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ROBERT LOUIS MECKLER

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Lynne C Weaver
Major professor

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ANATOMICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF RENAL AND SPLENIC SYMPATHETIC NERVES IN CATS

By

Robert Louis Mackler

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ABSTRACT

ANATOMICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS
OF RENAL AND SPLENIC SYMPATHETIC NERVES IN CATS

By

Robert Louis Meckler

The sympathetic nervous system can control different organs or target tissues individually. The purpose of the present studies was to compare and contrast 1) ganglionic distributions of renal and splenic sympathetic neuronal cell bodies, 2) dependence of spontaneous multiunit discharge of renal and splenic sympathetic nerves upon supraspinal sources of excitatory drive, and 3) responses of single renal and splenic axons to stimulation of afferent nerves. Retrograde axonal transport of horseradish peroxidase was employed to identify ganglionic distributions of cell bodies of postganglionic nerves supplying the spleen and kidney. Most labeled cell bodies of renal nerves were clustered in groups within ganglia of the solar plexus. The remainder of labeled renal neurons were located in upper lumbar or lower thoracic paravertebral sympathetic ganglia. In contrast, 90% of labeled cell bodies of splenic neurons were scattered throughout the left and right celiac poles of the solar plexus. In electrophysiological studies of multifiber sympathetic nerve activity, it was demonstrated that ongoing activity of splenic nerves is less dependent

upon supraspinal sources of excitation than is activity of renal or cardiac nerves. Neither increased nor decreased arterial pressure, systemic hypercapnia and acidosis, nor thoracolumbar dorsal rhizotomy revealed specific inputs responsible for preferential maintenance of splenic nerve activity in spinal cats. Potential heterogeneity of ongoing activity and reflex responses of individual fibers in splenic and renal nerves to stimulation of visceral receptors was assessed. Half of the splenic population and all renal fibers had cardiac-related discharge patterns. Of those tested for respiratory-related activity, 30% of splenic and 69% of renal fibers exhibited this pattern. Activity of splenic fibers was influenced less than that of renal fibers by changes in arterial pressure. Chemical stimulation of splenic afferent nerves caused large excitatory responses in activity of all splenic fibers and smaller excitatory responses in discharge of 75% of the renal fibers. Intestinal afferent nerve stimulation caused excitation, inhibition, or no change in splenic fiber discharge, whereas renal unit activity was almost always excited by this stimulation. These results indicate that the sympathetic system is organized to provide different viscera with discrete control.

This dissertation is dedicated with love to my mother, Anne Meckler, and to my family.

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INTRODUCTION

In the early twentieth century, the sympathetic nervous system was considered to respond to internal or external stimuli in a unitary fashion to provide a homeostatic mechanism for the maintenance of the internal environment of vertebrates (Cannon, 1930). This view influenced subsequent investigations in which the responses the sympathetic nerves to one organ, or responses of the adrenal medulla, were considered representative of the sympathetic nervous system as a whole. Had it been realized that sympathetic nerves could respond nonuniformly to various inputs, the Sherringtonian approach to studying nervous system function ("application of the reflex concept" in the analyses of neural systems) could have given information regarding the functional structure of the sympathetic nervous system. However, this was not understood until relatively recently.

The concept of sympathetic mass-activation, rendered by Cannon (1930), initially was challenged by Folkow, Johansson, and Löfving (1961) and Löfving (1961). These investigators made simultaneous measurements of the reflex changes in blood flow to hindlimb muscle and skin, kidney, and intestine that were initiated by stimulation of arterial chemoreceptors or by unloading of carotid baroreceptors. In these studies, renal and cutaneous blood flows were relatively unaffected whereas vessels of skeletal muscle constricted in response

to these perturbations; responses of intestinal blood flow were intermediate compared to the other vascular responses. These results were interpreted as an indication of reflex specificity among the sympathetic nerves supplying each vascular bed. However, the differences in regional distribution of blood flow may have arisen from heterogeneity of the neuroeffectors' sensitivities to equivalent magnitudes of sympathetic excitation. For example, blood vessels of different regions may have varying receptor densities (Bevan, 1979) leading to differences in vascular responses despite similar discharge rates of the sympathetic innervations. Therefore, simultaneous recordings of activity from different sympathetic nerves are necessary to distinguish between target responsiveness and specificity of the neural organization.

More recently, electrophysiological studies have demonstrated nonuniform reactions of different sympathetic nerves in a variety of circumstances, such as during chemoreceptor or baroreceptor stimulation, or during exercise (Grignolo, Koepke, and Obrist, 1982; Iriki, Walther, Pleschka, and Simon, 1971; Jänig, 1982; Kollai and Koizumi, 1977; Ninomiya and Irasawa, 1975). These studies have been interpreted, keeping in mind the different responses of various vascular beds mentioned above, as indications of the ability of the sympathetic nervous system to influence the vasculature of different organs in a differential manner, while ignoring the fact that not all sympathetic nerve fibers influence vascular function. Most studies designed to investigate nonuniformity of visceral sympathetic responses have entailed multifiber recording techniques. This kind of data is difficult to interpret due to the lack of information pertaining to the

heterogeneity or homogeneity of responses of individual neurons within a given fiber bundle. Different fibers within individual visceral nerves may influence different target tissues within the innervated organ. For example, renal nerves are known to influence blood flow, renin secretion, and tubular reabsorption of electrolytes (DiBona, 1982); splenic nerves innervate arterioles and trabeculae, as well as the splenic capsule (Cleland and Tait, 1927; Fillenz, 1970; Utterback, 1944). Therefore, populations of axons within a nerve trunk may exhibit different responses to a given stimulus based on the type of target tissues innervated by the particular neurons in question.

The following investigations will be included in this dissertation:

1. The distributions of renal and splenic sympathetic neuronal cell bodies within pre- and paravertebral sympathetic ganglia were compared and contrasted to provide the topographic organization of renal and splenic neural cell bodies. This may be useful for future investigations in which horseradish peroxidase may be injected into specific ganglionic sites to label preganglionic neurons which project to different organs.

2. The origins of spontaneous sympathetic neural discharge of renal and splenic nerves were ascertained because it is not known whether discharge of these sympathetic nerves is dependant upon supraspinal sources of excitatory drive.

3. The reflex responses of single renal and splenic nerve fibers were compared following stimulation of systemic pressoreceptors and chemical stimulation of splenic and intestinal afferent nerves. This was done to quantify reflex responses of renal and splenic sympathetic

nerves in absolute units of measurement (number of action potentials per second). In addition, these data may provide information about heterogeneity or homogeneity of populations of individual axons contained within renal and splenic nerves.

The literature review is focussed primarily upon data which provides evidence for the discrete neural control of individual vascular beds, individual organs or target tissues. The original data which comprises the body of the dissertation has been divided into three chapters. Each chapter contains an introduction, methods, results and discussion section pertaining to the project described in that chapter. A fourth chapter contains brief conclusions and a general discussion of the importance of the three studies.

LITERATURE REVIEW

EARLY HALLMARKS IN THE STUDY OF THE SYMPATHETIC NERVOUS SYSTEM

Since Galen's time (130-200), the peripheral components of the sympathetic system have been known, by Western civilization, to innervate the viscera (Ackerknecht, 1974; Sheehan, 1936). During the next millenium, little seems to have been done to extend Galen's anatomical observations. Most studies of the nervous system during the 16th, 17th, and 18th centuries were purely descriptive anatomical analyses, the interpretations of which were largely metaphysical. In those times, the brain was thought to be the source of "vital humours" or "sympathies" which were distributed throughout the organism to bring about harmonious action in, and reaction to, the external environment (Ackerknecht, 1974; Langley, 1916; Sheehan, 1936). Despite the lack of empirical data regarding the functions of the sympathetic system, valuable anatomical data was generated which served as the foundation for further study. Willis, in 1664, proposed that the viscera are not controlled voluntarily since he thought that the sympathetics descended from the cerebellum, rather than from the cerebrum where volition was thought to reside (Ackerknecht, 1974; Sheehan, 1936). Remak was probably the first to recognize that the sympathetic fibers that innervate the viscera originate in cell bodies located in the sympathetic ganglia rather than in the central nervous system, although

it was widely accepted by this time that the ganglia were able to communicate with the central nervous system via the rami communicantes (Ackerknecht, 1974; Sheehan, 1936).

Willis was the first to recognize the influence of sympathetic nerves on blood vessels. He thought that sympathetic nerves wrapped around blood vessels and mechanically caused constriction of the arteries resulting in a pulsatile increase in blood flow through the vessels (Sheehan, 1936). However, it wasn't until 1840 that Henle discovered muscle tissue in the walls of blood vessels and the close association of sympathetic nerves appearing to terminate in the muscle (Heymans and Neil, 1958; Sheehan, 1936). That same year, Stilling coined the term "vasomotor system". However, eleven years elapsed before Claude Bernard and Brown-Sequard demonstrated independently that the sympathetics exert ongoing vasoconstrictor influences on blood vessels (Olmsted and Olmsted, 1952). Bernard later severed the spinal cord, observed the ensuing decreased arterial pressure, and concluded that ongoing activity of the sympathetic system which maintains vasomotor tone, is dependant upon activity arising from the brain. Owsjannikow and Dittmar, in the 1870's, extended these investigations by trying to locate a vasotonic center in the brain by observing the changes in arterial pressure produced by serial transections of the neuraxis (Gebber, 1982; Heymans and Neil, 1958). No consistent changes in blood pressure resulted from transections anterior to the pons. However, as transections were made progressively more posterior through the pons and medulla oblongata, arterial pressure dropped more and more, until the neuraxis was severed at the junction of the medulla and spinal cord. At this point, arterial pressure was the lowest; further

posteriorly placed transections did not result in a further decrease in blood pressure. The interpretation of these data was that a vasotonic center was located in the brain between midpontine and caudal medullary levels of the neuraxis. A table summarizing important hallmarks in the study of the sympathetic nervous system control of cardiovascular function, up to the work of Cannon, is presented in Appendix A.

HOMEOSTASIS AND UNITARY ACTION OF THE SYMPATHETIC NERVOUS SYSTEM

Focus upon the sympathetic system changed to encompass the pathways by which these brain areas exerted their influences on peripheral neural components as well as the influences of sympathetic nerves on visceral and cardiovascular function. Claude Bernard emphasized the physiological importance of maintaining the uniformity of the "milieu interne" or internal environment (Cannon, 1930). Cannon proposed the use of the term "homeostasis" to describe the "general idea of uniformity or stability in the organism". He regarded the sympathetic division of the nervous system as an instrument indispensable for preservation of constant composition of this internal environment. Cannon reasoned that the sympathetic nervous system is organized to produce "widespread changes in smooth muscle and glands throughout the organism" (Cannon, 1930). This diffuse action of the sympathetic system is possible because of its "arrangement for simultaneous and unified action" (ibid.). Particularly, the extensive divergence of preganglionic innervation of postganglionic neurons, as well as of secretory cells of the adrenal medulla, would seem to ensure concordant physiological responses required to preserve homeostasis during mammals'

reponses to changes in the external environment. Cannon's postulated "en masse" action of the sympathetic nervous system has been used in many undergraduate, graduate, and medical textbooks to describe the "fight or flight" reaction in which the sympathetic system is known to participate. Although views of the uniformity of sympathetic action were strictly interpreted for decades, Cannon was aware of the dearth of information concerning influences of individual sympathetic nerves on particular target organs, as indicated by this statement in the Linarre Lecture (Cannon, 1930): "In the present state of our knowledge of the nature of nerve impulses in the autonomic system no decisive conclusion can be drawn regarding the possible function of the outlying neurones as 'transformers,' adapting the nerve impulses received from the brain and spinal cord to the peculiar visceral structures which are affected. More information is needed. May I venture the suggestion that here lies an attractive realm for research, which may yield highly interesting new facts."

ONGOING ACTIVITY OF SYMPATHETIC NERVES

Under control conditions, unevoked, repetitive discharge can be recorded from sympathetic nerves in anesthetized and unanesthetized animals and humans. This activity has been called tonic (e.g., Alexander, 1946), background (e.g., Beacham and Perl, 1964), basal (e.g., Gebber, 1982), and ongoing (e.g., Skok and Ivanov, 1983), and is necessary for the maintenance of arterial pressure. The presence of ongoing nerve activity enables the system to be finely controlled because activity can be increased or decreased to meet momentary demands

of the organism. In addition, ongoing activity of nerves increases the efficiency of target organ responses by "priming" the effectors, thereby enabling quick target responses "without the lag inherent in building up a response in quiescent tissue" (Polosa, Mannard, and Laskey, 1979).

Rhythmicity of Sympathetic Nerve Activity

Cardiac-related Rhythms

In the first electrophysiological recordings of mammalian sympathetic nerve activity, Adrian, Bronk, and Phillips (1932) observed waxing and waning of sympathetic nerve discharge occurring in approximate synchrony with the period of cardiac or respiratory cycles. The sources of these rhythmic fluctuations in ongoing discharge observed in recordings of sympathetic activity have been a matter of speculation and much investigative effort ever since (e.g., Gebber, 1982). Adrian, et al. thought the cardiac rhythm was due to the controlling influences of the phasic stimulation of arterial baroreceptors produced by systolic arterial pressure. This view was supported by Cohen and Gootman (1970) using crosscorrelation analyses. However, Gebber and coworkers (Barman and Gebber, 1980, 1981a,b; Gebber and Barman, 1980, 1981, 1982; McCall and Gebber, 1975; Taylor and Gebber, 1975) demonstrated that a periodic rhythm in sympathetic discharge persisted even after surgical denervation of carotid and aortic baroreceptors. The frequency of the rhythm was approximately the same as that of the cardiac cycle (2-6 Hz, in cats) but the fluctuations of sympathetic activity were no longer locked in a 1:1 relationship with the cardiac cycle. In addition, when

heart rate was slowed by a variety of pacing techniques in baroreceptor intact animals, baroreceptor afferent nerves continued to discharge in synchrony with the cardiac cycle, whereas rhythmic discharge of the sympathetic nerves was no longer locked to the cardiac cycle, but continued to be in the 2-6 Hz range. On the basis of these observations, Gebber has proposed that sympathetic rhythmicity can occur in the absence of peripheral inputs and that baroreceptor afferent activity entrains this rhythm so as to produce a 1:1, or locked, relationship between the cardiac cycle and sympathetic activity.

Respiratory-related Rhythms

Most studies indicate that sympathetic nerves have a higher probability of firing during inspiration (Adrian et al. 1932; Barman and Gebber, 1976; Cohen and Gootman, 1970; Gootman and Cohen, 1971) and it has been presumed that brain stem inspiratory neurons facilitate (and/or brain stem expiratory neurons inhibit) sympathetic activity. Although lung stretch receptors with afferent axons in the vagi probably play a role in the genesis of respiratory rhythmicity of sympathetic nerves, this rhythm can still be observed when these sympathoinhibitory effects are eliminated by vagal transection (Adrian, et al., 1932). Prior to 1976, the respiratory periodicity of sympathetic discharge was thought to be due to direct coupling between the brainstem respiratory oscillator and neural circuits that contribute to ongoing sympathetic excitation (Cohen and Gootman, 1970; Gootman and Cohen, 1971; Koizumi, Seller, Kaufman, and Brooks, 1971; Preiss, Kirchner, and Polosa, 1975). If respiratory rhythmicity of sympathetic activity is simply due to

direct influences of the respiratory oscillator, then anything which disrupts the rhythmicity of phrenic nerve activity also should disrupt the respiratory-related rhythmicity of sympathetic activity identically. For example, the latency between peak phrenic discharge and peak sympathetic discharge should remain constant during changes in the length of intervals between the bursts of phrenic activity. In addition, elimination of phrenic nerve activity (e.g. hyperventilation) should abolish the respiratory rhythmicity of sympathetic nerve discharge. However, Barman and Gebber (1976) provided evidence that the brainstem respiratory oscillator can be uncoupled from a presumedly separate sympathetic oscillator. First, changes in the phrenic activity cycle caused changes in the phase relationship between phrenic and sympathetic activities, which should not occur if the rhythm originates in the respiratory oscillator. Second, although the respiratory rhythmicity of sympathetic discharge should be eliminated by hyperventilation-induced inhibition of phrenic nerve activity, these rhythms persist after hyperventilation sufficient to cause cessation of phrenic nerve activity. Therefore, these authors assert that the "periodic components of sympathetic and phrenic nerve activity are generated by independent oscillators that normally are entrained to each other". Since that time, others have provided evidence that at least a proportion of sympathetic pre- or postganglionic neurons are more likely to be active during the expiratory phase of the respiratory cycle (Bachoo and Polosa, 1986; Bainton, Richter, Seller, Ballentyne, and Klein, 1985). Therefore, some brain stem expiratory neurons may serve

to facilitate excitatory inputs to sympathetic neurons, either directly or indirectly. The functional significance of inspiratory-related versus expiratory-related sympathetic activity is not known.

Functional Significance of Rhythmic Nerve Activity

Ongoing activity of sympathetic nerves has physiological advantages, as mentioned above. The rhythmicity of nerve activity also may have functional consequences. A few investigators have compared the vascular effects of stimuli delivered to sympathetic nerves at constant rates to the effects of stimuli delivered in trains (to mimic the bursts of activity observed in ongoing discharge of sympathetic nerves). The average contractile responses (i.e. force of contraction) of arteries were significantly greater when stimuli were delivered in bursts at irregular frequencies than when stimuli were applied continuously at regular intervals (Nilsson, Ljung, Sjoblom, and Wallin, 1983). In addition, the magnitude of blood flow response were shown to be dependent upon 1) the frequency of impulses within a burst, and 2) the intervals between successive bursts (Andersson, 1983). If stimuli were delivered in bursts, maximal and maintained blood flow responses were produced, but if stimuli were delivered at regular intervals, responses were submaximal and faded. Andersson proposes that during the periods of quiescence, nerve terminals can take up or synthesize enough transmitter for repeated release, whereas continuous stimulation leads to depletion of transmitter stores due to the inadequate time for repletion.

Sources of Sympathetic Activity

Maintenance of normal arterial pressure depends on intact pathways from the brainstem to spinal sympathetic centers; this has been known since the experiments of Bernard, Dittmar, and Owsjannikow (Gebber, 1982). Ranson and Billingsley (1916) consequently probed the floor of the fourth ventricle with stimulating electrodes and measured the evoked changes in arterial pressure in an attempt to locate medullary areas important for maintenance of arterial pressure. When the medial medulla was stimulated near the obex, arterial pressure decreased, and when more lateral and more rostral sites were stimulated, arterial pressure increased. Wang and Ranson (1939) extended these observations by electrical stimulation of deeper medullary regions in addition to the dorsal surface. These investigators produced decreases in blood pressure by stimulation of the medial reticular formation and increased arterial pressure was elicited by stimulation of more lateral reticular areas. Although vasoactive points were not confined to specific nuclear groups in the reticular formation, these results were interpreted as evidence for the existence of discrete pressor and depressor areas. These results were confirmed by Alexander (1946) who monitored cardiac sympathetic nerve activity as well as arterial pressure. In addition to the pressor and depressor areas defined by previous investigators, Alexander elicited increases in arterial pressure and nerve activity from stimulation of the dorsomedial reticular formation. Since that time, others have explored the brainstem with stimulating electrodes while recording discharge from sympathetic preganglionic neurons. Gootman and Cohen (1971) stimulated the dorsomedial reticular formation

(nucleus reticularis parvocellularis) and found short latency, large amplitude excitatory responses in splanchnic nerve activity. The next year, Nathan (1972) was able to elicit responses of even shorter latency from a more caudal area in the nucleus reticularis ventralis and proposed that neurons in this region comprised at least part of the final descending bulbospinal sympathetic pathway. However, as electrical stimulation can affect axons of passage as well as somata, this interpretation is equivocal.

In addition to studies employing electrical stimulation of brainstem sites, lesions "discretely placed" in the medulla have been used to identify loci important in cardiovascular control. Schlaefke and Loeschke (1967) used focal cooling of the rostral ventrolateral medulla to produce large decreases in arterial pressure. Dampney and Moon (1980) placed lesions in small areas of the rostral ventrolateral medulla and confirmed these results. Electrical stimulation of this region caused large increases in arterial pressure. Kumada et al. (1979) found that destruction of dorsal medullary neurons results in lowered arterial pressure. Dampney and Moon (1980) suggested that the ventrolateral medullary neurons project through the dorsomedial reticular formation as the pathway descends to spinal sympathetic preganglionic loci because lesions placed in the dorsomedial reticular formation attenuated the increase in blood pressure elicited by stimulation of the ventrolateral medulla. To eliminate the confounding effects of lesioning or stimulating axons of passage, Guertzenstein and Silver (1974) used local applications of chemicals which act selectively on cell bodies and elicited decreases (glycine) and increases (glutamate) in arterial pressure. These results have been interpreted

as evidence that the ventrolateral medulla is at least one significant source of sympathetic activity (Dampney, 1981). Barman and Gebber (1983) have challenged the premise that ongoing sympathetic activity is generated exclusively in the ventrolateral medulla. Although these authors agree that these neurons may represent the final common descending pathway to sympathetic preganglionic neurons, they propose that other, presumably medullary, areas are antecedant to the ventrolateral medullary areas and therefore the ventrolateral medulla is more a relay station than a source for descending excitatory influences on the sympathetic system. This suggestion is based on studies in which the time lag between medullary unit action potentials and the peak in sympathetic activity have been compared when activity was recorded from neurons in dorsomedial and ventrolateral medullary areas. Ventrolateral neurons fired, on average, approximately 50 ms later than dorsomedial neurons in relation to the peak discharge of sympathetic postganglionic nerves (Barman and Gebber, 1983). In addition, different pathways descend in parallel from a number of levels of the neuraxis to innervate neurons in the spinal sympathetic nuclei (Peiss, 1965).

Determination of direct projections from supraspinal neurons to sympathetic preganglionic neurons has proved to be difficult despite advances in anatomical technology. Utilizing the retrograde transport of horseradish peroxidase (HRP) from injection sites restricted to the intermediolateral cell columns of thoracic and lumbar segments of spinal cord, Amendt, et al. (1979) located cell bodies of bulbospinal neurons in the nucleus tractus solitarius, ventrolateral reticular formation, and ventral portions of the raphe nuclei. Neurons near the surface of the ventrolateral medulla were labeled with horseradish peroxidase

following injections into both the intermediolateral cell column and central autonomic nuclei of the spinal cord (Caverson, et al., 1983b). The descending projections from these areas have been confirmed using orthograde transport of radio-labeled amino acids (see Barman, 1984).

Another experimental approach used to identify potential sources of ongoing sympathetic activity has been to stimulate sites within the preganglionic cell columns of the lateral horn and record antidromic action potentials from brain sites. Henry and Calaresu (1974) were able to antidromically activate cells in several brainstem areas: nucleus reticularis parvicellularis, gigantocellularis, paramedian reticular nucleus, lateral reticular nucleus, raphe nuclei, and the inferior olivary nucleus. Electrical stimulation of these areas caused increased arterial pressure and heart rate (Henry and Calaresu, 1974). Others have antidromically activated neurons near the surface of the ventrolateral medulla by stimulation of the intermediolateral cell column or central autonomic area (Barman and Gebber, 1985; Caverson, Ciriello, and Calaresu, 1983a,b). Many of these ventrolateral medullary neurons responded to stimulation of the carotid sinus and aortic depressor nerves (Caverson, et al., 1983a), indicating their possible role in cardiovascular control. The possibility that discrete medullary areas are dedicated to particular vascular beds is discussed in section E.2.

In addition to descending excitatory drive of sympathetic nerve activity, evidence indicates that ongoing inhibitory influences also participate in the control of sympathetic discharge. Although many investigators have demonstrated the existence of descending inhibitory pathways from the medulla by electrical stimulation of the classical

depressor areas of the mediocaudal medulla (e.g., Coote and Macleod, 1974a; Gootman and Cohen, 1971; Henry and Calaresu, 1974), few have investigated ongoing influences of descending inhibition. Alexander (1946) made serial transections of the brain stem and observed that arterial pressure was decreased progressively until maximal depression was achieved as the lower medulla was transected. Then, when the neuraxis was severed at the first cervical spinal cord segment, arterial pressure and sympathetic activity actually increased by a small amount. Thus he showed that the lower medulla exerted a tonic descending inhibitory influence on sympathetic outlow. These results have been confirmed, in principle, by others using more modern techniques. Dembowski et al. (Dembowski, Czachurski, Amendt, and Seller, 1980; Dembowski, Lackner, Czachurski, and Seller, 1981) showed that the spinal components of somatosympathetic reflexes were exaggerated following reversible cold block of descending pathways within the cervical spinal cord. Furthermore, these effects were not altered by baroreceptor denervation or by midcollicular decerebration, indicating that this descending inhibition was not of baroreceptor origin (Dembowski, et al. 1980). These ongoing inhibitory influences were thought to be mediated by descending catecholaminergic neurons situated in ventrolateral areas of the medulla (Dembowski, et al. 1981). In addition, chemical inhibition of neurons in the caudal ventrolateral medulla can cause increased arterial pressure and heart rate (Blessing, West, and Chalmers, 1981) as well as increased activity of renal nerves (Pilowsky, West, and Chalmers, 1985). The ongoing sympathoinhibition

deduced from these studies was thought to originate in the medullary region of A1, noradrenaline-containing neurons (Blessing, Chalmers, and Howe, 1978).

REFLEX CHANGES IN SYMPATHETIC ACTIVITY

Pressoreceptors

Carotid Sinus and Aortic Arch

Slowly adapting mechanoreceptors are embedded in the adventitia of the walls of the aortic arch and at the bifurcation of the common carotid arteries. These receptors are sensitive to deformations of the vessel walls caused by increased transmural pressure which results in circumferential stretch of the vessels (Korner, 1979). Changes in arterial pulse pressure produce changes in vessel radius (deformation) due to the elasticity and geometry of the vessel walls, and, consequently, stimulation of the mechanoreceptors. The receptors have been described morphologically as diffuse arborizations or "circumscribed glomerular-like structures" (Heymans and Neil, 1958) at the ends of the afferent nerve fibers, the cell bodies of which are located in the petrosal ganglia. Axons of the sinoaortic baroreceptors can be myelinated (ca. two thirds of the fibers) or unmyelinated. Stimulus thresholds of the myelinated axons are between 40 and 70 mmHg, and the neurons increase their rates of activity in direct, linear proportion to the intensity of stimulation at arterial pressures of 75 to 150 mmHg; maximum discharge rates are elicited by pressures of 175 to

200 mmHg (Abboud and Thames, 1983). Unmyelinated fibres exhibit higher thresholds, higher saturation pressures, and lower sensitivities than do the myelinated afferent fibers (Abboud and Thames, 1983). As not all baroreceptor afferent neurons exhibit ongoing discharge at normal arterial pressures (Kircheim, 1976), increased activity of these nerves can be caused by recruitment of inactive neurons by increasing arterial pressure past the stimulus thresholds, as well as by increasing the discharge rates of active fibers by suprathreshold increases in blood pressure.

The sympathoinhibition produced by stimulation of arterial baroreceptors, first directly assessed by Adrian, et al. (1932), is known (Abboud and Thames, 1981; Heymans and Neil, 1958; Kircheim, 1976; Korner, 1979; Spyer, 1981). The location of the first synapse in the baroreceptor mediated sympathoinhibitory reflex arc is the least controversial link in the entire pathway. Whereas recent authors agree that the primary baroreceptor afferents in the cranial nerves terminate in the NTS, the precise pathway(s) by which pressoreceptor-mediated inhibition of sympathetic activity remain in dispute or are unknown (Abboud and Thames, 1981; Dampney, 1981; Kircheim, 1976; Korner, 1979; Spyer, 1981). Although central projection sites of the sinoaortic activity (beyond the NTS) have been investigated by numerous investigators, results are equivocal simply due to technical problems. For example, it is very difficult, if not impossible, to lesion cell bodies of neurons without also destroying fibers of passage. Therefore, if lesions are placed in the dorsomedial NTS with the intention of correlating the paths of fiber degeneration with specific NTS projection sites, destruction of passing fibers may lead to an incorrect

assessment. The same caveats apply to tract-tracing techniques utilizing HRP and radiolabeled amino acids. This necessitates the use of electrophysiological studies to corroborate anatomical investigations. However, care must also be taken to interpret electrophysiological data because baroreceptor activity may have effects on parts of the nervous system which seem to have nothing to do with the inhibition of sympathetic nerve activity (Spyer, 1981). Therefore, it is not possible to define the precise pathway(s) by which baroreceptor stimulation results in inhibition of sympathetic activity. Despite these problems associated with the identification of central baroreceptor pathways affecting sympathetic outflow, many investigations have been carried out to describe baroreceptor projections within the central nervous system. Projections from medial NTS to various areas have been demonstrated, including a direct projection to sympathetic preganglionic nuclei in the lateral horns of the thoracolumbar spinal cord (Loewy and Burton, 1978). In addition, neurons in the medial NTS, with activity related to the period of the cardiac cycle, project to the rostral ventrolateral medulla and to dorsomedial reticular areas. However, Barman and Gebber (1978) have demonstrated sympathoinhibition of baroreceptor origin following large lesions placed in the dorsomedial medulla (encompassing the paramedian reticular nucleus as well as the caudal raphe nuclei). Granata et al. (1985) have provided evidence that baroreceptor-mediated sympathoinhibition utilizes rostral ventrolateral medullary neurons (the C1 area) containing phenylethanolamine-N-methyl transferase (PNMT), the enzyme necessary for the synthesis of epinephrine. Neurons in the C1 area project to spinal cord locations of sympathethic preganglionic cell bodies (Ross et al., 1984) In addition,

electrolytic lesions in the C1 area abolish the depressor response to carotid sinus baroreceptor stimulation (Granata, et al., 1985). Similar results of studies on rabbits (Dampney, 1981a, 1981b) support the conclusion that this area mediates the barorecptor reflex. That these PNMT-containing neurons do not seem to comprise a specific cytoarchitecturally defined nucleus may well be the reason for the lack of precise anatomical data pertaining to the baroreflex. Investigators have attempted to define locations of the inhibitory synapses in the pathways. Spinal (Coote and Macleod, 1981; McCall, Gebber, and Barman, 1977; Taylor and Gebber, 1973), as well as supraspinal (Biscoe and Sampson, 1970; Kirchner, Sato, and Weidenger, 1971), inhibitory sites have been documented.

Reflex Influences of Baroreceptors on Peripheral Targets

Folkow, Johansson, and Löfving (1961) made the first systematic investigation of baroreceptor influences on different vascular beds. These investigators made simultaneous measurements of the reflex changes in blood flow to hindlimb muscle and skin, kidney, and intestine that were initiated by unloading of carotid arterial baroreceptors. In these studies, renal and cutaneous blood flows were relatively unaffected whereas skeletal muscle vessels constricted in response to these perturbations; intestinal blood flow decreased less than skeletal muscle blood flow. These results were confirmed by Kendrick et al. (1972) and by Pelletier and Shepherd (1975). Brender and Webb-Peploe (1969) found that baroreceptor stimulation caused decreased blood flow in hindlimb resistance vessels, decreased venous pressure in the isovolumetric

spleen, and produced no consistent changes in hindlimb capacitance vessels. The greater effects of baroreceptor stimulation on renal than cutaneous vasoconstrictors have been confirmed in recordings of renal and cutaneous sympathetic discharge (Ninomiya, et al., 1973). Cardiac sympathetic activity can be more inhibited than renal by stimulation of arterial baroreceptors (Ninomiya, et al., 1971). In addition to the differential effects of baroreceptor influences on distant components of the cardiovascular system, Ninomiya and coworkers have provided evidence that different vascular beds within the abdomen are affected differently by these cardiovascular afferent nerves. Baroreceptor stimulation caused greater inhibition of splenic than renal sympathetic nerve discharge (Ninomiya, et al., 1971). These results have been challenged by Tobey and Weaver (1987) who elicited greater inhibition of renal than splenic sympathetic activity by phenylephrine-induced increases in arterial pressure. It now is clear that baroreceptor reflexes must be considered individually with each particular target organ in mind. In addition, recording activity of single sympathetic neurons during baroreceptor stimulation may resolve differences of opinion regarding the organization of baroreceptor influences on individual target organs or, possibly, even on different tissues within an individual organ.

Cardiopulmonary and Thoracic Vasculature Pressoreceptors

Terminals of sensory fibers that innervate the heart are unencapsulated nerve endings within the subendocardium (Malliani, 1982). The axons of these afferent neurons are contained in the vagi (cell

bodies in the nodose ganglia) as well as in cardiac sympathetic nerves (cell bodies in spinal dorsal root ganglia), and may be either myelinated or unmyelinated.

The myelinated vagal afferent axons terminate at the junctions of the great veins and atrium and increase their rates of discharge in response to increased cardiac volume. Other myelinated vagal afferent axons innervate the atria and respond more to increased contractility than to volume loads per se; these fibers exhibit ongoing activity during atrial systole. In addition, myelinated vagal afferent fibers also terminate in the ventricular endocardium and discharge in synchrony with ventricular systole; these fibers respond to changes in cardiac distention or contractility. Approximately three fourths of cardiac vagal afferent fibers are unmyelinated (Donald and Shepherd, 1978). These C fibers are either mechanoreceptive or chemically sensitive, but are not thought to be polymodal. Mechanoreceptor C fibers innervate the ventricles to a greater extent than the atria and their discharge rates are proportional to end-diastolic pressure. Some C fibers terminate in the epicardium of the left ventricle and respond to changes in contractility or ventricular volume. The pulmonary vasculature also is innervated by vagal afferent nerves that respond to changes in arterial and venous pressures. The pulmonary arterial baroreceptors exhibit characteristics of ongoing and reflex discharge similar to those of sinoaortic baroreceptors. These pulmonary afferent nerves have small, myelinated axons (A-delta) and are active at normal pulmonary arterial pressure. Pulmonary vagal afferent neurons with unmyelinated (C) fibers

innervate pulmonary capillaries and/or veins and exhibit low discharge rates that have no consistent relationship to the cardiac cycle (Abboud and Thames, 1983).

Cardiopulmonary vagal afferent nerves exert ongoing influences on sympathetic nerve activity; interruption of these afferent nerves causes increased vascular resistance in kidney, intestine, and skeletal muscle (Donald and Shepherd, 1978). This tonic sympathoinhibition is believed to be mediated by unmyelinated vagal afferent fibers. Mechanical stimulation of vagal afferent neurons causes increased cardiac, decreased renal, and no change in splenic or lumbar sympathetic nerve activity (Karim, Kidd, Malpus, and Penna, 1972), as well as diuresis (Gauer and Henry, 1976).

Cardiac afferent axons contained within sympathetic nerves (cardiac sympathetic afferent nerves; cardiac spinal afferent nerves) are similar to those within the vagi, but many have been shown to be associated with polymodal receptors responsive to chemical and mechanical stimuli. Spinal afferent innervation of the heart is distributed predominantly to the ventricles, although the atria do have some receptors with spinal afferent axons (Donald and Shepherd, 1978). Pulmonary spinal afferent nerves exhibit ongoing activity which occurs during the systolic phase of pulmonary arterial or venous pressure. The arterial afferent nerves have lower discharge rates than do the venous afferents which "appear particularly suitable to sense pulmonary congestion" (Malliani, 1982). In addition, ongoing, pulse-synchronous activity has been recorded from spinal afferent neurons that innervate the aorta; these neurons respond to increased blood pressure (Malliani, 1982).

In contrast to the effects of vagal afferent stimulation, cardiopulmonary spinal afferent nerves generally are thought to mediate increased sympathetic efferent activity and arterial pressure (Donald and Shepherd, 1978). These effects can be observed in animals following acute transection of the spinal cord (Malliani, Lombardi, Pagani, Recordati, and Schwartz, 1975). Chemical stimulation of cardiac spinal afferent nerves can produce increased renal nerve activity and, consequently, antidiuresis and increased retention of sodium and potassium (Meckler, Macklem, and Weaver, 1981). However, Weaver, et al. have demonstrated that cardiac spinal afferent nerves have high stimulus thresholds, as very large intracardiac pressures or deformations of the ventricular walls are necessary to evoke reflex effects in recordings of renal nerve activity (Weaver, Macklem, Reimann, Meckler, and Oehl, 1979). It has been suggested that these afferent nerves exert more control during conditions of myocardial ischemia (Weaver, Danos, Oehl, and Meckler, 1981) or during chemical stimulation (Reimann and Weaver, 1980) of the afferent nerves, than during increased intracardiac pressure.

Injections of vasoactive drugs such as phenylephrine and nitroprusside evoke changes in activity of many cardiovascular afferent nerves. The summation of reflexes caused by such perturbations is important, and individual reflex effects of stimulation of isolated groups of receptors probably are not particularly meaningful under normal physiological circumstances. Therefore, it is as important to evaluate the effects of general increases in pressure as it is to assess the individual contributions of each group of pressoreceptors to cardiovascular control. The combined effects of stimulation of carotid,

aortic, and cardiopulmonary vagal afferent pressoreceptors by large increases in arterial pressure all contribute to the ensuing sympathoinhibition (Guo, Thames, and Abboud, 1982). The carotid sinus baroreceptor afferent nerves appear to be dominant in this response (Guo, et al., 1982; Mancia, Donald, and Shepherd, 1976). However, any one set of pressoreceptors does compensate for the absence of the other set of receptors in producing sympathoinhibition (Guo, et al., 1982). This apparent contradiction has been resolved partially by Thames and Ballon (1984) who have presented descriptive evidence that concomitant activation of carotid and aortic baroreceptors does not produce much more sympathoinhibition than does activation of either input separately because activation of each input alone can cause almost maximal inhibition.

Although pressoreceptive cardiac spinal afferent nerves are less likely to exert ongoing control of sympathetic activity when cardiac vagal afferent innervation remains intact (Abboud and Thames, 1983), cardiac spinal afferent nerves can have significant excitatory influences despite simultaneous inhibitory influences of vagal afferent nerves. Chemical stimulation of cardiac vagal and spinal afferent nerves simultaneously can produce inhibition, excitation, or no change in renal nerve activity (Reimann and Weaver, 1980). In addition, when both groups of afferent nerves are stimulated simultaneously by application of algogenic substances to the heart, renal nerve activity often is inhibited, while splenic nerve activity is increased (Weaver, Fry, and Meckler, 1984). These results indicate that sympathetic outflow to different viscera can respond selectively to summation of multiple afferent influences.

Abdominal Visceral Pressoreeptors

Gammon and Bronk (1935) described pacinian corpuscles closely associated with arteries supplying the small intestine. Many of the afferent axons innervating these receptors were shown to discharge during the systolic phase of the cardiac cycle, suggesting that they might play a role in transmitting information related to the arterial pressure in this region. Since that time, others have reported the presence of "baroreceptors" in a variety of viscera, including the kidney (Beacham and Kunze, 1969), liver (Kostreva, Castaner, and Kampine, 1980), and spleen (Herman, et al., 1982). Although cardiovascular and sympathetic neural responses have been elicited by stimulation of these visceral pressure receptors, the significance of such reactions during systemic injections of pressor agents (such as phenylephrine) remains obscure (Thames and Ballon, 1984). Recently, Martin and Longhurst (1986) have conducted experiments to compare abdominal visceral afferent nerve fibers to arterial baroreceptors and refute the existence of "high pressure baroreceptors" in the abdominal viscera. Although these authors describe 19 afferent fibers with ongoing activity related to the cardiac cycle, similar to the afferents described by Gammon and Bronk (1935), Martin and Longhurst could not produce consistent responses in these neurons by alterations in hemodynamic variables such as increased or decreased arterial pressure. Martin and Longhurst (1986) assert that these afferent neurons most likely respond to deformation of nearby tissue, changes in tissue fluids, and/or chemicals released into the local environment.

Viscerosympathetic Reflexes

The vasculature and parenchyma of abdominal organs are innervated by afferent nerves with axons in the splanchnic nerves and vagi. Stimulation of visceral afferent nerves innervating gall bladder, intestine, kidney, liver, pancreas, spleen, and stomach, with few exceptions, causes excitation of sympathetic nerve activity, increased heart rate and dP/dt, total peripheral resistance, and arterial pressure. The primary afferent pathway for the majority of these reflexes is the greater and lesser splanchnic nerves. Afferent axons contained in the splanchnic nerves of cats have somata in the dorsal root ganglia of spinal segments T2 through L2 (Jänig and Morrison, 1986). These afferent nerves project to Lissauer's tract in the dorsolateral spinal cord and travel rostrally and caudally to adjacent spinal segments where they terminate in Rexed's laminae V-VII and X. Cells in these areas, in turn, project to the brain in spinoreticular and spinothalamic tracts. Some of the secondary afferents project rostrally in the dorsal columns and terminate in the gracile and cuneate nuclei. Termination of at least some renal primary afferent neurons occurs in the medulla oblongata (Simon and Schramm, 1984). In addition to their supraspinal projections, visceral afferent nerves are known to influence spinal neurons which affect sympathetic preganglionic neurons via solely spinal pathways. It is not known whether the ascending pathways are viscerotopically organized, but some authors doubt the specifity of visceral afferent projections on the basis of the convergence of visceral sensory neurons upon cells with somatic afferent inputs, and the phenomenon of referred pain. However, at least the

autonomic components of some reflexes initiated from the viscera can be engaged preferentially. The present discussion will be limited to splenic and intestinal spinal afferent nerves. The interested reader will find information regarding the sensory innervation of these and other abdominal and pelvic organs in the reviews contained in a recent volume of Progress in Brain Research (Cervero and Morrison, 1986).

Injections of algogenic chemicals into splenic or superior mesenteric arteries of lightly anesthetized animals produces the "pseudaffective response": hyperpnea, increased arterial pressure, and vocalization (Guzman, Braun, and Lim, 1962; Moore and Singleton, 1933a). Floyd and Morrison (1974) recorded activity of splanchnic afferent fibers during mechanical perturbations of the spleen and intestine and described punctate receptive fields usually associated with blood vessels. The intestinal and splenic afferent fibers in the greater splanchnic nerve were unmyelinated as well as myelinated and often exhibited ongoing discharge which sometimes had cardiac or respiratory rhythms. The primary afferent pathways are the greater splanchnic nerves and lumbar splanchnic nerves for the spleen and intestine, respectively (Moore and Singleton, 1933b). Supraspinal neural circuits apparently are not necessary for some reflex responses to mechanical or electrical stimualtion of these nerves since intestinal vasoconstriction and increased arterial pressure can be elicited after the spinal cord is severed (Downman and McSwiney, 1946). Beyond that, little seems to be known about the anatomy of the afferent pathways.

Electrical stimulation of the mesenteric afferent nerves causes greater excitation of visceral (splanchnic preganglionic and mesenteric efferent) sympathetic activity than cutaneous or skeletal muscle

vasoconstrictor discharge (Koizumi and Suda, 1963). Johansson and Langston (1964) found that electrical stimulation of superior mesenteric afferent nerves caused vasoconstriction in the kidney and dilation of skeletal muscle vasculature. Later, others recorded activity of single preganglionic neurons during electrical stimulation of the mesenteric nerve and found that not all preganglionic neurons were involved in the reflex, even if the preganglionic neurons exhibited spontaneous activity (Fedina, Katunskii, Khayutin, and Mitsanyi, 1966; Franz, Evans, and Perl, 1966). Distension of the small intestine to excite mechanoreceptors causes greater excitation of mesenteric than renal or splenic sympathetic activity (Ninomiya and Irisawa, 1975; Ninomiya, Irasawa, and Woolley, 1974).

Few studies of the effects of splenic afferent nerve stimulation on sympathetic outflow have been done. Electrical stimulation of splenic afferent nerves causes increased arterial pressure, heart rate, dP/dt, and excitation of renal and cardiopulmonary sympathetic nerve discharge (Herman, Kostreva, and Kampine, 1982). In addition, these investigators could elicit similar responses by pinching the spleen to alter intrasplenic pressure, leading them to conclude that splenic afferent nerves could be the afferent limb of a low-pressure baroreceptor reflex. Calaresu, Tobey, Heidemann, and Weaver (1984) demonstrated that chemical stimulation of splenic afferent nerves can produce greater excitation of splenic than renal sympathetic efferent nerve activity. Similar results could be achieved whether arterial baroreceptor and cardiopulmonary vagal afferent nerves remained intact or were severed (Tobey and Weaver, 1987), indicating that the pressoreceptor stimulation, produced by the reflex increase in arterial pressure, was not necessary for unequal

splenic and renal nerve responses. Calaresu et al. speculated that the greater splenic than renal sympathetic responses to splenic stimulation may reflect the functional organization of these reflexes to provide "the largest output to the organ from which the reflex originates". These authors gave no indication of the meaning of this interpretation: do a greater number of splenic than renal sympathetic neurons participate in the excitatory reflex, or is the activity of individual splenic neurons increased more than that of individual renal neurons?

FUNCTIONAL SPECIFICITY OF THE SYMPATHETIC NERVOUS SYSTEM

Selective Connections in Sympathetic Ganglia

Langley was the first to demonstrate that different target organs could be activated selectively by electrical stimulation of the preganglionic axons contained within white rami of different spinal segments. Thus, electrical stimulation of the white rami of spinal segments T1 and T2 caused pupilary constriction, and contraction of the nictitating membrane; stimulation of T2 and T3 white rami led to vasoconstriction in the head; stimulation of T2 through T4 produced cardioacceleration; stimulation of T5 and T6 caused piloerection in the face and neck; stimulation of T6 through T8 produced responses of the sweat glands in the ipsilateral forefoot (Langley, 1892). Although segmental distributions of these effects were overlapping, it was clear that preganglionic fibers originating from different spinal segments preferentially influenced particular target areas. These results were confirmed later by Njå and Purves (1977) and extended by analyses of

intracellular recordings of postganglionic neuronal discharge. In addition to the segmental organization of preganglionic influences on different neuroeffector targets, individual postganglionic neurons are innervated by preganglionic neurons from distinctive sets of spinal cord segments (Purves, 1978). A particular postganglionic neuron is innervated predominantly by preganglionic neurons originating from one spinal segment. Additional preganglionic inputs are provided to this ganglion cell by spinal segments immediately adjacent to the dominant segment. Thus, for example, a neuron can be dominated by input from preganglionic cells from T4, and fewer preganglionic inputs to this ganglion cell originate from segments T3 and T5. As one proceeds farther and farther from the dominant spinal segment, activation of a postganglionic cell becomes progressively less likely. The selectivity of segmental influences of preganglionic cells on individual ganglion cells may be organized functionally to provide the selective activation of end organs with similar function (such as vasoconstrictor or pilomotor). This possibility will be discussed further in the following section.

Does the position of postganglionic neurons within a given ganglion determine the preganglionic inputs received by the ganglion cells? Purves offers evidence that position of cells in a ganglion is not an important factor in the determination of pre- to postganglionic connectivity. He found that injections of horseradish peroxidase into particular targets (e.g., eye or ear) produced an apparently random distribution of labeled cells within the superior cervical ganglion. Therefore, selective segmental inputs to this ganglion were shown to exist in the absence of topographical orientation of postganglionic

neurons within the ganglion. However, others have speculated that clustering of postganglionic cell bodies which innervate individual targets does exist and that the topographical arrangement of these cell bodies may be conducive to selective preganglionic inputs (Archakova, Bulygin, and Netukova, 1982; Kelts, Whitlock, Ledbury, and Reese, 1979; Kuntz, 1938). Celiac ganglion cells in close proximity to one another often have dendrites which are incorporated into common fiber bundles (Archakova, et al., 1982; Kuntz, 1938). Terminal branches of preganglionic axons arborize within these bundles to provide circumscribed groups of postganglionic cells with a common preganglionic innervation (Archakova, et al., 1982; Kelts, et al., 1979). Therefore, the position of postganglionic neurons within a given ganglion may be important in the determination of preganglionic influences on peripheral targets.

The distribution of neurons among different ganglia may be another important factor which determines the selectivity of preganglionic innervation of the ganglion cells. Postganglionic neurons in different thoracic paravertebral ganglia are innervated selectively by preganglionic neurons originating from different segments of the spinal cord (Lichtman, Purves, and Yip, 1980). Thus, thoracic chain ganglia from the stellate ganglion to the T5 ganglion receive preganglionic innervation from different sets of spinal cord segments. For example, the stellate ganglion is innervated by preganglionic neurons originating from spinal cord segments more rostral than segments which provide inputs to the T5 ganglion. Interestingly, postganglionic neurons also are segmentally distributed in sympathetic ganglia with respect to the

target tissues. Thus, the cell bodies of hindlimb muscle vasoconstrictors are located in ganglia of segments T13 to L3; cutaneous vasoconstrictors to the hindpaw are in L1 to L3; cutaneous vasoconstrictors to the tail are in L3 and L4; postganglionic neurons which innervate the lower abdominal and pelvic viscera are distributed in ganglia L3 to L5 (Jänig, 1986). In addition, preganglionic cell bodies of individual spinal cord segments may be organized anatomically with respect to the target tissue innervated by their postganglionic counterparts. For example, preganglionic neurons situated in the border region between the grey matter of the lateral horn and the lateral funiculus of spinal cord segment L4, project to the caudal sympathetic trunk and innervate neurons which supply hindlimb vasculature (Jänig and McLachlan, 1986; McLachlan, Oldfield, and Sittiracha, 1984). In contrast, preganglionic neurons in more medial areas of the L4 grey matter innervate postganglionic neurons in the inferior mesenteric ganglion, many of which regulate visceral motility of the lower abdominal organs (Jänig and McLachlan, 1986; McLachlan, et al., 1984).

In summary, preganglionic neurons originating from different spinal cord segments make selective connections with postganglionic neurons based upon 1) the particular ganglion containing the ganglion cell, 2) the position of the postganglionic soma within a sympathetic ganglion, and 3) the particular target organ innervated by the postganglionic neuron.

Electrophysiological Evidence for Functional Organization of the Sympathetic Nervous System

Whereas the sympathetic nervous system is known to be organized to provide discrete control of blood flow to different organs, Jänig and coworkers have presented much evidence for the functional organization of sympathetic outflow to different target tissues, even within an individual organ (Jänig, 1985, 1986). Initially, investigations were confined to sympathetic control of hindlimb blood vessels (cutaneous and skeletal muscle vascular beds), sweat glands, and hair. By careful comparison of responses of effector organs and single sympathetic fiber activity to natural stimulation of afferent nerves, and by the ongoing discharge patterns of the neurons, Jänig has formulated criteria for the functional identification of sympathetic neurons. For example, sudomotor neurons and vasoconstrictor fibers to skin and skeletal muscle exhibit ongoing discharge whereas pilomotor neurons and vasodilator fibers to skin and skeletal muscle do not discharge under resting conditions. Further, ongoing activity of muscle vasoconstrictors shows strong correlation to the arterial pressure pulse and is strongly inhibited by stimulation of carotid sinus baroreceptors. In contrast, ongoing activity of cutaneous vasoconstrictors is only weakly correlated with arterial pressure, and does not respond to increased carotid sinus pressure; activity of sudomotor neurons has no correlation with arterial pressure and is not affected at all by baroreceptor stimulation. The hindlimb pilomotor and vasodilator neurons can be activated only by very specific stimuli or in specific behavioral contexts. For example, stimulation of hypothalamic defence areas or emotional stimuli (such as

confrontation) elicits activity from previously quiescent muscle vasodilator neurons, whereas no reflex effects are observed in response to stimulation of baroreceptors, chemoreceptors, or any somatic stimuli (Jänig, 1985). Cutaneous vasodilator neurons could be activated only by warming of the spinal cord (Jänig, 1985). In addition to the functional classification of postganglionic neurons, Jänig has been able to find preganglionic neurons with activity patterns to match those of the postganglionic fibers. These data suggest that pre- and postganglionic neurons comprise functionally dedicated pathways to different effector organs.

Jänig has broadened the scope of his investigations by considering lumbar sympathetic outflow to the lower abdominal and pelvic viscera. His interpretations are the same for the sympathetic innervation of these areas as for hindlimb sympathetic outflow (Jänig, 1986a,b). Neurons with pulse-rhythmic activity and that are weakly excited by visceral afferent stimulation are vasoconstrictors. In contrast, cells that respond strongly to visceral stimuli and have no pulse wave correlation are thought to mediate regulation of visceral motility and secretion. Apparently, there are functionally dedicated pathways to lower abdominal and pelvic visceral vasculature and parenchyma. Therefore, according to Jänig, the sympathetic nervous system has a more complex organization than had been anticipated previously, to provide discrete control of different effector organs under the differing demands encountered by behaving mammals during challenges to homeostasis.

The possibility that medullary, as well as spinal, neurons are dedicated to particular vascular beds, has been investigated in

relatively few laboratories. Barman, Gebber, and Calaresu (1984) provided evidence that medullary reticular and raphe neurons nonuniformly affect the discharge patterns of renal, cardiac, and external carotid sympathetic nerves. A given brain stem neuron could be temporally related more strongly to the activity pattern of one than another of two sympathetic nerves. Although many medullary units were related similarly to discharge patterns of more than one sympathetic nerve, 50% of reticular and 30% of raphe neurons were more tightly correlated with the discharge of a particular sympathetic nerve. In addition, Barman and Gebber (personal communication) demonstrated that many ventrolateral medullary neurons project to the intermediolateral areas of particular spinal cord segments, whereas some neurons project to multiple segments of the spinal cord, indicating some degree of specificity of descending pathways. Similar descending trajectories of bulbospinal sympathoinhibitory neurons of the raphe nuclei have been described (Morrison and Gebber, 1985). Utilizing injections of excitant amino acids (such as D,L-Homocysteic acid, DLH) into the ventrolateral medulla (nucleus paragigantocellularis lateralis, PGL) to excite cell bodies without affecting axons of passage, Lovick (1985) demonstrated patterned changes in heart rate and vascular conductance of hindlimb arteries. Differing patterns of responses were elicited from chemical stimulation of different sites within PGL. Lovick proposed that specific neurons in PGL are dedicated to control of particular functional components of sympathetic outflows; the response pattern apparently were dependent upon the relative numbers of functionally different neurons excited during particular injections of the excitant amino acids. During simultaneous recordings of activity of sympathetic

fibers supplying cutaneous and skeletal muscle vasculature, McAllen and Dampney (1986) chemically stimulated sites in the ventrolateral medulla and produced differentiated sympathetic responses. Stimulation of medial areas of the subretrofacial nucleus produced excitation of cutaneous sympathetic activity, whereas stimulation of lateral areas of the nucleus caused excitation of sympathetic outflow to skeletal muscle; chemical stimulation of neurons in intermediate sites activated both components of sympathetic outflow. These results indicate the topographical organization of these "pre-sympathetic" medullary neurons.

As well as the identification of functional specificity of reflex discharge in vivo, it has been possible to classify sympathetic neurons on the basis of their firing characteristics produced in vitro. By their responses to intracellular depolarizing current, sympathetic ganglion cells of the guinea pig and cat have been classified as 'tonic' or 'phasic' (Cassell, Clark, and McLachlan, 1986; Decktor and Weems, 1981, 1983; Hartman and Krier, 1984; Kreulen and Szurszewski, 1979; Weems and Szurszewski, 1978). Tonic cells are characterized by maintained repetitive discharge throughout a period of injecting a pulse of suprathreshold depolarizing current. In contrast, phasic cells respond to this stimulation with a transient burst of action potentials at the onset of current injection. The distributions of these two cell types in lower lumbar sympathetic paravertebral and prevertebral ganglia of guinea pigs coincide with the differential distribution of functionally specific postganglionic neurons (Baron, Jänig, and McLachlan, 1985; Cassell et al., 1986). In guinea pigs, virtually all neurons in the caudal lumbar sympathetic chain can be classified as phasic, whereas a majority of neurons in the inferior mesenteric

ganglion (distal lobe) are tonic (Cassell, Clark, and McLachlan, 1986). Interestingly, most neurons in the caudal sympathetic chain probably are vasoconstrictors that innervate the vasculature of hindlimb skin and skeletal muscle (McLachlan and Jänig, 1983). In contrast, neurons in the distal lobe of the inferior mesenteric ganglion send axons in the hypogastric nerves (Baron, Jänig, and McLachlan, 1985) and are involved with regulation of motility and secretion of the pelvic viscera as well as with pelvic vasculature (Baron, Jänig, and McLachlan, 1985; Langley and Anderson, 1895a,b,c).

Central preganglionic input may also differ between tonic and phasic postganglionic neurons (Jänig, 1986). Postganglionic neurons in the lower sympathetic chain of guinea pigs discharge action potentials only when "dominant" preganglionic axons are stimulated (see Jänig, 1986). In this respect, cells of these ganglia appear to be similar to neurons in ganglia situated on pelvic nerves (Szurszewski, 1981). In contrast, neurons in the inferior mesenteric ganglion require convergence of many presynaptic inputs before these postganglionic neurons will discharge (Crowcroft and Szurszewski, 1971; Szurszewski, 1981). Therefore, perhaps preganglionic and peripheral synaptic inputs are required for activity to occur in lower lumbar, prevertebral ganglion cells, whereas preganglionic input alone may be sufficient for discharge of neurons in the chain ganglia. If it is true that phasic neurons innervate the vasculature, whereas tonic neurons regulate visceral motility, then these data may indicate that vascular reflexes require intact connections between the spinal cord and sympathetic ganglia, whereas reflexes directed at visceral parenchyma can occur in the absence of these connections. Some data indicate that reflexes affecting the

vasculature apparently do not occur in the absence of connections of sympathetic ganglia with central neural structures (Calaresu, Kim, Nakamura, and Sato, 1978; Johansson and Langston, 1964). However, the occurrance of peripheral reflexes is common to both superior and inferior mesenteric prevertebral outflow (Szurszewski, 1981). After interruption of all afferent and efferent connections between the central nervous system and the celiac plexus, distention of one intestinal segment elicits inhibition of motility in other intestinal segments (Kuntz, 1940; Kuntz and Saccomanno, 1944; Semba, 1954). In addition, the gastric inhibitory effect of acid in the duodenum persists following decentralization of the celiac plexus (Schapiro and Woodward, 1959). These results suggest that sensory fibers in the intestine do make synaptic contact with neurons in prevertebral ganglia. Synaptic input from intestinal afferent neurons to prevertebral postganglionic neurons has been demonstrated electrophysiologically (Crowcroft and Szurszewski, 1971; Kreulen and Szurszewski, 1979) as well as immunohistochemically (Costa and Furness, 1983; Dalsgaard, Hökfelt, Schultzberg, Lundberg, Terenius, Dockray, and Goldstein).

Neurochemical Evidence for Specificity of Target Tissue Innervation

The proportion of phasic neurons in the lower abdominal sympathetic system corresponds to the proportion of noradrenergic neurons showing immunoreactivity to neuropeptide Y (Macrae, Furness, and Costa, 1986; McLachlan, 1986). This peptide has been co-localized with noradrenaline in sympathetic neurons which specifically innervate submucosal blood

vessels of the intestine (Sundler, Moghimzadeh, Hakanson, Ekelund, and Emson, 1983). Immunohistochemical evidence indicates that axons of sensory neurons from the intestine terminate selectively around celiac neurons which regulate intestinal motility, but not around neurons which are thought to innervate mesenteric blood vessels (Lindh, et al., 1986; Macrae, et al., 1986; Sundler, et al. 1983). Recent neurochemical studies have shown that the majority of neurons in the posterior lobes of the celiac ganglion (adjacent to the splanchnic input) are neuropeptide Y-positive. In the rest of the ganglion, some neuropeptide Y-positive cells are present but more neurons are stained for somatostatin in these regions; other cells do not contain either of the two peptides (Lindh, Hökfelt, Elfvin, Terenius, Fahrenkrug, Elde, and Goldstein, 1986; Macrae, et al. 1986).

COMPARISON OF THE DISTRIBUTIONS OF RENAL AND SPLENIC NEURONS IN SYMPATHETIC GANGLIA

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INTRODUCTION

Stimulation of visceral afferent nerves has been shown to cause differential responses in splenic and renal sympathetic efferent nerve activity. Stimulation of splenic receptors with algogenic chemicals, such as capsaicin or bradykinin, produces reflex excitation of splenic nerve activity which is greater than the increase in renal nerve activity (Calaresu, et al., 1984). Stimulation of afferent fibers within cardiac sympathetic nerves also causes greater reflex increases in splenic than renal nerve activity; this pattern of differential responses can be elicited in spinal cats as well as in animals with intact neuraxes (Weaver, et al., 1983). Since supraspinal neural pathways are not always necessary for these reflexes to occur, the autonomic organization responsible for at least some of these differential responses must reside within spinal or ganglionic neural circuits. The distribution of postganglionic neurons among different sympathetic ganglia may determine the specificity with which afferent and preganglionic neurons innervate the postjunctional cells. For example, postganglionic neurons in different thoracic paravertebral

ganglia are innervated selectively by preganglionic neurons originating from different segments of the spinal cord (Lichtman, Purves, and Yip, 1980).

Also, the arrangement of cells within any ganglion may be conducive to interaction among individual postganglionic neurons (Archakova, Bulygin, and Netukova, 1982; Decktor and Weems, 1981; Kelts, Whitlock, Ledbury, and Reese, 1979; Kuntz, 1938). Celiac ganglion cells in close proximity to one another often have dendrites which are incorporated into common fiber bundles (Kuntz, 1938). Terminal branches of preganglionic axons arborize within these bundles to provide circumscribed groups of postganglionic cells with a common preganglionic innervation. This arrangement could contribute to specificity of reflex responses; and, consequently, the relative densities of specific types of postganglionic neurons within ganglia is important. Although the ganglionic location of efferent renal neurons has been demonstrated anatomically (Kuo,Krauthamer, and Nadelhaft, 1982) and electrophysiologically (Decktor and Weems, 1981), the origin of splenic efferent nerves has been studied only cursorily (Kuo and Krauthamer, 1981). Therefore, retrograde transport of horseradish peroxidase (HRP) was employed to compare the inter- and intra-ganglionic distributions of cell bodies of sympathetic efferent neurons supplying the kidney and spleen in cats.

METHODS

Twenty-three cats (2.7 $\pm$ 0.2 kg) used in this study were anesthetized with 30-35 mg/kg sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL) administered intravenously. A left or right flank incision was made and splenic (6 cats), left renal (6 cats), or right renal (7 cats) nerves were dissected from surrounding tissue and cut very close to the spleen or kidney. The central ends of the nerves were moistened and placed on a small sheet of parafilm. Surrounding tissue was covered with gauze soaked in 0.9 % NaCl to prevent fluid loss by evaporation and to avoid contamination of the tissue with peroxidase. Ten to fifteen milligrams of crystalline HRP (Sigma VI; Sigma Chemical, St. Louis, MO) was applied to the central nerve stumps for one hour. Afterwards, excess HRP was blotted, and the nerves were rinsed with warm buffered physiological saline solution. In four additional animals the nerves (2 splenic, 2 renal) were transected 1-2 cm central to the site of HRP application to test for nonspecific ganglionic labelling and endogenous peroxidase activity. The incisions were sewn closed; the cats were given fluids subcutaneously and allowed to recover from the anesthesia. Following survival times ranging from 24-48 h, the animals were administered 40 mg/kg sodium pentobarbital, 500 units heparin (ICN Pharmaceuticals, Inc., Cleveland, OH), and 5 mg/kg hexamethonium (Mann Research Laboratories, Inc., New York, NY) intravenously to prevent blood clotting and to produce maximal

vasodilation for optimal tissue fixation. Animals then were perfused transcardially with 1-2 l warm, heparinized saline, followed first by 1.5 l of a fixative solution composed of 0.15 M phosphate buffer, 1% (w/v) paraformaldehyde, and 2% gluteraldehyde, and then 1.5 l of the fixative with 10% (w/v) sucrose added. The perfusion schedule was a modified version of that described by Mesulam (Rosene and Mesulam, 1978).

Immediately after perfusion, left and/or right thoraco-lumbar sympathetic chain ganglia (stellate through L3) and upper abdominal prevertebral ganglia (left and right celiac, superior mesenteric) were removed and placed in 30% sucrose in 0.15 M phosphate buffer and refrigerated for 1-4 h. Tissue sections were cut at 50 microns on a freezing stage at -20°C (Cryo-Histomat MK2, Hacker Instruments, Inc., Fairfield, NJ) and processed utilizing tetramethylbenzidine (Sigma Chemical Co., St. Louis, MO) as the chromogen following a modification of Mesulam's method (Mesulam, 1978; 1980). Details of the histological procedure can be found in Appendix B. Tissue was mounted on gelatinized slides, allowed to dry overnight, counterstained with neutral red or toluidine blue, coverslipped, and systematically inspected for reaction product using light- and/or dark-field microscopy. Labelled neurons were counted, and the range of cell diameters was noted using a calibrated ocular reticle under light-field illumination at a magnification of 200X. Every labelled cell in every section was counted and all numbers were corrected for double counting using the method of

Abercrombie (1946): $P = (AM)/(L+M)$; where P = corrected number of cells, A = uncorrected number of cells, M = thickness of the tissue sections in microns, and L = average length of the cells.

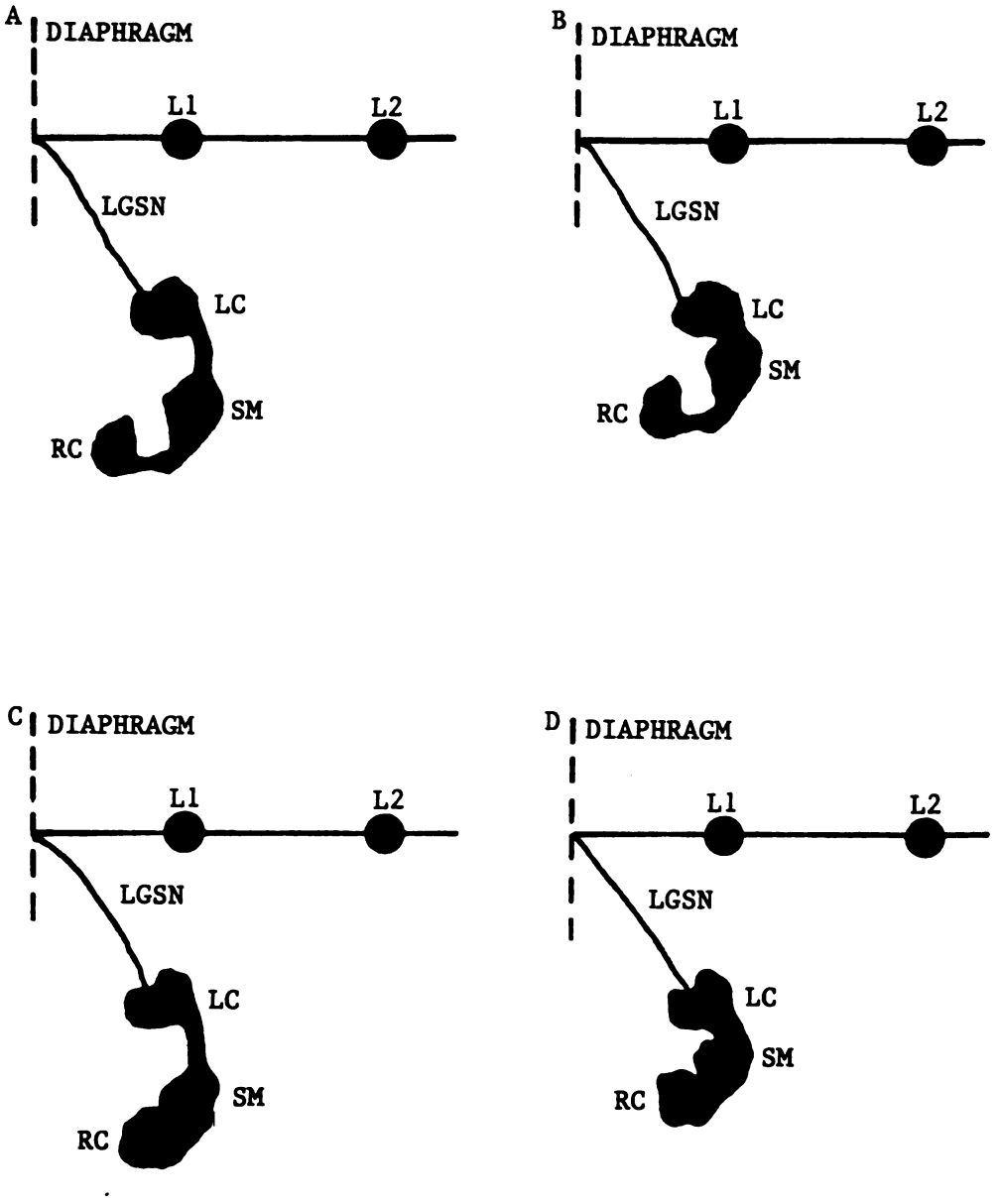
RESULTS

Variation was noted in the extent to which the left celiac, superior mesenteric, and right celiac ganglia were fused into a single complex ganglion (solar plexus; Figure 1). Even when the plexus appeared as three separate ganglia (n = 4), neuron cell bodies were observed in the connective strands between the ganglionic masses. Therefore it was impossible to delineate precise borders among the three ganglia. Junctions of greater splanchnic nerves and the solar plexus will be referred to as the left and right celiac poles of the solar plexus. Regardless of the configuration of the solar plexus, the relative distributions of cell bodies of renal and splenic nerves were consistent from animal to animal.

Both labelled and unlabelled cells in the sympathetic ganglia were round, oval, or fusiform. The cell shapes likely were dependent upon the plane of the sections. Long axes of all cells measured from 10 to 50 microns.

A total of 10,140 splenic, left renal, and right renal neurons (corrected counts) were labelled with HRP (Table 1). Labelled cells usually ranged in size from 30 to 50 microns. However, in two experiments the labelled perikarya (of one right renal nerve and of one left renal nerve) were 10-20 microns in size. HRP reaction product was packed densely within the soma and often filled cell processes (Figure 2). Ganglionic cells were never labelled in the 4 cats in which the

Figure 1. Schematic diagrams of variations in the gross anatomy of the solar plexus ganglia. A. (n = 4) Left celiac (LC), superior mesenteric (SM), and right celiac (RC) ganglia appear as 3 distinct ganglionic masses connected by nerve trunks. B. (n = 3) Left celiac and superior mesenteric ganglia are fused; distinct right celiac ganglion is connected by a nerve trunk. C. (n = 2) Distinct left celiac ganglion connected by a nerve trunk to fused superior mesenteric and right celiac ganglia. D. (n = 14) All three ganglia are fused together into a single ganglionic mass. LGSN, left greater splanchnic nerve; L1 and L2, first and second ganglia of the left lumbar sympathetic chain; Position of the diaphragm is indicated by the interrupted line.


A
DIAPHRAGM
L1
L2
LGSN
LC
SM
RC
B
DIAPHRAGM
L1
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DIAPHRAGM
L1
L2
LGSN
LC
SM
RC
D
DIAPHRAGM
L1
L2
LGSN
LC
SM
RC

FIGURE 1.

TABLE 1. Numbers of labelled cells in sympathetic ganglia.

		TCG	L1	L2	L3	Sol. plex.	Totals
SPLENIC (n=6)							
# cells	(total)	90	42	7	556	6,072	6,767
# sections	(total)	229	16	12	25	143	425
# cells/cat	(mean)	18	14	2	185	1,216	----
	(range)	0-46	0-40	0-6	0-556	512-2,168	----
LEFT RENAL (n=6)							
# cells	(total)	335	513	480	142	1,459	2,929
# sections	(total)	330	36	24	25	164	579
# cells/cat	(mean)	57	85	80	28	243	----
	(range)	0-157	1-293	1-268	5-70	0-784	----
RIGHT RENAL (n=7)							
# cells	(total)	118	151	201	139	569	1,178
# sections	(total)	278	28	26	21	356	709
# cells/cat	(mean)	17	22	33	28	81	----
	(range)	0-111	0-93	0-98	1-120	0-441	----

TCG: thoracic chain ganglia (stellate through T13), L1: first lumbar chain ganglion, L2: second lumbar chain ganglion, L3: third lumbar chain ganglion, Sol. plex.: solar plexus (left celiac, superior mesenteric, and right celiac ganglia); n: number of cats, # cells: number of labelled cells from all animals (numbers are corrected for double counting), # section: number of 50 micron sections from all animals.

Figure 2. High contrast photomontage of HRP label within two splenic sympathetic neurons. The perikarya and processes of both cells are labeled densely with HRP product. Calibration in the upper right corner is 40 microns.

specific nerve bundles treated with HRP were severed central to the site of HRP application.

Ganglionic distribution of splenic neurons

A small percentage of labelled splenic neurons were located within the sympathetic chain ganglia (Table 1, Figure 3). Fewer than 3 labelled splenic neurons per ganglion were located in the thoracic chain from the stellate ganglion through T11. Only 90 splenic nerve cell bodies were labelled in thoracic chain ganglia T12 and T13. Ganglia of lumbar segments contained 605 splenic neurons, but 99% of these cells were labelled in only one experiment. Most labelled splenic neurons (6072 cells) were distributed randomly throughout the left and right celiac poles of the solar plexus (Table 1, Figure 3, Figure 4A, Figure 5). The center of the plexus (superior mesenteric region) was virtually devoid of labelled splenic cells.

To determine if the splenic nerve axons were located only in nerves emanating from the left celiac pole of the solar plexus, nerves close to the left celiac pole of the ganglionic plexus were severed in one cat. HRP was applied, as usual, to splenic nerves close to the spleen. Labelled splenic neurons of this cat were distributed uniformly throughout both celiac poles, illustrating that the spleen is well innervated by nerves orginating from the right side of the solar plexus.

Ganglionic distribution of left renal neurons

Renal neurons were distributed more evenly between the chain ganglia and solar plexus (Table 1, Figure 3) than were splenic neurons. Fewer

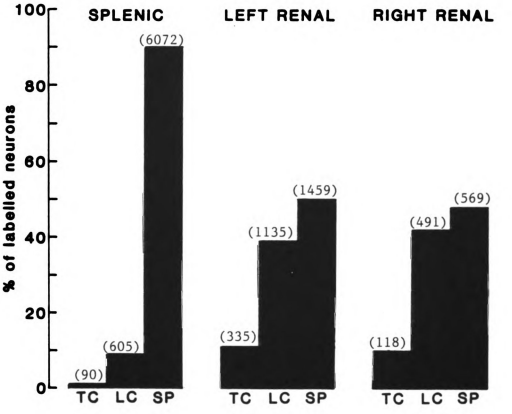

Figure 3. The percent distributions of splenic and renal neurons among sympathetic ganglia of the thoracic chain (TC), lumbar chain (LC), and solar plexus (SP). Numbers of labeled neurons are given in parentheses above each bar.

Figure 4. Photomicrographs of peroxidase-labeled splenic (A) and renal (B) sympathetic neurons in the left celiac pole of the solar plexus ganglia of two cats. Calibrations in the upper right corners are 0.5 mm.

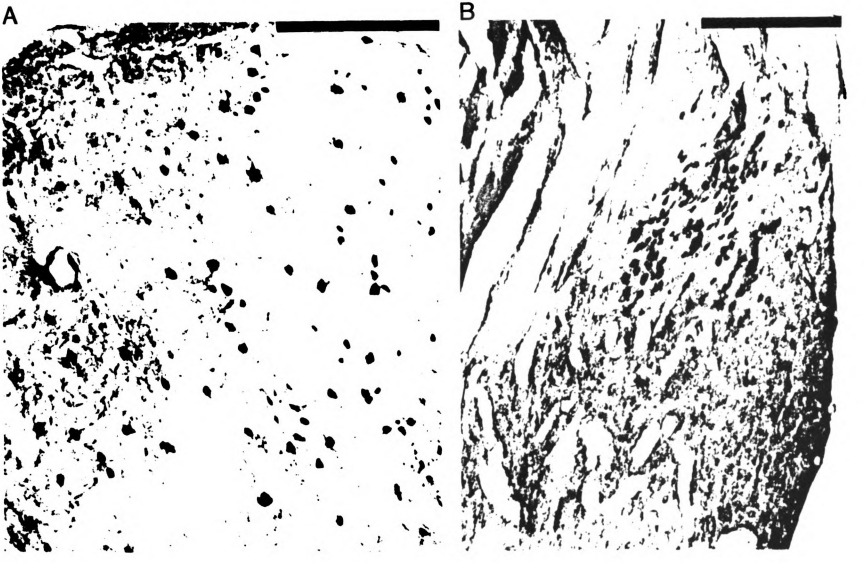

Figure 4.

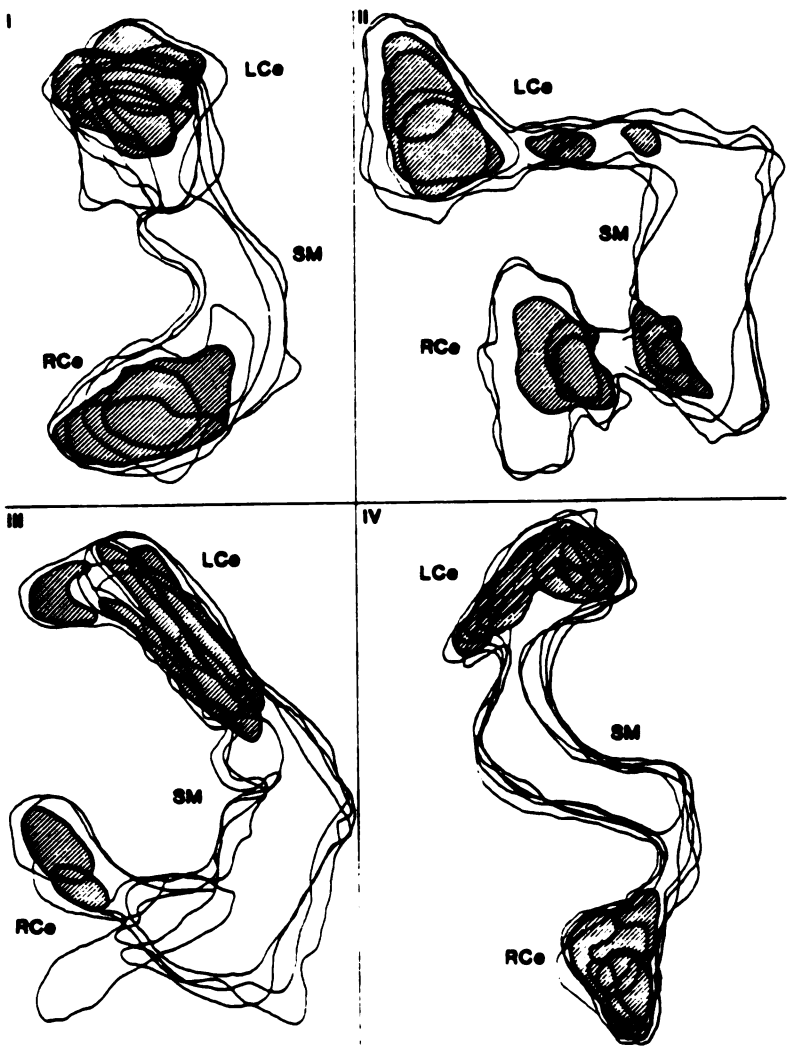

Figure 5. Camera lucida drawings showing the arrangement of labeled splenic neurons in the solar plexus ganglia of four cats. Shaded areas are tracings of the positions of splenic neurons. I: 5 sections; II: 6 sections; III: 6 sections; IV: 8 sections.

than 7 labelled left renal neurons per ganglion were located in the thoracic chain from the stellate ganglion through T10. The thoracic sympathetic chain (T11-T13) contained 335 labelled renal nerve cell bodies. Most renal neurons were located in ganglia of lumbar segments (1135 cells) and in the solar plexus (1459 cells). Left renal nerve cells within the solar plexus were grouped together in the border region between superior mesenteric and left celiac ganglia (Figure 4B, Figure 6), usually within a few millimeters of the origin of the renal nerves. In two cats, the left renal neurons were clustered very closely together.

Ganglionic distribution of right renal neurons

Only 118 labelled right renal neurons were detected in the thoracic paravertebral sympathetic chain from stellate through T13. Most labelled right renal nerve somata were located in the right celiac pole of the solar plexus (569 cells) or within lumbar segments of the right sympathetic chain (491 cells), as illustrated in Table 1 and Figure 3.

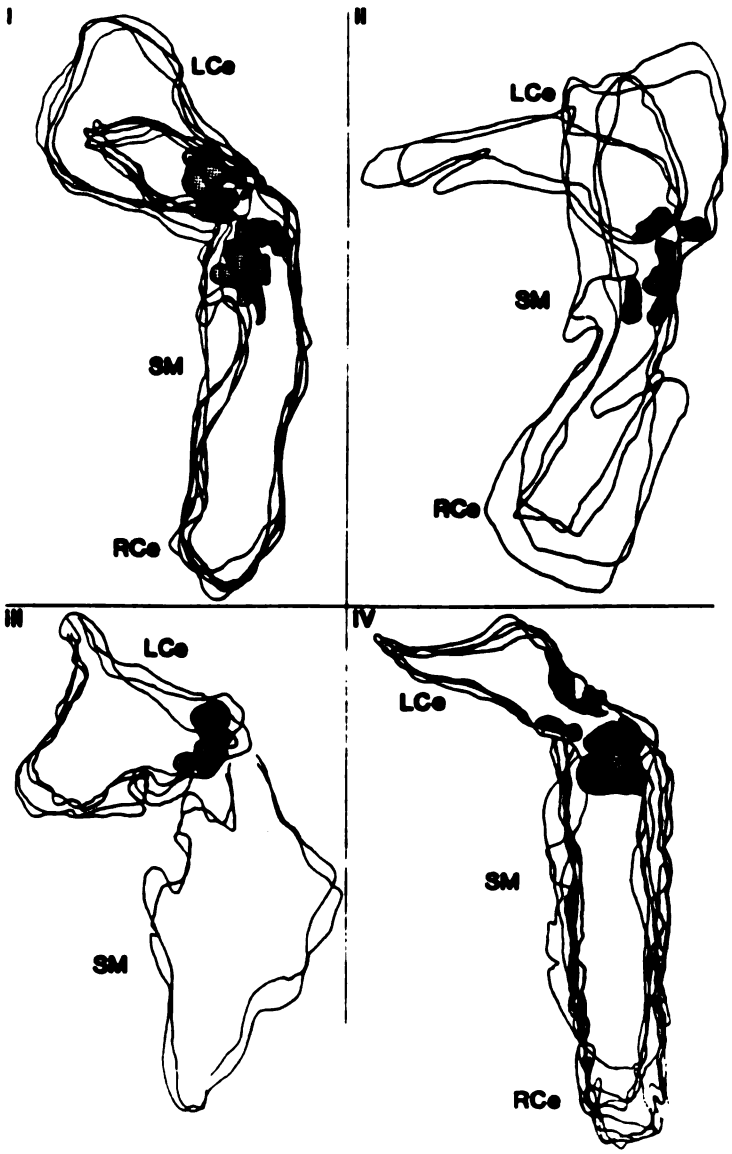

Figure 6. Camera lucida drawings showing the arrangement of labeled left renal neurons in the solar plexus ganglia of four cats. Shaded areas are tracings of the positions of left renal neurons. I: 9 sections; II: 4 sections; III: 4 sections; IV: 9 sections.

DISCUSSION

These data demonstrate different distributions of renal and splenic postganglionic neurons both within the solar plexus and among different ganglia of the sympathetic chain. Left and right renal nerve cell bodies are located primarily in lumbar paravertebral ganglia and are situated in clusters within the solar plexus. In contrast, splenic nerve perikarya are scattered homogeneously throughout the celiac poles of the solar plexus and are relatively scarce in the thoracolumbar paravertebral chain ganglia.

Labelling of ganglion cells did not result from nonspecific accumulation of HRP (Broadwell and Brightman, 1976) nor of endogenous peroxidase activity because no labelled cells were observed if the nerves treated with HRP were severed central to the site of HRP application. The number of labelled splenic neurons was greater than the number of renal neurons probably because splenic nerves are grouped into large bundles close to the bifurcation of the splenic artery where the peroxidase was applied. In contrast, the left renal nerve plexus is diffuse and nerve bundles had to be gathered together to apply the HRP. Right renal nerves were even more difficult to expose than the other nerves, because the position of right kidneys typically limited access to the right renal nerves. Consequently, more left than right renal nerve cells were labelled. Although an attempt was made to label all renal or splenic nerves in each cat, it cannot be assumed that

peroxidase was applied to all nerves in each animal, and the numbers of labelled neurons cannot be interpreted as the total number of cells innervating these viscera. However, the distributions of labelled cells were consistent within each group (i.e., renal or splenic). The conclusions drawn from observations in all ganglia of all cats are likely to describe accurately a representative distribution of the whole population.

Clustering of labelled splenic neurons within the solar plexus as reported by Kuo and Krauthamer (1981), was not observed in these experiments. Nerves emerging from the ganglia of the solar plexus diverge to intermingle in a diffuse web of fibers before fasciculating into large bundles near each target organ. In this study HRP was applied to well-defined bundles of splenic nerves a few millimeters from the spleen, peripheral to the plexus of nerve fibers. In contrast, Kuo and Krauthamer applied peroxidase to the nerves very close to the celiac ganglion and therefore possibly labelled gastric or hepatic as well as splenic nerves. Reports of the organization of the superior cervical ganglion (Lichtman, Purves, and Yip, 1980; Nja and Purves, 1977; Purves and Wigston, 1983) are consistent with this explanation. Clusters of labelled superior cervical ganglion cells were observed in those investigations only if HRP was applied to a nerve very close to its point of exit from the ganglion. However, if individual target areas (e.g., the ear) were injected with HRP, labelled cells were observed randomly distributed throughout the ganglion. These findings illustrated

that cells which had axons contained in a specific nerve were located near the point of exit of that nerve. In contrast, ganglion cells which innervated a particular target tissue were distributed randomly throughout the rostral portion of the superior cervical ganglion. Since HRP was applied to all nerves very close to the respective organs in our study, the differential distributions of renal and splenic nerves which we observed did not result from differences in experimental protocol.

The data concerning renal nerves are qualitatively in agreement with those of Kuo, et al. (Kuo, deGroat, and Nadelhaft, 1982), who described a topographic arrangement of renal postganglionic neurons in the solar plexus. However, since the left and right celiac and superior mesenteric ganglia usually are fused, the location of renal neurons could not be ascribed to any specific ganglion. Likewise, these findings are similar to those of Kuo regarding the segmental distribution of renal efferent neurons within the paravertebral chain (Kuo, deGroat, and Nadelhaft, 1982; Kuo, Nadelhaft, Hisamitsu, and deGroat, 1983). The numbers of labelled cells reported by Kuo were greater than those reported in this investigation. However, Kuo, et al., did not state the thickness of their tissue sections, and since their numbers were not corrected for double counting of labelled cells, their counts would be much greater than those of this study if the thickness of their tissue sections were less than 50 microns. In addition, if Kuo, et al., used larger cats than those used in this investigation, they may have labelled more neurons due to the larger size of the ganglia (Jerison, 1963).

Because most renal neurons are located in lumbar ganglia or are situated in clusters within the solar plexus, and the majority of splenic nerve cells are scattered throughout the celiac poles of the solar plexus, it is likely that renal and splenic neurons are innervated by different sets of preganglionic axons. Selective innervation of a ganglion cell from particular segments of the spinal cord can be highly correlated with the rostrocaudal location of the ganglion (Lichtman, Purves, and Yip, 1980; Nja and Purves, 1977) without regard to particular position of that cell within the ganglion (Lichtman, Purves, and Yip, 1979). Accordingly, differences in the rostrocaudal position of the ganglia in which splenic and renal neurons are located may provide one basis for selective innervation of these cells.

The topographical arrangement of renal neurons within the solar plexus demonstrated by electrophysiological (Decktor and Weems, 1981) and anatomical (Kuo, deGroat, and Nadelhaft, 1982) techniques and by the data in this report may also contribute to selective synaptic inputs to these neurons. Histological studies of the solar plexus indicate that a variety of dendrodendritic synapses occur among postganglionic neurons which lie in close proximity to each other (Archakova, Bulygin, and Netukova, 1982; Kelts, Whitlock, Ledbury, and Reese, 1979; Kuntz, 1938). Decktor and Weems (1981) were able to elicit postsynaptic responses in renal neurons by electrically stimulating renal nerves. They attributed these responses either to reno-renal ganglionic reflexes or to dendro-dendritic coupling of renal postganglionic neurons. Ganglionic

dendro-dendritic synapses are more likely to occur among neurons situated closely together than among distant cells. Clustering of renal neurons could facilitate such localized synaptic events which would enhance responses of renal nerves specifically. In addition, the dendrites of groups of cells may be incorporated into bundles of fibers (Archakova, et al., 1982; Kelts, et al., 1979; Kuntz, 1938) which could facilitate innervation of these cells by a common population of preganglionic axons (Kuntz, 1938). Finally, because Purves has demonstrated that postganglionic neurons which are randomly distributed within a ganglion can receive selective preganglionic innervation (Purves and Lichtman, 1978), the random distribution of splenic neurons does not preclude the possibility that they, too, receive selective synaptic inputs.

In conclusion, the distributions of renal and splenic neurons in the sympathetic chains and solar plexus are different. This organization is consistent with current ideas of selective connections between pre- and postganglionic sympathetic neurons and may provide an anatomical substrate for selective reflex responses of renal and splenic nerves.

SPLENIC, RENAL, AND CARDIAC NERVES HAVE UNEQUAL DEPENDENCE UPON TONIC SUPRASPINAL INPUTS

Brain Research 338:123-135

INTRODUCTION

Stimulation of visceral receptors often produces unequal reflex responses in splenic, renal, and cardiac sympathetic nerves (Calaresu, et al., 1984; Weaver, et al., 1984; Weaver, et al., 1983). Activity of splenic nerves can be more excited or less inhibited than that of cardiac or renal nerves (Calaresu, et al., 1984; Weaver, et al., 1983, 1984). Such nonuniformity among these three populations of sympathetic nerves can exist before and after high cervical spinal cord transection, suggesting that a portion of this specificity is generated in the spinal cord or peripheral nervous system (Weaver, et al., 1983). The cause of these differences, particularly between splenic and renal nerves, is not known. For example, spinal reflexes initiated at the heart are not confined to the approximate spinal segments of their origin, but instead can involve more distant lower thoracic and lumbar spinal cord segments (Weaver et al., 1983). Therefore segmental organization of spinal reflexes does not provide the basis for the inequality of cardiac, splenic, and renal excitatory responses to stimulation of cardiac receptors in spinal cats. Splenic sympathetic responses to this stimulus are as great or greater than cardiac nerve responses and are much greater than those of renal nerves. Greater splenic nerve

excitation may be due to stronger afferent inputs to pathways affecting splenic nerves, in contrast to pathways affecting cardiac or renal nerves. Alternatively, some components of the neural pathways leading to the spleen may be more excitable than those innervating the kidney. For example, tonic excitatory afferent inputs, active excitatory supraspinal, spinal, or ganglionic neural circuits, or inherent characteristics of splenic neurons may maintain these neurons closer to firing threshold.

The inequality of reflex responses among cardiac, splenic, and renal nerves leads to questions concerning differences in the spontaneous rates of discharge of these nerves. Do they differ only in their receptivity to reflex inputs, or do their sources of ongoing drive differ, as well? Do they depend equally upon tonic supraspinal sources of excitation or inhibition? A comparison of resting discharge rates of these three nerves after interruption of supraspinal inputs would reveal possible differences in their dependence upon descending excitation or inhibition. Such information would provide another step toward understanding the complex organization of splenic and renal visceral sympathetic outflow.

This study was undertaken to make such a comparison in chloralose-anesthetized cats. The decrease in splenic nerve activity after high cervical spinal cord transection was consistently less than the decreases in activity of cardiac and renal nerves. To determine if the greater splenic sympathetic activity in the spinal state resulted from

greater responsiveness to potential external sources of excitation, neural responses to factors known to affect sympathetic discharge in spinal animals were compared. Factors tested were 1) changes in arterial pressure (Alexander, 1945; Beacham and Perl, 1964; Brooks, 1933, 1935; Fernandez de Molina and Perl, 1965; Malliani, Pagani, Recordati, and Schwartz, 1971; Pagani, Schwartz, Banks, Lombardi, and Malliain, 1974), 2) direct effects of high arterial carbon dioxide pressure and low pH on preganglionic or postganglionic neurons (Alexander, 1945; Franz, Evans, and Perl, 1966; Johnson, Smith, and Walker, 1969; Preiss and Polosa, 1977; Szulczyk and Trzebski, 1976; Zhang, Rohlicek, and Polosa, 1982), and 3) excitation by tonically active spinal afferent inputs (Dostrovsky, Millar, and Wall, 1976; Mannard and Polosa, 1973; Polosa, 1968).

METHODS

General procedures. Cats (n = 30; 3.0 ± 0.1 kg) used in this study were anesthetized with 60 to 80 mg/kg alpha-chloralose (Sigma Chemical Company, St. Louis, MO) administered intravenously. A tracheostomy tube was inserted, a cannula was passed into the inferior vena cava via a femoral vein for delivery of drugs and solutions, and cannulae were passed into the thoracic aorta from the femoral arteries for monitoring systemic arterial pressure and for withdrawal of blood for analysis of arterial pH, pO_2 and pCO_2 (165 Blood Gas Analyzer, Corning Medical, Medfield, MA). To eliminate influences of the vagi and carotid sinus and aortic arch baroreceptors and chemoreceptors, ninth and tenth cranial nerves were severed bilaterally as they passed into the jugular foramen. After assessment of the animal's plane of anesthesia, gallamine triethiodide (5 mg/kg, Flaxedil, Davis Geck, New York, NY) was administered intravenously to ensure adequate muscle relaxation during surgical and experimental procedures, and the lungs were ventilated with a mixture of room air and 100% O_2 by a Harvard respirator. Esophageal temperature was monitored and maintained at approximately 37° C. In some experiments, end-tidal CO_2 was monitored (Infra-red CO_2 analyzer, type 901-MK2, P.K. Morgan, Ltd., Chatham, England). Heart rate was monitored using a Grass EKG Tachograph (7P4FG) which was triggered by the arterial pressure pulse.

A laminectomy was performed to expose the first cervical segment of

the spinal cord for later transection in all 30 cats. Additionally, in 6 cats, a dorsal laminectomy was performed from the second thoracic to the fifth or sixth lumbar vertebra to expose the spinal cord from T4 to L5. Sutures were threaded beneath the dorsal rootlets on the dorsal surface of the spinal cord from T4 to L5 on both the right and left sides for later transection of the dorsal rootlets. In these 6 animals the thoracic paravertebral sympathetic chains were severed bilaterally between ganglia corresponding to spinal segments T4 and T5, to ensure complete isolation of splenic and renal nerves from outflow of preganglionic neurons located in the rostral thoracic spinal cord. In all cats the solar plexus was exposed by a left retroperitoneal approach, and bundles of splenic and/or left renal nerves were traced to the respective organs, severed, and the central ends were placed on bipolar platinum-iridium electrodes for recording electrical activity. In 18 cats the left scapula was reflected, the first and second ribs were removed, and the stellate ganglion was exposed retropleurally. The left inferior cardiac nerve or ansa subclavia was cut, and activity was recorded from the central end. Exposed nerves were immersed in warm mineral oil, and surrounding tissue was coated with petroleum jelly and moistened gauze to prevent tissue dehydration. A pneumothorax was made to eliminate artifacts in the neural recordings caused by movement associated with ventilation. Nerve activity was amplified within a bandwidth of 30 Hz to 3 kHz, monitored on an oscilloscope, and recorded on magnetic tape. Systemic arterial pressure was recorded (Statham P23

pressure transducers, Oxnard, CA) and stored on magnetic tape, as well.

Experimental protocol. Multifiber activity was recorded simultaneously from two of the three nerves (splenic, renal, and cardiac) in each cat. After the recordings were stable for at least 15 min, the following procedures were performed (not necessarily in the order given): 1) The animal was ventilated for 5 to 10 min with a mixture of room air and 95% O_2 and 5% CO_2 to assess the contributions of $PaCO_2$ to ongoing sympathetic activity. Arterial blood samples were taken for analysis of pH, PaO_2, $PaCO_2$ before and during each period of CO_2 inspiration. 2) Approximately 5 to 10 ml blood was withdrawn from an arterial cannula to decrease blood pressure (by 30 $\pm$ 8 mmHg) for 1 to 2 min and then reinfused. 3) Ten to 15 micrograms/kg phenylephrine (NeoSynephrine, Winthrop Laboratories, New York, NY) was injected intravenously to increase blood pressure (by 50 $\pm$ 4 mmHg) for 15 to 20 s. Animals were allowed to recover for 5 to 30 min between experimental procedures.

Next, cats were readministered 5 mg/kg Flaxedil. When recordings were stable, the dura mater was cut and reflected to expose the first segment of the spinal cord and the spinal cord was severed with fine micro-dissecting scissors. Completeness of the transection was verified by lifting and examining the oral end of the severed spinal cord. A small pleget of oxidized cellulose was placed in the incision to minimize loss of blood from the spinal cord transection. Mean arterial pressure (MAP = [1/3 (systolic - diastolic)] + diastolic) was maintained

above 70 mmHg by intravenous infusion of phenylephrine at a rate of 5 to 10 micrograms/min (0.05-0.10 ml/min of 100 micrograms/ml phenylephrine in saline). Nerve activity was tape-recorded throughout the periods of spinal cord transection and for 1 to 2 min at 15, 30, 60, and 120 min following this procedure in 24 cats. As the purpose of these experiments was to ascertain contributions of supraspinal neural circuits to the generation of spontaneous sympathetic nerve discharge, it was considered necessary to monitor sympathetic discharge soon after the spinal cord was severed, but before compensatory mechanisms of generation of sympathetic discharge would be likely to occur. Four time periods were chosen so that stability of the discharge rates could be examined over the 2 h period following spinal cord transection. In addition, the animals were subjected to 1) ventilation with the CO_2, O_2, and air mixture, 2) hemorrhage of small amounts of blood, and 3) transient increases in the rate of phenylephrine infusion (to deliver a bolus of 47 $\pm$ 7 micrograms) to produce increases in arterial pressure equal to those tested before spinal cord transection.

In 6 additional animals, at 30 min after C1 spinal transection, an isolated segment of spinal cord was prepared by transecting the cord at T4 and at L5. Nerve activity was allowed to stabilize for 15 to 60 min, and then the dorsal roots were severed by applying traction to the sutures placed loosely beneath the rootlets. Nerve activity again was allowed to stabilize for 10 to 60 min.

All tape-recorded nerve activity was quantified in counts of spikes

per second after window discrimination (Frederick Haer, New Brunswick, ME). The thresholds for window discrimination were set very close to the maximum amplitude of background electrical noise during meticulous inspection of nerve activity with an oscilloscope and and audiomonitor to exclude background noise and to include as much nerve activity as possible in the quantification procedures. The short-acting ganglionic blocking agent, trimethaphan camsylate (0.2 mg/kg Arfonad, Hoffman-La Roche, Nutley, NJ), was administered intravenously prior to spinal cord transection in some cats to ascertain the amplitude of background noise in the recordings of nerve activity. Since the amplitude of background noise obtained with Arfonad did not differ appreciably from the noise levels determined at the end of the experiments by intravenous administration of 1 to 5 mg/kg hexamethonium (Mann Research Laboratories, Inc., New York, NY) or after carefully crushing the nerves central to the recording electrodes, it was concluded that noise levels in recordings of any given nerve did not change during the course of the experiments. The threshold of the amplitude analyzer was monitored carefully throughout each analysis to ensure that background electrical noise was never included in the quantitation of nerve activity. Activity recorded in 9 cats also was analyzed by voltage integration, in addition to the frequency analysis, to provide a qualitative comparison of the results obtained with each technique. Neural activity was digitized and then rectified and integrated during 10-s periods by a LSI 11/23 microprocessor (Digital Equipment Corporation, Maynard, MA).

Integrated background electrical noise, determined after ganglionic blockade or after crushing the nerves, was subtracted from the integrated nerve activity; and, therefore, the reported values of integrated nerve activity do not include electrical noise.

Data analysis. Group comparisons. A factorial analysis of variance (Sokal and Rohlf, 1969) was used to compare ongoing rates of spike counted discharge of the three nerves of all cats during the four time periods: intact neuraxis, 30, 60, and 120 min following spinal cord transection. The pooled standard deviation based on the error mean square from this analysis was used to calculate values of standard error of the mean used to illustrate the variability in the figures. Therefore, one standard error bar is used to represent the standard error for the whole group in the figures. A Student-Newman-Keuls test of least significant ranges (SNK; Sokal and Rohlf, 1969) was used to compare the mean rates of discharge among the three nerves at each time period as well as to compare discharge rates of each individual nerve among the four time periods. Differences were considered significant when $P \leq 0.05$. Values of mean arterial pressure during these same time periods were analyzed similarly. Voltage-integrated nerve activity in the intact state and at 1 hr after cord transection was compared by an analysis of variance using a completely random design. The standard error calculated for this analysis also was obtained from the error mean square.

Changes in spike counted nerve activity and mean arterial pressure during manipulations of blood pressure or respiratory gas composition were evaluated with an analysis of variance using complete block design, and mean values during treatment were compared to mean control values with a test of least significant differences ($P \leq 0.05$). The percentage changes in splenic and cardiac nerve discharge caused by hemorrhage in spinal animals were compared using a completely random analysis of variance after logarithmic transformation (Sokal and Rohlf, 1969).

Paired comparisons. To provide a more rigorous analysis of the effects of spinal cord transection on the ongoing discharge of the sympathetic nerves under study, comparisons were made between nerves which were recorded simultaneously (i.e., splenic and renal; splenic and cardiac; renal and cardiac). Discharge rates of nerves recorded simultaneously were analyzed with a factorial analysis of variance during the two time periods: 1) when the neuraxis was intact, and 2) 60 min following spinal cord transection. Mean values of discharge rates among the three nerves (cts/sec) were compared with a SNK test when the neuraxis was intact to evaluate potential differences in the baseline activity among the nerves. In addition, the effects of spinal cord transection on activity of individual nerves were examined with a SNK test. The relative effect of spinal transection on each nerve within these pairs also was evaluated by comparison of the percent changes in activity of simultaneously recorded nerves. Percentages were logarithmically transformed (Sokal and Rohlf, 1969), a completely

randomized analysis of variance was done, and a SNK test was used to compare the average changes in activity of each nerve of the simultaneously recorded pairs.

RESULTS

Ongoing sympathetic discharge. Group comparisons. Multifiber discharge of splenic, renal, and cardiac nerves was monitored before and for 2 hr after transection of the spinal cord at the first cervical segment. As illustrated in Figure 7 the discharge of these nerves before cord transection was characterized by synchronized bursts of activity. As the spinal cord was severed, sympathetic discharge and mean arterial pressure increased transiently. Following transection of the spinal cord, the discharge rates of renal and cardiac nerves were significantly depressed by more than 50%, whereas splenic nerve activity was not decreased significantly (Figures 7 and 8). Activity of splenic, renal, and cardiac nerves was less synchronized, and signal-to-noise ratios were smaller in the spinal than in the intact state. Splenic nerve activity was unchanged or actually increased after cord transection in 4 of 16 cats; an example from one of these cats is shown in Figure 7. Discharge rates within each group (splenic, renal, or cardiac) did not change significantly during the 2 hrs monitored after spinal cord transection (Figure 8). Similar responses to cord transection were obtained when nerve activity of 9 cats was quantified by voltage integration (Table 2). Average mean arterial pressure did not differ statistically among the three groups of cats (splenic, renal, cardiac) in the intact or spinal state, although pressure in the spinal state sometimes was significantly different from that in the intact

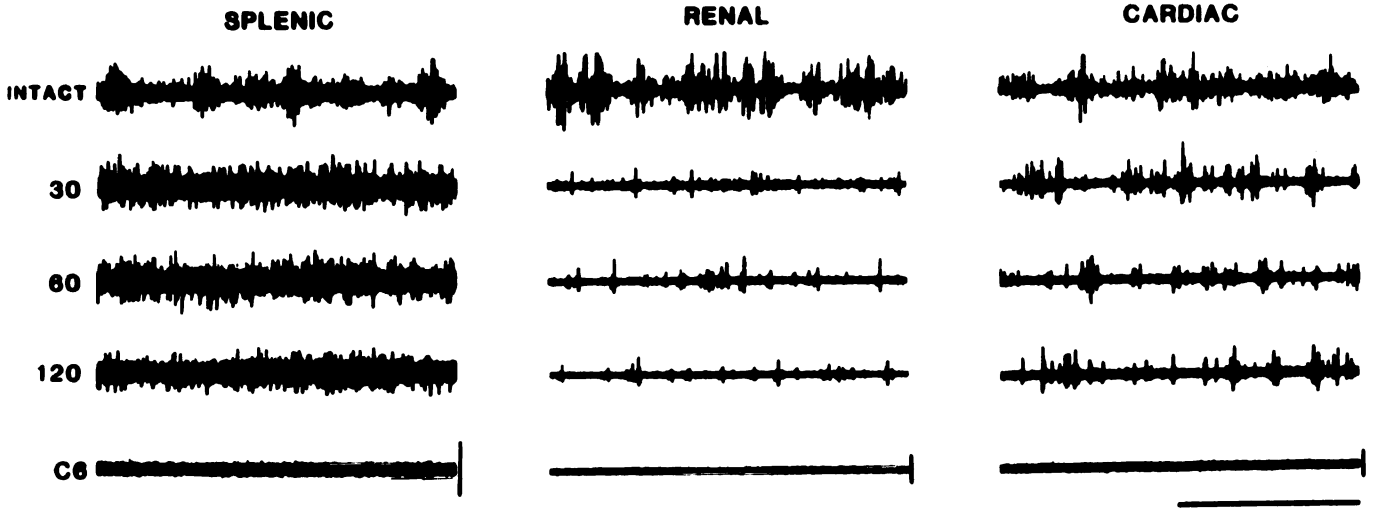

Figure 7. Photographs of oscillograph tracings of sympathetic nerve activity before and after spinal cord transection. Activity of splenic, renal, and cardiac nerves was sampled when the neuraxis was intact (Intact), at 30, 60, and 120 min after cord transection, and following administration of hexamethonium (C6). Vertical calibrations are 30 microvolts, horizontal calibration is 1 s.

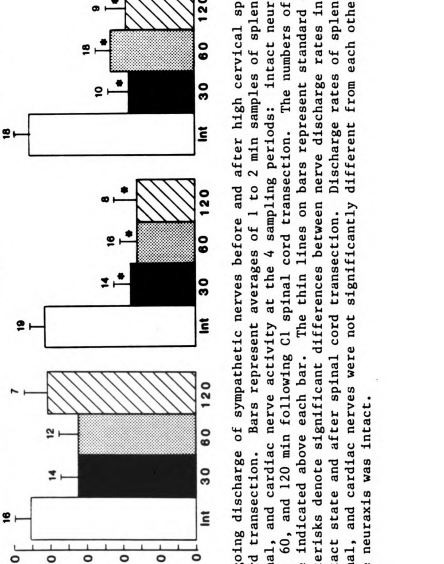

Figure 8. Ongoing discharge of sympathetic nerves before and after high cervical spinal cord transection. Bars represent averages of 1 to 2 min samples of splenic, renal, and cardiac nerve activity at the 4 sampling periods: intact neuraxis (Int), 30, 60, and 120 min following C1 spinal cord transection. The numbers of animals are indicated above each bar. The thin lines on bars represent standard error. Asterisks denote significant differences between nerve discharge rates in the intact state and after spinal cord transection. Discharge rates of splenic, renal, and cardiac nerves were not significantly different from each other when the neuraxis was intact.

TABLE 2. Effect of spinal cord transection on integrated sympathetic discharge.

Experimental group	INT (V s/10s)	C1X (V s/10s)	S.E.
Splenic (n = 4)	40	26	11
Renal (n = 4)	42	8*	10
Cardiac (n = 4)	56	11*	16

Values of integrated splenic, renal, and cardiac nerve activity during the intact state (INT) and at 1 hr after spinal cord transection (C1X). Asterisks denote significant differences between INT and C1X. Values of splenic, renal, and cardiac experimental groups were not significantly different from each other in the intact state.

state (Table 3, Table 4). After spinal transection, heart rate decreased by approximately 39 beats/min and did not change for the duration of the experiment (Table 4).

Paired comparisons. Paired analyses were done to allow comparisons of simultaneously recorded responses of pairs of nerves (i.e., splenic and renal; splenic and cardiac; renal and cardiac) to spinal cord transection. The results of these paired comparisons were considered to be most meaningful if discharge rates of both nerves of each pair were similar in the intact state. Rates of discharge of simultaneously recorded splenic and renal nerves were similar when the neuraxis was intact (Table 3). However, splenic nerve activity was unaffected statistically by spinal transection, whereas renal nerve activity was significantly depressed. Likewise, simultaneously recorded splenic and cardiac nerves had similar rates of activity before spinal transection. Again, splenic nerve activity was not affected, while cardiac nerve activity decreased significantly after severing the cord.
Simultaneously recorded discharge rates of renal and cardiac nerves both were decreased significantly following spinal transection. However, activity of renal and cardiac nerves differed from each other when the neuraxis was intact. Therefore, percent changes in the activity of simultaneously recorded nerves were recorded, as well.
As illustrated in Figure 9, spinal cord transection caused a greater percent decrease in renal and cardiac nerve activity than in splenic nerve activity. Furthermore, spinal transection produced similar

TABLE 3. Effect of spinal cord transection on discharge rates of sympathetic nerves recorded simultaneously.

	INTACT NEURAXIS	SPINAL CORD TRANSECTED	S.E.
SPLENIC/RENAL PAIRS (n = 7)			
Splenic nerve activity	77 (N.S.)	64 (N.S.)	9
Renal nerve activity	77 (*)	23 (*)	9
Mean arterial pressure	110 ± 10	86 ± 7	—
SPLENIC/CARDIAC PAIRS (n = 6)			
Splenic nerve activity	97 (N.S.)	57 (N.S.)	13
Cardiac nerve activity	75 (*)	25 (*)	13
Mean arterial pressure	102 ± 7	87 ± 6	—
RENAL/CARDIAC PAIRS (n = 9)			
Renal nerve activity	75 (*)	27 (*)	6
Cardiac nerve activity	100 (*)	54 (*)	6
Mean arterial pressure	121 ± 14	94 ± 7	—

Sympathetic nerve activity is expressed in cts/sec. Mean arterial pressure is expressed in mmHg. N.S.: not significantly different; *: significant difference; S.E.: standard error of the mean. See further description in text.

TABLE 4. Average mean arterial pressure and heart rate before and after spinal cord transection.

Experimental groups	Intact neuraxis		Minutes after spinal cord transection					
			30		60		120	
	MAP	HR	MAP	HR	MAP	HR	MAP	HR
Splenic	108 ± 6	227 ± 6	93 ± 6	189 ± 7*	87 ± 7	195 ± 7*	105 ± 9	182 ± 11*
Renal	117 ± 6	229 ± 7	97 ± 6*	186 ± 7*	91 ± 6*	189 ± 7*	111 ± 9	187 ± 11*
Cardiac	115 ± 6	220 ± 6	97 ± 8	183 ± 9*	88 ± 6*	187 ± 6*	93 ± 8*	184 ± 9*

Values are means ± S.E. All values for mean arterial pressure (MAP) and heart rate (HR) are expressed in mmHg and beats/min, respectively. Asterisks denote significant differences between intact state and after spinal cord transection. "Experimental groups" refers to animals in which activity of splenic, renal, or cardiac nerves was recorded. Neural responses of these groups are illustrated in Figure 2. Number of animals per group is indicated on Figure 2.

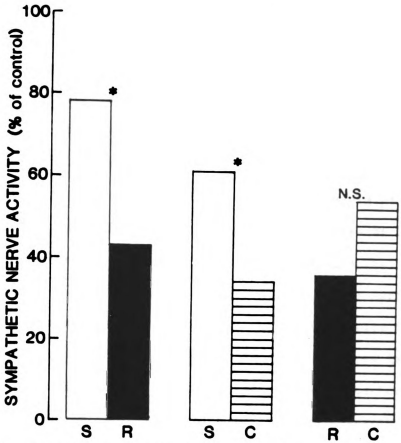

Figure 9. Effects of spinal cord transection on simultaneously recorded discharge rates of pairs of sympathetic nerves. Bars represent rates of discharge of nerves expressed as a percent of control (intact neuraxis) activity. Percentages were logarithmically transformed prior to analysis of variance, because the percentages were not normally distributed. Therefore coefficients of variation (C.V.) from the analyses of variance are given (below) rather than the standard errors. S: splenic; R: renal; C: cardiac; *: significant difference between the two nerves; N.S.: not significant; S/R C.V.: 74%; S/C C.V.: 28%; R/C C.V.: 15%.

percent changes in rates of activity of renal and cardiac nerves (Figure 9).

Sympathetic responses to hypercapnia. To assess the contribution of $PaCO_2$ to differential generation of ongoing activity of the three nerves under study, the lungs were ventilated with a mixture of room air, 95% O_2, and 5% CO_2 for 5 to 10 min, making the animals (n = 11) hypercapnic and acidotic (Table 5). The levels of hypercapnia achieved did not cause significant changes in the rates of discharge of renal or cardiac nerves either prior to or following spinal cord transection (Figure 10). Splenic nerve activity increased during hypercapnia while the neuraxis was intact but did not change during this procedure after the spinal cord had been severed. The decreases in mean arterial pressure induced by the hypercapnia were statistically significant only in the group of cats in which renal nerve activity was recorded (Table 5). Heart rate was unaffected by inspiration of CO_2, both prior to and following transection of the spinal cord. Hyperventilation of the lungs with a mixture of room air and 100% O_2 did not cause changes in the discharge rates of splenic, renal, or cardiac nerves.

Sympathetic responses to increases or decreases in arterial pressure. Sensitivity of sympathetic activity to changes in blood pressure was evaluated in 9 cats to appraise the contribution of systemic arterial pressure to differing levels of ongoing activity of the three nerves. A transient 38 to 65 mmHg increase in arterial pressure was produced before spinal transection by intravenous

TABLE 5. Values of pH, $PaCO_2$ and mean arterial pressure during normocapnia and hypercapnia before and after spinal cord transection.

INTACT NEURAXIS

Experimental groups	Normocapnia			Hypercapnia		
	pH	$PaCO_2$ (mmHg)	MAP (mmHg)	pH	$PaCO_2$ (mmHg)	MAP (mmHg)
Splenic (n = 7)	7.39 ± 0.01	27.9 ± 1.9	108 ± 5	7.15 ± 0.02	56.3 ± 3.5	105 ± 5
Renal (n = 6)	7.39 ± 0.02	30.0 ± 2.4	134 ± 4	7.15 ± 0.01	55.0 ± 2.8	127 ± 4
Cardiac (n = 8)	7.36 ± 0.01	32.3 ± 1.6	132 ± 7	7.15 ± 0.03	60.9 ± 2.1	128 ± 7

SPINAL CORD TRANSECTED

Experimental groups	Normocapnia			Hypercapnia		
	pH	$PaCO_2$ (mmHg)	MAP (mmHg)	pH	$PaCO_2$ (mmHg)	MAP (mmHg)
Splenic (n = 7)	7.36 ± 0.01	24.5 ± 2.0	87 ± 5	7.10 ± 0.01	52.3 ± 1.9	78 ± 5
Renal (n = 6)	7.34 ± 0.02	29.5 ± 2.4	98 ± 3	7.10 ± 0.02	54.0 ± 2.7	86 ± 3*
Cardiac (n = 8)	7.32 ± 0.01	28.4 ± 2.5	96 ± 4	7.10 ± 0.04	57.3 ± 1.2	88 ± 4

Values are means ± S.E. Asterisk denotes significant difference in mean arterial pressure (MAP) between normocapnia and hypercapnia. "Experimental groups" refers to animals in which activity of splenic, renal, or cardiac nerves was recorded. Neural responses of these groups are illustrated in Figure 4.

Figure 10. Effect of hypercapnia on sympathethic discharge before and after spinal cord transection. Bars represent averages of 1 to 2 min samples of activity of splenic, renal, and cardiac nerves during normocapnia and hypercapnia before (INTACT) and after (SPINAL) spinal cord transection. Numbers of animals are given in parentheses. The thin lines on bars represent standard error. Asterisk indicates significant difference between discharge rates during normocapnia and those during hypercapnia.

Figure 11. Effect of increased arterial blood pressure on sympathetic discharge before and after spinal cord transection. Upper bars indicate rates of discharge of splenic, renal, and cardiac nerves, and lower bars illustrate mean arterial pressure (MAP) during periods of control (C), phenylephrine injection (PE), and recovery (R). The upper panels illustrate responses in the presence of an intact neuraxis (INTACT); the lower panels responses after spinal cord transection (SPINAL). The numbers of animals are given in parentheses. The thin lines on bars represent standard error. Asterisks denote significant differences between the control values and those during the maximum response to phenylephrine.

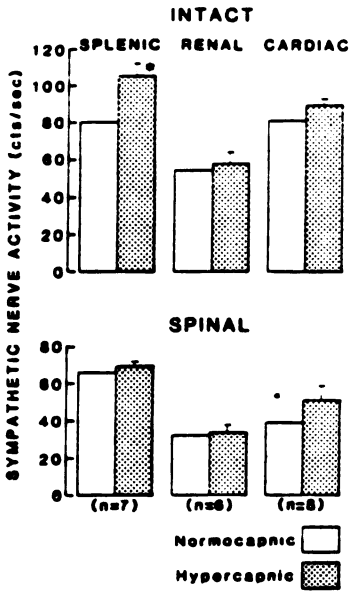

Figure 10.

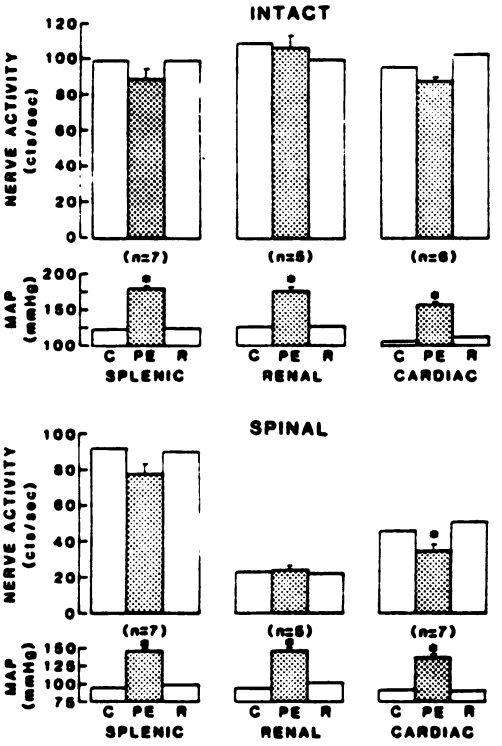

Figure 11.

injections of 10 to 15 micrograms/kg phenylephrine. Activity of splenic, renal, and cardiac nerves remained relatively constant in the presence of this increase in blood pressure (Figure 11). Following spinal cord transection, increases in blood pressure were achieved by increasing the rate of intravenous phenylephrine infusion momentarily to deliver a bolus of 47 $\pm$ 7 micrograms. Cardiac nerve activity was decreased during the maximum increase in blood pressure in each cat following transection of the spinal cord. In contrast, activity of renal and splenic nerves was not significantly changed by increases in arterial pressure.

Prior to spinal cord transection, 11 to 40 mmHg decreases in blood pressure produced by hemorrhage had no effect on the discharge rates of splenic, renal, or cardiac nerves (Figure 12). However, similar decreases in blood pressure after cord transection caused significant excitation of splenic and cardiac nerves. The excitatory responses of cardiac nerves were significantly greater than those of splenic nerves. Renal nerve activity also tended to be increased during this hypotension.

Sympathetic responses to dorsal rhizotomy. To evaluate the possibility that activity of spinal afferent nerves tonically or preferentially contributed to the ongoing discharge of splenic or renal nerves in the spinal cat, the following procedures were performed in 6 cats. The spinal cord was severed at the first cervical segment as usual. Thirty minutes later, the spinal cord was transected at L5 and

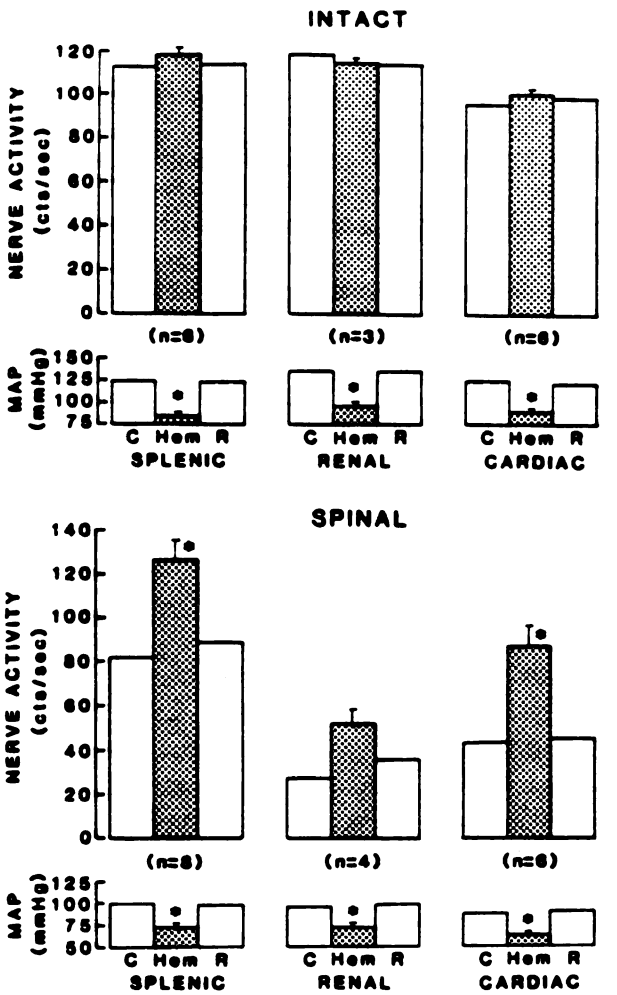

Figure 12. Effect of hemorrhage on sympathetic discharge before and after spinal cord transection. Format is the same as that of Figure 4. HEM: hemorrhage. The excitation of cardiac nerve activity was significantly greater than that of splenic nerve activity.

T4. The paravertebral sympathetic chains had been transected previously between T4 and L5 in these animals to isolate the renal and splenic nerves from influences of upper thoracic (T1 to T4) sympathetic preganglionic outflow. Isolation of this T4-L5 segment from the rest of the spinal cord did not cause changes in splenic or renal nerve activity (Figure 13). Fifteen to 60 min after isolation of the spinal cord segment, the dorsal roots from T4 to L5 were severed, thus depriving this isolated section of spinal cord of spinal afferent input. No changes were observed in the rate of splenic nerve discharge. Renal nerve activity tended to be slightly increased, but this change was not statistically significant. Blood pressure was not affected by any of these procedures.

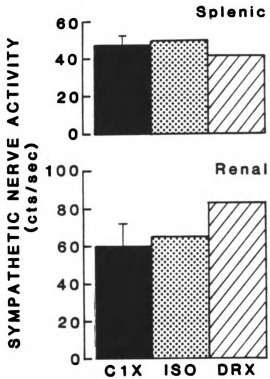

Figure 13. Ongoing discharge of sympathetic nerves following dorsal rhizotomy in 6 cats. Bars represent averages of 1 to 2 min samples of activity of splenic and renal nerves following C1 spinal cord transection (C1X), after transection of the spinal cord at T4 and L5 (ISO), and following dorsal rhizotomy from T4 to L5 (DRX). The thin lines on bars represent standard error. Activity of both nerves was unaffected by dorsal rhizotomy. Mean arterial pressure was 92 ± 4, 91 ± 4, and 90 ± 4 mmHg following C1X, ISO, and DRX, respectively.

DISCUSSION

These data indicate that the continuous electrical discharge recorded from splenic nerves is less dependent upon supraspinal pathways than is activity of renal or cardiac nerves. Although discharge of all three postganglionic nerves persisted after high cervical spinal cord transection, activity of renal and cardiac nerves decreased by more than fifty percent, whereas splenic nerve activity did not change significantly. The differences in dependence of the three nerves upon supraspinal inputs did not result from hypoxemia, hypercapnia, low levels of arterial pressure, or from tonic activity of afferent nerves entering the spinal cord through dorsal roots.

The most valid comparisons possible in this investigation are the relative degrees of depression of ongoing activity produced in each of the nerves by cord transection. Limitations of multifiber recording techniques complicate comparison of discharge rates or absolute voltages among the nerves. Because spike counting is subject to frequency saturation, the discharge rates of the 3 nerves were compared in the intact state to be certain that one nerve was not more likely to be affected by quantitation errors than another. Despite the limitations of spike counting, this technique was considered preferable to voltage integration because, after spinal cord transection, renal and cardiac nerves often have only a few active fibers firing, and these fibers discharge asynchronously with widely different voltages. The values

obtained by voltage integration are greatly affected by the amplitudes of single spikes in few-fiber preparations and, therefore, are difficult to interpret. However, to provide a comparative analysis which did not include errors inherent to spike counting, results from a few animals were quantified by voltage integration. Even though limitations are inherent in either technique of analysis, both yielded the same relative results and detected a lesser decrease in activity of splenic than cardiac and renal nerves upon spinal cord transection. Paired comparisons of the effects of spinal cord transection on discharge of nerves recorded simultaneously also led to the conclusion that splenic nerve activity is less dependent upon supraspinal sources of excitation than is discharge of renal or cardiac nerves. Since tonically active spinal afferent neurons were not responsible for the differing amounts of depression of nerve activity caused by spinal transection, it is possible that supraspinal neurons directly influence sympathetic outflow differentially. Barman, et al., (1984) have demonstrated nonuniformity of the temporal relationship between the discharge of medullary reticular formation neurons and that of different sympathetic nerves.

The source of the differences among these three nerves could not be determined in this study. Since mean arterial pressure did not differ statistically among the groups of cats during any single time period, small differences in blood pressure were unlikely to have contributed to inequalities in discharge rates among the nerves. Furthermore, results of the paired comparisons yielded the same qualitative conclusions as

did the group comparisons. If the especially well-maintained spontaneous activity in splenic nerves were caused by hypoxia or by compromised perfusion of the spinal cord or sympathetic ganglia after cord transection, then increasing arterial perfusion pressure would result in a decrease of the anoxia-induced nerve activity. However, activity of splenic and renal nerves did not respond to increases in arterial pressure produced by intravenous infusion of phenylephrine. Although cardiac nerve activity was inhibited by increased arterial pressure, this response probably was caused by stimulation of thoracic spinal afferent nerves rather than by increased spinal or ganglionic perfusion. Malliani, et al., (1971) were able to abolish similar responses of upper thoracic preganglionic nerve activity in acutely spinalized cats by removal of the stellate ganglion to eliminate cardiopulmonary afferent pathways.

In our study mean arterial pressure was kept above 70 mmHg, and the cats were ventilated with a mixture of room air and 100% O_2 (PaO_2 = ca. 300 mmHg). Rohlicek and Polosa (1981) demonstrated that discharge of cervical sympathetic preganglionic nerves was not sensitive to arterial pO_2 between values of 40 and 400 mmHg. Although pO_2 of the spinal cord was not measured in our experiments, it is likely that spinal tissue O_2 tension is directly related to arterial oxygen content (Leniger-Follert, Lübbers, and Wrabetz, 1975; Metzger and Heuber, 1977; Nair, Whalen, and Buerk, 1975). Furthermore, blood flow in the transected spinal cord is autoregulated between arterial pressures of 50

to 125 mmHg (Metzger and Heuber, 1977). Therefore, hypotension and hypoxia did not appear to be sources of ongoing sympathetic activity in the spinal cats, although these stimuli were capable of causing responses in these experiments. Because the hypotension produced by hemorrhage approached the lower limits of autoregulation of blood flow in our experiments, the excitatory responses of splenic and cardiac nerves to hemorrhage in spinal cats probably resulted from decreased vascular perfusion of spinal tissue during the hypotension. The greater responses of cardiac nerves may again have been caused by a specific effect of decreased influences from a population of cardiopulmonary pressoreceptors.

Hypercapnia and acidosis are other possible sources of maintained discharge of sympathetic nerves in spinalized animals. Gootman and Cohen (1981) described CO_2-induced increases in splanchnic nerve activity in spinal cats. In addition, Zhang, et al., (1982) reported a direct relationship between $PaCO_2$ and discharge rate of sympathetic preganglionic nerves in acutely spinalized cats. However, since neither splenic, renal, nor cardiac nerve activity responded to increases in $PaCO_2$, it could not have been responsible for the ongoing nerve activity or the differences among nerves observed in our study. The discrepancy between our data and those of Zhang, et al. (1982) may relate to differences in the arterial pressure changes induced by hypercapnia in the two studies. Zhang, et al. (1982) reported hypercapnia-induced decreases in blood pressure, hypotension which could have contributed to

the increased nerve activity in that study. In contrast, hypercapnia usually did not cause significantly lower blood pressure in our study. In addition, Zhang, et al. (1982) reported a sigmoidal relationship between $PaCO_2$ and firing frequency of cervical sympathetic neurons. The values for hypercapnic $PaCO_2$ in our experiments were on the lower portion of the rising phase of this curve. Therefore, the significant increases in $PaCO_2$ produced in our study were not sufficient to cause consistent excitation of the nerves. The values of $PaCO_2$ for normocapnic cats in this investigation were similar to those reported elsewhere (Fink and Schoolman, 1963; Herbert and Mitchell, 1971).

Activity of renal and cardiac nerves did not respond to increased tidal CO_2 when the neuraxis was intact, perhaps because chemoreceptor afferent nerves were severed in our experiments. Excitatory neural responses to increased $PaCO_2$ or asphyxia have been documented in cats with intact neuraxes (Cohen and Gootman, 1972; Preiss and Polosa, 1977), if peripheral chemoreceptor pathways remained intact. However, Priess and Polosa (1977) reported that rates of discharge in 38% of cervical sympathetic preganglionic neurons either decreased or did not change in response to increased end-tidal CO_2, even though carotid body chemoreceptors remained intact.

Since this study was conducted in animals within 2 hr after spinal cord transection, the absence of sensitivity of sympathetic discharge to increased $PaCO_2$ or to increased blood pressure could be attributed to hyporeflexia ("spinal shock"), which has been observed in acutely

spinalized preparations (Mukherjee, 1957; Sherrington, 1906). However, spinal shock probably is not responsible for generating the ongoing sympathetic activity and is likely to be depressing it. If inhibitory influences of afferent or propriospinal neurons were more powerful than excitatory influences, then the result of hyporeflexia could be increased basal nerve activity. If this were the case, one would expect sympathetic activity to decrease with time after spinal cord transection. However, nerve activity did not change significantly during the 2-hr period monitored after spinal cord transection. In addition, the duration of spinal shock was minimized by averting a large fall in blood pressure after spinal cord transection by phenylephrine infusion (Mukherjee, 1957).

Activity of splenic and renal nerves did not change significantly following dorsal rhizotomy, indicating that spinal afferent nerve activity did not tonically excite or inhibit sympathetic discharge. Effects of dorsal rhizotomy on cardiac nerve activity were not assessed. Since cardiac and renal nerve activity reacted similarly throughout other aspects of the study, and a search for the source of splenic nerve hyperactivity was the impetus for this experiment, it was considered adequate to monitor only renal nerve activity as a contrast to splenic nerve activity.

It is possible that the anesthetic used in this study (chloralose) may have affected some of the observed results. For example, chloralose does attenuate the respiratory response to CO_2-induced stimulation of

central chemoreceptors (Baylis and Monroe, 1964). Similarly, chloralose may have diminished the neural responses to CO_2 in animals with intact neuraxes, which could account for the inconsistent effects of increased $PaCO_2$ on sympathetic nerve activity in this study. However, no evidence suggests that chloralose produces unequal effects on the ongoing activity of different sympathetic nerves. Confirmation of the results of this investigation in decerebrate, unanesthetized cats awaits further investigation.

Although nerve activity persisted after spinal cord transection, the functional consequences of the ongoing discharge are not known. In rats, renal nerve activity can increase by two-fold after spinal cord transection (Taylor and Schramm, 1986). Under these conditions, the increased renal nerve activity contributes to decreased excretion of sodium and water (Osborn and Schramm, 1987). However, the increased renal nerve activity appears to have little effect on renal vascular resistance due to the ability of the renal vascular bed to autoregulate renal blood flow (Osborn and Schramm, 1987). Two days after spinal transection at the sixth cervical segment, Ardell, et al. (1982) observed minimal activity of renal and external carotid sympathetic nerves and failed to elicit a fall in blood pressure by intravenous administration of hexamethonium until one week later. This suggests that ongoing sympathetic activity does not support blood pressure in acute spinal animals. In contrast, data of Johnson, et al. (1969) demonstrate that intravenous administration of hexamethonium can cause a

fall in blood pressure in acute spinal cats. In our study, blood pressure had to be supported artificially by intravenous infusions of phenylephrine, indicating that the observed ongoing discharge was not adequate to support blood pressure completely. The decreased heart rate in the spinal animals reflected the sparse cardiac sympathetic activity. The remaining renal nerve activity probably would not be adequate to cause renal vasoconstriction but could contribute to tonic control of sodium reabsorption and renin secretion (DiBona, 1982). The exceptional splenic nerve activity and splenic sympathetic reflexes (Weaver et al., 1983) in spinal animals would prevent pooling of blood in this capacitive reservoir and would facilitate extrusion of stored erythrocytes into the circulation (Greenway and Lister, 1974). Thus, the varying levels of tonic discharge to different visceral organs may contribute to cardiovascular support in the spinal animal.

In summary, these experiments have identified another characteristic of splenic sympathetic nerves which distinguishes them from cardiac and renal nerves. Splenic nerves are not only more reactive to many excitatory inputs (Calaresu, et al., 1984; Weaver, et al., 1983, 1984) but also are less dependent upon tonic supraspinal excitation than are cardiac and renal nerves. This characteristic may be a general property of the innervation of splanchnic capacitive circulation, as mesenteric nerve activity also is minimally affected by spinal cord transection (Stein and Weaver, 1987). It was not possible to determine sources of external excitation driving splenic more than

cardiac or renal nerves in the spinal animals. Possible explanations for the lesser dependence of splenic nerves upon supraspinal excitation are that: 1) splenic nerves are more dependent upon excitation by local neural circuits in the spinal cord or sympathetic ganglia than are renal or cardiac nerves, 2) activity of splenic nerves may be generated by peripheral sources of tonic afferent nerve activity which is not of dorsal root origin, 3) pacemaker potentials in pre- or postganglionic neurons directed toward the spleen
may generate the ongoing discharge in splenic nerves, 4) splenic nerves receive greater descending inhibitory or less descending excitatory influences than renal or cardiac nerves, or 5) components of neural circuits innervating the spleen are inherently more excitable (e.g., closer to threshold) than those innervating the heart or kidney. Possibly, fractionated bulbospinal outflow and spinal or ganglionic mechanisms all contribute to differences in the ongoing or reflex activity of splenic, renal, and cardiac nerves.

CHARACTERISTICS OF ONGOING AND REFLEX DISCHARGE OF SINGLE SPLENIC AND RENAL SYMPATHETIC POSTGANGLIONIC FIBERS IN CATS

Provisionally accepted for publication in Journal of Physiology

INTRODUCTION

Much of our current understanding of the way in which neural discharge to different organs can be affected dissimilarly by afferent inputs is based on experiments in which electrical discharge has been recorded from whole nerves innervating different organs (Karim, Kidd, Malpus, and Penna, 1971; Weaver, et al., 1984). For example, stimulation of splenic or cardiac spinal afferent nerves by algogenic substances such as capsaicin and bradykinin causes reflex excitation of splenic and renal sympathetic nerve activity and increased arterial pressure (Calaresu, Tobey, Heidemann, and Weaver, 1984; Weaver, Fry, Meckler, and Oehl, 1983). Although excitatory responses of multifiber renal nerve activity are significant, excitation of multifiber splenic nerve discharge is consistently of greater magnitude than that of renal nerve activity. Excitatory responses of splenic nerve activity may be greater than those of renal, because more of the splenic fibers are engaged in these excitatory reflexes. Alternatively, similar proportions of fibers in each nerve may be engaged in the reflexes, but the magnitude of excitation of each splenic fiber may exceed the magnitude of excitation of each renal fiber.

Differential responses of multifiber splenic and renal nerve activity to cardiovascular pressoreceptor stimulation also have been reported (Tobey and Weaver, 1987). Activity of renal nerves was more inhibited than that of splenic nerves in response to phenylephrine-

induced increases in arterial pressure. In contrast, Ninomiya et al. (Ninomiya, Nisimaru, and Irisawa, 1971) observed greater inhibition of splenic than renal multifiber nerve activity in response to pressoreceptor stimulation. However, Ninomiya et al. attempted to analyse responses of only the cardiac rhythmic components of the multifiber nerve activity. Therefore, these investigators may have underestimated contributions of the responses of non-rhythmic components of sympathetic nerve activity. For example, perhaps the non-rhythmic component of renal nerve activity responds much more to pressoreceptor stimulation than does the non-rhythmic component of splenic nerve activity. The best way to separate rhythmic from non-rhythmic components of sympathetic nerve activity is to test activity of individual fibers for cardiac rhythmicity. In this way, the potential heterogeneity of responses of individual fibers, with or without cardiac rhythmic activity, can be evaluated accurately.

In addition to the non-uniform splenic and renal nerve responses to reflex inputs, ongoing activity of whole splenic nerves is less dependent upon supraspinal sources of tonic excitation than is ongoing activity of whole renal nerves (Meckler and Weaver, 1985). Again, one can only speculate whether a greater number of splenic than renal neurones remains active following spinal cord transection, or whether activity of each renal neurone is depressed relative to that of each splenic neurone.

In this paper we describe ongoing and reflex activity of single fibers within splenic and renal sympathetic nerves. The purpose of the present study was to investigate the organization of differential splenic and renal sympathetic outflow by assessment of the heterogeneity

of reflex responses and ongoing activity of individual splenic and renal nerve fibers. Therefore, experiments were designed to allow: 1) correlation of ongoing activity of single splenic and renal fibers with peak systolic arterial pressure, 2) correlation of ongoing discharge of single fibers with integrated phrenic nerve discharge, 3) quantitation of reflex changes in single fiber activity caused by stimulation and unloading of systemic pressoreceptors, and 4) quantitation of reflex changes in activity of single fibers elicited by selective chemical stimulation of splenic or intestinal afferent nerves before and after high cervical spinal cord transection.

METHODS

General procedures. Experiments were done on 16 adult cats (3.0 ± 0.2 kg) of either sex, anaesthetized with intravenously administered alpha-chloralose (80 mg/kg; Sigma Chemical Company, St. Louis, MO). Supplemental doses (20 mg/kg) were given when necessary throughout the experiments, as determined by assessment of the cat's palpebral reflex and response to paw pinch, the stability of blood pressure, and the size of the pupils. A tracheostomy tube was inserted, and cannulae were passed into the inferior vena cava via the femoral veins for delivery of drugs and solutions. Cannulae were passed into the thoracic aorta via the femoral arteries for monitoring arterial pressure and for withdrawal of blood for analysis of pH, PaO_2, and $PaCO_2$ (Blood Gas Analyser, Model 165; Corning Medical, Medfield, MA). A catheter was passed through the urethra to allow continuous emptying of the urinary bladder. After careful assessment of the animals' plane of anaesthesia (as stated above), gallamine triethiodide (Flaxedil; Davis Geck, New York, NY) was administered, to ensure adequate muscle relaxation for surgical and experimental procedures, and the animals were respired with room air by a Harvard respirator. Initial and supplemental doses of gallamine were 5.0 mg/kg and 2.0 mg/kg, respectively. Oesophageal temperature was monitored and maintained at approximately 37°C. A solution of 5% dextrose w/v in half-strength normal saline (pH adjusted to 7.3) was infused slowly throughout the experiments to compensate for fluid loss. Blood gas composition was maintained (pH = 7.35-7.45, PaO_2 > 85 mmHg) by administration of sodium

bicarbonate, changing respiratory rate or depth, or by addition of 100% O_2 to the inspired air.

A laminectomy was done to expose the first cervical segment of the spinal cord for later transection. Following a midline laparotomy, a few loops of the small intestine were placed gently in a small plastic dish. All vascular and neural connections between the intestine and central structures remained intact. The dish was then filled with normal saline and covered with plastic wrap to prevent cooling and dehydration of the intestine. The splenic artery was cannulated via the left gastric artery without obstructing blood flow to the spleen. Snares were placed around the splenic artery and vein central to the cannulation site, being careful to avoid damaging the splenic nerves. Vascular connections of the spleen with other organs were ligated and cut to provide isolation of the splenic vasculature. A pneumothorax was made to minimize artifacts in the neural recordings caused by movement associated with artificial respiration.

Nerves to the kidney (7 cats) and spleen (9 cats) were identified close to the respective organs, dissected from surrounding tissue, and severed (Figure 14). The central ends of the nerves were desheathed, separated into small bundles, and placed on a small black plastic platform for further splitting. Nerve bundles subsequently were teased apart progressively and placed on fine bipolar platinum-iridium electrodes until ongoing activity of single fibers could be distinguished (amplifier bandwidth: 30 Hz to 3 kHz). Activity of single cells was discriminated from that of other active fibers by the uniform amplitude of action potentials, and occasionally also by spike duration, with a slope/height window discriminator (Frederick Haer, New

Figure 14. Neuronal recording arrangement and methods of chemical stimulation of visceral afferent nerves. Efferent single fiber recordings were obtained from filaments teased from splenic or renal sympathetic nerves (A). Splenic receptors were stimulated selectively by injection of 10 micrograms bradykinin (BK) into the artery of the vascularly isolated spleen (B). Serosal receptors were stimulated selectively by superfusion of the small intestine with 10 micrograms bradykinin in 25 ml warm saline solution (C).

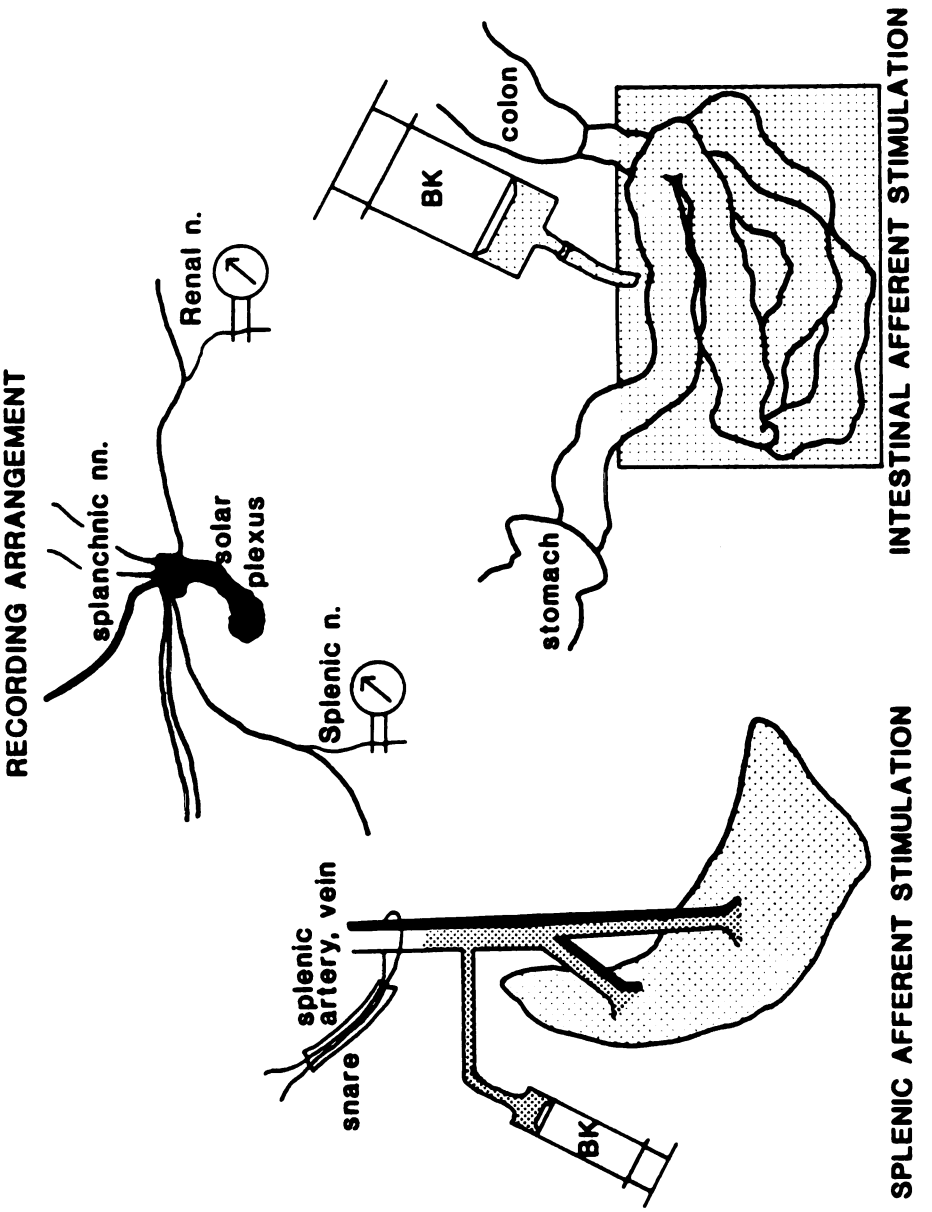
RECORDING ARRANGEMENT
splanchnic nn.
solar plexus
Renal n.
Splenic n.
splenic artery, vein
snare
BK
SPLENIC AFFERENT STIMULATION
BK
colon
stomach
INTESTINAL AFFERENT STIMULATION

Figure 14.

Brunswick, ME). Thresholds were considered acceptable if more than 95% of interspike intervals were greater than 20 ms. Output pulses of the window discriminator were counted in 10-s periods with a PDP 11/23 computer (Digital Electronics Corporation, Maynard, MA).

The phrenic nerve was dissected from the sternohyoid muscle, desheathed, and placed on a bipolar electrode for multifiber recording of activity (amplifier bandwidth: 30 Hz to 3 kHz). Neural activity was monitored on an oscilloscope and recorded on magnetic tape. Arterial pressure was recorded (P23 Db pressure transducer; Gould Statham, Oxnard, CA) on a Grass polygraph (Grass Instruments, Quincy, MA) and stored on magnetic tape.

Experimental protocol. After the neuronal recordings were stable for approximately 30 min, ongoing activity of the fiber(s), phrenic nerve discharge, and arterial pressure was tape recorded for 5 to 20 min. Parameters recorded during this period were used to characterize ongoing activity of the fibers (see Data Analysis, below). Then the following procedures were done, not necessarily in the order given: 1) Systemic pressoreceptor stimulation and "unloading" by graded changes in arterial pressure produced by bolus i.v. injections of phenylephrine (1.0 to 10.0 micrograms/kg, Neo-Synephrine; Winthrop Laboratories, New York, NY) and sodium nitroprusside (1.0 to 10.0 micrograms/kg; Sigma Chemical Company, St. Louis, MO), respectively. 2) Following occlusion of the splenic artery and vein with snares, 10 micrograms bradykinin triacetate (Sigma Chemical Company, St. Louis, MO) in 0.1 ml saline was flushed into the spleen (Figure 13) with 0.5 ml saline (Calaresu et al., 1984). 3) After removing the saline from the

intestinal container, 10 micrograms bradykinin in 25 ml warm saline was superfused over the small intestine (Figure 13). Two min later the bradykinin was removed, and the intestine was rinsed with 300 ml warm saline. One to 2-min recovery samples of unit discharge and arterial pressure were recorded 5 to 10 min following each procedure. Animals were allowed to recover for 5 to 45 min between various periods of stimulation, but bradykinin was administered to a given organ only once per hr to prevent the development of tachyphylaxis to bradykinin.

The spinal cord was transected at the first cervical segment in 13 cats (from which activity of 13 renal and 9 splenic sympathetic fibers was recorded). Mean arterial pressure was maintained above 90 mmHg by i.v. infusions of phenylephrine at a rate of 5 to 10 micrograms/kg/min (0.05 to 0.15 ml/min of 200 micrograms/ml phenylephrine in saline). After a 1-hr period of stabilization, chemical stimulation of splenic and intestinal afferent nerves was repeated as before spinal cord transection. If ongoing discharge of fibers was absent, and if discharge could not be elicited by chemical stimulation of splenic or intestinal afferent nerves following spinal cord transection, then 3 to 5 ml of saturated KCl solution was given intravenously, at the end of the experiment, to cause depolarization of neurones and action potential propagation in the living fibers on the electrode. Fast-sweep oscillographic recordings of KCl-induced action potentials were compared to the spontaneously occurring action potentials recorded in the intact state. Units were identified on the basis of waveform and peak-to-peak amplitude. If activity of a fiber could not be elicited by KCl injection, it was assumed that the electrode had moved during spinal cord transection or that the axon was

no longer capable of impulse conduction; and, therefore, we could not assess the sources of its ongoing activity. The short-acting ganglionic blocking agent, trimethaphan camsylate (0.2 mg/kg Arfonad; Hoffman-LaRoche, Nutely, NJ), was administered intravenously prior to spinal cord transection, or hexamethonium (Mann Research Laboratories, New York, NY) was administered at the end of experiments, to verify that postganglionic neuronal activity was being recorded.

Data analysis, ongoing activity patterns. Arterial pressure, integrated phrenic nerve discharge, and window-discriminated activity of single sympathetic fibers were digitized and analysed, using an IBM AT computer. Interspike interval histograms were plotted to ascertain whether fibers were discharging at constant or irregular intervals. Postevent interval histograms of unit activity were triggered by 1) peak integrated phrenic nerve activity (window length = 5 s) and 2) peak systolic arterial pressure (window length = 500 ms). These histograms were compared with those triggered by stimulator output pulses (with stimulation frequency approximating the frequency of the real event triggers). Unit activity was considered to be positively correlated with the period of cardiac or respiratory cycles if the amplitude of the neural histogram was greater than that of the stimulator histogram by at least a factor of three.

Reflex changes in sympathetic neuronal activity. Ninety-five per cent confidence limits were calculated from 6 to 12 periods of control discharge rates (10 s/period; c.i. = $t_{[0.05]}(\bar{x}/n)$). Responses of individual fibers were considered significant if values for the maximum 10-s responses exceeded the confidence limits. Mean responses of groups

of fibers were tested with a Least Significant Differences test after a completely blocked analysis of variance. Values were considered significant if $P \geq 0.05$. Responses (% of control) of renal fibers were compared to those of splenic fibers by the nonparametric Wilcoxon test for unpaired data. Paired comparisons of neuronal responses to splenic and intestinal stimulations were done with a nonparametric Friedman's test (Sokal and Rohlf, 1969).

Significant differences from control are indicated by asterisks in the figures. Illustrated standard errors were calculated from the pooled standard deviations derived from the analyses of variance.

RESULTS

Ongoing activity. Average rates of ongoing discharge of 33 splenic and renal single fibers ranged from 0.03 to 3.2 spikes per second. The difference between the mean discharge rate of 1.2 $\pm$ 0.2 spikes/s and the median rate of 0.9 spikes/s indicates that the distribution of average discharge frequencies is skewed toward lower rates. Distributions of ongoing discharge rates of the 17 splenic and 16 renal fibers were similar (Figure 15), and interspike interval histograms of ongoing splenic and renal sympathetic activity revealed no differences between these 2 populations. Histograms were either flat or unimodal but broad. Minimum interspike intervals ranged from 18 to 236 ms (mean = 72 $\pm$ 14 ms), and the instantaneous frequency of any neurone was never constant.

To reveal one possible source of differences in the 2 populations of neurones, ongoing activity of 14 splenic and 16 renal fibers was tested for correlation with the arterial pressure pulse. Sixteen renal fibers exhibited continuous action potentials correlated in time with the arterial pressure pulse (Figure 16A). In addition, activity of 9 of 13 renal fibers also was correlated with phrenic nerve activity (Figure 16B). Splenic fibers, in contrast, could be divided into rhythmic and non-rhythmic populations. Only 8 of the 16 splenic fibers tested for cardiac rhythmicity had activity correlated with the arterial pressure pulse. Examples of the presence and absence of cardiac rhythmicity in the activity of 2 splenic fibers are illustrated in the upper and lower panels of Figure 17, respectively. Discharge of only 3 splenic fibers had respiratory-related rhythmicity. Respiratory rhythmicity of

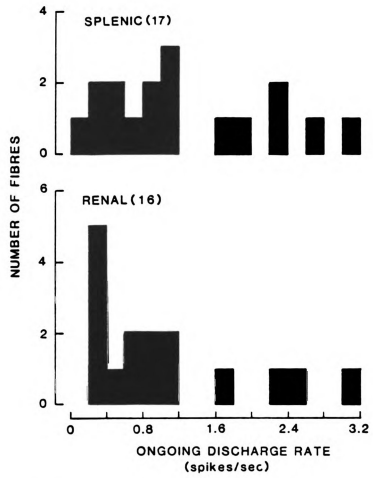

Figure 15. Distributions of ongoing discharge rates of splenic and renal sympathetic fibers. Ten-second periods of ongoing activity of splenic (upper panel) and renal (lower panel) fibers were averaged over 1 to 2 min. Mean and median of the splenic distribution are 1.2 and 1.0 spikes/s, respectively. Those for the renal distribution are 1.2 and 0.7 spikes/s, respectively. Numbers of fibers are given in parentheses.

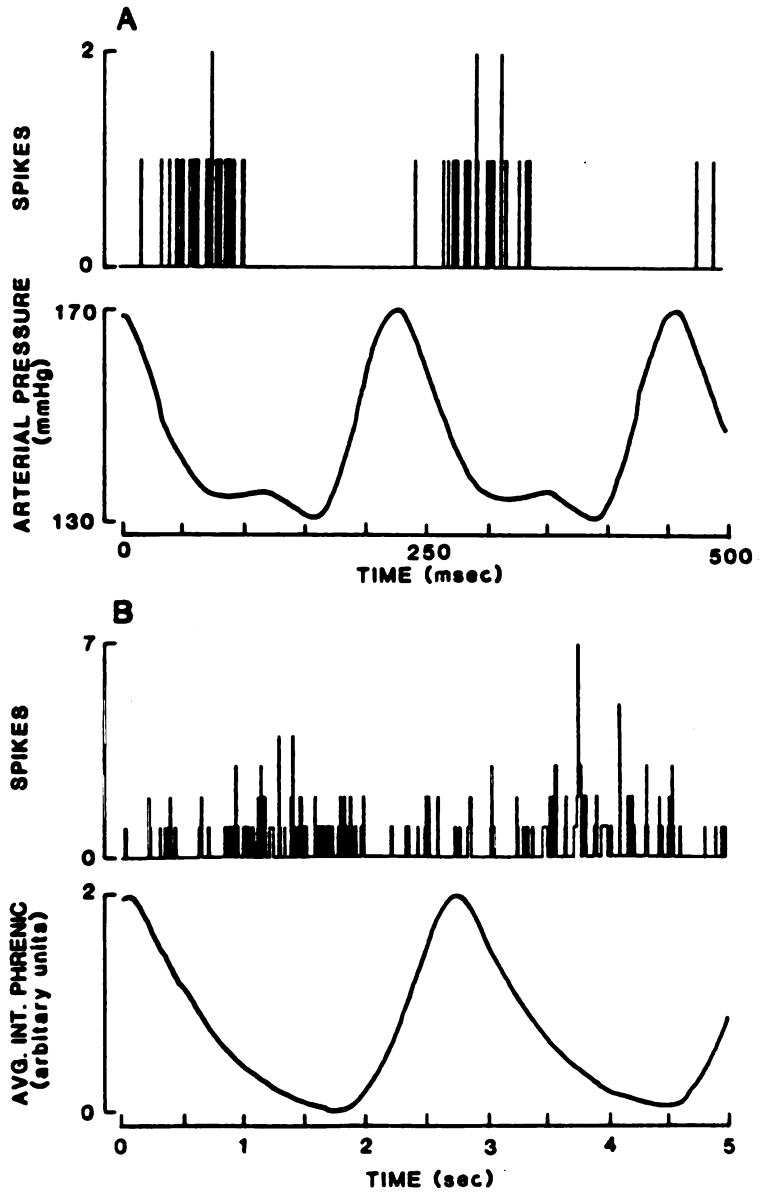

Figure 16. Cardiac and respiratory rhythmicities of a renal sympathetic fiber. Histograms of neuronal activity are displayed above an average of the arterial pressure pulse (A) and an average of integrated phrenic nerve activity (B). Sampling of unit activity was triggered by peak systolic arterial pressure or the inspiratory peak of integrated phrenic nerve discharge, respectively. Activity of all renal fibers was correlated to the arterial pressure pulse. Activity of 9 of 13 renal neurons was correlated to the period of phrenic nerve discharge.

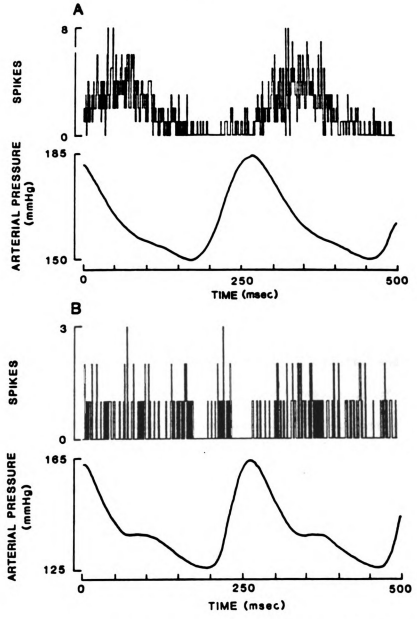

Figure 17. Assessment of cardiac rhythmicity in activity of two splenic sympathetic fibers. Format is the same as that for Figure 16A. Correlation and absence of correlation of splenic unit activity with the arterial pressure pulse are shown in panels A and B, respectively. Activity of 8 of 16 splenic fibers was related to the arterial pressure pulse.

neuronal activity was never observed in activity of fibers without cardiac rhythmicity. Ongoing discharge rates did not differ between the rhythmic and non-rhythmic groups of splenic fibers.

Pressoreceptor unloading and stimulation. In addition to testing unit activity for cardiac rhythmicity, responses to graded decreases and increases in arterial pressure were studied. The responses of 16 renal and 9 splenic fibers to virtually identical blood pressure changes are illustrated in Figure 18. According to the confidence range tests, discharge rates of all fibers changed significantly whether or not the activity was correlated with the arterial pressure pulse. However, as a group, renal neuronal excitation exceeded that of splenic fibers in response to the 20 mmHg decrease in arterial pressure, and when pressure was decreased further by 40 mmHg, excitatory responses of splenic and renal neuronal activity were equivalent. Similar magnitudes of inhibition were produced in both groups of fibers by 20 mmHg blood pressure increases. However, increases of 40 mmHg caused significantly greater inhibition of activity of renal than splenic fibers.

Reflex responses to chemical stimulation of visceral afferent nerves: Intact neuraxis. Chemical stimulation of splenic afferent nerves also caused different magnitudes of excitation of activity of renal and splenic fibers. Discharge rates of all 10 splenic fibers increased up to 7 fold following injection of 10 micrograms bradykinin into the isolated spleen, whereas activity of 6 of 8 renal fibers was increased by 50 to 100% by splenic receptor stimulation (Figure 19, upper panel; Table 6); activity of 2 renal fibers did not change. This stimulation never produced inhibition of renal or splenic neuronal discharge. Pressor responses of 8 to 27 mmHg (renal average =

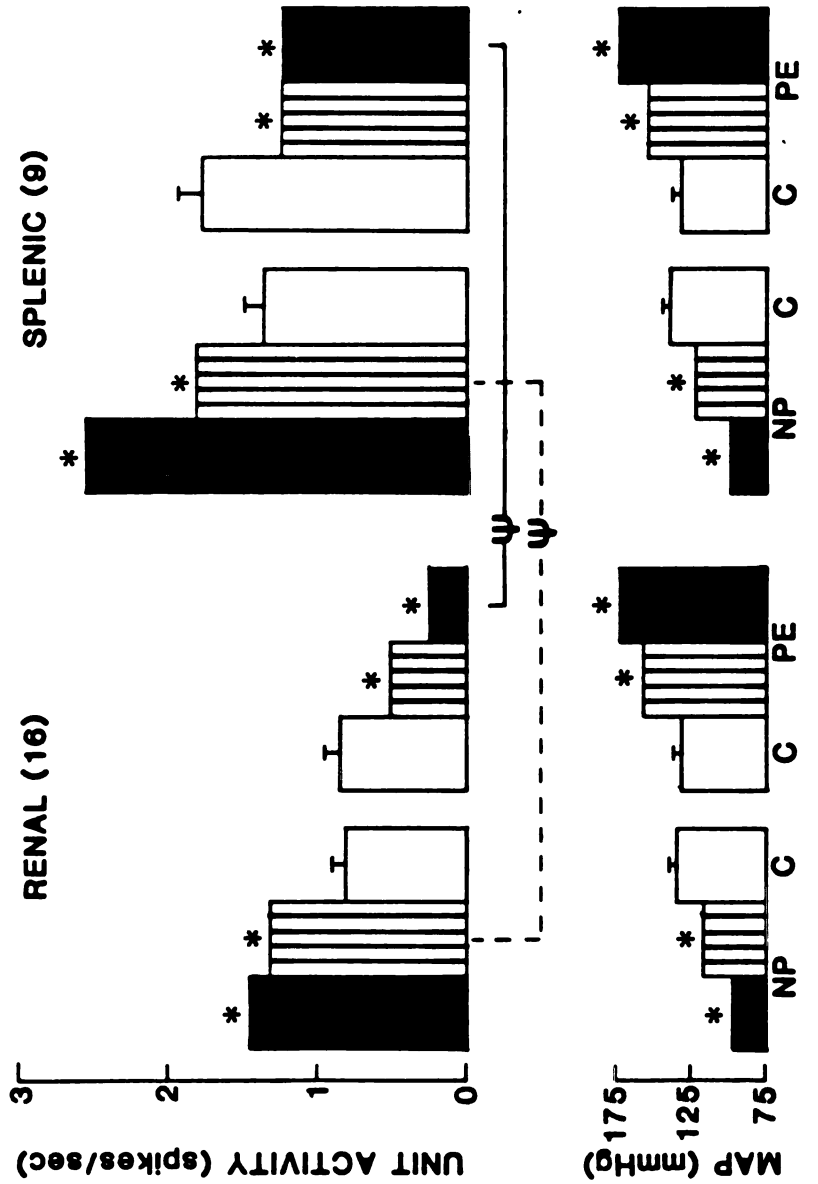

Figure 18. Effects of arterial and cardiopulmonary pressoreceptor stimulation and unloading on discharge rates of renal and splenic sympathetic fibers. Each bar represents the average discharge rate of renal (upper left panel) and splenic (upper right panel) fibers and arterial pressure (lower panels) during periods of control (C), nitroprusside injection (NP), and phenylephrine injection (PE). Thin lines on the control bars indicate pooled standard errors. Large psi symbols in the center indicate differences between renal and splenic responses. Numbers of fibers are given in parentheses.

Table 6. **Summary of the effects of splenic and intestinal receptor stimulation on discharge of splenic and renal sympathetic fibers.**

		SPLENIC FIBERS				RENAL FIBERS			
		Total tested	Excitation	Inhibition	No change	Total tested	Excitation	Inhibition	No change
INTACT	Splenic stimulation	10	10	0	0	8	6	0	2
	Intestinal stimulation	17	9	1	7	16	14	0	2
SPINAL	Splenic stimulation	4	4	0	0	5	4	0	1
	Intestinal stimulation	9	6	3	0	7	7	0	0

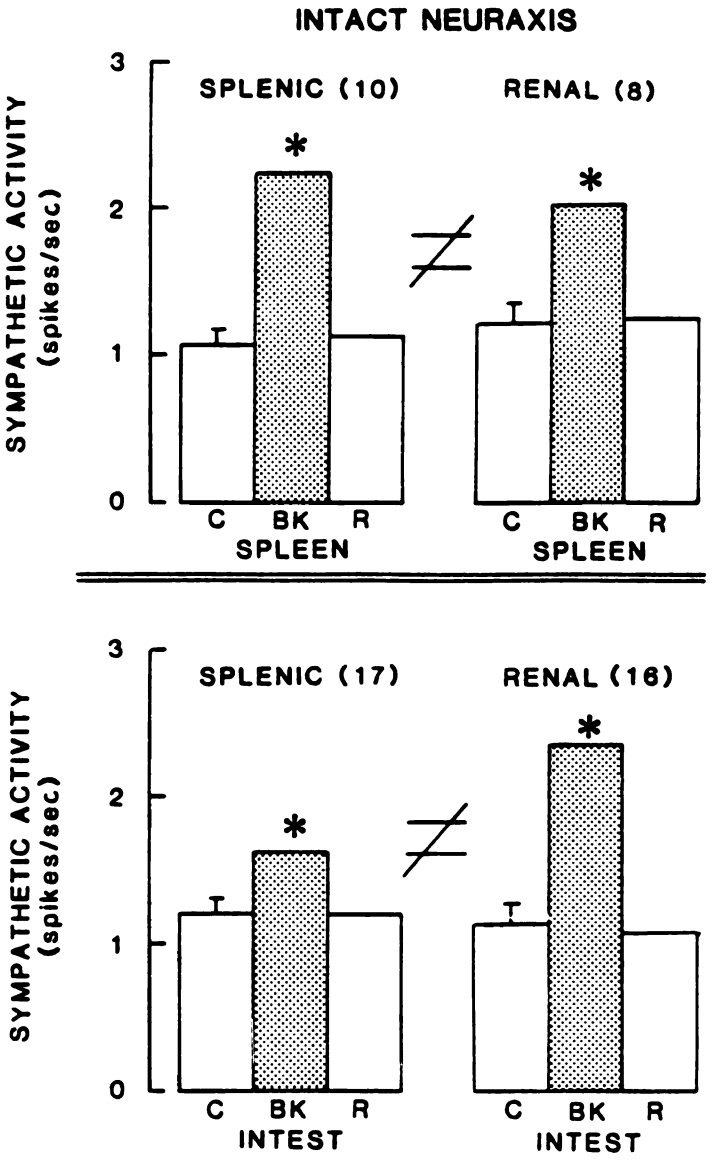

Figure 19. Group comparisons of splenic and renal unit responses to visceral stimulation. Bars represent average discharge rates of splenic (left panel) and renal (right panel) fibers during the 60-s control period (C), the 10-s period of maximum response to bradykinin (BK), and during the 60-s recovery period (R). Thin lines represent pooled standard errors. Numbers of fibers are given in parentheses. Splenic stimulation (upper panels) caused greater excitation of splenic than renal fiber activity ($\neq$). Intestinal stimulation (lower panels) elicited greater excitation of renal than splenic fiber activity ($\neq$). See text for details.

12 ± 3 mmHg; splenic average = 16 ± 4 mmHg) accompanied the neural responses. Average excitation of the 10 splenic fibers was greater than that of the 8 renal fibers. The average excitation of the 6 renal fibers that did respond was less than that of the splenic fibers. Therefore, it appears that splenic stimulation resulted in differential splenic and renal sympathetic reflexes, because responses of individual splenic fibers exceeded those of individual renal fibers.

Differential splenic and renal responses also were observed following application of bradykinin to the intestinal serosa (Figure 19, lower panel; Table 6). Stimulation of intestinal afferent nerves caused 2 to 3-fold increases in activity of 14 of 16 renal fibers; activity of 2 renal fibers did not change. However, splenic neuronal responses varied from 30% inhibition of one fiber's discharge to the 1 to 2-fold excitation observed in the activity of 9 of the 17 fibers; activity of 7 splenic fibers did not change. Despite the heterogeneity of the splenic responses, the mean response of all 17 fibers was significant, though of lesser magnitude than the average excitation of activity of the renal population. However, when only excitatory responses of renal and splenic fibers were compared, intestinal receptor stimulation caused equivalent magnitudes of renal and splenic excitation. Therefore, the inequality of total renal and splenic neuronal responses can be accounted for only by the heterogeneity of splenic units' responses to intestinal stimulation.

Reflex responses of fibers with cardiac rhythmic activity were not different from those of fibers with non-rhythmic activity. To determine whether the magnitude of an individual fiber's response to visceral stimulation was related to the rate of ongoing discharge, responses of

units with low discharge rates (below median rate) were compared to those of units with higher discharge rates (above median rate). There were no consistent differences between responses of fibers with ongoing discharge rates greater and less than the median.

Comparison of responses to stimulation of splenic and intestinal receptors: Intact neuraxis. Activity was recorded from 10 splenic and 7 renal fibers during separate stimulations of both splenic and intestinal afferent nerves, to allow comparisons of the responses of single units to both of these stimuli.

The excitation of splenic fiber activity elicited by splenic injection of bradykinin was always greater than the excitation produced by intestinal superfusion with bradykinin (Figures 20 and 21). In contrast, the renal neuronal responses to both stimuli consistently were equivalent (Figures 20 and 21; Table 6).

Reflex responses to stimulation of visceral receptors: Spinal cord transected. Electrical recordings from 9 splenic and 13 renal fibers were maintained throughout high cervical spinal cord transection and for 1 to 2 hr afterward. Ongoing activity of 7 of 9 splenic fibers still could be observed 1 hr after the spinal cord was severed. Average discharge rate of the 7 active fibers was 2.1 $\pm$ 0.2 spikes/s before and 1.0 $\pm$ 0.2 spikes/s after spinal cord transection. Activity of 2 splenic fibers ceased after severing the spinal cord. Activity of 2 additional splenic fibers that could be identified but not discriminated before spinal transection was discriminated and analysed for responses to visceral stimulation after the spinal cord was transected. In contrast to the splenic population, only 7 of 13 renal fibers had ongoing activity in the spinal state. Activity of 6 fibers could be elicited in

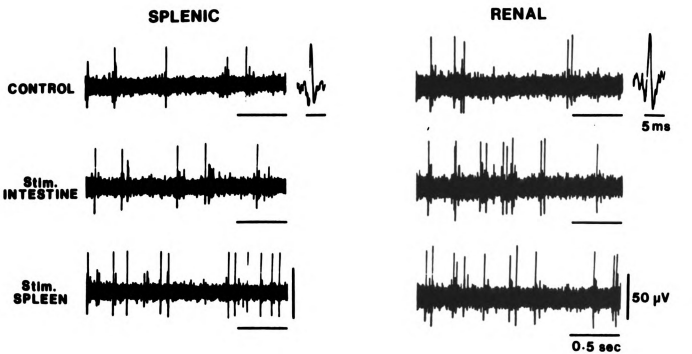

Figure 20. Oscillographic records of responses of discharge of one splenic and one renal sympathetic fiber to chemical stimulation of visceral afferent nerves. Splenic (left panel) and renal (right panel) single fiber activity is shown during periods of control and maximum response to stimulation of intestinal and splenic receptors with bradykinin. Fast-sweep traces of the analyzed action potentials are shown to the right of the control tracings. Calibrations are noted in the lower right corner of the figure. Activity of this splenic fiber was increased more by splenic stimulation than by intestinal stimulation. Activity of the renal fiber, in contrast, was excited to the same degree by both stimulations.

Figure 21. Paired comparisons of responses of sympathetic fibers to chemical stimulation of splenic and intestinal afferent nerves. Bars represent average discharge rates of splenic neurons (upper panels) and renal neurons (lower panels) during periods of control (C), bradykinin administration (BK), and recpvery (R). Numbers of fibers are given in parentheses. Splenic stimulation consistently elicited greater responses of splenic unit activity than did intestinal stimulation ($\neq$, upper panels). Both stimulations produced equivalent responses of renal fiber activity (=, lower panels).

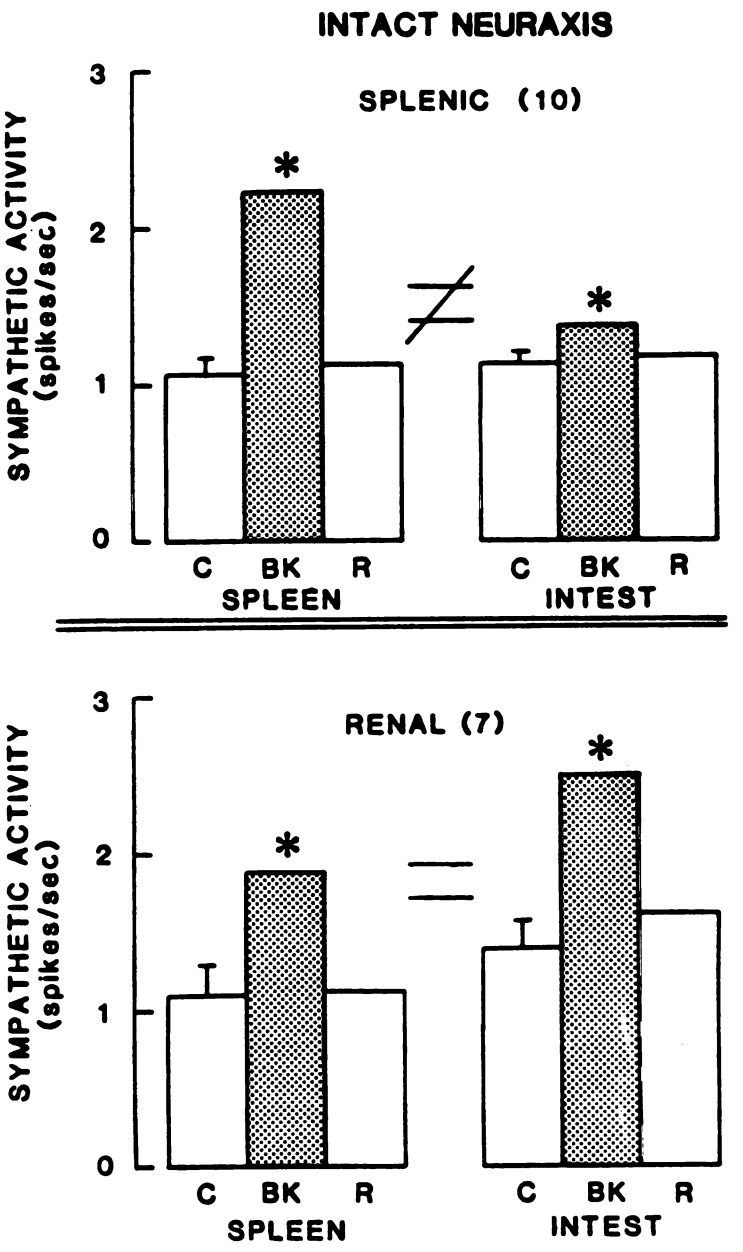
INTACT NEURAXIS
SPLENIC (10)
SYMPATHETIC ACTIVITY (spikes/sec)
3
2
1
0
*
*
≠
C BK R
SPLEEN
C BK R
INTEST
RENAL (7)
SYMPATHETIC ACTIVITY (spikes/sec)
3
2
1
0
*
*
=
C BK R
SPLEEN
C BK R
INTEST

Figure 21.

the spinal state only by KCl administration. The average discharge rate of the 7 active renal neurones was 1.4 $\pm$ 0.4 spikes/s before and 1.5 $\pm$ 0.5 spikes/s after spinal cord transection. Following spinal cord transection, activity of splenic and renal fibers no longer had cardiac rhythmicity even though some of these neurones did have rhythmic activity when the neuraxis was intact.

After spinal cord transection, activity of 4 splenic and 5 renal fibers was monitored during splenic receptor stimulation. This stimulation produced changes in splenic and renal fiber activity that were similar to those elicited prior to transecting the spinal cord. Average excitation of activity of 4 splenic fibers exceeded that of 5 renal fibers (Figure 22, upper panel; Table 6). All 4 of the spontaneously active splenic fibers which were tested for responses to splenic stimulation responded significantly, and activity of 4 of 5 renal fibers was excited significantly by the splenic injection of bradykinin; activity of 1 renal fiber did not change.

Activity of 9 splenic and 7 renal fibers was monitored during chemical stimulation of intestinal afferent nerves following spinal cord transection. Excitation was elicited in activity of all 7 renal fibers and 6 of the 9 splenic fibers. With one exception, excitatory responses of fibers to intestinal stimulation were the same before and after spinal cord transection. Activity of 1 splenic fiber, that was excited by this stimulation before spinal transection, was reflexly inhibited after the spinal cord was transected; activity of 2 other splenic fibers, which could not be discriminated when the neuraxis was intact, was inhibited following spinal cord transection. Comparison of average responses in the 2 groups yielded a significant difference between

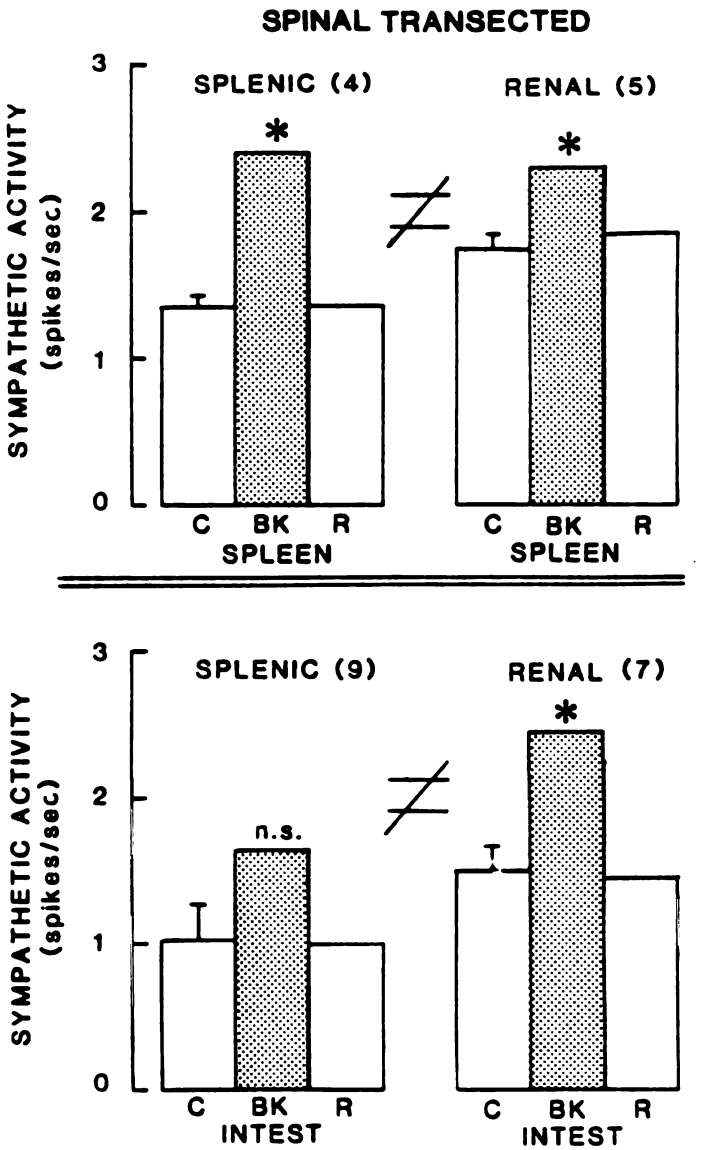

Figure 22. Group comparisons of responses to visceral stimulation one hour after spinal cord transection. Format is the same as that for Figure 19. Splenic stimulation (upper panels) caused greater excitation of activity of splenic than renal fibers (≠) after the spinal cord was transected. Intestinal stimulation still produced excitation of average renal fiber activity and no change in average splenic unit activity after spinal transection. n.s.: not significant.

average responses of 9 splenic and 7 renal fibers (Figure 22, lower panel).

Comparison of responses to stimulation of splenic and intestinal receptors: Spinal cord transected. Activity was recorded from 4 splenic and 5 renal fibers during separate stimulations of both splenic and intestinal receptors, as was done prior to spinal cord transection. Again, average excitation of splenic neuronal activity (n = 4) produced by splenic stimulation was greater than that caused by intestinal stimulation (Figure 23, upper panel). As when the neuraxis was intact, average activity of the 5 renal fibers was excited similarly by either stimulation (Figure 23, lower panel; Table 6).

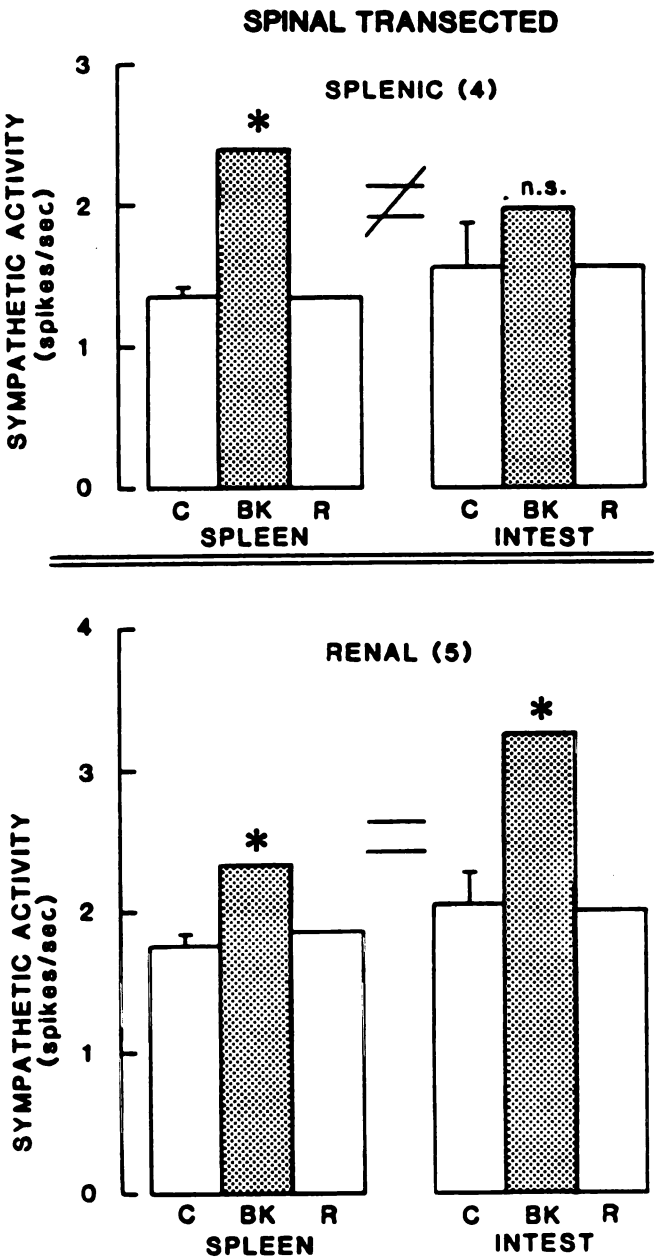

Figure 23. Paired comparisons of responses to visceral stimulation following spinal cord transection. Format is the same as that for Figure 21. Splenic fiber activity was always increased more by splenic stimulation than by intestinal stimulation (≠, upper panels). Again, activity of renal fibers responded similarly to both stimulations (=, lower panels).

DISCUSSION

The mechanisms of non-uniform responses of splenic and renal nerve activity to chemical stimulation of visceral afferent nerves were investigated. Stimulation of splenic receptors causes greater excitation of splenic than renal nerve activity by eliciting large responses in all splenic fibers and smaller responses in approximately three-fourths of the renal fibers. In contrast, stimulation of intestinal receptors produces greater renal than splenic nerve excitation by increasing the activity of most renal fibers, whereas the population of splenic fibers responds heterogeneously to this input. Although all fibers of the splenic and renal nerves respond to pressoreceptor stimulation and unloading, activity of renal units is more inhibited and more excited, respectively, than that of splenic fibers. As visceral stimulation following spinal cord transection elicits response patterns in the renal and splenic populations that are similar to those produced when the neuraxis is intact, supraspinal neural pathways are not required for these differential reflexes to occur.

The range of ongoing discharge rates of splenic and renal fibers in this study is in agreement with those of sympathetic postganglionic fibers innervating the kidney (Kidd, Linden, and Scott, 1981; Rogenes, 1982), blood vessels of skeletal muscle (Dean and Coote, 1986; Jänig and Kümmel, 1981; Koizumi and Sato, 1972), and cutaneous blood vessels (Blumberg, Jänig, Rieckmann and Szulczyk, 1980). In the present study, activity of only half of the splenic fibers was correlated with the arterial pressure pulse, whereas almost all renal units discharged with

cardiac rhythm. Adrian, Bronk, and Phillips (1932) first observed the cardiac rhymicity of sympathetic nerve activity, and since then others have reported the presence (Chalazonitis and Gonella, 1971; Gregor, Jänig and Wiprich, 1977; Kollai and Koizumi, 1980) or absence (Skok and Ivanov, 1983; Rogenes, 1982; Blumberg et al., 1980) of cardiac rhythmicity in the activity of single sympathetic neurones. It is tempting to speculate that differences in responses of splenic and renal nerves reflect functional differences in subpopulations of fibers within the nerves. Jänig and coworkers (Jänig, 1986) have examined the hypothesis that function of single sympathetic fibers in the lower abdomen can be inferred from relationships of ongoing activity with the arterial pressure pulse and from their relative responsiveness to stimulation of various afferent nerves. Accordingly, neurones with pulse-rhythmic activity and that are only weakly excited by visceral afferent nerve stimulation were assumed by these investigators to be vasoconstrictors. In contrast, cells that respond strongly to visceral stimulation but that lack pulse-rhythmicity were thought to mediate regulation of visceral motility and secretion (Jänig, 1986). However, despite Jänig's logical interpretation of the available data, it still is not possible to determine with certainty which individual neurones synapse with each type of target tissue. Although cardiac rhythmicity of sympathetic activity is thought to reflect influences of pulse-related baroreceptor stimulation on the activity of vasomotor neurones (Taylor and Gebber, 1975), some neurones that innervate blood vessels of the skin, and are thought to be vasoconstrictors, do not have cardiac rhythmic activity and are relatively isolated from baroreceptor influences (Blumberg et al., 1980). Therefore, the function of

individual neurones probably should not be inferred from the data of the present study. However, renal and splenic populations of sympathetic nerve fibers certainly receive different degrees of influence from pressoreceptors, splenic receptors, and intestinal receptors.

The proportion of renal fibers having respiratory-related activity in this study (70%) corresponds to the proportion of preganglionic fibers having this rhythmic activity (70-80%) reported by Priess and Polosa (1977). In the present study, activity of fewer splenic than renal fibers had respiratory-related rhythms. In general, the higher proportion of renal than splenic fibers with ongoing activity related to respiratory or cardiac cycles parallels differences in dependence of renal and splenic nerve activity on supraspinal sources of tonic excitatory drive (Meckler and Weaver, 1985). Indeed, activity of 78% of the splenic fibers could still be observed after spinal cord transection, whereas activity of more than half of the renal fibers ceased. This suggests that ongoing activity of neurones exhibiting rhythms originates in the brain and ongoing activity of non-rhythmic neurones is generated in the spinal cord. However, our data demonstrated that discharge of a given neurone can exhibit cardiac- or respiratory-related rhythmicity and ongoing activity of that neurone still may be generated in the spinal cord. Many neurones exhibiting rhythmic discharge when the neuraxis was intact, were still active following transection of the spinal cord. Therefore, the source of a neurone's activity cannot be predicted from relationships of its ongoing discharge to the cardiac or respiratory cycle.

Previous studies of baroreceptor reflex influences on sympathetic outflow have demonstrated different degrees of sensitivity of activity

of nerves innervating vascular beds of skin, skeletal muscle, kidney, and intestine (Lofving, 1961; Kendrick, Öberg, and Wennergren, 1972a,b). The two studies in which the reflex influences of pressoreceptor stimulation on activity of renal and splenic nerves were compared are conflicting. Ninomiya et al. (1971) reported greater inhibition of splenic than renal nerve activity in response to pressoreceptor stimulation by intravenous noradrenaline. In contrast, Tobey and Weaver (1987) reported that stimulation of pressoreceptors by intravenous injection of phenylephrine caused greater inhibition of renal than splenic nerve activity. The results of the single fiber experiments of the present study confirm the results of Tobey and Weaver. In the present study, 90% of the renal fibers sampled discharged with cardiac-related rhythms, but only 50% of the splenic fibers had this rhythmic activity. Ninomiya et al. (1971) estimated that 90% of renal and 90% of splenic multifiber nerve activity discharge with cardiac-related rhythms, and only the rhythmic components of the nerve activity were analysed with respect to pressoreceptor inputs. Therefore, Ninomiya and coworkers may have overestimated the proportion of splenic nerve activity that was rhythmic, due to the inability to separate responses of the two components of multifiber nerve activity. In addition, neurones that do not have cardiac rhythmic activity do respond to phenylephrine- and nitroprusside-induced changes in arterial pressure. Therefore, it is possible that responses of non-rhythmic as well as rhythmic components of splenic nerve activity were included in Ninomiya's analysis of responses to pressoreceptor stimulation.

Bolus injections of phenylephrine were used to produce transient changes in arterial pressure, to allow consistent stimulation of

pressoreceptors among the different experiments. More prolonged infusions, necessary for extended periods of increased arterial pressure, potentially can lead to resetting of arterial baroreceptors (Abboud and Thames, 1982). In addition, prolonged infusions of phenylephrine can have direct effects on central nervous system networks in the baroreceptor reflex pathway (Imaizumi, Brunk, Gupta, and Thames, 1984). As these changes can cause variability of sympathetic responses to pressoreceptor stimulation, only bolus injections were used in these experiments.

Previous studies of splenic receptor stimulation demonstrated that activity of whole splenic nerves increased more than whole renal nerve discharge (Calaresu et al., 1984; Tobey and Weaver, 1987). However, the multifiber recording methods used in those studies only allowed analysis of the relative changes in nerve activity which cannot be precisely quanitified. Therefore, it could be argued that the reflexes are not really differential; rather, the reflex may be distributed equally to subpopulations of neurones in both nerves, and the splenic nerve may contain more fibers engaged in the reflex. The present study confirms the differential nature of the effects of splenic receptor stimulation on renal and splenic sympathetic outflow. It appears that the population of splenic neurones reacts homogeneously to splenic stimulation. Although 6 of 8 renal fibers were excited by splenic stimulation, the magnitudes of these responses were consistently less than those of the splenic neuronal responses. Pressoreceptor reflex influences probably contribute to the inequality of renal and splenic nerve responses to splenic receptor stimulation. However, these spleno-sympathetic reflexes are unequal after vagotomy and sinoaortic

denervation (Tobey and Weaver, 1987) indicating that pressoreceptor influences are not solely responsible for the non-uniformity. Results of the present study confirm this interpretation, because although stimulation of intestinal and splenic receptors causes equivalent pressor responses, stimulation of intestinal receptors causes greater excitation of renal than splenic nerve activity; and stimulation of splenic receptors causes greater excitation of splenic than renal nerve activity. Therefore, the secondary activation of pressoreceptors during these visceral receptor stimulations cannot be responsible for the unequal pattern of splenic and renal responses.

Intestinal receptor stimulation caused greater excitation of average renal than splenic fiber activity. However, if inhibitory responses and non-responding splenic fibers were disregarded, and only excitatory responses of renal and splenic fibers were compared, the magnitudes of excitation of splenic and renal fibers were equivalent. Therefore, intestinal afferent nerves exert similar influences upon the activity of all renal and some splenic neurones. In contrast, activity of a substantial proportion of splenic neurones (41%) was not affected by intestinal stimulation. These results explain those of multifiber electrophysiological recording experiments in which the average excitatory responses of splenic and renal nerve activity to intestinal stimulation are equivalent (Meckler and Weaver, unpublished observations). Apparently, determination of differential reflex responses with multifiber recording techniques may underestimate the potential non-uniformity compared with the use of single unit methodology.

Although supraspinal neural pathways may participate in these viscero-sympathetic reflexes, these reflexes are still differential after spinal cord transection. Previous investigations of the role of supraspinal neural circuitry in the non-uniformity of viscero-sympathetic reflexes have been difficult to interpret, due to the changes in baseline activity of sympathetic nerves produced by spinal cord transection (Meckler and Weaver, 1985; Weaver et al., 1983). These baseline problems were avoided in the present study, because the renal and splenic fibers that were still active following spinal cord transection had similar ongoing discharge rates. Therefore, complete spinal reflex pathways are likely to play an important role in the expression of differential viscero-sympathetic reflexes in intact animals.

Localized injections or applications of the peptide, bradykinin, were used to initiate the viscerosympathetic reflexes in this study. Bradykinin stimulates small myelinated (A-delta) and unmyelinated (C) afferent fibers (Lew and Longhurst, 1986; Longhurst, Kaufman, Ordway, and Musch, 1984). The use of this peptide was preferred, compared to electrical stimulation of visceral afferent nerves, to increase the probability of eliciting reflexes, especially after spinal cord transection. Sherrington (1906) was the first to notice that electrical stimulation of afferent nerves from the pinna does not always produce reflex contraction of the pinna whereas mechanical stimulation of the pinna's overlying skin consistently causes the pinna to contract. In addition, the classic scratch reflex can be elicited in dogs easily by electrical or mechanical stimulation prior to spinal cord transection, However, following spinal cord transection, often only mechanical

stimulation elicits the scratch reflex (Sherrington, 1906). More recently, Weaver, et al. (1983) could not demonstrate a cardio-renal reflex after spinal cord transection by electrical stimulation of cardiac afferent nerves, although the reflex could be observed if it were initiated by chemical stimulation of the cardiac afferent nerves.

The functional significance of these differential reflexes is not clear. As splenic vasoconstriction cannot be elicited independent of splenic capsular contraction (Greenway and Stark, 1969), classification of splenic neurones on the basis of their responses to visceral or pressoreceptor stimulation may not be meaningful. In addition, as responses of the renal fibers to visceral stimulation were homogeneous, it is difficult to infer that different neurones have selective influences on different target tissues within the kidney. However, one investigation of the effects of exercise on renal and splenic function provides evidence that renal excretion of sodium and water can be increased (via inhibition of renal nerve activity) simultaneously with increased hematocrit (via excitation of splenic nerve activity) (Grignolo, Koepke, and Obrist, 1982). In addition, Weaver, Stella, Genovesi, Golin, and Zanchetti (1986) have demonstrated that greater excitation of mesenteric than renal nerve activity during intestinal receptor stimulation results in greater decreases in mesenteric than renal vascular conductance. Therefore, we speculate that the differential responses of these sympathetic nerves probably result in non-uniform redistributions of blood flow to the kidney and spleen.

PRINCIPAL RESULTS AND CONCLUSIONS

1. Half of the peroxidase-labeled cell bodies of renal neurons were clustered in groups within the solar plexus. The remainder of renal nerve cell bodies were situated in upper lumbar (L1-L3) and lower thoracic (T12-T13) paravertebral sympathetic ganglia.
2. Most peroxidase-labeled cell bodies of splenic neurons were scattered randomly throughout the left and right celiac poles of the solar plexus.
3. After spinal cord transection, discharge rates of cardiac and renal nerve bundles were depressed significantly to less than 50% of pre-transection values, indicating the dependence of activity of these nerves upon supraspinal sources of excitatory drive.
4. Discharge rates of splenic nerve bundles were not altered significantly by spinal cord transection, indicating spinal or peripheral sources of ongoing activity.
5. The maintenance of splenic nerve activity following spinal cord trasection was <u>not</u> affected by increased or decreased arterial pressure, systemic hypercapnia and acidosis, nor by potential ongoing activity of dorsal afferent nerves.
6. Half of the single splenic nerve fibers and all renal nerve fibers had cardiac-related discharge patterns. Of those tested for respiratory-related activity, 30% of the splenic fibers and 69% of the renal fibers exhibited this discharge pattern.

7. Activity of splenic fibers was less inhibited than that of renal fibers by stimulation of pressoreceptors with phenylephrine-induced increases in arterial pressure. Activity of splenic fibers also was less excited than that of renal fibers by unloading pressoreceptors with depressor doses of sodium nitroprusside.
8. Chemical stimulation of splenic afferent nerves with bradykinin consistently elicited more excitation of average splenic than average renal unit activity by causing large excitatory responses in activity of all splenic fibers and smaller excitatory responses in the discharge of 75% of the renal fibers.
9. Application of bradykinin to the intestinal serosa produced greater excitation of average renal than average splenic unit activity by causing excitation, inhibition, or no change in splenic fiber discharge, whereas renal unit activity was almost always excited by this stimulation.
10. A greater number of splenic than renal fibers continued to exhibit ongoing activity after spinal cord transection.
11. Responses of splenic and renal fibers to chemical stimulation of splenic and intestinal afferent nerves after spinal cord transection were similar to those responses elicited when the neuraxis was intact.

The results of these studies indicate that activity of renal and splenic sympathetic nerves exhibit differential behavior with regard to control by supraspinal and reflex mechanisms. In addition, the results of the single fiber experiments confirm and extend results of previous studies in which multifiber sympathetic activity was recorded (Calaresu,

et al., 1984; Stein and Weaver, 1987; Tobey and Weaver, 1987). Splenic receptor stimulation produces greater excitation of multifiber splenic than multifiber renal nerve activity by causing greater magnitudes of responses in a greater number of splenic than renal nerve fibers. Pressoreceptor influences are directed more strongly toward individual renal than to individual splenic fibers. These differential characteristics may be facilitated by the differential anatomical distributions of cell bodies of renal and splenic neurons within sympathetic ganglia. The relative homogeneity of single renal fiber activity to the ongoing influences of pressoreceptors, and responses to chemical stimulation of visceral afferent nerves, may depend, in part, upon the clustered anatomical distribution of cell bodies of renal neurons within the solar plexus. This is in contrast to the potential for heterogeneity of splenic fiber responses (eg., with regard to chemical stimulation of intestinal afferent nerves) which correlates with the random distribution of splenic neuronal cell bodies within sympathetic ganglia.

The differences in central nervous system sources of ongoing activity between splenic and renal nerves is reflected in the relative insensitivity of the splenic population, compared with the sensitivity of the renal population, to the influences of supraspinal neural pathways, such as cardiac and respiratory rhythmicities and baroreceptor reflexes. The lack of dependence of splenic sympathetic activity upon supraspinal sources of excitatory drive is similar to the independence of mesenteric nerve activity from tonic descending excitation (Stein and Weaver, 1987). As splenic and mesenteric nerves supply components of the splanchnic vasculature considered to play a major role as a

capacative reservoir in the cat, the similarity of sources of ongoing activity of these nerves may reflect the functional organization of the sympathetic nervous system to provide functionally similar targets with congruous sympathetic outflow.

Although reflex responses of splenic and renal vasculature have been compared only infrequently, differential responses of splenic and renal nerve activity can contribute significantly to nonuniformity of the vascular responses in some circumstances. For example, during exercise renal blood flow does not change whereas contraction of splenic vasculature and capsule can decrease blood flow through the spleen (Grignolo, et al., 1982). Therefore the differences in ongoing and reflex discharge of splenic and renal nerves are likely to contribute to discrete control of splenic and renal function.

APPENDICES

APPENDIX A

Table A. Historical sketch of investigations of neural control of the cardiovascular system.

Galen	130-200	1) identifies vagosympathetic trunks 2) ganglia are butresses to strengthen nerves innervating the viscera 3) nerves are responsible for "sympathy" or "consent" between body parts.
Eustachius	1563	distinguishes between vagi and sympathetic trunks
Willis	1664	1) involuntary nature of visceral innervation 2) involuntary nerves mechanically cause vasoconstriction 3) explains visceral sensation (pain) 4) identifies aortic depressor nerve and realizes it is afferent
du Petit	1727	sympathetics don't simply descend from brain; Horner's syndrome
Winslow	1732	1) coins "sympathetic" nerve 2) ganglia equated to "little brains"
Whytt	1751, 1765	1) local stimulation leads to reflex movements of organs 2) adequate stimulus and referred pain 3) nerves are made up of fibers
Meckel	1751	nerves entering ganglia are smaller than those exiting ganglia
Johnstone	1764	nerves entering ganglia appear different from those exiting ganlia (myelinated//unmyelinated fibers)
Bichat	1800	1) autonomy of sympathetic system of ganglia that control the viscera 2) somatic functions controlled by the brain and spinal cord 3) rami communicantes allow emotions to influence visceral functions 4) sympathetic nerves closely associated with blood vessels
Ehrenberg	1833	saw isolated cells within ganglia

Table A (con't.).

Remak	1838	1) distinguishes white and grey rami 2) grey rami are made up of unmyelinated fibers of ganglion cells
Henle	1840	1) finds muscle in walls of blood vessels 2) sympathetic nerves associated with vascular muscle
Stilling	1840	coins "vasomotor system"
Weber brothers	1845	vagal inhibition of the heart
Budge & Waller	1851	cervical sympathetic trunk contains outflow from spinal cord
Brown-Sequard	1852	electrical stimulation of cervical sympathetic trunk elicits vasoconstriction in the ear
Bernard	1851 1858 1859	1) obtains vasodilation by severing cervical sympathetic trunk (but thought due to metabolic effects) 2) severs spinal cord and observes fall in arterial pressure 3) severs splanchnic nerves and observes increased urine flow; stimulates splanchnics--decreased urine flow 4) concept of the internal environment
Hall	1856	"reflex sensibility" in the absence of conscious sensation
von Bezold	1863	cardiac left ventricular stretch produces bradycardia and vasodilation
Owsjannikow Dittmar	1871 1873	serial transection of the neuraxis used to locate "vasomotor center" in medulla oblongata
Gaskell	1885-1889	1) sympathetics communicate with the spinal cord via white rami 2) spinal sympathetic outflow originates in lateral horns of thoracic and lumbar spinal segments 3) describes innervation of renal blood vessels

Table A (con't.).

Langley	1873-1925	1) coins "autonomic nervous system" 2) classifies and coins: "sympathetic or thoracolumbar division"; "parasympathetic or crainiosacral division"; "enteric system" 3) coins "preganglionic and postganglionic nerves" 4) nicotine blocks conduction of transmission from pre- to postganglionic nerves, but does not affect afferent conduction through the ganglia 5) autonomic system is purely motor 6) sympathetic system is segmentally organized with respect to the longitudinal axis of the body 7) epinephrine mimics sympathetic stimulation; pilocarpine mimics parasympathetic stimulation 8) reciprocal nature of sympathetic vs. parasympathetic innervations 9) sympathectomy results in the sensitization of responses to epinephrine injections
Bainbridge	1915	cardiac right atrial stretch causes reflex tachycardia
Ranson & Billingsley	1916	probe floor of the fourth ventricle and find pressor and depressor areas
Cannon	1920's	1) concept of homeostasis 2) unitary nature of sympathetic action

APPENDIX B

The enzyme, peroxidase, is a protein with a molecular weight of approximately 44 kD. It is abundant in the root of the horseradish plant which contains three isoenzymes. If an aqueous solution of this enzyme is introduced into an area of axon terminals or damaged neuronal fibers, endocytosis typically occurs within 15 min. (Jones and Hartman, 1978). The amount of peroxidase taken up by the nerve fibers is directly related to the amount of surface area of membrane to which the enzyme is exposed. Peroxidase applied to the cut ends of nerve fibers diffuses a short distance into the axoplasm where it is packaged into oval or tubular lysosomal sacs and multivesicular bodies. The vesicles then become associated with transport filaments which are carried toward the cell body via the neuron's microtubule systems. Although the velocity of transport may vary, this speed ranges from 70 to 120 mm/day (Jones and Hartman, 1978; Kristensson and Olsson, 1971; Ochs, 1976). The oxidative reaction catalyzed by peroxidase results in the polymerization of a variety of chromagens, including tetramethyl benzidene. Hydrogen peroxide, the primary substrate, donates an oxygen atom which is accepted by the chromagen. Accumulations of oxidized chromagen polymers then can be visualized with the aid of a microscope.

The compositions of the incubation media, and the experimental protocol, used in the present study to demonstrate distributions of labeled sympathetic neuronal cell bodies, are given below.

SOLUTIONS

Acetate Buffer (pH = 3.3)	100 ml 1.0 M sodium acetate 100 ml water 95 ml 1.0 M hydrochloric acid q.s. to 500 ml with water check final pH
Solution A (less than 2 hours)	92.5 ml water 5 ml acetate buffer (pH = 3.3) 100 mg sodium nitroprusside
Solution B (less than 2 hours)	5 mg tetramethyl benzidine 2.5 ml absolute alcohol may be heated to 40 ^{o}C
Hydrogen peroxide (0.3%)	0.1 ml 3% hydrogen peroxide q.s. to 1 ml with water
Cold, 5% acetate buffer	5 ml acetate buffer (ph = 3.3) q.s to 100 ml with water

PROTOCOL

The frozen ganglia (-20 ^{o}C) are cut into sections of the desired thickness. The sections are collected in a small basket made from "cocktail-ice cube trays" (each cube measures approximately a half

inch on each side) with the bottoms replaced by fine nylon mesh. This basket is situated in phosphate buffer during collection of the sections. The basket of tissue sections then can be transferred easily from vessel to vessel as described below. All incubation vessels are standard, rectangular staining dishes, and 100 ml of solution in each staining dish is sufficient to immerse the tissue in their baskets without floating over the tops of their compartments. Therefore, each compartment of the tissue basket can be used for a separate paravertebral sympathetic ganglion, or for a single section of a prevertebral ganglion if specific distributions and topography are to be investigated.

Combine solutions A and B in the incubation vessel only seconds before introducing the tissue. Allow the tissue to incubate in the solution for 20 min, providing constant agitation to achieve complete infiltration of the tissue. Add 0.6 ml 0.3% hydrogen peroxide and incubate for 5 to 15 min. As time passes, once the H_2O_2 is introduced into the incubation medium, the reaction proceeds and cells begin to appear "labeled"; if too much time elapses, a blue, flocular material will precipitate throughout the solution. Immediately transfer tissue into cold, 5% acetate buffer and refrigerate for 5 min. Transfer tissue into fresh, cold, 5% acetate buffer for another 5 min. Mount tissue sections on gelatinized slides directly from the acetate buffer. Allow to dry (overnight) and counterstain with Neutral Red or Toluidine Blue, using standard techniques (Sheehan and Hrapchak, 1980), with only one exception: when dehydrating the mounted sections during the counterstaining procedure, NEVER expose the tissue to alcohol (the reaction product will disappear). Instead, dehydrate the

tissue sections with acetone. Coverslip using standard procedures (xylene does not destroy the reaction product). Coverslipped tissue can be stored in the refrigerator for "very long periods of time" without fading of the reaction product, however if the tissue is not refrigerated, the reaction product will fade. Therefore, photography of desired tissue sections is recommended before storing for any length of time.

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