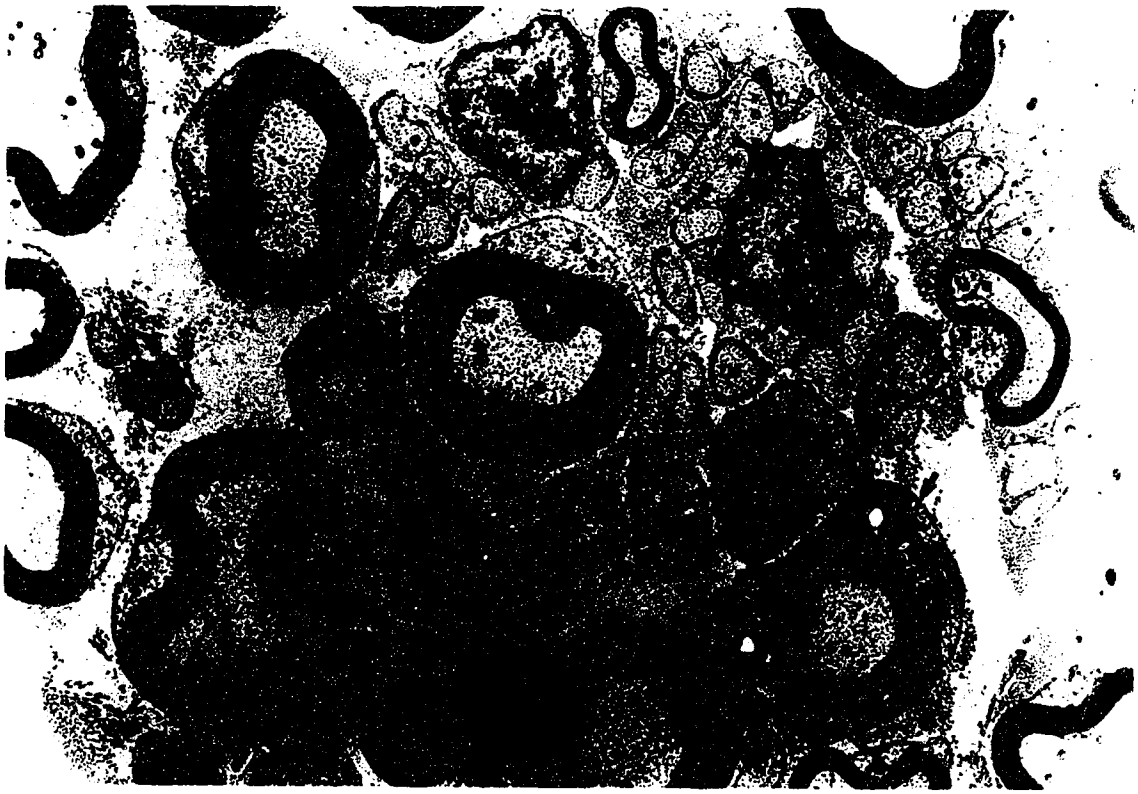


Figure 4-3. Higher magnification (X9,000) of cross section of the aortic depressor nerve showing myelinated (MF), unmyelinated fibers (UF), node of Ranvier (NR), and Schwann cells (SC). Note a cleft of Schmidt-Lanterman (arrow).



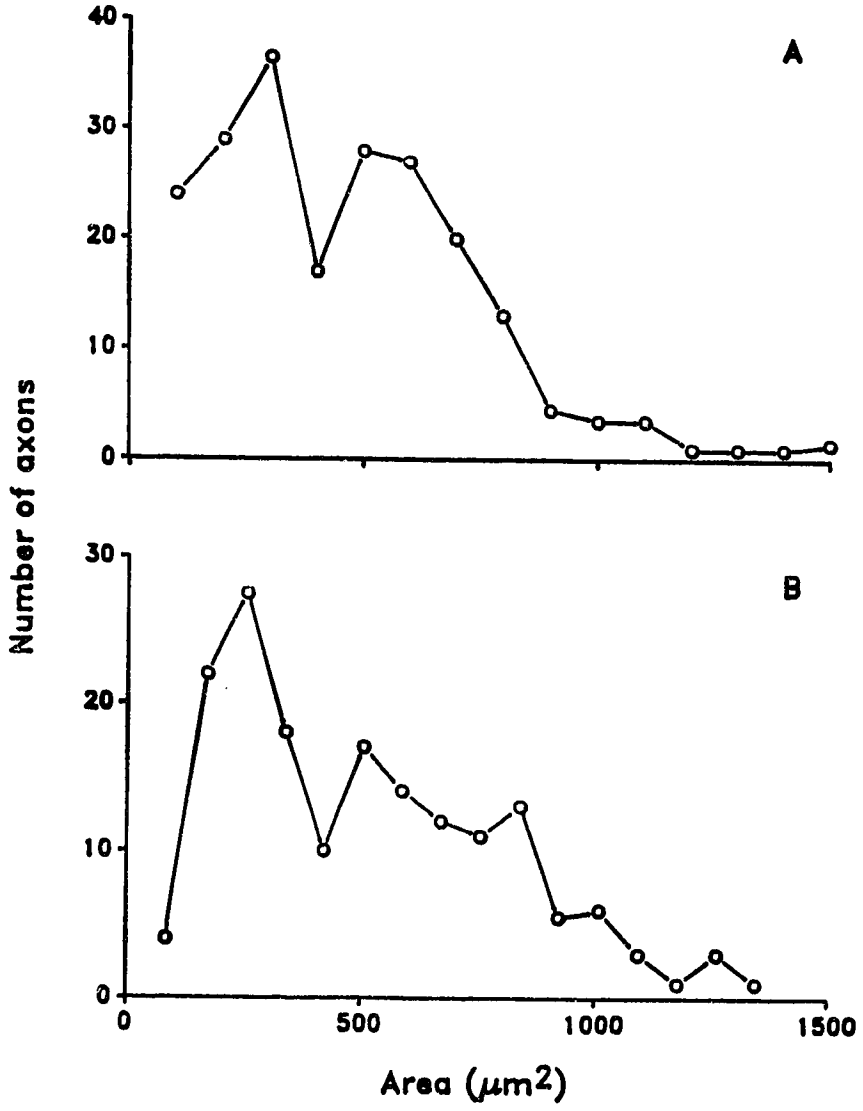


Figure 4-4. The distribution of cross-sectional areas of A: 206 and B: 167 myelinated fibers in the aortic depressor nerve of a guinea pig.

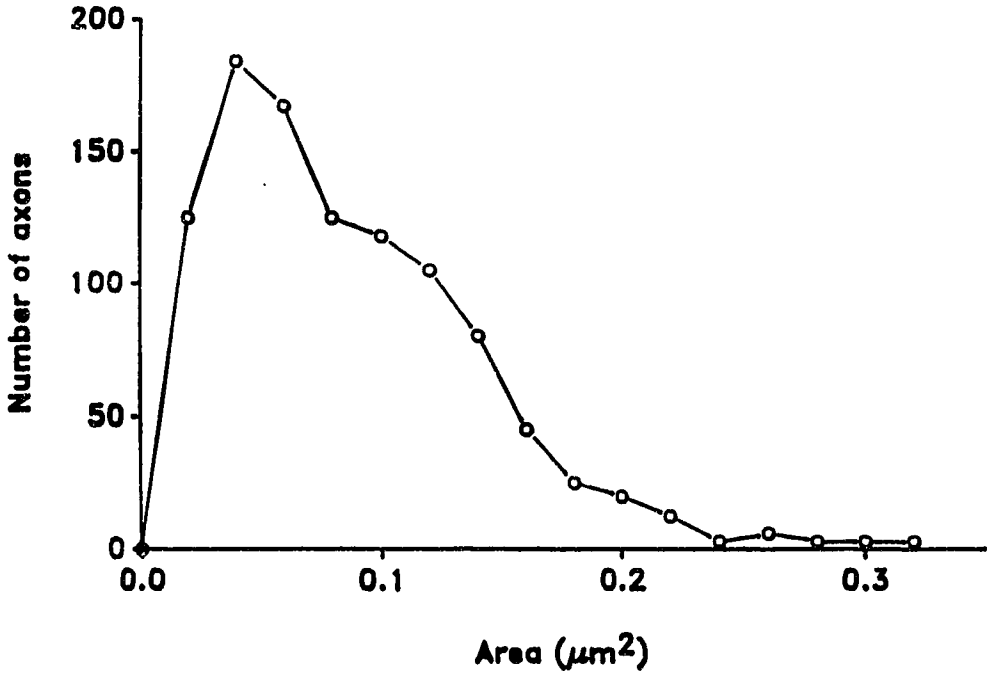


Figure 4-5. The distribution of cross-sectional areas of 1006 unmyelinated fibers in the aortic depressor nerve of a guinea pig.

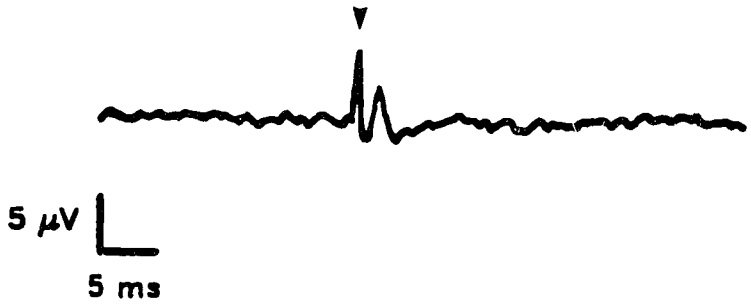


Figure 4-6. Typical compound action potential recorded from the aortic depressor nerve. Photograph shows potential recorded with stimulus (5 V) above threshold for myelinated fibers (threshold = 3 V; conduction velocity = 8.5 m/s). First spike is stimulus artifact (arrow). Electrode separation: 17 mm.

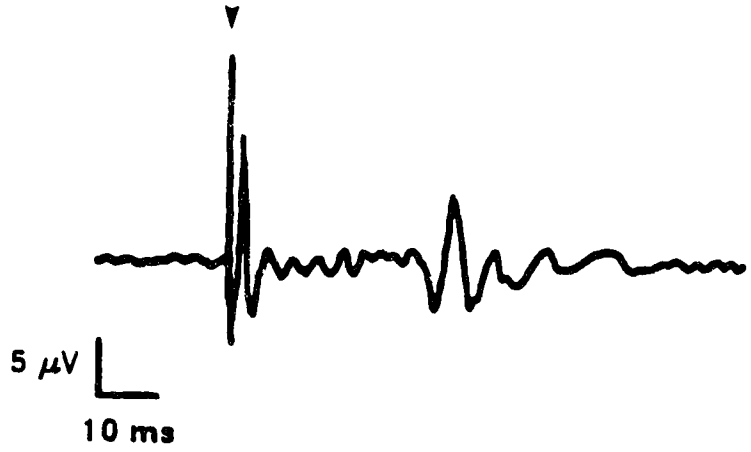


Figure 4-7. Typical compound action potentials recorded from the aortic depressor nerve. Increasing stimulus strength (8 V) caused a slowly conducted wave to appear about 33 ms after that shown in Fig. 4-6. This is due to firing of unmyelinated fibers (threshold = 6 V; conduction velocity = 0.45 m/s). First spike is stimulus artifact (arrow). Electrode separation: 15 mm.

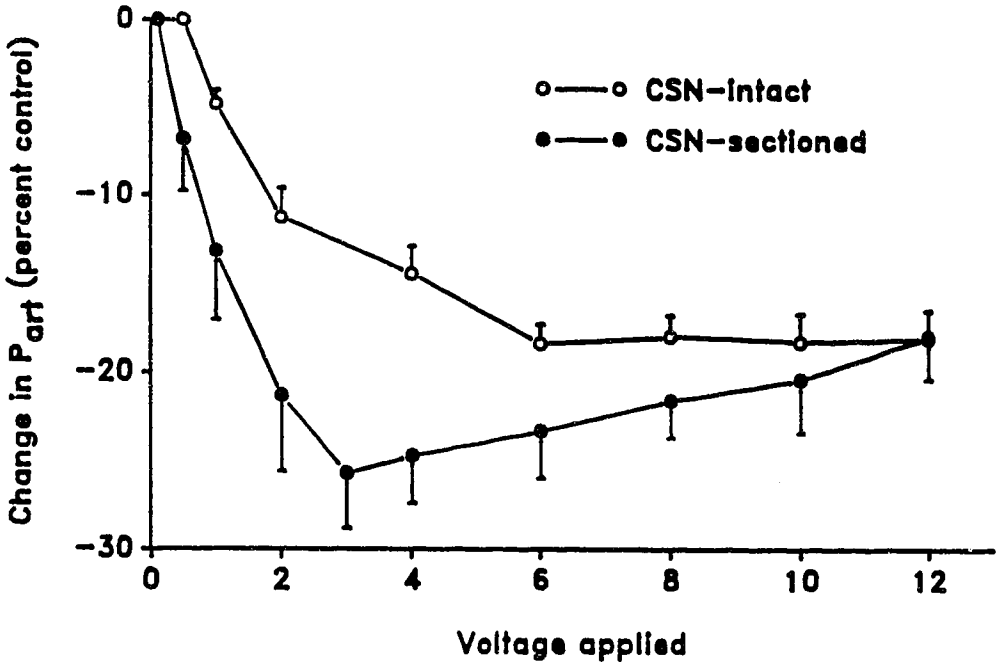


Figure 4-8. Pentobarbital anesthetized animals after 1) aortic depressor nerve denervation (open symbols), and 2) both aortic depressor nerve and carotid sinus nerve denervation (filled symbols): effects of increasing stimulus voltage (10 Hz, 1 ms, 30 s) on the depressor and bradycardiac responses to bilateral aortic depressor nerves stimulation. Responses (mean ± SE, n = 4) are shown as percent change from control.

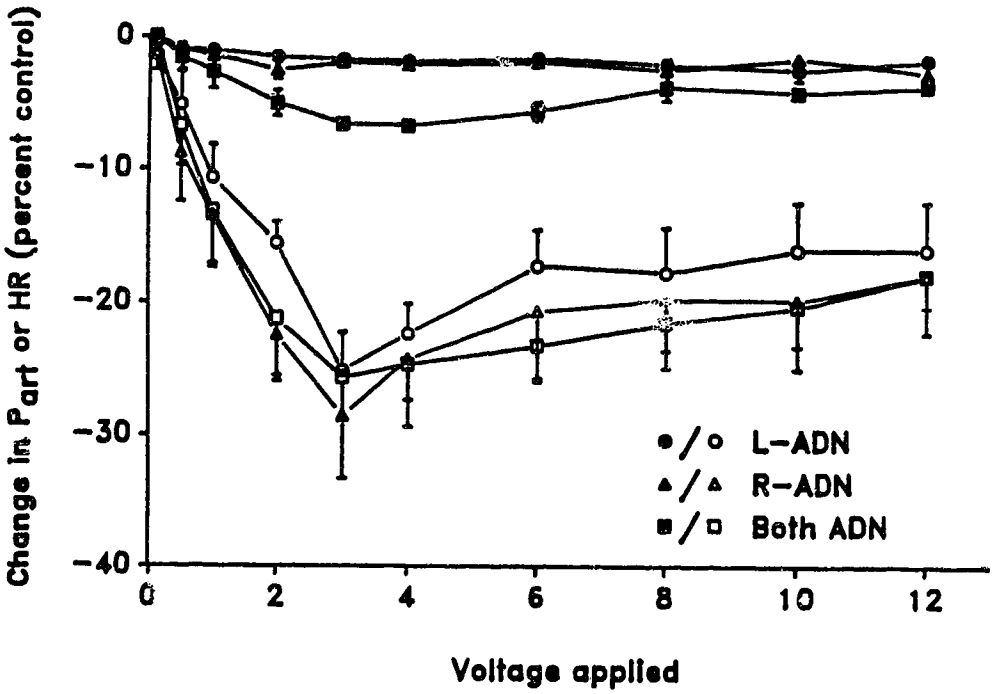


Figure 4-9. Pentobarbital anesthetized animals, both aortic depressor and carotid sinus nerves cut: effects of gradually increasing stimulus voltages (10 Hz, 1 ms, 30 s) on the depressor responses and bradycardia to left, right, and bilateral aortic depressor nerve stimulation. Responses (mean ± SE, n = 4) are shown as percent change from control. (Open symbols: mean arterial blood pressure; filled symbols: heart rate.)

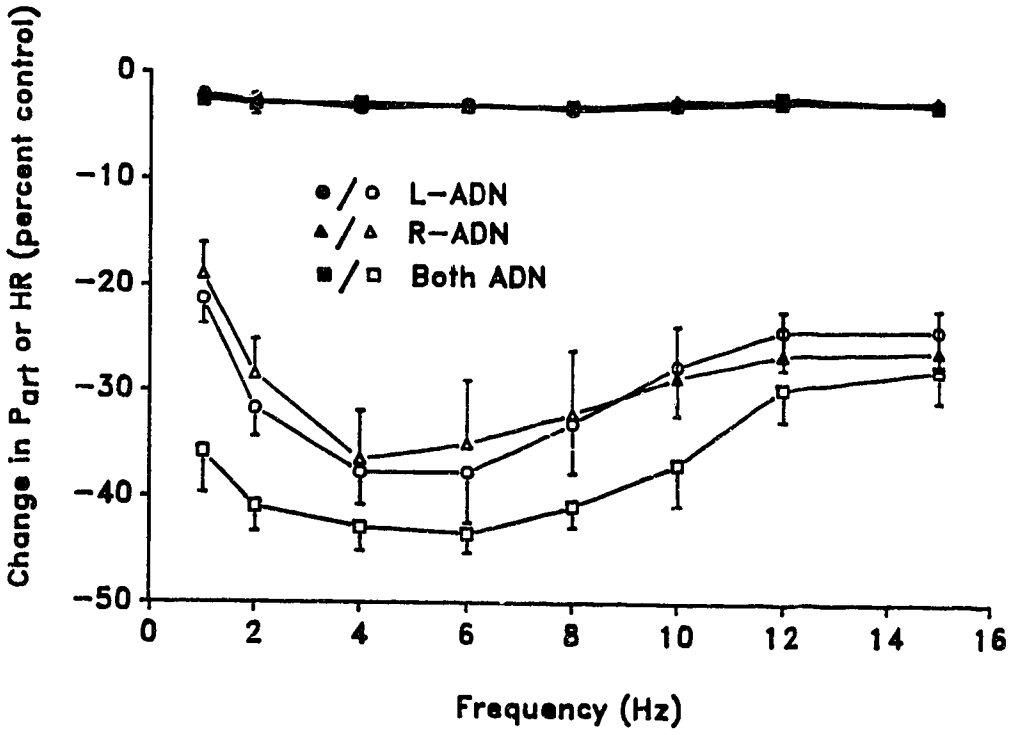


Figure 4-10. Pentobarbital anesthetized animals, both aortic depressor and carotid sinus nerves cut: effects of gradually increasing stimulus frequency (10 v, 1 ms, 30 s) on the depressor responses and bradycardia to the left, right, and bilateral aortic depressor nerve stimulation. Responses (mean ± SE, n = 4) are shown as percent change from control. (Open symbols: mean arterial blood pressure; filled symbols: heart rate.)

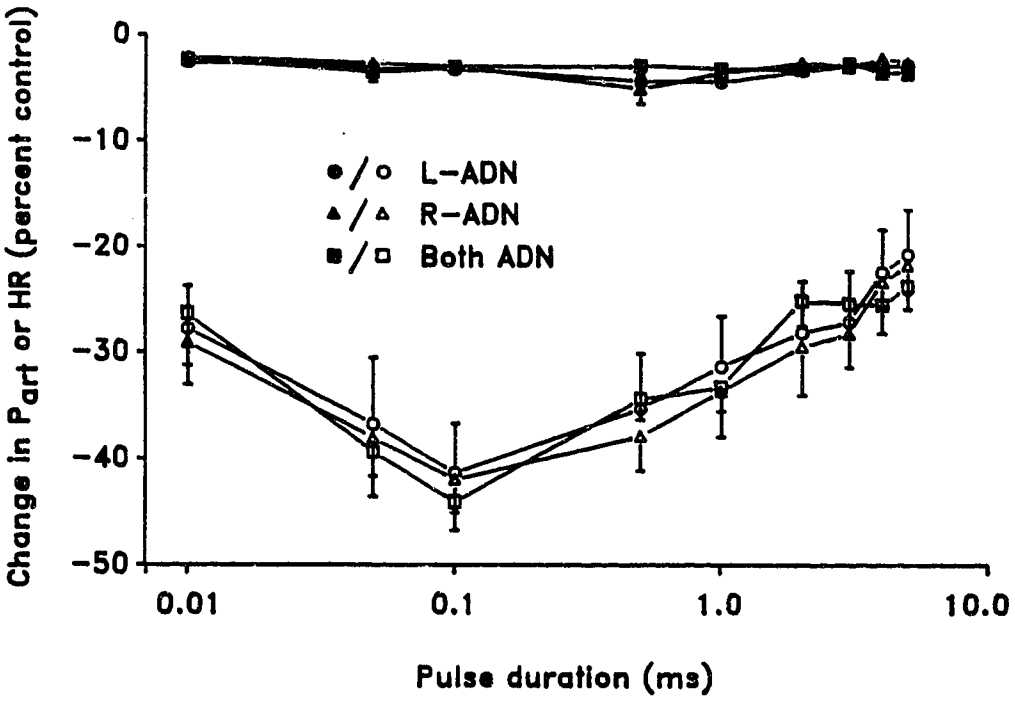


Figure 4-11. Pentobarbital anesthetized animals, both aortic depressor and carotid sinus nerves cut: effects of gradually increasing stimulus pulse width (10 v, 10 Hz, 30 s) on the depressor responses and bradycardia to the left, right, and bilateral aortic depressor nerve stimulation. Responses (mean ± SE, n = 4) are shown as percent change from control. (Open symbols: mean arterial blood pressure; filled symbols: heart rate.)

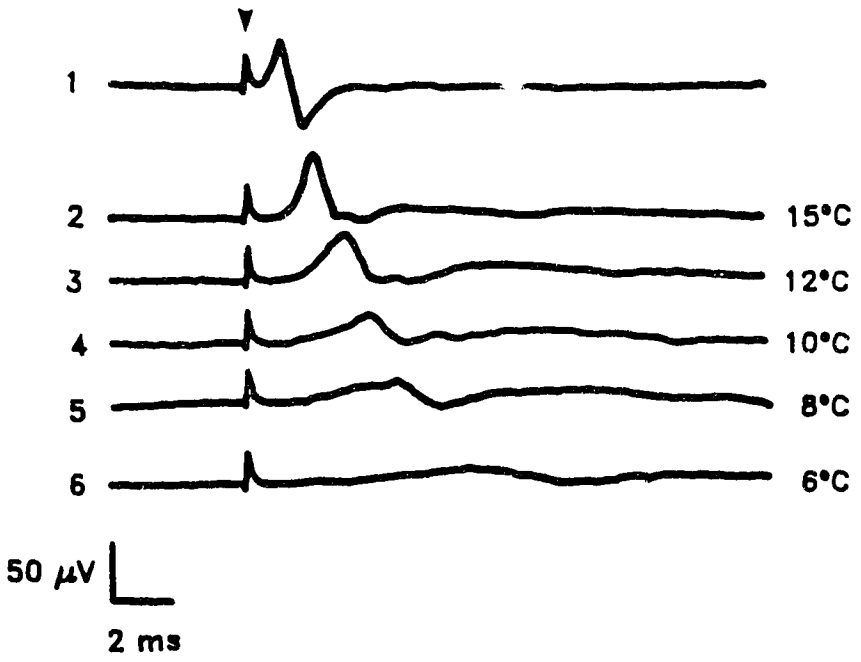


Figure 4-12. Effect of cooling on conduction in myelinated fibers in the aortic depressor nerve. Cold block temperature was 6°C. (stimulus: 3 V, 0.1 ms, single pulse), (trace 1: control; traces 2-6: nerve cooled to 15, 12, 10, 8, and 6°C, respectively.) First spike is stimulus artifact (arrow).

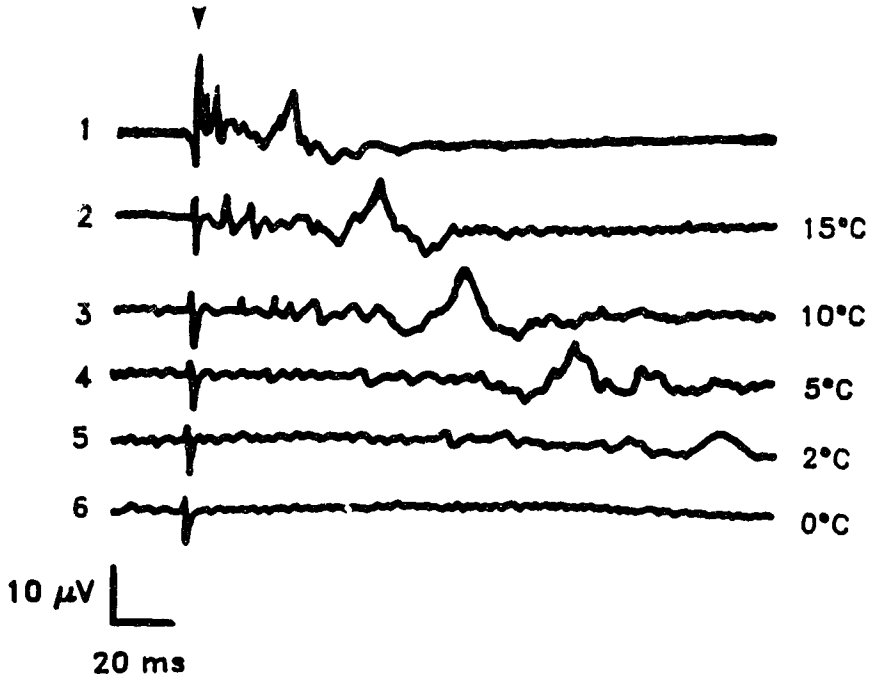


Figure 4-13. Effect of cooling on conduction in unmyelinated fibers in the aortic depressor nerve. Cold block temperature was 0°C. (stimulus: 3.5 V, 1 ms, single pulse), (trace 1: control; traces 2-6: nerve cooled to 15, 10, 5, 2, and 0°C, respectively.) First spike is stimulus artifact (arrow).

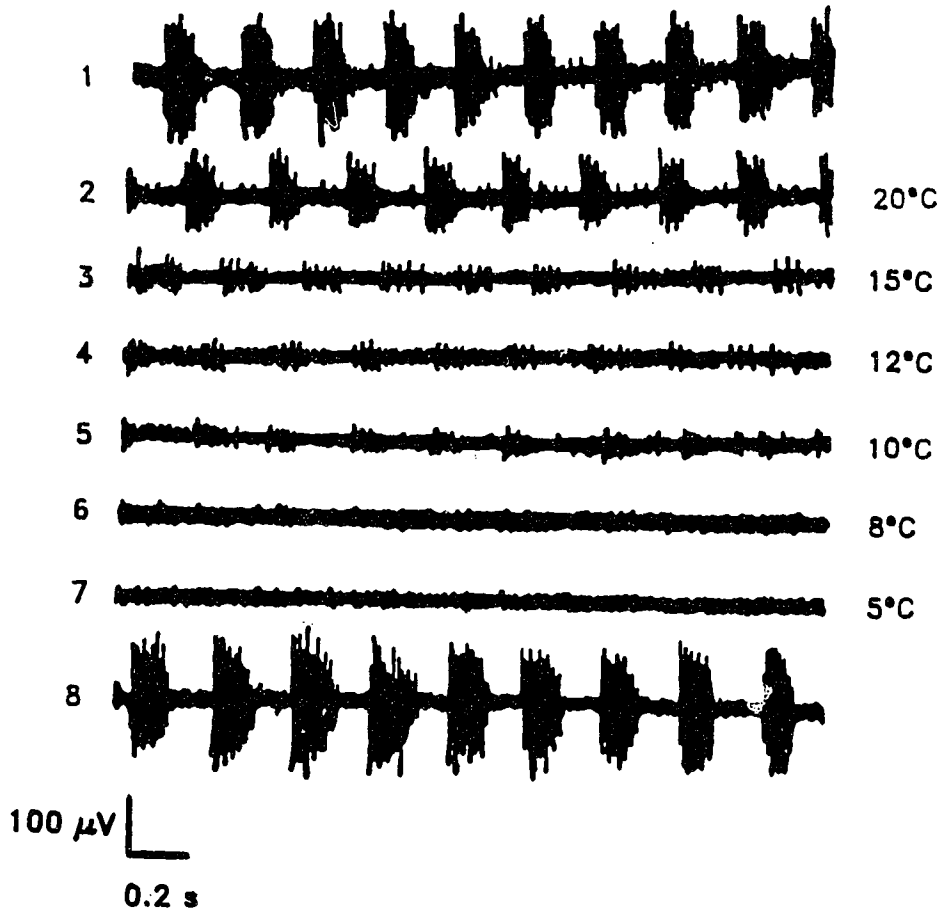


Figure 4-14. Effects of cooling the aortic depressor nerve on the conduction of aortic baroreceptor activity. The baroreceptor activity disappeared after the nerve was cooled to 8°C. (Trace 1: control; traces 2-8: nerve cooled to 20, 15, 12, 10, 8, and 5°C, then rewarmed, respectively.)

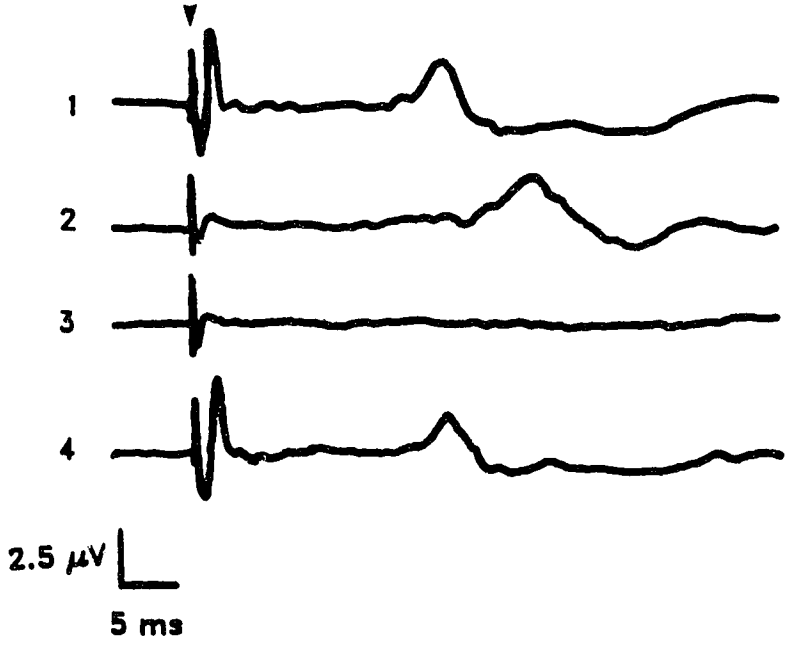


Figure 4-15. Effect of cooling the aortic depressor nerve on conduction of stimulation. 1st trace: conduction by A: myelinated fibers (5 m/s), and C: unmyelinated fibers (0.5 m/s) upon nerve stimulation. 2nd trace: A wave disappeared after the nerve was cooled to 5°C, note the C wave remained but conduction was slower. 3rd trace: both A and C waves were eliminated after the nerve was cooled to 0°C. 4th trace: both A and C waves reappeared after the nerve was warmed. (Stimulus: 6 V, 1 ms, single pulse)

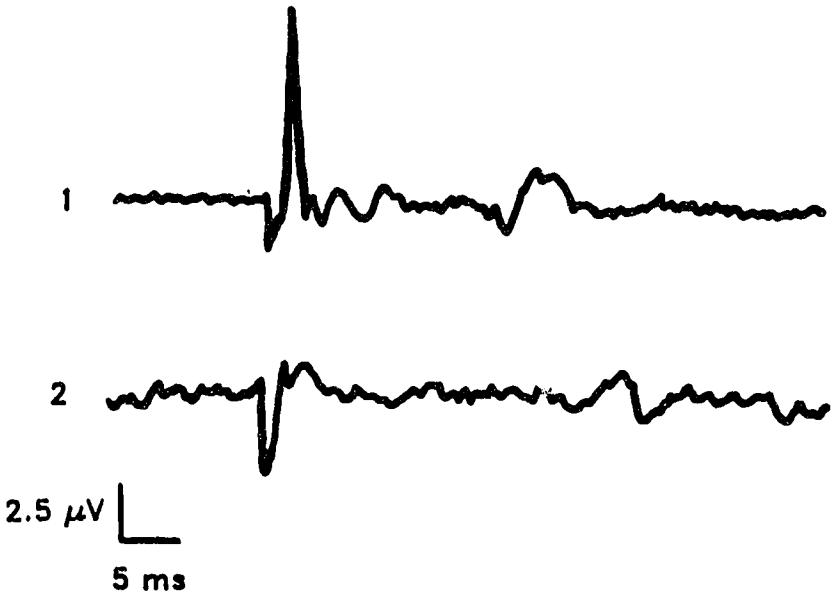


Figure 4-16. Anodal block: upper trace - stimulation induced both fast and slow conducting waves; lower trace - a small blocking current (2 μA) selectively eliminated myelinated afferent conduction.

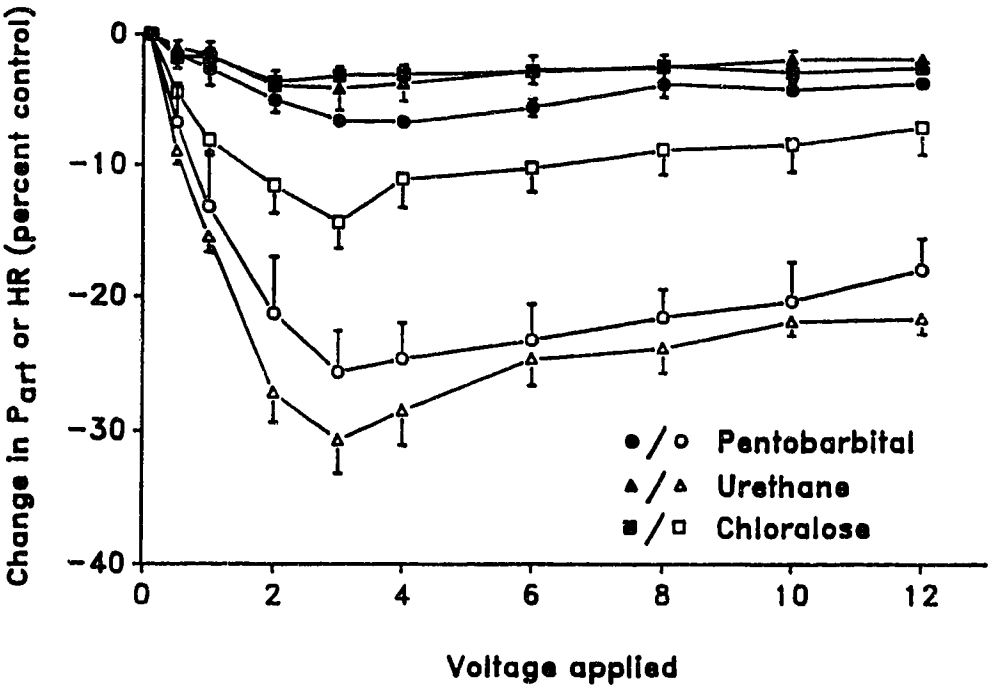


Figure 4-17. Both aortic depressor and carotid sinus nerves sectioned. Effects of pentobarbital, urethane or chloralose on the depressor responses and bradycardia to aortic depressor nerve stimulation (0.1-12 V, 10 Hz, 1 ms, for 30 s). Responses (mean ± SE, n = 4) are shown as percent change from control. (Open symbols: mean arterial blood pressure; filled symbols: heart rate.)

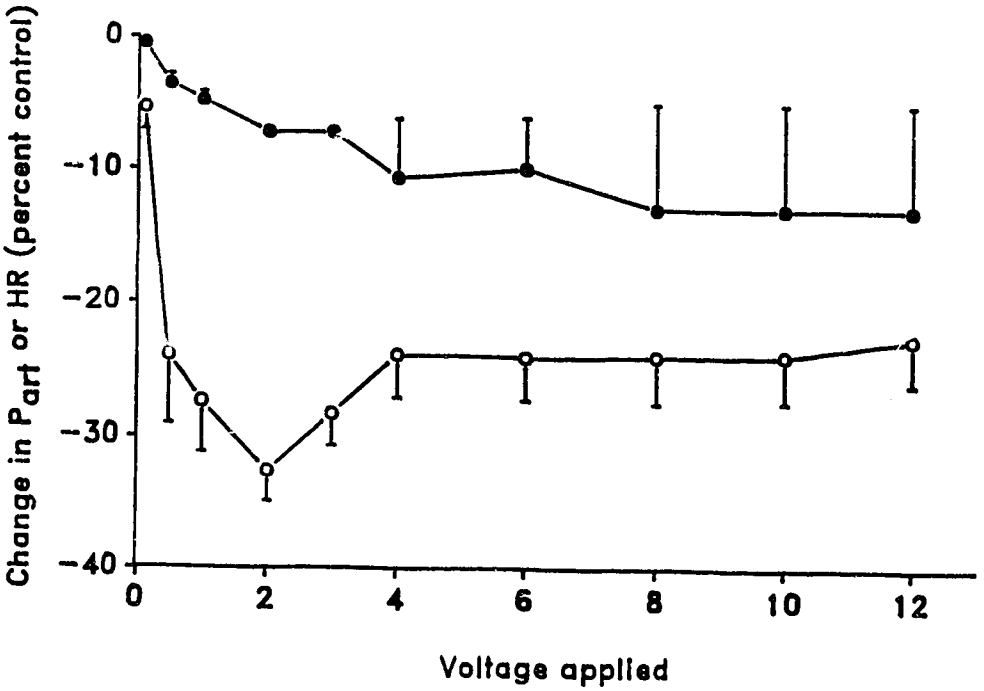


Figure 4-18. Pentobarbital anesthetized, adrenalectomized animals, both aortic depressor and carotid sinus nerves cut: effects of gradually increasing stimulus strength (10 Hz, 1 ms, 30 s) applied bilaterally to the aortic depressor nerves on arterial blood pressure and heart rate. Responses (mean $\pm$ SE, n = 4) are shown as percent change from control. (Open symbols: mean arterial blood pressure; filled symbols: heart rate.)

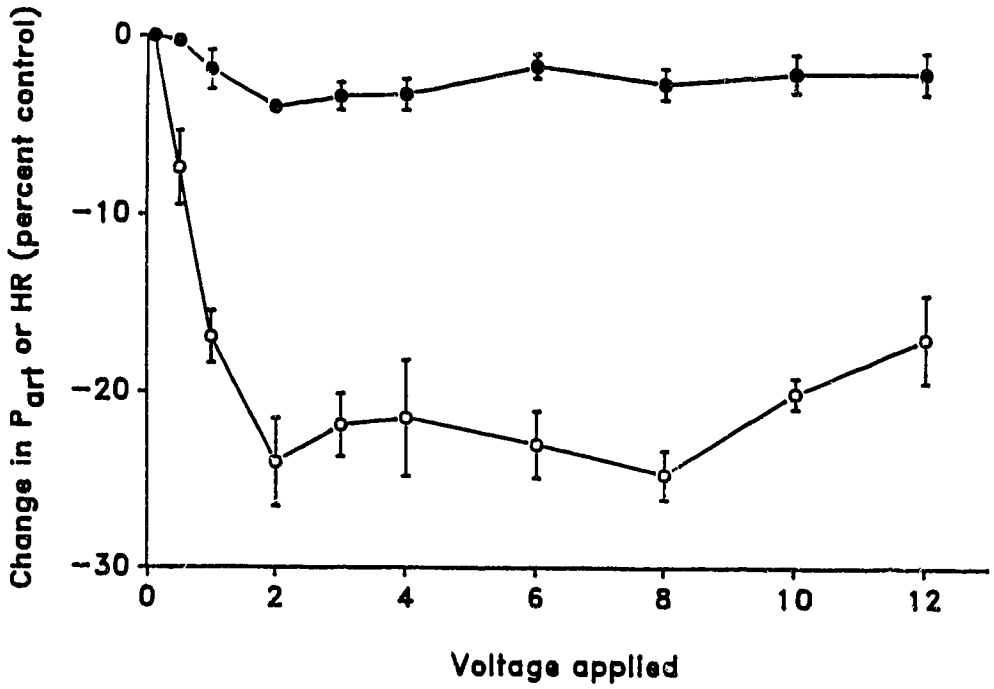


Figure 4-19. Pentobarbital anesthetized animals, both carotid sinus and aortic depressor nerves cut: effects of gradually increasing stimulus strength (10 Hz, 1 ms, 30 s) applied bilaterally to the carotid sinus nerves on arterial blood pressure and heart rate. Responses (mean ± SE, n = 4) are shown as percent change from control. (Open symbols: mean arterial blood pressure; filled symbols: heart rate.)

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CHAPTER V. THE FUNCTIONS OF THE UNMYELINATED NERVE FIBERS IN THE AORTIC DEPRESSOR NERVES OF GUINEA PIGS.*

* A version of this chapter will be submitted to the American Journal of Physiology: Heart and Circulatory Physiology. The results of this chapter have been published in abstract form (SUN, H.S., and BIGGS, D.F. 1989. "Are there sympathetic fibers in the aortic depressor nerves of guinea pigs?" The FASEB J. 3(3): A434, Abstract #1205.)

5.1. INTRODUCTION

Baroreceptors (mechanoreceptors) are sensory receptors that form part of the acute (short-term) cardiovascular homeostatic-control mechanism. In the guinea pig, there are two groups of arterial baroreceptors: 1) aortic baroreceptors - located in the aortic arch and brachiocephalic artery where it joins the right subclavian and right common carotid arteries, and 2) carotid-sinus baroreceptors.

Nonidez (1935) reviewed the early literature on the anatomy and physiology of aortic baroreceptors. The aortic depressor nerve was first described in rabbits, by Cyon & Ludwig in 1866. In mice, cats, dogs, swine and humans, after a variable course, the aortic nerve afferents pass cephalad in the vagi (Hashimoto & Hirohata, 1936). In some rats, aortic baroreceptor afferents pass centrally only via the vagi; in others, they pass via the cervical sympathetic nerves to the nodose ganglia and then via the vagi; and in yet others, via the recurrent laryngeal nerves to the superior laryngeal nerves and then via the vagi (Sun & Biggs, 1986, 1987). However, in rabbits, aortic afferents pass cephalad in well-defined, separate aortic nerves that join the vagi via the superior laryngeal nerves (Alexander and Cuir, 1963; Angell, 1971a, 1971b, 1971c, 1973; Douglas and Ritchie, 1956; Douglas et al., 1956; Kardon et al., 1973; Nonidez, 1935; Numao et al., 1983; Yao and Thoren,

1983). Sun and Biggs (1986, 1987) determined the pathways for the aortic depressor nerves in guinea pigs (Fig.5-1) and found them to be similar to those in rabbits. In all species, the aortic nerves are composed of myelinated and unmyelinated fibres, many of which subserve baroreceptors and pass cephalad to the cardiovascular centers of the medulla. In some species, other aortic-nerve fibers subserve chemoreceptors in the aortic glomi and aortic body (paraganglion aorticum supracardiale [Nonidez, 1935]). In studies of guinea-pig aortic nerves, Sun and Biggs (1986, 1987, 1988) described both myelinated and unmyelinated fibers. They showed, by selectively blocking myelinated afferents in the aortic nerves by cooling or by passing an anodal current, that only myelinated fibers subserve baroreceptors in these nerves. Also, they demonstrated the absence of chemoreceptor afferents, as there was no increase in nerve activity after inducing hypoxia or injecting sodium cyanide (intra-aortically) .

The carotid-sinus and aortic pressure-sensing regions receive a rich efferent innervation. Most of this is derived from the sympathetic branch of the autonomic nervous system (Nonidez, 1935), although some may be of parasympathetic origin (Eyzaguirre et al., 1983; Majcherczyk et al., 1980). Experiments, in vitro, with a rat aortic-arch preparation modified to separate receptor effects from vessel effects showed conclusively that

noradrenaline increased baroreceptor discharge via alpha-adrenoceptors (Kunze et al., 1984; Kunze, 1985). Thus, the sensitivity of baroreceptors may be controlled by a feedback burst of sympathetic activity during diastole; this would accord the pulse a major role in reflex control of the cardiovascular system. This was termed "sympathetic modulation" of baroreceptor activity (Nonidez, 1935). Clearly, the notion of sympathetic modulation of baroreceptor activity is not new (Nonidez, 1935).

Kunze (1985) assessed conflicting views of neuro- and drug-induced modulation of baroreceptor activity. Kunze and co-workers (1984, 1985) showed that alpha-adrenoceptor agonists increase the baroreceptors' sensitivity and that beta-adrenoceptor stimulants had no effect. These findings were confirmed by Munch et al. (1987), who showed that "baroreceptor responses to vasoactive agents reflect not only changes in wall dimension but perhaps changes in wall tension and/or the coupling relation between baroreceptors and smooth muscle structures." The alpha-adrenoceptors on these baroreceptors have been pharmacologically characterized: they are blocked by nonspecific alpha-blockers such as tolazoline, phentolamine, and phenoxybenzamine (Landgren et al., 1952; Brattstrom et al., 1980; Brattstrom, 1980, 1981; Kunze et al., 1984), and the specific $alpha_1$-blocker prazosin (Munch et al., 1987; Munch & Brown, 1987). As responses to alpha-adrenoceptor

agonists are usually cGMP-mediated and slow, lasting minutes, any rapid pulse-by-pulse modulatory effects of these receptors must occur via another, possibly channel-mediated, mechanism.

Numao et al. (1983) reported three subsystems in the aortic depressor nerves of rabbits: 1) aortic baroreceptor A-fiber afferents with sympatho-inhibitory function, 2) aortic baroreceptor C-fiber afferents with sympatho-inhibitory function, and 3) the nociceptive C-fiber afferents with sympatho-excitatory function. They showed that the reflex excitatory component elicited by activating aortic C-fibers was eliminated selectively by treatment of animals with capsaicin. They confirmed that rabbit aortic nerves contained afferent fibers of non-barosensory modality by acute intracisternal injection of opioid peptides.

Sun and Biggs (1986, 1987, 1988, 1989, and Chapter IV) showed that their guinea-pig model of aortic baroreceptors was unique because, 1) the aortic depressor nerves in guinea pigs appeared as separate nerves in the cervical region, that were readily distinguishable from the vagi and the cervical sympathetic trunks; 2) chemoreceptor afferents were absent; and 3) baroreceptors were subserved by only myelinated afferents. However, they did not determine the origin and function of the many unmyelinated fibers in the aortic nerves.

In this functional study of the unmyelinated fibers in the aortic depressor nerves of guinea pigs, I sought to determine whether 1) some were sympathetic efferent fibers that modify aortic baroreceptor activity; and 2) others were nociceptive afferent fibers that mediate brief pressor responses that precede the depressor responses to aortic depressor nerve stimulation.

5.2. METHODS

5.2.1. Animals

The guinea-pig model of aortic depressor nerve activity was developed in our laboratory and is in routine use. Animals weighing 250-350 g were used because they lack fatty tissue in the cervical region. Male or female, Hartley-strain guinea pigs (Charles River, St Constant, Quebec, Canada) were purchased and housed in laminar-flow units (Bioclean, Hazleton, MD) on grids in cages suspended over trays of rock salt, at 25°C, on a 12-hour dark-light cycle. They were allowed water ad lib, and fed normal chow supplemented with apples.

5.2.2. Animal Preparation

The animals were anesthetized with pentobarbital (30 mg/kg i.p., with additional doses (5 mg/kg, i.v.) p.r.n.; urethane was not used because of its alpha$_2$- blocking actions (Armstrong et al., 1982; Moore et al., 1984).

Trachectomy was performed and the animals breathed room air spontaneously. Artificial respiration was applied only if needed.

Arterial blood pressure was monitored via a tapered cannula (PE90) inserted into a carotid artery (Statham P23Dd pressure transducer, HP8805B carrier amplifier, and Hewlett-Packard 7702B physiograph), and mean arterial blood pressure was determined by averaging the systolic and diastolic pressures. Heart rate was monitored from ECG lead II, or the systolic pulse (HP8812A rate computer). Drugs were injected via a catheter (PE50) placed into a jugular vein.

Under a dissecting microscope the carotid-sinus nerves were located and severed in the jugular foramen on both sides. The aortic depressor nerves, superior laryngeal nerves, and vagi were located and isolated. In some experiments, the nodose (vagal) and superior cervical ganglia, and cervical sympathetic trunks were isolated. The cervical region was flooded with mineral oil to insulate the electrodes and to prevent drying of the tissues.

Nerve potential recordings were made from the cut central and peripheral ends of one aortic depressor nerve, using established techniques (Sun & Biggs, 1986, 1987, 1988). Nerve activity was quantified using a full-wave rectifier and an integrator.

5.2.3. Experiments

5.2.3.1. Anatomic Studies

Under a dissecting microscope, the cervical region was dissected carefully, and nerves were visualized. The relationship among the nerves in cervical regions, especially the aortic depressor nerves, the superior laryngeal nerves, the sympathetic and vagi were examined. Special attention was paid to connections among the aortic depressor nerves, the superior laryngeal nerves, the superior cervical ganglia and the cervical sympathetic trunks.

5.2.3.2. Recording of Efferent Activity

The aortic depressor nerves were sectioned. Recordings were made from the caudal and cranial cut ends of one aortic depressor nerve using the techniques established in our laboratory. Nerve potentials were recorded via a pair of bipolar platinum electrodes connected to an amplifier system (Sun and Biggs, 1986, 1987) built in our laboratory. Nerve activity was displayed on a Tektronix 5113 dual-beam oscilloscope, and permanent records were made on Polaroid film (Type 667) with a Tektronix C-5A oscilloscope camera. We sought to record efferent activity and to determine its origin by recording from the cranial cut end of the aortic depressor

nerve and: 1) injecting a ganglionic blocker hexamethonium (10 mg/kg, i.v.); 2) mechanically separating the ipsilateral links between the superior laryngeal nerve and the superior cervical ganglion; and 3) sectioning the ipsilateral superior laryngeal nerve adjacent to the nodose ganglion.

5.2.3.3. Stimulation of Efferent Fibers

The left cervical sympathetic trunk and the right aortic depressor nerve were sectioned in mid-cervical regions, and the left aortic depressor nerve was isolated and left intact. The cranial cut end of the left cervical sympathetic trunk was laid upon a pair of bipolar platinum stimulating electrodes connected via an isolation unit (SIU5) to a Grass S44 stimulator. The nerve was stimulated (10 V, 4.5 Hz, 0.1 ms for 20 s), and left aortic nerve activity was recorded concomitantly. Arterial blood pressure and heart rate were monitored. Changes in the aortic afferent (baroreceptor) activity, and the arterial blood pressure, in response to cervical sympathetic trunk stimulation were monitored and recorded.

5.2.3.4. Determination of the Efferent Pathways

In 8 animals, one aortic depressor nerve and the ipsilateral cervical sympathetic trunk were sectioned in mid-cervical region close to the thorax. The cranial cut

end of the cervical sympathetic trunk was stimulated electrically (1-15 V, 0.1 ms) with a single pulse and activity in the ipsilateral cranial cut end of the aortic depressor nerve was recorded simultaneously (Fig. 5-2). Stimulus thresholds and conduction velocities were measured to determine the type of nerve fiber conduction that connected the cervical sympathetic trunk and the aortic depressor nerve. There would be a degree of synaptic delay in the transmission of the sympathetic pulses through the cervical sympathetic ganglia, and this would affect the measurement of the threshold and condition velocities. If an electrical connection was detected between the cervical sympathetic trunk and the aortic depressor nerve, the ganglionic blocker hexamethonium (10 mg/kg, i.v.) was injected, or the fine rami connecting the superior laryngeal nerve and the superior cervical ganglion were mechanically separated.

5.2.3.5. Orthodromic Electrical Stimulation

Both carotid sinus and aortic depressor nerves were sectioned. The cranial cut ends of the aortic depressor nerves were stimulated electrically (10 V, 10 Hz, 0.1 ms for 20 s). Arterial blood pressure and heart rate were monitored while stimulating the nerves. The responses to stimulation were compared after 30 min and 4 h of anesthesia with pentobarbital sodium (30 mg/kg, i.p.).

This procedure was repeated in guinea pigs that had been pretreated with capsaicin.

5.2.3.6. Capsaicin Treatment and Histological Studies

Capsaicin treatment: Five guinea pigs were used. Animals were anesthetized with pentobarbital (20-30 mg/kg, i.p.). 10 min after injection of salbutamol (0.6 mg/kg s.c.), capsaicin (20 mg/kg, s.c., 12.5% solution in equal parts of 95% ethanol and Tween-80, diluted to 25 mg/ml with normal saline) was given. After 2 h, another dose of pentobarbital (10-20 mg/kg, i.p.) was injected, the same dose of salbutamol was repeated, and more capsaicin (30 mg/kg, s.c.) was given. If respiratory distress developed, epinephrine (0.1 mg/kg, s.c.) was injected. After recovery from the anesthesia (about 2 h later), animals appeared "normal" and were returned to their housing. They were used for experiments 7-10 days after treatment.

Histological studies: the characteristics of nerve fibers in the left aortic depressor nerve of a single capsaicin-treated guinea pig were examined. Tissues were fixed, initially in vivo, with conventional transmission electron microscopy techniques (Bancroft and Stevens, 1982). After animals were anesthetized with pentobarbital, a 1-cm length of the left aortic depressor nerve was exposed in the mid-cervical region, and 3 ml of cold (4°C) freshly prepared 3% glutaraldehyde solution (in phosphate

buffer, pH 7.2) was poured into the area and left in contact with the nerve for 10 min. Then, the nerve was dissected free, removed, and fixed in more glutaraldehyde solution for 3 h. After postfixing in 1% osmium tetroxide solution for 2 h, and dehydration with graded ethanols, the tissue was embedded in LX-112 epoxy resin, and sectioned using a Reichert ultramicrotome. Ultrathin sections (60-90 nm) were cut with diamond knives. Sections were mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (Siemens Elmiskop 102). The numbers of myelinated and unmyelinated fibers were counted.

5.2.4. Statistical Analyses

A minimum of 4 replicates was obtained in each series of experiments. All values are expressed as mean $\pm$ SE. Differences were examined using Student's (paired) t test. Significance was assumed at the 5% level.

5.2.5. Drugs

Drugs used were epinephrine injection (Parke Davis Canada, Scarborough, Ont., Canada), hexamethonium bromide (K & K Laboratories, Plainview NY, USA), N-vanillyl-pelargonamide [synthetic capsaicin, recrystallized in our laboratory] (Fluka AG, Ronkonkoma, NY, USA), sodium pentobarbital (Euthanyl^{R}, 3 ml in 117 ml distilled water,

M.T.C. Pharmaceuticals, Mississauga, Ont., Canada), salbutamol hemisulfate (Sigma, St Louis, USA).

5.3. RESULTS

5.3.1. Anatomic Studies

There were close connections between the superior laryngeal nerves and the superior cervical ganglia. Thus, superior laryngeal nerves laid upon the superior cervical ganglia on both sides (Fig. 5-3), and, after lifting the superior laryngeal nerves, we saw some fine rami that ran from the superior cervical ganglia and joined the superior laryngeal nerves.

5.3.2. Recordings of Efferent Activity

Efferent activity was recorded from the cranial cut end of either aortic depressor nerve. There were 3 types of activity: 1) Type A (Fig. 5-4 and 5-5) related to cardiac events as it was synchronized with cardiac systoles. This type of activity was most common. 2) Type B related to respiratory events as it appeared only during inspiration (data not shown). And 3) Type C (Fig. 5-6 and 5-7) that had a scattered and random firing pattern.

Activities related to cardiac and respiratory events were eliminated after injection of the ganglionic blocker hexamethonium or after the ipsilateral links between the superior laryngeal nerve and the superior cervical ganglion

were mechanically separated. Type C activity was unaffected by either of these procedures, but was abolished by sectioning ipsilateral superior laryngeal nerve close to the nodose ganglion.

5.3.3. Stimulation of Efferent Fibers

If the cranial cut end of left cervical sympathetic trunk was stimulated electrically, baroreceptor activity in the left aortic nerve was enhanced (Fig. 5-8 and 5-9). Changes in arterial blood pressure and heart rate were variable: sometimes they increased (Fig. 5-9) and sometimes they were unchanged (Fig. 5-8). All changes in baroreceptor activity and arterial blood pressure in response to stimulation were eliminated by mechanically separating the ipsilateral links between the superior laryngeal nerve and the superior cervical ganglion.

5.3.4. Determination of Efferent Pathways

In 8 animals, stimulating the cranial cut end of the cervical sympathetic trunk evoked a slowly conducted potential (0.55 ± 0.1 m/s, n=8) in the ipsilateral cranial cut end of the aortic depressor nerve. The mean stimulus threshold of the evoked potentials was 6.9 ± 0.6 V, n=8. The potentials were eliminated after injection of the ganglionic blocker hexamethonium (10 mg/kg, i.v.), or after the ipsilateral links between the superior laryngeal nerve

and the superior cervical ganglion were mechanically separated (Fig. 5-10 and 5-11). Hexamethonium (1 mg/kg, i.v.) did not alter evoked potentials recorded in the aortic depressor nerve in response to cervical sympathetic trunk stimulation (Fig. 5-11).

5.3.5. Orthodromic Electrical Stimulation

After 30 min anesthesia with pentobarbital, orthodromic electrical stimulation of the aortic depressor nerves caused only depressor responses and slight bradycardia. However, after 4 h of anesthesia with pentobarbital, aortic depressor nerve stimulation resulted in a brief pressor component that preceded the depressor responses. Heart rate did not change significantly (Fig. 5-12). The brief pressor responses to stimulation were absent in animals pretreated with capsaicin 10 d before experiments (Fig. 5-13).

5.3.6. Histological Studies

Histological findings revealed striking changes in the ratio of myelinated to unmyelinated nerve fibers seen in cross sections of the aortic depressor nerves in the capsaicin-treated animal. The ratio was 1:1 (compared to 1:3-4 in the control group). Some myelinated fibers revealed dense changes that filled in the central area (Fig. 5-14) and were not seen in normal animals.

5.4. DISCUSSION

In the aortic depressor nerves of guinea pigs, we showed that the aortic baroreceptor afferent activity was conducted via the myelinated nerve fibers and that the functions of the remaining unmyelinated fibers was unknown (Sun and Biggs, 1988, 1989, and Chapter V). We report here some of the functions of the unmyelinated fibers.

We noted close connections between the superior cervical ganglion of the cervical sympathetic trunk and the superior laryngeal nerve which, in turn, merged with the aortic depressor nerve. Thus, some sympathetic nerve fibers could run from the cervical sympathetic trunk to the superior cervical ganglion, then to superior laryngeal nerve, and finally to the aortic depressor nerve. Also, there may be some parasympathetic nerve fibers in the aortic depressor nerves, as the aortic depressor nerves join the superior laryngeal nerves, en route to the vagi. These sympathetic and parasympathetic nerve fibers should be of efferent origin. Therefore, the aortic depressor nerves of guinea pigs could contain both sympathetic and parasympathetic efferents.

Recordings from the efferent activity of the aortic depressor nerves of guinea pigs revealed three different types of activity. Type A activity was related to cardiac events. As the ipsilateral carotid sinus nerves and the aortic depressor nerves were sectioned, the activity

recorded from the cranial cut end of the aortic depressor nerve must be of efferent origin. The activity was of lower amplitude than the afferent activity recorded in the caudal cut end of the aortic depressor nerve. This suggested that the cardiac-cycle-associated efferent activity in the aortic depressor nerve was conducted via unmyelinated nerve fibers. Type B activity was related to respiratory events, and it appeared only during inspiration. This suggested that efferent fibers yielding Type B activity might travel into the thorax, and innervate the respiratory organs, as the aortic depressor nerve runs into the thoracic cavity. Types A and B activities were abolished after injection of the ganglionic blocking agent hexamethonium or after mechanically separating the links between the superior laryngeal nerve and the superior cervical ganglion of the cervical sympathetic trunk. This indicated that: 1) both Type A and B activities were of sympathetic origin, 2) the sympathetic nerve fibers yielding Type A and B activity originated from the paravertebral ganglionic chains, passed rostrally via the cervical sympathetic trunk to the superior cervical ganglion, then to the superior laryngeal nerve, and caudally to the aortic depressor nerve, and 3) these fibers in the cervical sympathetic trunk were preganglionic fibers (most likely myelinated), that synapsed in the superior cervical ganglion, and then became postganglionic

unmyelinated fibers that ran into the superior laryngeal nerve, and then to the aortic depressor nerve. The sympathetic efferent fibers in the aortic depressor nerve appeared to modify aortic baroreceptor activity in guinea pigs, as has been reported in other species (Brattstrom, 1980, 1981; Brattstrom et al., 1980; Kunze, 1985; Kunze et al.,1984; Landgren et al., 1952; Munch and Brown, 1987; Munch et al.,1987; Neil and O'Regan, 1971; Nonidez 1935). Isolated aortic depressor nerve-aortic arch preparations and single fiber recording would confirm this.

Type C efferent activity was of parasympathetic preganglionic origin, as it was not eliminated by hexamethonium, or by mechanically separating the links between the superior laryngeal nerve and the superior cervical ganglion. It was eliminated by sectioning the superior laryngeal nerve near the nodose ganglion. It may emanate from preganglionic parasympathetic efferent fibers travelling in the aortic depressor nerve to the thorax. The function of these fibers is unknown.

In the third series of experiments, the effect of stimulating the sympathetic efferent fibers in the cervical sympathetic trunk on the aortic baroreceptor activity and arterial blood pressure was determined. Stimulation of the efferents in the cervical sympathetic trunk enhanced afferent baroreceptor activity. This effect was abolished after the links between the superior laryngeal nerve and

the superior cervical ganglion were mechanically separated. Thus, the sensitivity of the aortic baroreceptors to pulse-induced changes in arterial blood pressure was increased by stimulating the sympathetic efferent fibers in the aortic depressor nerve.

We determined whether there were sympathetic efferent pathways between the cervical sympathetic trunk and the aortic depressor nerve by stimulating the cranial cut end of the cervical sympathetic trunk and recording from the ipsilateral cranial cut end of the aortic depressor nerve. Slowly conducted compound action potentials were revealed. These were eliminated after giving the ganglionic blocker hexamethonium, or after mechanically separating the links between the superior laryngeal nerves and the superior cervical ganglion.

In the last series of experiments, we determined whether there were visceral nociceptive C-fiber afferents in the aortic depressor nerves. Numao et al. (1983) have reported that the aortic depressor nerves in rabbits contained visceral nociceptive C-fiber afferents with sympatho-excitatory function. We found the similar sympatho-excitatory effects upon orthodromic stimulation of the aortic depressor nerves. The pressor responses to the stimulation appeared after 4 h of anesthesia with pentobarbital, and differ from the pressor component seen if chemoreceptor afferents in the aortic nerves are

stimulated in swine, dogs and cats (Douglas and Schaumann, 1956; Pelletier et al., 1972; Schmidt, 1968). The latter remain constant throughout the course of experiments. The pressor responses we noted were absent in animals pretreated with capsaicin. In histological studies of an aortic depressor nerve from a capsaicin-pretreated guinea pig, the ratio of myelinated to unmyelinated nerve fibers changed from 1:3-4 in controls to 1:1 in the capsaicin-treated animal. Thus, large numbers of the unmyelinated nerve fibers in the aortic depressor nerves were destroyed by capsaicin. These findings suggest that there are many visceral nociceptive afferent fibers in the aortic depressor nerves of guinea pigs. The remaining unmyelinated nerve fibers in the aortic depressor nerves of capsaicin treated animals appear to be sympathetic efferent fibers.

In summary, we found that in the unmyelinated fibers of the aortic depressor nerves in guinea pigs are, 1) sympathetic efferent nerve fibers, that originate in the paravertebral ganglionic chains, pass rostrally via the cervical sympathetic trunks to the superior sympathetic ganglia, then to the superior laryngeal nerves and finally pass caudally to the aortic depressor nerves; 2) visceral nociceptive afferent nerve fibers, that have a sympatho-excitatory function and travel within the aortic depressor nerves to the central nervous system.

5.5. ACKNOWLEDGEMENTS

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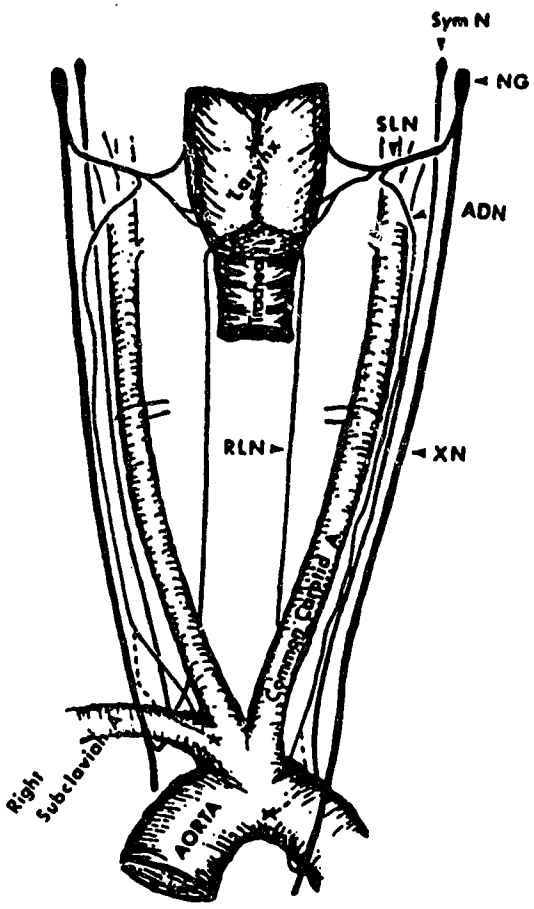

Figure 5-1. Anatomy of the cervical region in the guinea pig showing relevant nerves: ADN - aortic depressor nerve; NG - nodose ganglion; RLN - recurrent laryngeal nerve; SLN - superior laryngeal nerve; Sym N - sympathetic nerve; and XN - vagus nerve.

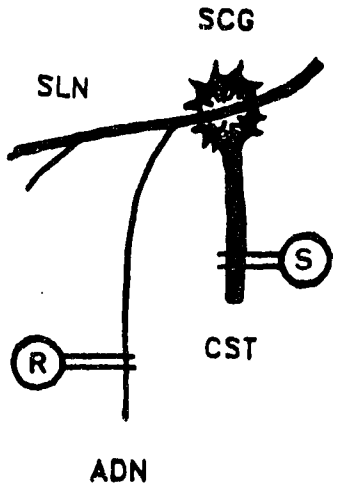

Figure 5-2. Diagram showing the stimulation site (S) of the cranial cut end of the cervical sympathetic trunk, and the recording site (R) of the ipsilateral cranial cut end of the aortic depressor nerve. Symbols: ADN - aortic depressor nerve; CST - cervical sympathetic trunk; SCG - superior cervical ganglion; SLN - superior laryngeal nerve.

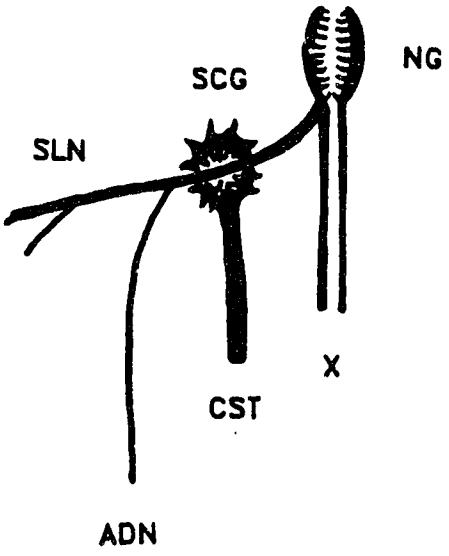

Figure 5-3. Local anatomy of the cervical region in guinea pigs showing the links between the superior laryngeal nerves and the superior cervical ganglion of the cervical sympathetic trunk. The superior laryngeal nerve laid upon the superior cervical ganglion (in ventral view). The aortic depressor nerve merges into the superior laryngeal nerve adjacent to this site. Symbols: ADN - aortic depressor nerve; CST - cervical sympathetic trunk; NG - nodose ganglion; SCG - superior cervical ganglion; SLN - superior laryngeal nerve; XN - vagus nerve.

Figure 5-4. Guinea pig, both carotid sinus and aortic depressor nerves sectioned: efferent activity recorded from the left aortic depressor nerve. The activity (upper trace) was related to systole (lower trace: arterial blood pressure).

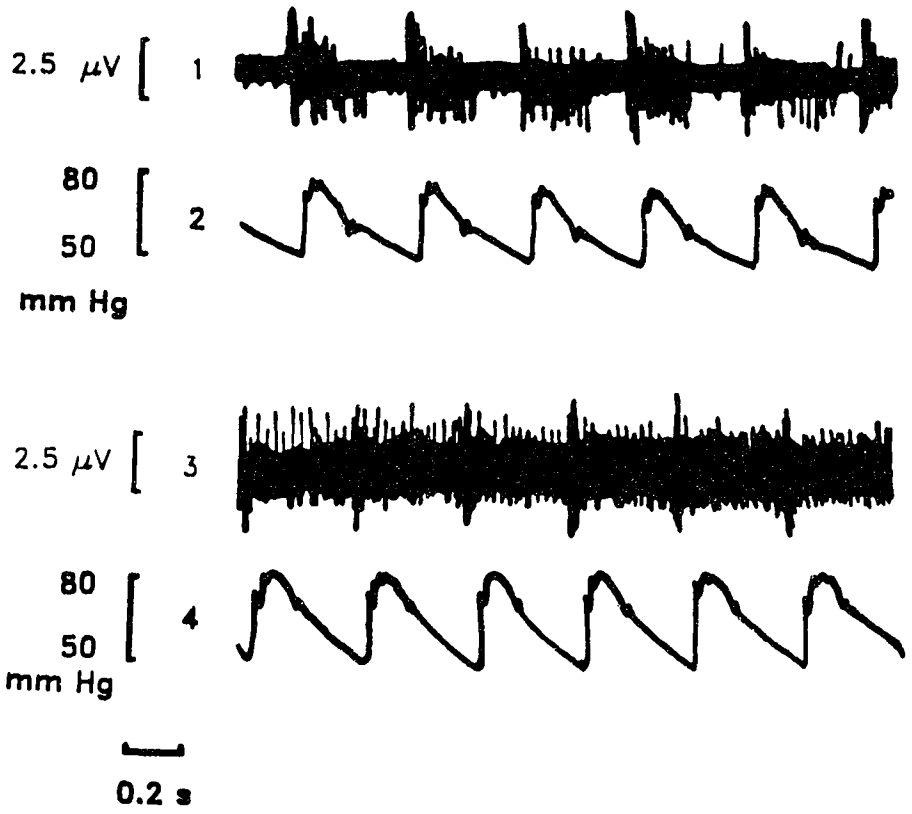

Figure 5-5. Guinea pig, both carotid sinus and aortic depressor nerves sectioned: efferent activity recorded from the right aortic depressor nerve. Activity related to systole (trace 1), and continuous activity (trace 3). Traces 2 and 4: arterial blood pressure. Traces 1 and 2, and 3 and 4 recorded simultaneously.

Figure 5-6. Guinea pig, both carotid sinus and aortic depressor nerves sectioned: afferent and efferent activities recorded from the left aortic depressor nerve. Trace 1: afferent activity synchronized with systole (trace not shown). Traces 2 and 3: scattered and random activity.

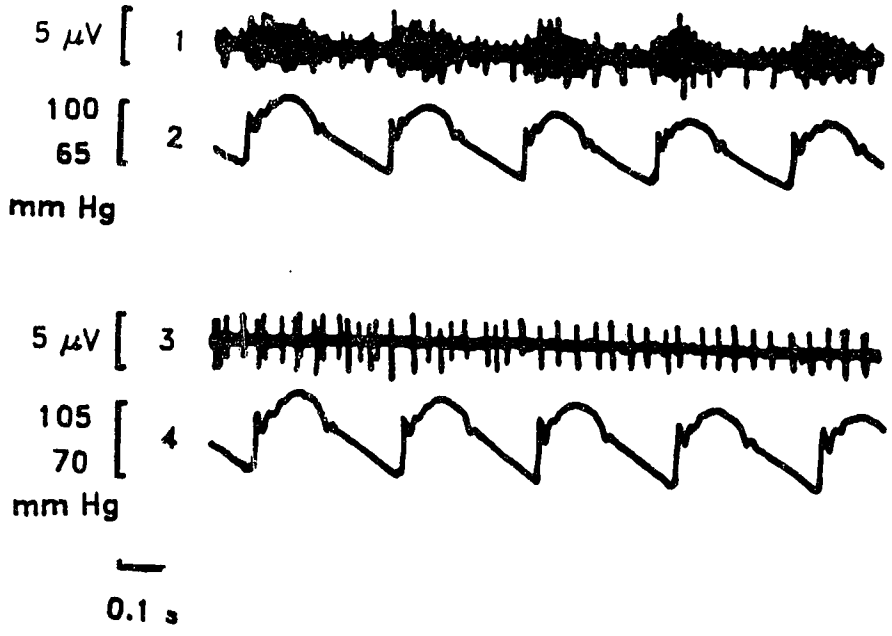

Figure 5-7. Guinea pig, both carotid sinus and aortic depressor nerves sectioned: afferent and efferent activities recorded from the right aortic depressor nerve. Trace 1: afferent activity synchronized with systole (trace 2, arterial blood pressure). Trace 3: scattered and random activity that was not related to systole (trace 4, arterial blood pressure). Traces 1 and 2, and 3 and 4 were recorded simultaneously.

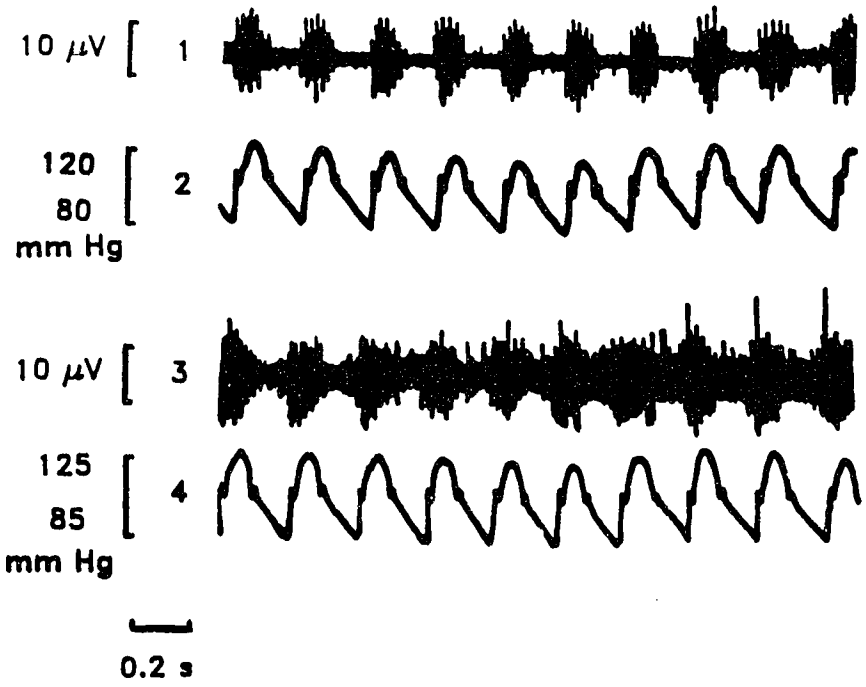

Figure 5-8. Guinea pig, carotid sinus nerves sectioned: aortic baroreceptor afferent activity (trace 3) increased after the cranial cut end of the cervical sympathetic trunk was stimulated electrically (10 V, 4.5 Hz, 0.1 ms for 20 s). Arterial pressure (trace 4) did not change. Trace 1: aortic baroreceptor afferent activity in control; trace 3: the increased activity after stimulation; trace 2 and 4: arterial blood pressure. Trace 1 and 2, and 3 and 4 were recorded simultaneously.

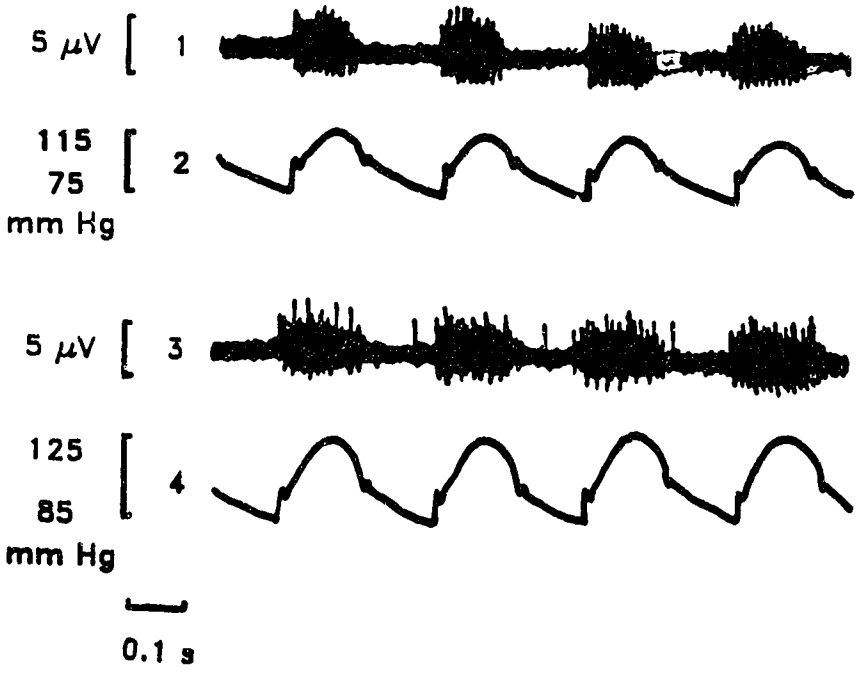

Figure 5-9. Guinea pig, carotid sinus nerves sectioned: both aortic baroreceptor afferent activity (trace 3) and the arterial blood pressure (trace 4) increased when the cranial cut end of the cervical sympathetic trunk was stimulated electrically (10 V, 4.5 Hz, 0.1 ms for 20 s). Trace 1: aortic baroreceptor afferent activity in control; trace 3: the increased activity after stimulation; trace 2: arterial blood pressure in control; trace 4: the increased arterial pressure after stimulation. Trace 1 and 2, and 3 and 4 were recorded simultaneously.

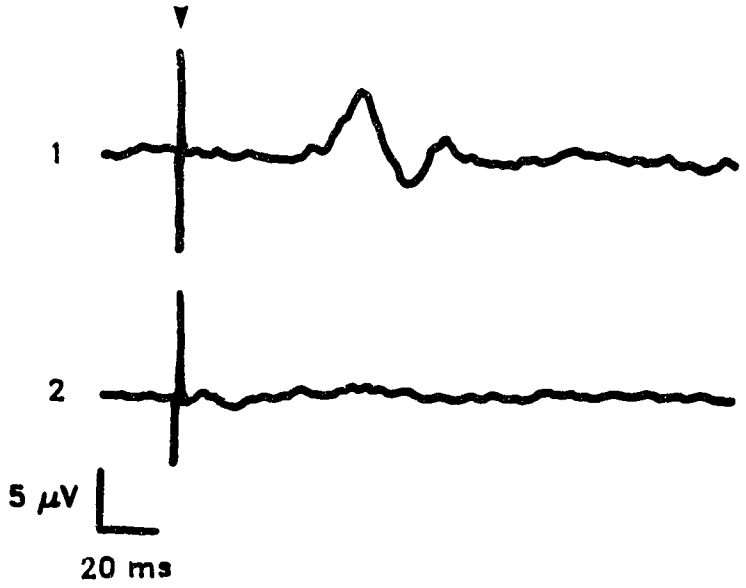

Figure 5-10. Guinea pig, both carotid sinus and aortic nerves sectioned: electrophysiological evidence of pathways between the cervical sympathetic system and the aortic depressor nerve. Upper trace: evoked slow wave (threshold = 8 V, conduction velocity = 0.33 m/s) recorded from the cranial cut end of the aortic depressor nerve, after stimulating the cranial cut end of the cervical sympathetic trunk. Lower trace: the slow wave was eliminated by hexamethonium (10 mg/kg, i.v.).

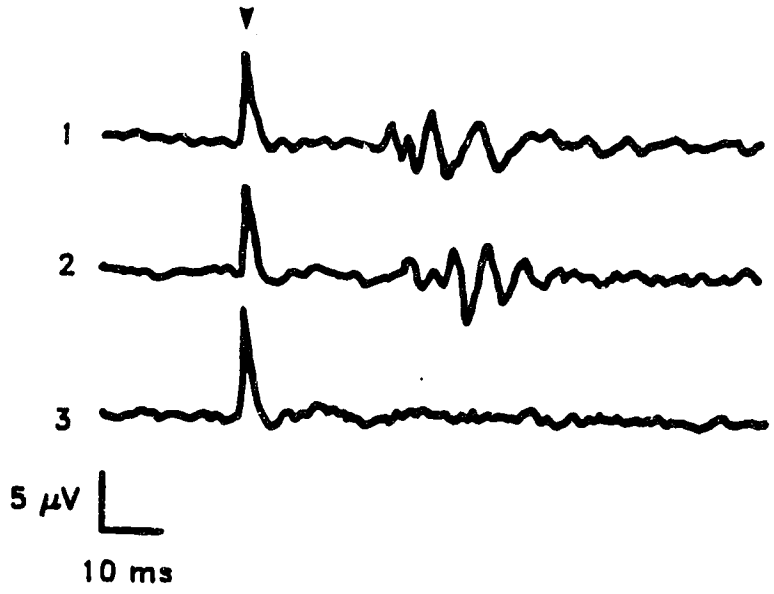

Figure 5-11. Guinea pig, both carotid sinus and aortic nerves sectioned: recordings showing links between the cervical sympathetic trunk and the aortic depressor nerve. Upper trace: evoked slow waves recorded as in Figure 5-10. Middle trace: after hexamethonium (1 mg/kg, i.v.) - (note dose too low to block the activity). Lower trace: the slow waves disappeared after the links between the superior laryngeal nerve and the superior cervical ganglion of the cervical sympathetic trunk were mechanically separated.

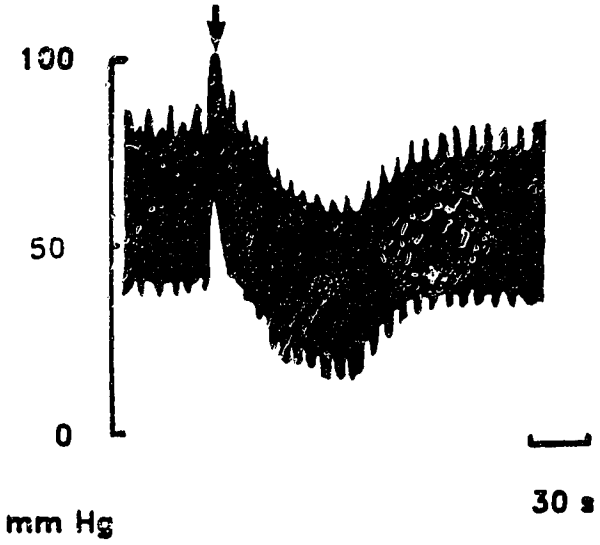

Figure 5-12. Guinea pig, both carotid sinus and aortic depressor nerves sectioned: orthodromic stimulation of the aortic depressor nerves induced a brief pressor component (at arrows) that preceded the depressor responses. This recording was made 4 h after induction of pentobarbital anesthesia.

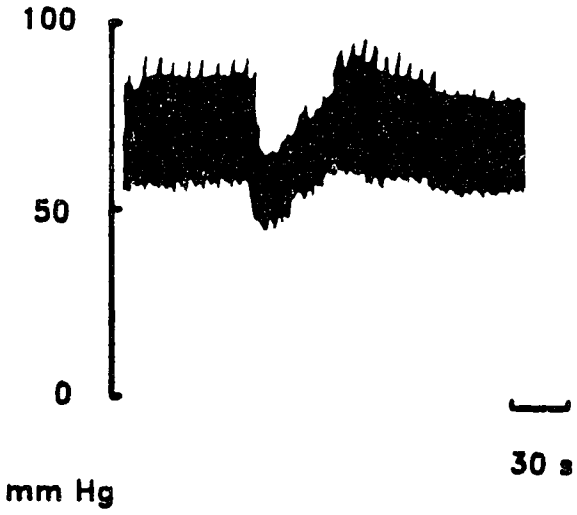

Figure 5-13. Guinea pig, both carotid sinus and aortic depressor nerves sectioned: the brief pressor responses to stimulation were absent in animals pretreated with capsaicin (50 mg/kg, s.c.). Only depressor responses to stimulation were recorded after 4 h anesthesia with pentobarbital in the capsaicin-pretreated animals.

Figure 5-14. Electron microscopic section of an aortic depressor nerve in a capsaicin-treated guinea pig shows: 1.) myelinated and unmyelinated nerve fibers, their ratio differs from that of a control group, and 2.) some dense changes in the central area of some myelinated fibers that were not seen in normal animals.

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CHAPTER VI. THE EFFECTS OF CAROTID OCCLUSION ON ARTERIAL BLOOD PRESSURE IN GUINEA PIGS*.

* A version of this chapter will be submitted to "Archives Internationales de Pharmacodynamie et de Therapie."

6.1. INTRODUCTION

Arterial baroreceptors are located in the carotid sinuses and the aortic arch (Kirchheim, 1976; Bagshaw, 1985). They are slowly-adapting mechanoreceptors that respond to mechanical stress in the arterial walls secondary to increases in arterial blood pressure (P_{art}) (Kirchheim, 1976; Brown, 1980; Sagawa, 1983; Bagshaw, 1985).

In humans and most other species, the carotid sinuses appear as anatomical enlargements at the origin of the internal carotid arteries (Bagshaw, 1985). However, in guinea pigs, the internal carotid arteries are absent and the carotid sinuses are located at the origin of occipital arteries (Heymans and Neil, 1958; Rees, 1967; Bagshaw, 1985). The carotid sinuses are highly elastic and contain less vascular smooth muscle than adjacent portions of the common carotid, external carotid, internal carotid and occipital arteries. They are richly innervated by myelinated and unmyelinated afferents, and by sympathetic efferents originating from the superior cervical ganglia (Bagshaw, 1985; Bock and Gorgas, 1976; Reis and Fuxe, 1968; Shin et al., 1987).

Carotid sinus and aortic baroreceptors help regulate P_{art}, and sectioning the carotid sinus and/or the aortic depressor nerves induces acute increases in P_{art}. By

contrast, stimulation of the carotid sinus or aortic depressor nerves induces a reflex fall in P_{art} by decreasing peripheral vascular resistance and inducing bradycardia (Sagawa, 1983; Daly, 1986).

Bilateral occlusion of the common carotid arteries has been widely used to investigate reflexes involving carotid sinus baroreceptors. In most species, carotid baroreflexes dominate over aortic baroreflexes (Bagshaw, 1985). Thus, decreasing intra-sinus pressures (P_{sinus}) by bilateral occlusion of the common carotid arteries increases P_{art} even if the aortic depressor nerves are intact (Kirchheim, 1976). However, in guinea pigs with intact aortic depressor nerves, Biggs et al. (1984) showed that carotid occlusion induced falls in P_{art} without any significant changes in heart rate. This study was performed to clarify and to confirm these paradoxical findings.

6.2. METHODS

6.2.1. Animals

Male or female Hartley-strain guinea pigs (Charles River, St Constant, Quebec) weighing 400-700 g were used. Animals were housed in laminar-flow units (Bioclean, Hazleton, MD) on grids in cages suspended over trays of rock salt, at 25°C, on a 12-h dark/light cycle. They were allowed water ad lib, and fed standard guinea-pig chow supplemented with apples.

6.2.2. Animal preparation

Animals were anesthetized with pentobarbital (30 mg/kg, i.p.) with additional doses (5 mg/kg, i.v.) as required. A tracheotomy was performed, and animals breathed room air spontaneously.

In all experiments, P_{art} and P_{sinus} were monitored (Gould P50 pressure transducers, Grass 7D polygraph) unilaterally via two cannulas (PE50) inserted into the left common carotid artery, one pointing caudally (P_{art}) and the other cranially (P_{sinus}). Mean P_{art} and P_{sinus} were determined as the average of the systolic and diastolic pressures. Heart rate was monitored via ECG lead II (HP8812A rate computer, Hewlett-Packard 7702B physiograph). Drugs were injected via a catheter (PE50) placed in the left jugular vein.

Under a dissecting microscope, the carotid sinus and aortic depressor nerves were isolated on each side. 3-5 cm of the right common carotid artery caudal to the carotid sinus was carefully dissected free. A small vascular clamp (about 2 mm wide and 20 mm long) was used to occlude the carotid artery. The cervical region was flooded with mineral oil to prevent drying of the tissues.

6.2.3. Experimental procedures

The left carotid sinus nerve was sectioned. Thus, as the left common carotid artery was cannulated, occlusion of the right common carotid artery represented "bilateral

carotid occlusion" and was termed "carotid occlusion" for the purposes of this study.

Carotid occlusion was performed for 2 min at a site 3 cm caudal (distal), and 1 cm caudal (proximal) to the carotid sinus. Occlusion at the distal site was assumed to be "non-stretching" as the clamp was placed as carefully as possible to avoid stretching the carotid artery. Occlusion at the proximal site was assumed to induce some stretching of the carotid sinus as the clamp was applied. The effects of both occlusions were compared before and after sectioning the aortic depressor nerves. As a control, both types of occlusion were repeated after sectioning the right carotid sinus nerve. P_{art}, P_{sinus}, and heart rate were monitored before, during and after occlusions.

The effect of pretreatment with capsaicin on responses to carotid occlusion was examined in 5 animals; 5 animals pretreated with the vehicle used to dissolve the capsaicin served as controls.

Capsaicin treatment: Animals were anesthetized with pentobarbital (20-30 mg/kg, i.p.). Then, 10 min after injection of salbutamol (0.6 mg/kg s.c.), capsaicin (20 mg/kg, s.c., 12.5% solution in equal parts of 95% ethanol and Tween-80, diluted to 25 mg/ml with normal saline) was given. 2 h later, more pentobarbital (10-20 mg/kg, i.p.) was injected, the dose of salbutamol repeated, and capsaicin (30 mg/kg, s.c.) was given. If respiratory

distress developed, epinephrine (0.1 mg/kg, s.c.) was injected. After recovery from the anesthesia (about 2 h later), animals appeared "normal" and were returned to their housing. Control animals were treated similarly, but received vehicle only. Both groups of animals were used for experiments 7-10 days after treatment.

6.2.4. Statistical analyses

All values are reported as mean ± S.E. A least 4 replicates were obtained in each series of experiments. Differences were examined using Student's (paired) t test. Significance was assumed at the 5% level.

6.2.5. Drugs

Drugs used were: epinephrine injection (Parke Davis Canada, Scarborough, Ont), N-vanillylpelargonamide [synthetic capsaicin, recrystallized in our laboratory] (Fluka AG, Ronkonkoma, NY), sodium pentobarbital (Euthanyl^{R}, 3 ml in 117 ml distilled water, M.T.C. Pharmaceuticals, Mississauga, Ont), salbutamol hemisulfate (Sigma, St Louis, MO).

6.3. RESULTS

Baseline values of mean P_{art}, P_{sinus}, and heart rate in guinea pigs under pentobarbital anesthesia taken after sectioning the left carotid sinus nerve and allowing time

for animals to stabilize were 65 ± 3 mm Hg, 40 ± 2 mm Hg, and 230 ± 7 beats/min, respectively.

6.3.1. Effects of carotid occlusion before sectioning the aortic nerves

Carotid occlusion distal to the carotid sinus induced either pressor (n=2) or depressor (n=2) responses. In animals that gave pressor responses, mean P_{art} increased from 60 to 70, and from 75 to 85 mm Hg, and P_{sinus} fell from 45 to 40, and from 40 to 30 mm Hg; heart rate was unchanged. In animals that gave depressor responses, mean P_{art} fell from 65 to 40, and from 60 to 55 mm Hg, and P_{sinus} fell from 40 to 20, and from 35 to 15 mm Hg; heart rate fell from 240 to 220 beats/min in one animal, but was unchanged in the other.

Carotid occlusion proximal to the sinus induced only depressor responses (n=4). Mean P_{art} fell from 65 ± 7 to 45 ± 7 mm Hg (NS), and P_{sinus} decreased from 41 ± 5 to 26 ± 7 mm Hg ($p<0.05$); heart rate decreased from 225 ± 10 to 210 ± 10 beats/min (NS).

6.3.2. Effects of carotid occlusion after sectioning the aortic nerves

Bilaterally sectioning the aortic depressor nerves increased mean P_{art} from 60 ± 4 to a maximum of 86 ± 10 mm Hg ($p<0.05$), and P_{sinus} from 40 ± 2 to a maximum of 47 ± 4 mm

Hg (NS). Mean heart rate was unchanged (230 ± 7 to 230 ± 7 beats/min). After 10 min, mean P_{art} and P_{sinus} fell slowly, and stabilized at 66 ± 6 and 41 ± 4 mm Hg respectively. Mean heart rate was unchanged.

Carotid occlusion distal to the carotid sinus now induced only pressor responses without changes in heart rate (Fig. 6-2). Mean P_{art} increased from 64 ± 6 to 75 ± 9 mm Hg ($p<0.05$), and P_{sinus} fell from 39 ± 6 to 31 ± 7 mm Hg ($p<0.05$). Upon releasing the occlusion, P_{art} and P_{sinus} returned to baseline levels almost immediately.

Carotid occlusion proximal to the carotid sinus induced depressor responses without changes in heart rate (Fig. 6-3). Mean P_{art} decreased from 61 ± 4 to 44 ± 5 mm Hg ($p<0.05$), and P_{sinus} fell from 38 ± 6 to 22 ± 5 mm Hg ($p<0.05$). The decreases in P_{art} showed "adaptation" and returned to baseline levels during the 2-min occlusion (Fig.6-3). Upon releasing the occlusion, P_{sinus} returned to baseline levels almost immediately.

6.3.3. Effects of carotid occlusion on P_{art}, P_{sinus} and heart rate after sectioning the right carotid sinus nerve

No changes in P_{art} or heart rate were observed during distal or proximal occlusion after the right carotid sinus nerve was sectioned (Fig. 6-4 and 6-5). However, P_{sinus} still fell from 38 ± 6 to 28 ± 5 mm Hg ($p<0.01$) during the

occlusion. Upon releasing the occlusion, P_{sinus} returned to baseline levels almost immediately.

6.3.4. Effects of capsaicin pretreatment on responses to carotid occlusion

In capsaicin-treated animals, distal occlusion increased mean P_{art} in 4 animals (75 ± 2 to 84 ± 2 mm Hg [$p<0.05$]) and decreased it in 1 animal (80 to 75 mm Hg). P_{sinus} fell from 42 ± 3 to 31 ± 3 mm Hg ($p<0.05$); heart rate increased slightly (257 ± 4 to 259 ± 4 beats/min [NS]). Proximal occlusion induced only depressor responses. Mean P_{art}, P_{sinus}, and heart rate fell from 76 ± 2 to 54 ± 5 mm Hg ($p<0.05$), 42 ± 3 to 24 ± 5 mm Hg ($p<0.05$), and 256 ± 4 to 243 ± 10 beats/min (NS), respectively.

After sectioning the aortic depressor nerves, distal occlusion only increased mean P_{art} (76 ± 4 to 92 ± 3 mm Hg [$p<0.05$]), and decreased P_{sinus} (41 ± 2 to 31 ± 4 mm Hg [$p<0.05$]). Heart rate was unchanged (254 ± 4 to 254 ± 4 beats/min). Proximal occlusion induced falls in P_{art} (77 ± 3 to 56 ± 5 mm Hg [$p<0.05$]), and P_{sinus} (41 ± 2 to 24 ± 4 mm Hg [$p<0.05$]) in all animals. Heart rate was unchanged (254 ± 4 to 254 ± 4 beats/min). After the right carotid sinus nerve was sectioned, no changes in P_{art} were observed and heart rate was unchanged (254 ± 4 to 254 ± 4 beats/min) during distal or proximal occlusions. However, P_{sinus} fell from 40 ± 4 to 28 ± 1 mm Hg ($p<0.05$) during occlusions.

In 5 control animals treated with vehicle only, distal occlusion induced increases in mean P_{art} in 3 animals (75 ± 3 to 85 ± 2 mm Hg), and decreases in 2 animals (75 to 70, and 70 to 65 mm Hg). P_{sinus} fell from 41 ± 2 to 30 ± 2 mm Hg ($p<0.05$), and heart rate increased slightly from 254 ± 4 to 256 ± 4 beats/min (NS). Proximal occlusion induced only falls in mean P_{art} 74 ± 2 to 52 ± 3 mm Hg ($p<0.05$). P_{sinus} and heart rate fell from 41 ± 2 to 25 ± 2 mm Hg ($p<0.05$), and from 254 ± 4 to 240 ± 6 beats/min (NS), respectively.

After sectioning the aortic depressor nerves, distal occlusion induced increases in mean P_{art} (74 ± 2 to 91 ± 2 mm Hg [$p<0.05$]), and decreases in P_{sinus} (41 ± 2 to 30 ± 4 mm Hg [$p<0.05$]). Heart rate was unchanged (252 ± 4 to 252 ± 4 beats/min). Proximal occlusion induced falls in P_{art} (74 ± 2 to 54 ± 3 mm Hg [$p<0.05$]), and P_{sinus} (41 ± 2 to 23 ± 3 mm Hg [$p<0.05$]). Heart rate was unchanged (252 ± 4 to 252 ± 4 beats/min). After the right carotid sinus nerve was sectioned, no changes in P_{art} were observed and heart rate was unchanged (252 ± 4 to 252 ± 4 beats/min) during distal or proximal occlusions. However, P_{sinus} fell from 40 ± 3 to 30 ± 3 mm Hg ($p<0.05$) during occlusions.

6.4. DISCUSSION

Occlusion of the common carotid arteries is widely used to study reflexes involving carotid sinus baroreceptors. In most species, such as in cats, dogs,

rabbits and rats, carotid occlusion results in pressor responses and tachycardia (Kirchheim, 1976). However, in guinea pigs, Biggs et al. (1984) reported that carotid occlusion induced paradoxical depressor responses without changes in heart rate.

In the first series of experiments, the aortic depressor nerves were left intact, and great care was taken to avoid stretching the carotid sinus during carotid occlusion. If the common carotid artery was occluded distal to the carotid sinus, either pressor or depressor responses were noted. Carotid occlusion proximal to the carotid sinus induced brief (<1 min) falls in P_{art}. The sinus appeared to be stretched as the clamp was applied; which could stimulate carotid baroreceptors despite the concomitant fall in P_{sinus}.

Depressor responses showed "adaptation" in that they slowly returned to baseline levels during occlusion although P_{sinus} remained at about 70% of baseline for 2 min. The adaptation noted could have arisen from the stimulus of applying the clamp, i.e., after the depressor stimulus waned, P_{art} would return to baseline. Then, one would expect to observe a pressor response mediated via the 30% decrease in P_{sinus}. This was never noted, even though there was usually enough time for it to appear. Biggs et al. (1984) and D'Souza (1990) noted similar adaptation of the depressor responses to bilateral occlusion.

The (relatively) small decrease in P_{sinus} noted after occlusion was surprising. On average, pressure fell by about 30%. D'Souza (1990), who examined the pressure-activity relationship of carotid baroreceptors in guinea pigs, showed that the baroreceptors' threshold was about 20 mm Hg and the curve relating the two parameters reached a maximum at about 100 mm Hg. Thus, although the decreases in P_{sinus} noted in these experiments could have induced physiologically significant decreases in baroreceptor activity, the changes may have been within the buffering capacity of the aortic baroreceptors. So, in the second series of experiments, the aortic depressor nerves were sectioned to prevent any interference from aortic baroreflexes. Distal occlusion now resulted only in pressor responses. During occlusion, P_{sinus} fell, and presumably resulted in an unapposed decrease in carotid-sinus baroreceptor activity that induced a reflex increase in P_{art}.

The responses to proximal occlusions were similar before and after the aortic depressor nerves were sectioned. As mentioned above, occlusion proximal to the sinus induced brief depressor responses, and the sinus appeared to be stretched as the clamp was applied.

In the last series of experiments, the occlusions were repeated after the right carotid sinus nerve was sectioned. Predictably, neither occlusion had any effect on P_{art},

although, P_{sinus} fell as before. Thus, in these experiments, both the pressor and the depressor responses to occlusion were mediated via the carotid sinus nerve. This suggests that the carotid sinus nerve contains sensory afferents that mediated responses to mechanical stimuli.

In most other species, e.g., cats, dogs, rabbits and rats, carotid occlusion induces reflex pressor responses, that are eliminated by cutting the carotid sinus nerves (Kirchheim, 1976). In guinea pigs anesthetized with pentobarbital after both aortic depressor nerves were sectioned, carotid occlusion induced pressor responses only if the carotid sinus was not stretched as the clamp was applied.

Comparison of these data with those reported by Biggs et al. (1984), revealed some differences. Firstly, these workers reported that depressor responses were most marked if urethane was used as the anesthetic, findings confirmed by D'Souza (1990). Urethane blocks $alpha_2$-adrenoceptors (Armstrong et al., 1982; Moore et al., 1984), and was not used this study. As chloralose was a potent respiratory depressant in guinea pigs, pentobarbital was selected as the anesthetic for this study. Secondly, aortic baroreflexes were intact in animals used in previous studies (Biggs et al., 1984); this would have "buffered" any carotid baroreflexes. I eliminated this potential problem by sectioning the aortic depressor nerves.

Thirdly, both Biggs et al. (1984) and D'Souza (1990) found that sectioning the carotid sinus nerves reduced, but did not abolish, the depressor responses to occlusion. Interestingly, they found that bilateral vagotomy eliminated them. In my study, both pressor and depressor responses to occlusion were eliminated by sectioning the carotid sinus nerves. Vagotomy was not required. Finally, in my study a fine vascular clamp was used for the occlusion, and stretching was minimized by occluding the artery at a site as far distal to the sinus as possible. My data showed clearly that the site of occlusion helped determine the response.

These discrepancies could result from differences among the guinea-pig preparations. Baroreceptor input into the central nervous system would be greatly reduced in my preparation after both aortic, and one carotid sinus, nerves were sectioned. However, in the absence of direct monitoring of baroreceptor activity in the intact carotid sinus nerve, one cannot determine the level of input into the central nervous system.

My records of P_{sinus} indicated that guinea pigs have well developed vertebral arteries and that the Circle of Willis was capable of maintaining (back) pressure in the sinus after the common carotid artery was occluded. Thus, under control conditions, P_{sinus}, monitored via the Circle of Willis, was 50-75% of P_{art}. Carotid occlusion reduced this

to 30-60% of P_{art}. The (relatively) high pressures recorded during occlusion and the absence of any responses after sectioning the carotid sinus nerve indicate that central hypoxia cannot be involved in any changes in P_{art} induced by occlusion. Peripheral chemoreceptors in the carotid body could be involved as they have afferents in the carotid sinus nerve. However, my records of P_{sinus} suggest that there is sufficient pressure to provide adequate pressure for perfusion of the carotid body during occlusion. Electrical stimulation of chemoreceptor afferents results in reflex pressor responses (Douglas and Schaumann, 1956; Edis and Shepherd, 1971; Pelletier et al., 1972; Schmidt, 1968). Thus, it is possible that the pressor responses I noted resulted from stretch-related stimulation of chemoreceptors in the carotid body. My experiments in which the carotid sinus nerve was cut cannot eliminate this possibility as both baroreceptor and chemoreceptor afferents run in this nerve. Goel and Biggs (1984) reported that injection of the chemoreceptor stimulant phenylbiguanide into the anterior vena cana induced reflex pressor responses. By contrast, the same drug injected intra-aortically induced reflex depressor responses. Phenylbiguanide injected via the latter route would go to the carotid body via the common carotid arteries, and these workers noted that depressor responses to phenylbiguanide intra-aortically were reduced by sectioning the carotid

sinus nerves. Thus, although these latter findings contrast with those noted with electrical stimulation in cats, dogs and swine (Douglas and Schaumann, 1956; Edis and Shepherd, 1971; Pelletier et al., 1972; Schmidt, 1968), they suggest that stimulation of chemoreceptors could result in reflex depressor responses.

Pretreatment with capsaicin had no effect on responses to carotid occlusion. Thus, capsaicin-sensitive nociceptive afferents cannot be involved in mediating the pressor or depressor responses. However, non-capsaicin-sensitive nociceptive afferents running in the carotid sinus nerve might be involved in the depressor responses; a possibility that was not ruled out by this study. Capsaicin did not appear to affect any sensory afferent nerves mediating carotid baroreflexes.

In summary, in pentobarbital anesthetized, spontaneously breathing guinea pigs with the aortic depressor nerves sectioned 1) carotid occlusion distal to the carotid sinus induced pressor responses, 2) carotid occlusion proximal to the carotid sinus induced depressor responses, that appeared to result from stretching the sinus, and 3) both pressor and depressor responses were abolished by sectioning the carotid sinus nerves.

6.5. ACKNOWLEDGEMENTS

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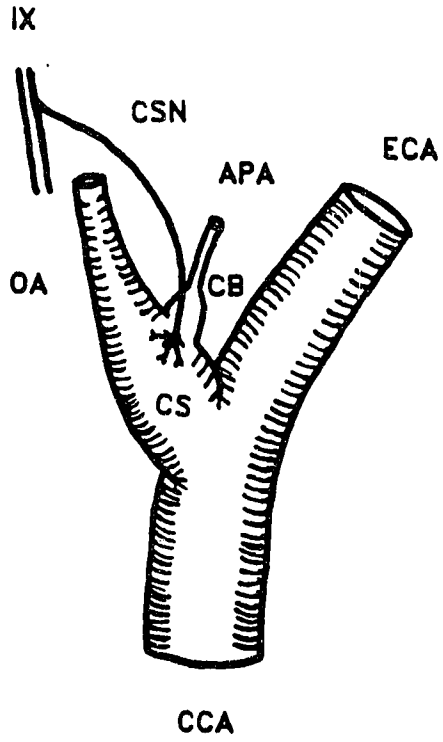

Figure 6-1. Anatomy of the right carotid bifurcation region (ventral view) in the guinea pig: APA - anterior pharyngeal artery; CB - carotid body; CCA - common carotid artery; CS - carotid sinus; CSN - carotid sinus nerve; ECA - external carotid artery; IX - glossopharyngeal nerve; and OA - occipital artery. Note the absence of an internal carotid artery.

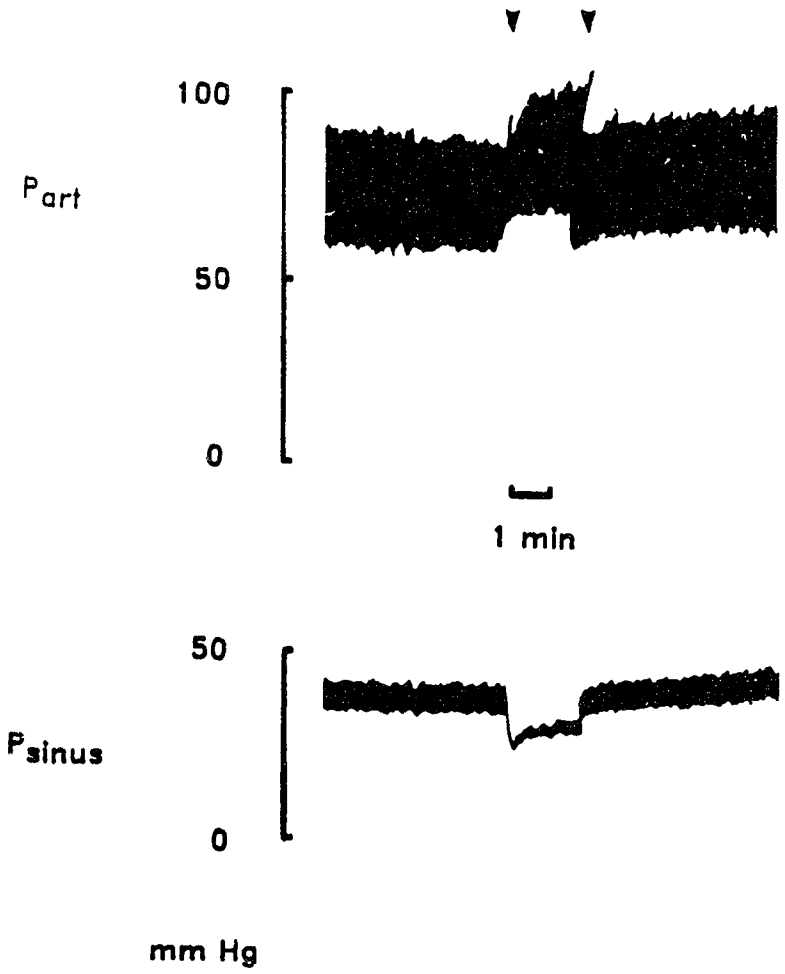

Figure 6-2. Guinea pig, pentobarbital anesthesia, both aortic depressor nerves and the left carotid sinus nerve sectioned: carotid occlusion (between arrows) distal to the carotid sinus induced an increase in arterial blood pressure (P_{art} - upper trace: recorded from the left common carotid artery), and decrease in intra-sinus pressure (P_{sinus} - lower trace: recorded from the left carotid sinus).

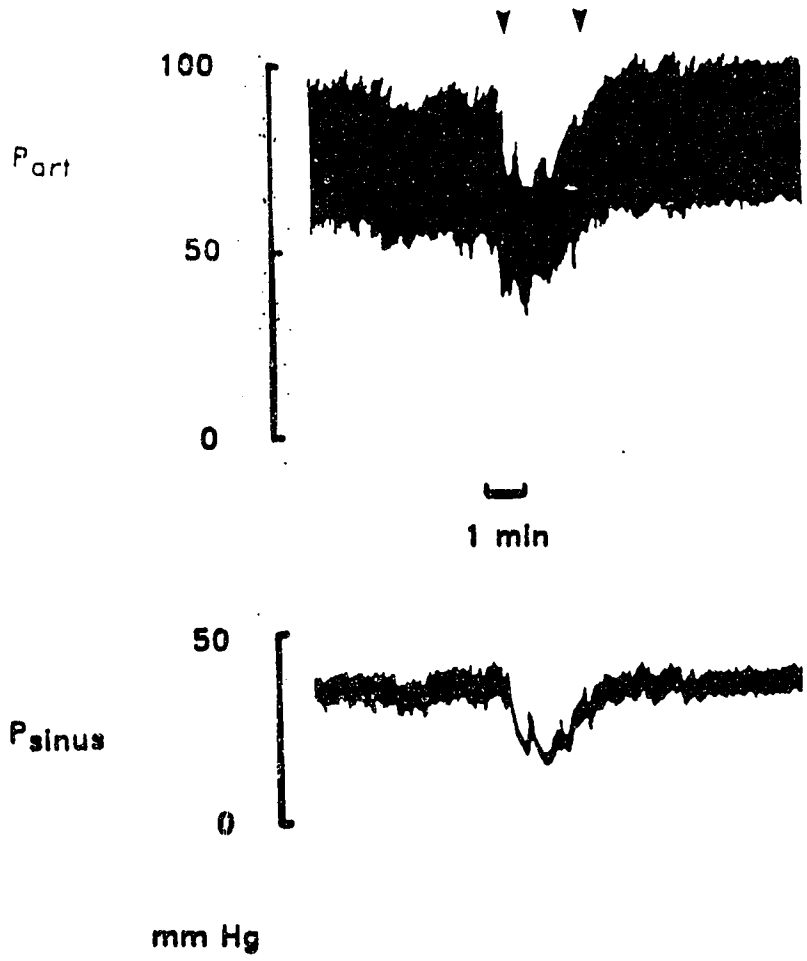

Figure 6-3. Guinea pig, pentobarbital anesthesia, both aortic depressor nerves and the left carotid sinus nerve sectioned: carotid occlusion (between arrows) proximal to the carotid sinus induced falls in P_{art} (upper trace: recorded from the left common carotid artery) and intra-sinus pressure (P_{sinus} - lower trace: recorded from the left carotid sinus).

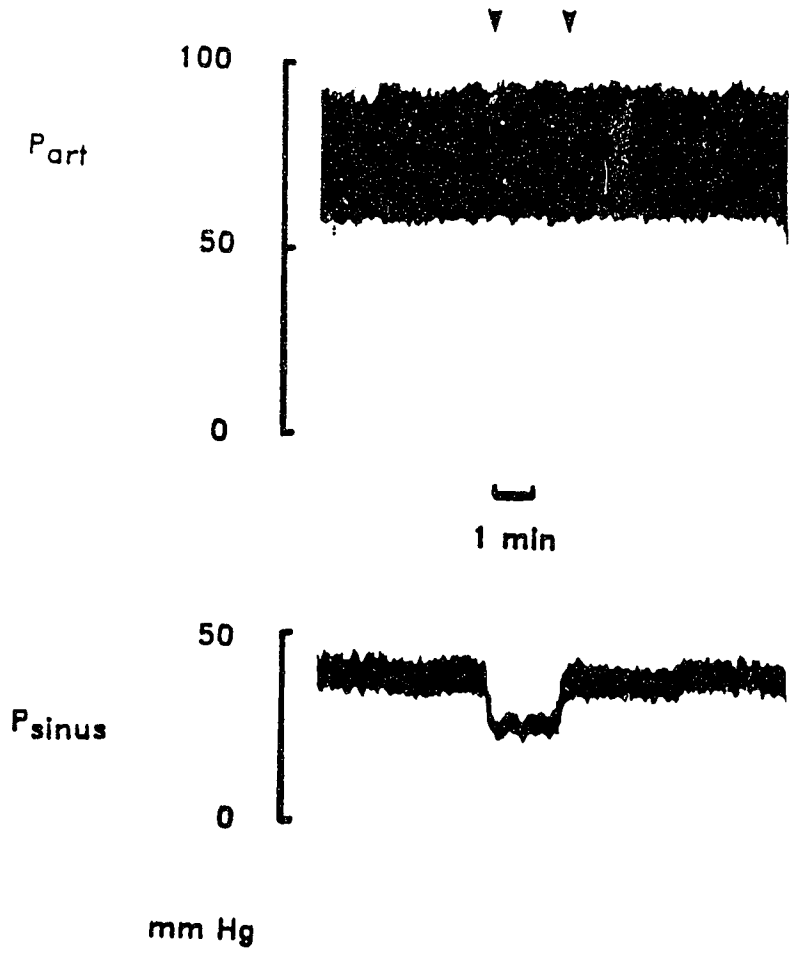

Figure 6-4. Guinea pig, pentobarbital anesthesia, both aortic depressor nerves and the left carotid sinus nerve sectioned: no changes in P_{art} (upper trace: recorded from the left common carotid artery) were observed during carotid occlusion (between arrows) distal to the carotid sinus after the right carotid sinus nerve was sectioned. However, intra-sinus pressure (P_{sinus} - lower trace: recorded from the left carotid sinus) fell during the occlusion.

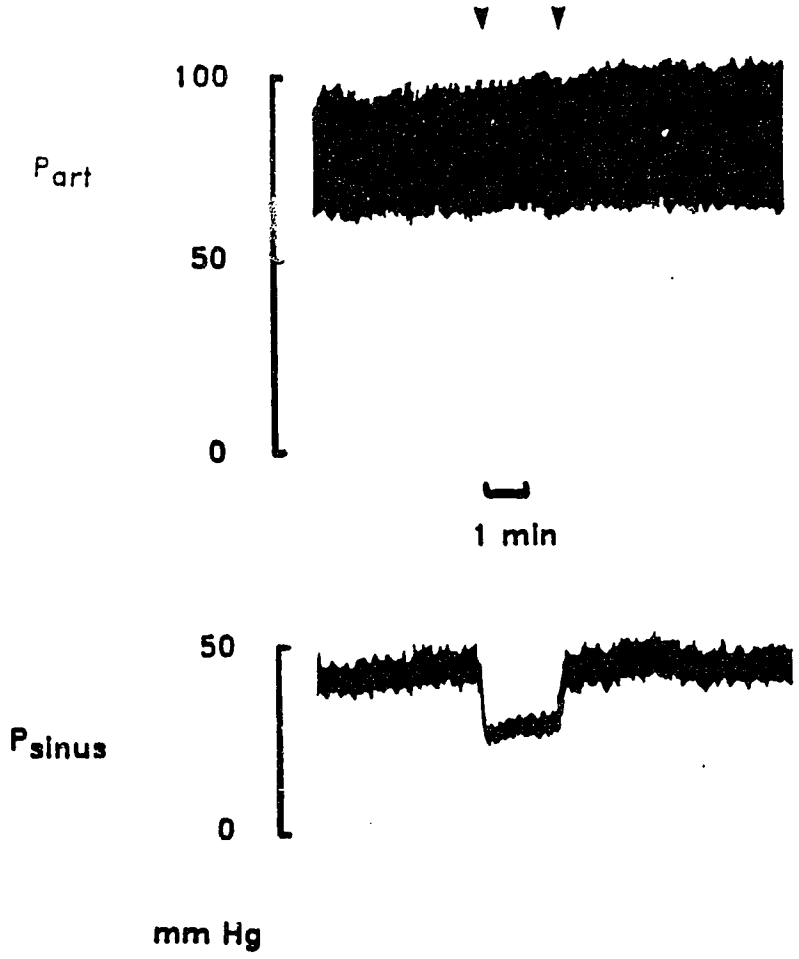

Figure 6-5. Guinea pig, pentobarbital anesthesia, both aortic depressor nerves and the left carotid sinus nerve sectioned: no changes in P_{art} (upper trace: recorded from the left common carotid artery) were observed during carotid occlusion (between arrows) proximal to the carotid sinus after the right carotid sinus nerve was sectioned. However, intra-sinus pressure (P_{sinus} - lower trace: recorded from the left carotid sinus) fell during the occlusion.

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CHAPTER VII. DISCUSSION AND CONCLUSIONS

In guinea pigs, recording from the superior laryngeal nerves revealed bursts of activity in synchrony with cardiac systole. This activity was unaltered after the internal and external branches of the superior laryngeal nerves to the larynx had been sectioned, but it disappeared after the ipsilateral aortic depressor nerves were transected.

Dissection revealed that the aortic depressor nerves emerged from the superior laryngeal nerves and ran caudally toward the heart as separate nerves adjacent to, but distinct from, the vagi and the cervical sympathetic trunks. They entered the aortic arch at the origin of the left common carotid artery on the left side, and at the bifurcation of the brachiocephalic artery into the right subclavian and common carotid artery on the right side. This pattern of innervation was consistent in 190 of 200 guinea pigs studied. The aortic baroreceptor afferent pathways of guinea pigs appear resemble those of rabbits, the species in which the aortic depressor nerves were first described (Nonidez, 1935).

Bursts of activity, similar to those seen in the superior laryngeal nerves, were recorded from these aortic depressor nerves. The pressor agent phenylephrine increased arterial blood pressure and aortic depressor nerve activity, and induced reflex bradycardia. Also, the depressor agent sodium nitroprusside decreased arterial

blood pressure and aortic depressor nerve activity. These findings suggest that baroreceptor afferents are present in the aortic depressor nerve.

Transmission electron microscopy was used to examine the histology of the aortic depressor nerves in guinea pigs. Sections revealed both myelinated and unmyelinated nerve fibers. Measurements of the electrophysiological characteristics of the aortic depressor nerves differentiated the two groups of fibers: one group had low thresholds to electrical excitation and high conduction velocities and represented the myelinated nerve fibers seen in the sections; the other had higher thresholds and lower conduction velocities and represented the unmyelinated nerve fibers noted. This histological and electrophysiological evidence confirmed that there were myelinated and unmyelinated nerve fibers in the aortic depressor nerves of guinea pigs.

In studies of the aortic baroreflexes in guinea pigs, aortic baroreceptor afferents in the aortic depressor nerves were stimulated electrically after sectioning the carotid sinus nerves on both sides to eliminate any reflex buffering effects of the carotid sinus baroreceptors. Orthodromic electrical stimulation induced voltage, frequency and pulse-width dependent depressor responses and slight bradycardia. The depressor responses were blocked by clonidine, hexamethonium, and prazosin, and the

bradycardia was blocked by atenolol. Thus, the efferent arcs of the aortic baroreflexes were mediated via central $alpha_2$-adrenoceptors, autonomic ganglia, and peripheral $alpha_1$- and $beta_1$-adrenoceptors. The aortic baroreflexes did not involve muscarinic receptors, peripheral $alpha_2$- or $beta_2$- adrenoceptors, or H_1- or H_2-histaminergic receptors, or vagal efferents. Both the depressor responses and the bradycardia resulted solely from a reflex reduction in sympathetic tone. Either aortic depressor nerve was capable of initiating baroreflexes in guinea pigs, as responses to stimulating the left, right, or both aortic depressor nerve(s) were similar in form and magnitude. The choice of anesthetic (pentobarbital, urethane or chloralose) did not alter the responses. As acute bilateral adrenalectomy did not affect the responses, the release of catecholamines from the adrenal medulla appeared to contribute little to sympathetic tone in guinea pigs.

The depressor responses and slight bradycardia evoked by stimulating afferents from aortic baroreceptors appeared to be mediated solely via the myelinated nerve fibers. Thus, maximal responses were noted at a stimulus of 3 V, the excitation threshold for myelinated fibers in the aortic depressor nerves (Hurch, 1939; Paintal, 1973). Also, findings in c[illegible]g and anodal block experiments, in which aortic barore[illegible]tor afferent activity and the response to aortic baroreceptor afferent stimulation were

recorded, confirmed that only myelinated afferents subserved aortic baroreceptors in guinea pigs. This was noteworthy, as in most other species (rabbits, cats, dogs, rats, and swine), aortic baroreceptors are subserved by myelinated and unmyelinated afferent fibers, and vagal efferents contribute to changes in heart rate (Angell James, 1971a, 1971b, 1971c, 1973; Douglas and Ritchie, 1956; Douglas and Schaumann, 1956; Douglas et al., 1956; Kardon et al., 1973; Pelletier et al., 1972; Schmidt, 1968).

Orthodromic stimulation of chemoreceptor afferents results in centrally mediated pressor responses (Douglas and Schaumann, 1956; Edis and Shepherd, 1971; Pelletier et al., 1972; Schmidt, 1968). In these experiments, aortic depressor nerve stimulation never induced pressor responses suggesting that chemoreceptor afferents were absent. The lack of any chemoreceptor-stimulant induced increases in aortic depressor nerve activity after these agents were given by intra-aortic injection supported this suggestion. Thus, there was no evidence of chemoreceptor afferents in the aortic depressor nerves. In this respect, guinea-pig aortic depressor nerves resembled those of rabbits (Angell, 1971a, 1971b, 1971c, 1973; Douglas et al., 1956; Numao et al., 1983). Interestingly, in most other species, chemoreceptor afferents run in the aortic nerves (Brown et al., 1976, 1978; Daly and Evans, 1953; Douglas and Ritchie,

1956; Douglas et al., 1956; Douglas and Schaumann, 1956; Edis and Shepherd, 1971; Kardon et al., 1973; Numao et al., 1983; Oberg and Thoren, 1973; Pelletier et al., 1972; Schmidt, 1968; Thoren et al., 1977).

As the only afferents from aortic baroreceptors were myelinated and chemoreceptor afferents were absent, I investigated the functions of the unmyelinated fibers. Anatomical studies of the cervical region of guinea pigs revealed connections between the superior cervical ganglia and the superior laryngeal nerves where they merged with the aortic depressor nerves. In ventral views, the main trunks of superior laryngeal nerves were directly adjacent to, and appeared to touch, the superior cervical ganglia, and there were fine rami that ran between the two structures. Thus, there appeared to be sympathetic fibers entering or leaving the aortic depressor nerves. Also, there may be parasympathetic efferent fibers in the aortic depressor nerves, as the vagi gave rise to the superior laryngeal nerves.

After sectioning the carotid sinus nerves bilaterally, recordings of efferent activity from the central ends of the aortic depressor nerves revealed significant activity. It was of lower amplitude than aortic afferent activity suggesting that it originated from unmyelinated nerve fibers. Two types of activity were of sympathetic origin and were associated with cardiac and respiratory events,

respectively. Another type of activity was of parasympathetic origin and had a scattered and random firing pattern.

Stimulating sympathetic efferents in the aortic depressor nerves enhanced baroreceptor activity in the aortic depressor nerve. Functional studies of the link between the superior laryngeal nerves and the superior cervical ganglia confirmed that: 1) there were unmyelinated fibers connecting these two structures; 2) their thresholds and conduction velocities were in the range of the unmyelinated nerve fibers; and 3) these unmyelinated sympathetic efferents synapsed in the superior cervical ganglia. Thus, some of the unmyelinated nerve fibers were sympathetic efferents. Others appeared to be visceral nociceptive afferent fibers, as stimulating the aortic depressor nerves induced brief, pressor, sympatho-excitatory effects that preceded any depressor responses. Typically, this effect appeared after 4 h of anesthesia with pentobarbital. These brief pressor responses were absent in animals pretreated with large doses of capsaicin, which destroys nociceptive afferent nerve fibers (Buck and Burks, 1986; Numao et al., 1983). The histology of aortic depressor nerves from these capsaicin-pretreated guinea pigs showed that the ratio of myelinated to unmyelinated nerve fibers changed from 1:3-4 in controls to 1:1. This indicated that many of the unmyelinated nerve fibers were

nociceptive afferents. Thus, there were many visceral nociceptive unmyelinated afferent fibers in the aortic depressor nerves of guinea pigs, and some of the remaining unmyelinated fibers were sympathetic efferents.

Occlusion of the common carotid arteries is widely used to investigate reflexes involving carotid sinus baroreceptors. In most species, carotid occlusion induces reflex hypertension and tachycardia, and responses are eliminated by sectioning the carotid sinus nerves (Kirchheim, 1976). However, in guinea pigs, this laboratory had previously reported paradoxical depressor responses to carotid occlusion (Biggs et al., 1984; D'Souza, 1990). I obtained pressor responses to carotid occlusion in guinea pigs anesthetized with pentobarbital, if: 1) both aortic depressor nerves were cut; and 2) the carotid sinus was not stretched as the clamp was applied. Thus, the guinea pig can respond to carotid occlusion in the same way as other species.

In summary, I have developed a unique, innervated aortic baroreceptor preparation in guinea pigs. My preparation makes it possible to record activity exclusively from baroreceptor afferents in the aortic depressor nerves as: 1) all fibers subserving aortic baroreceptors appear to be myelinated afferents; 2) chemoreceptor afferents are absent from the aortic nerves;

and 3) unmyelinated fibers in the aortic nerves are either nociceptive afferents or sympathetic efferents.

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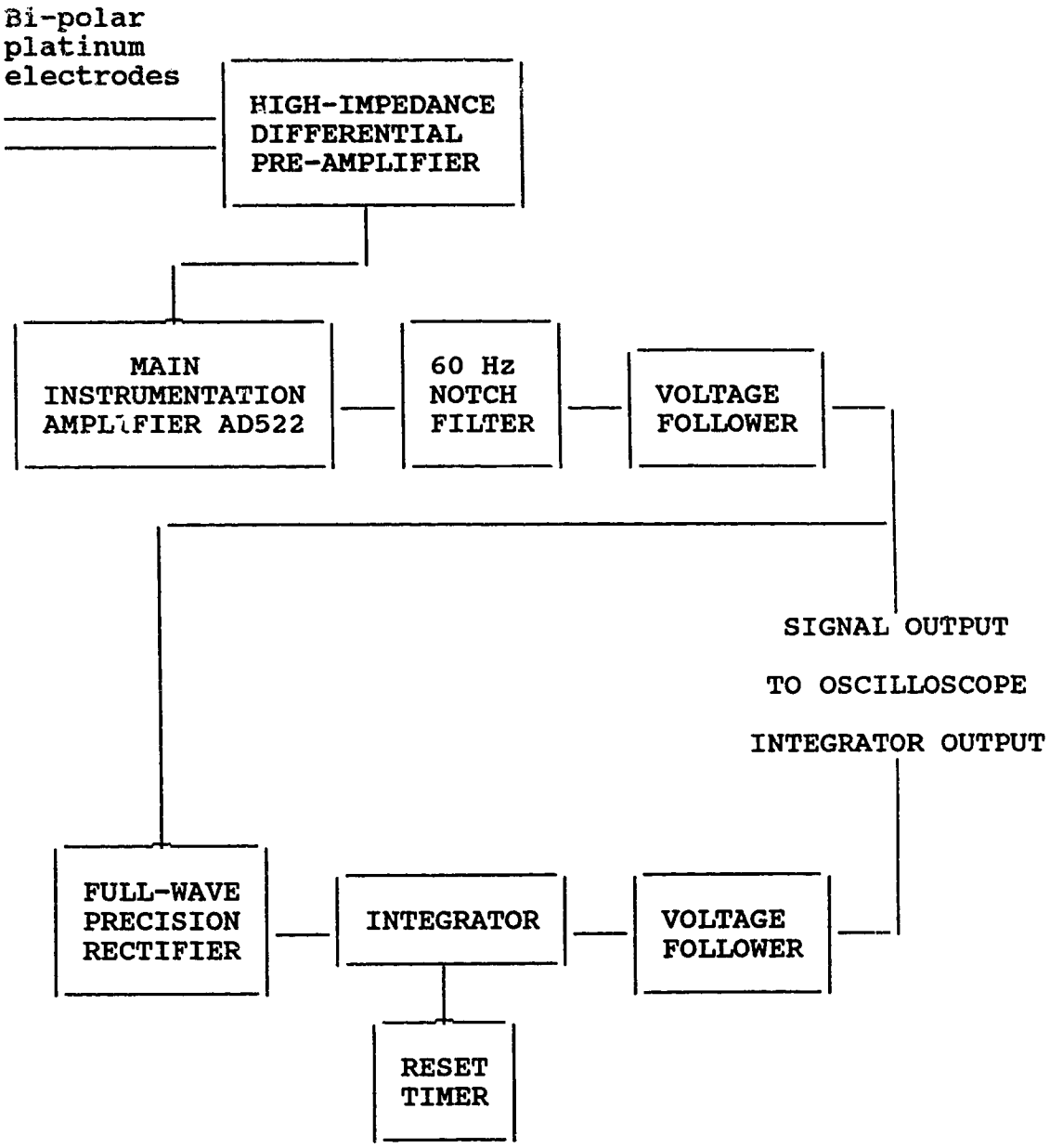
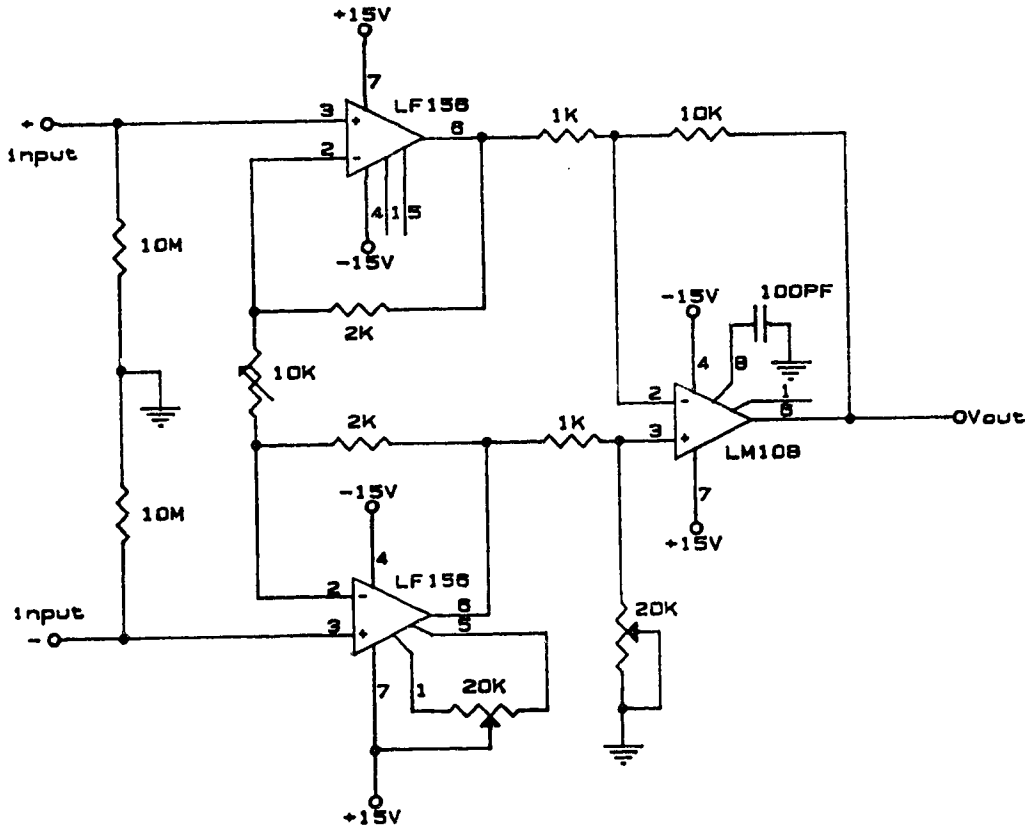
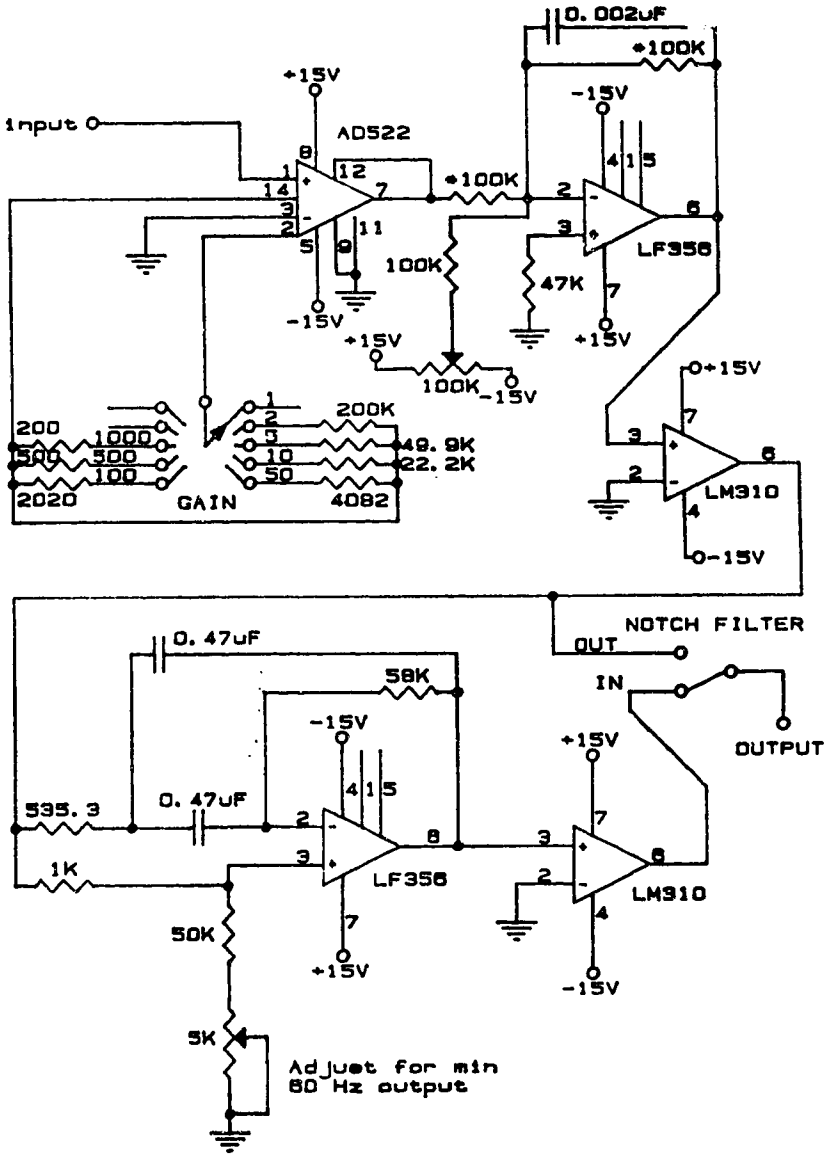


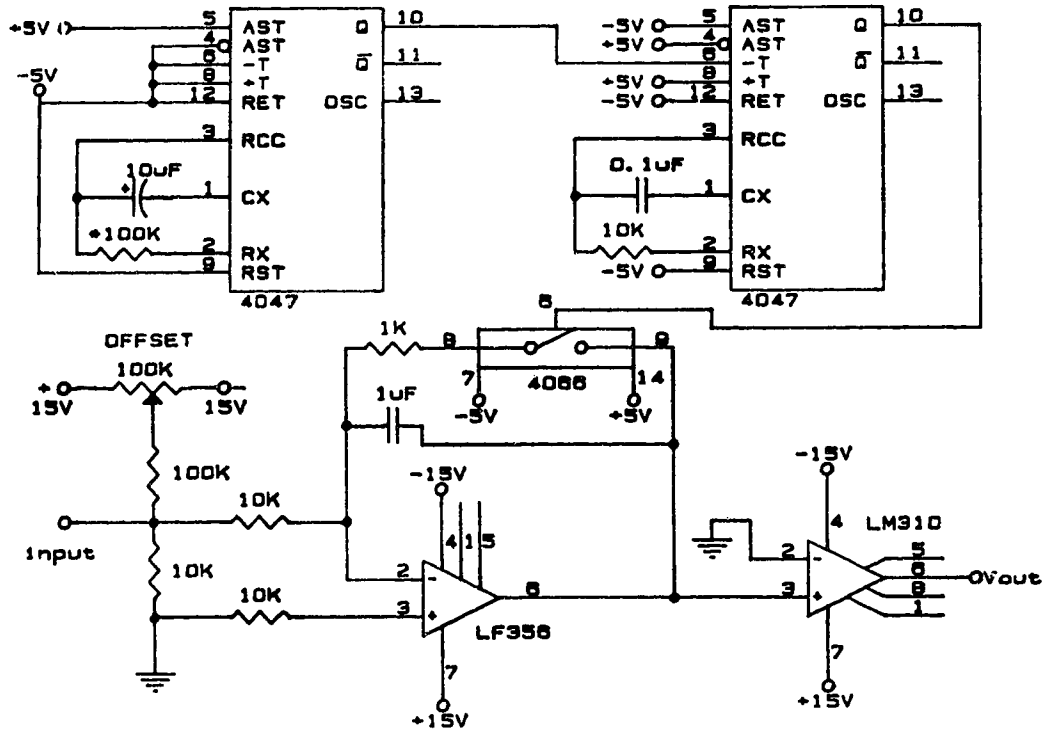
Appendix A-1. Block diagram of a bio-electric amplifier system. The system was used for recording the nerve activity.



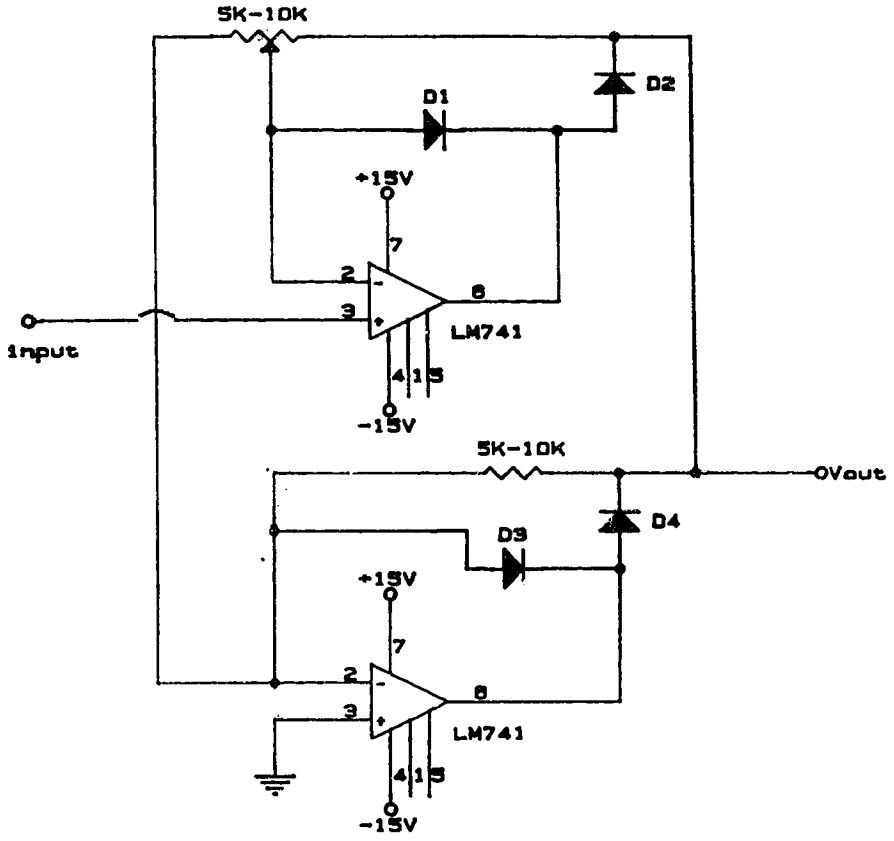
Appendix A-2. Schematic circuit of the high-input impedance differential amplifier (pre-amplifier).



Appendix A-3. Schematic circuit of the main instrumentation amplifier.



Appendix A-4. Schematic circuit of the full-wave precision rectifier.



Appendix A-5. Schematic circuit of the integrator.