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MESENTERIC AND RENAL NERVES DIFFER IN THE ORIGINS OF THEIR ONGOING DISCHARGE AND IN THEIR RESPONSES TO AFFERENT INFLUENCES

By

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ABSTRACT

MESENTERIC AND RENAL NERVES DIFFER IN THE ORIGINS OF THEIR ONGOING DISCHARGE AND IN THEIR RESPONSES TO AFFERENT INFLUENCES

By

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The sympathetic nervous system regulates the functions of various effector organs selectively. The present studies used electrophysiological techniques to compare discharge patterns of mesenteric and renal efferent nerves in anesthetized cats in response to 1) stimulation and unloading of systemic pressoreceptors, 2) stimulation of intestinal receptors, 3) high cervical spinal cord transection and 4) inhibition of discharge of neurons within the rostral ventrolateral medulla (RVLM blockade). Mass discharge of renal nerves was inhibited more than that of mesenteric nerves following stimulation of pressoreceptors. Recordings from individual fibers revealed that all renal fibers, but only 60% of mesenteric fibers were engaged in reflexes elicited by stimulation and/or unloading of pressoreceptors. Similarly, all renal fibers, but only approximately 60% of mesenteric fibers had discharge which was correlated with the arterial pulse wave. Multifiber recordings demonstrated that

stimulation of intestinal receptors with bradykinin consistently caused greater excitation of mesenteric than renal nerve discharge. This stimulus excited the same proportion of fibers within both nerves. Stimulation of intestinal receptors in anesthetized rats also caused significant excitation of mesenteric and renal nerve activity. Chronic capsaicin treatment in rats caused significant depletion of substance P-like immunoreactivity in dorsal root ganglia and significant attenuation of the neural responses, implying a role for substance P or other capsaicin-sensitive peptides in the central transmission of this reflex. Discharge of renal nerves was influenced more than that of mesenteric nerves by supraspinal inputs. Severing the spinal cord caused significant decreases in tonic mass discharge of renal nerves without altering the ongoing discharge of mesenteric nerves; firing of 75% of renal, but only 11% of mesenteric fibers ceased after spinal transection. RVLM blockade caused greater reductions in the overall discharge of renal than mesenteric nerves, while reducing the 1-6 Hz periodicity in firing of both nerves similarly. Transection of the spinal cord during RVLM blockade did not affect the level of renal nerve activity. In contrast, mesenteric nerve activity increased following spinal cord transection, returning to control levels. These data indicate that mesenteric and renal nerves may respond differentially to supraspinal and visceral afferent influences.

This dissertation is dedicated in solidarity with
my comrades in the upper deck bleachers of Tiger Stadium.

And to Jonathan Adiv and Noah David.
It's not Babar Visits Another Planet (Brunhoff, 1972),
but it's the best I could do.

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INTRODUCTION

The sympathetic nervous system is capable of producing distinct patterns of nerve discharge which selectively control blood flow to various effector organs. Non-uniform sympathetic outflow often serves important homeostatic functions. This is demonstrated clearly by the differential pattern of sympathetic responses to thermal stress, which leads to antagonistic blood flow adjustments in the splanchnic and cutaneous vascular beds. On exposure to heat, blood flow is diverted from the inner vascular beds to the periphery, preserving temperature homeostasis (Riedel and Iriki, 1979; Simon and Riedel, 1975). Differential patterns of sympathetic outflow can be elicited by a variety of environmental and behavioral stimuli. Many investigators have demonstrated that baroreceptor and chemoreceptor influences can unequally affect sympathetic nerve discharge or blood flow to different organs (Folkow, Johansson and Löfving, 1961; Hadjiminas and Öberg, 1968; Irisawa, Ninomiya and Woolley, 1973; Jänig, 1985; 1986; Kendrick, Öberg and Wennergren, 1972; Kollai and Koizumi, 1977; Löfving, 1961; Ninomiya and Irisawa, 1975). These unequal sympathetic responses may contribute to the maintenance of cardiovascular

homeostasis during the defense response in cats (Baccelli, Albertini, Del Bo, Mancia and Zanchetti, 1981). Stimulation of visceral afferent nerves also may elicit non-uniform patterns of sympathetic discharge (Jänig, 1985; 1986; Johansson and Langston, 1964; Ninomiya, Irisawa and Woolley, 1974). In addition, fractionated sympathetic discharge patterns can be generated within the central nervous system. For example, not all sympathetic preganglionic neurons are spontaneously active (Dembowsky, Czachurski and Seller, 1985b; Janig, 1985). Also, the patterned vascular changes which occur during desynchronized sleep (Mancia, Baccelli, Adams and Zanchetti, 1971) are apparently caused by non-uniform patterns of sympathetic activity originating in the central nervous system (Futuro-Neto and Coote, 1982).

Most reports of fractionated sympathetic discharge have stressed differences between visceral, skeletal muscle and cutaneous sympathetic activity. The viscera generally have been considered as a collective unit, and, therefore, ongoing and reflex activity patterns of different components of abdominal visceral sympathetic outflow rarely have been compared. Is the selective organization of the sympathetic nervous system sufficient to produce discrete patterns of discharge among nerves innervating different abdominal viscera? Recent investigations by Weaver and co-workers (Weaver, Meckler, Tobey and Stein, 1986) have demonstrated

that splenic and renal sympathetic activity is selectively regulated.

The present studies were designed to provide information about the organization of sympathetic outflow to the small intestine and kidney. The characteristics of ongoing and reflex discharge of mesenteric and renal nerves were compared. The research consisted of the following investigations:

1. Multi- and single fiber mesenteric and renal nerve responses to the stimulation of systemic (arterial and cardiopulmonary) pressoreceptors and intestinal receptors were characterized in anesthetized cats with intact neuraxes and after high cervical spinal cord transection. The following hypotheses were tested.

a. Stimulation of systemic pressoreceptors causes greater inhibition of multifiber renal than mesenteric nerve discharge.

b. More renal than mesenteric nerve fibers are engaged in reflexes originating from systemic pressoreceptors.

c. Chemical stimulation of intestinal receptors causes greater excitation of multifiber mesenteric than renal nerve activity.

d. More mesenteric than renal nerve fibers have discharge which is excited by chemical stimulation of intestinal receptors.

e. Spontaneous activity of mesenteric, but not renal, nerves can be generated within the spinal cord. Excitatory sympathetic responses elicited by intestinal receptor stimulation can be mediated by exclusively spinal circuits.

2. Reflex responses of mesenteric and renal sympathetic nerves also were evaluated in anesthetized rats. Two hypotheses were tested.

a. Chemical stimulation of intestinal receptors with bradykinin evokes greater excitation of mesenteric than renal nerve activity in rats.

b. Chronic subcutaneous administration of capsaicin causes a depletion of substance P-like immunoreactivity in dorsal root ganglia and in the dorsal horn of the spinal cord. Capsaicin pretreatment attenuates the intestino-sympathetic excitatory responses, implying a role for substance P or other capsaicin-sensitive peptides in the central transmission of this reflex. Capsaicin treatment does not affect baroreceptor reflexes.

3. The role of the rostral ventrolateral medulla in the maintenance and in the generation of rhythm of ongoing activity of mesenteric and renal nerves was determined. The following hypotheses were tested.

a. Inhibition of tonic activity of neurons within the RVLM (blockade) by bilateral application of the inhibitory

amino acid glycine causes greater reductions in overall discharge of renal nerves than that of mesenteric nerves.

b. RVLM blockade reduces the 2-6 Hz rhythm in firing of both mesenteric and renal nerves similarly.

c. Blockade of the RVLM unmasks tonically active sympathoinhibitory systems which may contribute to the decreases in nerve discharge. This hypothesis was tested by comparing magnitudes of nerve discharge observed after spinal cord transection to those seen following RVLM blockade.

The following review of the literature concentrates on topics pertinent to these investigations. Sympathetic influences on the intestine and kidney are described. The early concepts of the organization of the sympathetic nervous system are reviewed briefly. The ongoing and reflex activity patterns of sympathetic nerves are discussed and, in each section, examples of specificity among different components of sympathetic outflow are reviewed. Because the investigations included in this dissertation involved stimulation of systemic (arterial and cardiopulmonary) pressoreceptors and intestinal receptors, the sections concerning reflex activity of sympathetic nerves concentrate on reflexes originating from these receptors.

LITERATURE REVIEW

Sympathetic Regulation of the Intestine

Preganglionic fibers supplying the small intestine course through the thoracic splanchnic nerves. The origin of these fibers has been determined by using the retrograde tracer horseradish peroxidase (HRP). Following application of HRP to the central cut end of the greater splanchnic nerve, peroxidase-labeled preganglionic cell bodies are found ipsilaterally, within the T1 - T13 spinal cord segments of cats (Kuo, Yamasaki and Krauthamer, 1980) and rabbits (Torigoe, Cernucan, Nishimoto and Blanks, 1985). Most labeled sympathetic preganglionic neurons are located in the intermediolateral cell column, but cells also are found in the lateral funiculus, nucleus intercalatus and the central autonomic area. Postganglionic mesenteric neurons are located mainly in the celiac and mesenteric ganglia, as well as the paravertebral ganglia (Costa and Furness, 1984; Kuo and Krauthamer, 1981; Langley, 1899).

Sympathetic innervation of the intestine regulates mucosal secretory processes, intestinal motility and blood flow. Activation of mesenteric nerves inhibits the flux of sodium from the mucosa to the lumen, leading to increased intestinal net fluid absorption (Sjövall, Jodal, and Lundgren, 1987). Direct stimulation of mesenteric nerves

also inhibits intestinal motility (Gonella, Bouvier and Blanquet, 1987). Sympathetic denervation causes significant increases in movement of the small intestine, indicating that these sympathetic nerves are tonically active. Moreover, many investigators have demonstrated intestino-intestinal inhibitory reflexes which "appear to be a reinforcement of the permanent sympathetic restraint on intestinal motility" (Gonella et al. 1987). Folkow and co-workers (Folkow, Lewis, Lundgren, Mellander and Wallentin, 1964) showed that electrical stimulation of mesenteric nerves caused frequency dependent decreases in intestinal blood flow and blood volume.

Costa and Furness (1984) have identified three types of adrenergic neurons supplying different tissues within the small intestine in the guinea pig which apparently subserve the three different functions described above. The first type contains norepinephrine co-localized with somatostatin. These neurons innervate the intestinal mucosa and may regulate mucosal secretory and absorption processes. A second group of postganglionic neurons, containing only norepinephrine, project to the myenteric ganglia and may regulate intestinal motility. Finally, a third type of mesenteric neuron, containing norepinephrine co-localized with neuropeptide Y, terminate on blood vessels and apparently subserve a vasomotor function.

The gastrointestinal tract (stomach and intestines) receives approximately 18-20% of the cardiac output and, thus, redistribution of blood flow to or from these organs may make important contributions to cardiovascular homeostasis (see Donald, 1983). Greenway and Lister (1974) estimated that during volume-loading in cats, the gastrointestinal tract pooled 40%, and the splanchnic circulation as a whole (GI tract, liver and spleen) pooled approximately 70%, of the volume infused. They also reported that during non-hypotensive hemorrhage the amount of blood mobilized from the gastrointestinal tract and the splanchnic circulation as a whole accounted for 23% and approximately 60%, respectively, of the volume removed. The mechanisms involved in the mobilization of blood have been debated. Greenway and Lister (1974) proposed that this process involved active constriction of the capacitance vessels mediated by a sympathetic reflex from atrial volume receptors (Pelletier, Edis and Shepherd, 1971). Other workers have shown that unloading aortic baroreceptors in dogs produces active constriction of abdominal capacitance vessels (Karim, Hainsworth and Pandy, 1978). However, Brooksby and Donald (1972) have suggested that the mobilization of blood from the splanchnic circulation during hemorrhage in dogs involves passive collapse of capacitance vessels, subsequent to arteriolar constriction and decreased transmural pressure. Although opinions differ regarding the precise mechanism

involved in the mobilization of blood from the capacitive circulation, there is general agreement that the sympathetic nervous system is engaged in this process; thus, sympathetic innervation of the intestine appears to make significant contributions to cardiovascular homeostasis.

Sympathetic Regulation of the Kidney

Renal postganglionic neurons in cats and rats are located in the celiac and superior mesenteric ganglia, in renal ganglia and in lower thoracic and upper lumbar paravertebral ganglia (Kuo, DeGroat and Nadelhaft, 1982; Meckler and Weaver, 1984; Sripairojthikoon and Wyss, 1987). Renal neurons in the celiac ganglion receive preganglionic input from the greater, lesser and least splanchnic nerves (Calaresu, Kim, Nakamura and Sato, 1978). Barajas and Wang (1979) have demonstrated that renal nerve fibers innervate renin-containing granular cells of the juxtaglomerular apparatus, proximal and distal tubules and afferent and efferent arterioles.

Physiological investigations have confirmed that renal nerves regulate renin release, tubular sodium and water reabsorption and renal blood flow (DiBona, 1982). Electrical stimulation of efferent renal nerves at very low frequencies (0.5 Hz) in anesthetized dogs cause increases renin release in the absence of changes in urinary sodium excretion, renal

blood flow or glomerular filtration rate (Osborn, DiBona and Thames, 1981). Stimulation of efferent renal nerves at frequencies of 1-3 Hz caused an antinaturesis and antidiuresis in the absence of changes in renal blood flow or glomerular filtration rate (Slick, Aguilera, Zambraski, DiBona and Kaloyanides, 1975). Higher frequencies of stimulation produce these changes in renal function and, in addition, changes in renal hemodynamics. Renal blood flow is reduced as a result of arteriolar vasoconstriction and there is a shunting of blood away from the outer cortex (DiBona, 1982).

EARLY CONCEPTS OF SYMPATHETIC ORGANIZATION

Widespread Influences

Claude Bernard, in 1878, stated that "the stability of the mileu interieur is the primary condition for freedom and independence of existence" (Sheehan, 1936). Later, Cannon (1929; 1930) introduced the term "homeostasis" to describe this concept of stability in the organism and proposed that homeostasis is preserved by "diffuse action of the sympathetic division" and the "particular action" of the parasympathetic division of the autonomic nervous system. "When we consider that the sympathetic, in emergencies, serves in a great variety of ways to preserve the stability of the organism, if that is threatened, the importance of

it's arrangement for simultaneous and unified action becomes evident" (Cannon, 1930). The general notion of widespread sympathetic influences was supported by anatomical investigations which revealed divergence within the sympathetic nervous system. Billingsley and Ranson (1918) hypothesized that one preganglionic nerve fiber may form synapses with up to thirty two postganglionic cells. Langley (1892) reported that sympathetic preganglionic neurons affecting the pupil, the nictitating membrane, the blood vessels of the head, the sub-maxillary gland and the heart originated in common thoracic spinal cord segments.

In recent years, it has become widely recognized that the sympathetic nervous system can produce distinct patterns of nerve discharge to control selectively, the functions of various effector tissues. Different components of sympathetic outflow are known to display different patterns of ongoing discharge and to be influenced selectively by descending supraspinal and various afferent nerve inputs (see e.g. Jänig, 1985).

ONGOING ACTIVITY OF SYMPATHETIC NERVES

Tonic Discharge of Sympathetic Nerves

Under control conditions, sympathetic nerves discharge spontaneously in animals and humans. This characteristic of

sympathetic nerves has been documented by numerous investigators, recording neural activity from preganglionic, as well as postganglionic nerve bundles, in both anesthetized and unanesthetized animals (Polosa, Mannard and Laskey, 1979). Such tonic activity of sympathetic nerves maintains tone within autonomic effector cells. This is demonstrated clearly by the pronounced decrease in arterial blood pressure seen after high cervical spinal cord transection (Sheehan, 1936). In addition, ongoing sympathetic activity contributes to precise regulation of effector function, as each nerve is capable of decreasing or increasing it's rate of discharge.

Although tonic activity of whole sympathetic nerves is readily demonstrated, in the anesthetized animal the majority of individual sympathetic preganglionic neurons do not spontaneously discharge (Coote and Westbury, 1979; Dembowsky et al. 1985; Gilbey, Numao, and Spyer, 1986; Jänig, 1985; McLachlan and Hirst, 1981; Polosa, 1968). Estimates of the number of preganglionic neurons that are spontaneously active range from 20% (Polosa, 1968) to 60% (Coote and Westbury, 1979). The presence or absence of spontaneous discharge may have functional significance. Jänig (1985) has reported that vasoconstrictor and sudomotor neurons to the hindlimb of the cat are spontaneously active, whereas vasodilator and pilomotor neurons are not. Sympathetic preganglionic and postganglionic neurons which discharge spontaneously, fire irregularly, at rates of approximately 1-4 Hz (Polosa et al.

1979). Polosa (1968), in discussing a physiological role for such slow discharge rates, proposed that "one of the functions of these neurons is to match the output frequency of the neural motor control system to that frequency which is adequate for an optimal muscle response." Indeed, Folkow et al. (1964) demonstrated that electrical stimulation of mesenteric nerves at frequencies almost as low as those occuring spontaneously (4-6 Hz) produced maximal steady-state increases in vascular resistance which ranged from 50-100% above control levels.

Synchronization of Sympathetic Discharge

The action potentials of sympathetic neurons are usually synchronized to produce oscillations in the mass discharge of sympathetic nerve bundles which are temporally related to the respiratory and cardiac cycles. Such patterns of sympathetic nerve firing were originally described by Bronk and co-workers (Adrian, Bronk and Phillips, 1932; Bronk, Ferguson, Margaria and Solandt, 1936) in recordings of cervical and abdominal sympathetic nerve activity in the cat and rabbit.

Synchronization of discharge of neurons within sympathetic nerves may have important physiological consequences. Such firing patterns may provide for spatial summation at the neuroeffector junction, to cause a greater release of neurotransmitter and a greater effector response.

Studies of the influence of sympathetic impulse patterns on contractile responses of rat mesenteric vessels *in vitro* (Nilsson, Ljung, Sjöblom and Wallin, 1985) and cat skeletal muscle vessels *in situ* (Andersson, 1983) have shown that nerve impulses which are delivered asynchronously produce less vascular tone than do phasically delivered impulses.

Respiratory-related Rhythms

Mass discharge of most sympathetic nerves increases during inspiration, reaching maximal levels at the end of inspiration and beginning of expiration (Adrian *et al*. 1932). The consistency of this pattern is demonstrated by generating post-stimulus histograms of nerve activity, triggered by recordings of phrenic nerve activity. Afferent nerves from the lungs, stimulated by mechanical stretch of the lungs, probably contribute to this firing pattern by reflexly inhibiting sympathetic nerve discharge (Cohen, Gootman and Feldman, 1980). However, respiratory-related activity patterns are present in vagotomized animals, indicating that this pattern is generated within the central nervous system (Adrian *et al*. 1932; Barman and Gebber, 1976).

Respiratory-related discharge patterns are not imposed on all components of sympathetic outflow uniformly. Mirgorodsky and Skok (1969) reported that approximately half of the spontaneously active neurons in the superior cervical

ganglion in cats discharged with a respiratory-related rhythm. Preiss, Kirchner and Polosa (1975) recorded activity from spontaneously firing fibers in the cervical sympathetic chain of the cat. They found that discharge of approximately 65% of the neurons displayed an inspiratory peak, discharge of approximately 5% of the neurons displayed an expiratory peak and firing of the remaining 30% of the neurons did not appear to be modulated by central respiratory-related inputs. In the rat, preganglionic neurons in the thoracic spinal cord projecting to the cervical sympathetic nerve also display these three distinct patterns of discharge, although the number of neurons which discharge maximally during phrenic nerve silence (i.e. expiration) is considerably higher (30%; Gilbey et al. 1986). Also, in the rat, there appear to be regional differences in the pattern of mass sympathetic outflow; mass discharge of cardiac, renal, splanchnic and adrenal nerves is maximal during inspiration, whereas that of cervical and lumbar sympathetic nerves is maximal during expiration (Numao, Koshiya, Gilbey and Spyer, 1987). Jänig's group has reported that tonic activity of vasoconstrictor neurons supplying cat hindlimb skeletal muscle, but not skin, displays strong repiratory-related rhythmicity (Blumberg, Jänig, Riekmann and Szulczyk, 1980; Gregor, Jänig and Wilprich, 1977). Thus, central respiratory-related drive is not distributed uniformly to all components of sympathetic

outflow. The functional significance of this specificity remains obscure.

Cardiac-related Rhythms

Strong cardiac-related rhythms in the mass discharge of sympathetic nerves have been reported by many investigators (Adrian et al. 1932; Bronk et al. 1936; Cohen and Gootman, 1970; Gebber, 1984; Kezdi and Geller, 1968; Ninomiya and Irisawa, 1975). Until the 1970's cardiac-related rhythms in firing of sympathetic nerves were thought to be a consequence of baroreceptor-induced inhibition of nerve activity during the systolic phase of the cardiac cycle. This idea was based on the knowledge that removal of baroreceptor afferent input caused a diminution of the phasic, pulse-sychronous component of nerve discharge and an increase in the continuous component of nerve discharge (Bronk et al. 1936; Ninomiya and Irisawa, 1975). Moreover, increased static pressures in an isolated carotid sinus attenuated the bursting pattern of renal nerve discharge, although increased sine wave pressures in the carotid sinus enhanced the pulse-synchronous firing pattern of the renal nerve (Kezdi and Geller, 1968). However, Gebber and co-workers (see Gebber, 1984 for review) have suggested that cardiac-related rhythms in sympathetic nerve firing are generated within brainstem neural circuits

and are entrained in a 1:1 relationship to the cardiac cycle by baroreceptor afferent nerve input. This hypothesis is based on the following observations. Shifts in the phase relations between carotid sinus baroreceptor nerve discharge and the cardiac-related rhythm in firing of efferent renal nerves were produced by slowing the heart rate by efferent vagal stimulation (Gebber, 1976), or by cardiac pacing (Barman and Gebber, 1983). A 2-6 Hz rhythm in the firing of sympathetic nerves persisted after sinoaortic denervation and vagotomy, but the oscillations in sympathetic nerve discharge were no longer phase-locked in a 1:1 relationship with the cardiac cycle (Barman and Gebber, 1980; Gebber and Barman, 1980; Taylor and Gebber, 1975). This rhythmicity in sympathetic nerve firing was present after mid-collicular decerebration, indicating that it was not dependent on forebrain structures (Barman and Gebber, 1980), a finding which differs from that of other investigators (Camerer, Stroh-Werz, Krienke and Langhorst, 1977). The oscillations in nerve discharge disappeared after high cervical spinal cord transection, indicating that the rhythm was generated supraspinally (Ardell, Barman and Geber, 1983; McCall and Gebber, 1975). Finally, neurons in the ventrolateral medulla which project to the intermediolateral cell column of the thoracic spinal cord, discharge with a 2-6 Hz periodicity in baroreceptor-denervated cats (Barman and Gebber, 1983; Barman, Gebber and Calaresu, 1984).

Some components of sympathetic outflow do not appear to fire with a cardiac-related rhythm. Only 20-30% of spontaneously active preganglionic neurons in the thoraco-lumbar spinal cord have cardiac-related activity patterns (Coote and Westbury, 1979; Gilbey, Peterson and Coote, 1982; Seller, 1973). Irisawa et al. (1973) reported that whereas pulse-synchronous bursts of action potentials characterized the ongoing mass activity of renal nerves, continuous discharges were dominant in the tonic activity of whole mesenteric nerves. Individual renal postganglionic neurons also have been shown to display strong cardiac-related rhythmicity in firing (Dorward, Burke, Jänig and Cassell, 1987; Meckler and Weaver, 1988). Ongoing activity patterns of individual fibers in mesenteric nerves have not been previously described. Jänig and his collaborators have shown that motility-regulating fibers innervating the pelvic viscera and sudomotor fibers directed to the hindlimb in cats do not fire with a cardiac-related rhythmicity (Jänig, 1985: Jänig, 1986; Jänig and McLachlan, 1987). In contrast, neurons innervating pelvic vessels and hindlimb skeletal muscle vessels display strong cardiac-related rhythmicity in firing. These studies suggest that it may be possible to ascertain the function of a neuron (i.e. vasomotor or not) based on it's pattern of ongoing activity (cardiac-related or not). However, Jänig and co-workers also have demonstrated that tonic activity of vasoconstrictor neurons to the

hindlimb cutaneous vasculature is very weakly correlated with the arterial pulse, indicating that neurons subserving similar functions (muscle and skin vasoconstrictor neurons) may have varying patterns of ongoing discharge (Blumberg et al. 1980; Gregor et al. 1977; Jänig, 1985).

Supraspinal Regulation of Sympathetic Tone

Descending Excitatory Projections

The experiments of Dittmar and Owsjannikow in the 1870's (see Gebber, 1984) established the importance of medullary structures in the maintenance of resting arterial pressure. These investigators made serial transections of the brain stem in the rabbit and observed the effects on blood pressure. They concluded that tonic activity of neurons located within the caudal one third of the pons and rostral two thirds of the medulla oblongata was crucial in maintaining the resting level of arterial blood pressure. Alexander (1946) later demonstrated that transection of the neuraxis at the level of the obex caused decreases in blood pressure which were accompanied by the cessation of ongoing cardiac nerve activity. Since that time, many investigators have attempted to locate specific medullary regions which generate or relay tonic vasomotor sympathetic drive (reviewed by Calaresu and Yardley, 1988; Hilton, 1986).

Early studies involved a search for sites in the bainstem which, when electrically stimulated, produced changes in blood pressure. Ranson and Billingsley (1916) stimulated the dorsal surface of the medulla and found a medial depressor region near the obex and a lateral pressor region just rostral to the obex. Wang and Ranson (1939) and Alexander (1946) extended these investigations by stimulating the depths of the medulla. They reported that the lateral pressor and medial depressor regions extended through the dorso-ventral extent of the brainstem, leading to the conclusion that the neurons generating tonic vasomotor tone were diffusely distributed throughout the lateral reticular formation.

Recent physiological and anatomical investigations of the central neural circuits involved in cardiovascular regulation have provided evidence that sympathetic vasomotor tone is generated within one or two specific sites within the medulla. Most attention has been focused on a group of neurons in the rostral ventrolateral medulla (RVLM) which appear to play a crucial role in the maintenance of tonic sympathetic nerve activity and resting blood pressure in cats (Dean and Coote, 1986; Guertzenstein, 1973; Guertzenstein and Silver, 1974; Hilton, Marshall and Timms, 1983; Kubo, Nugura, Kihara and Misu, 1986), rats (Benarroch, Granata, Ruggiero, Park and Reis, 1986; Ross, Ruggiero, Park, Joh, Sved, Fernandez-Pardal, Saavedra and Reis, 1984b) and rabbits

(Pilowsky, West and Chalmers, 1985). These cells, which are located superficially, in the region of the nucleus paragigantocellularis lateralis of the reticular formation (Guyenet and Brown, 1986; Lovick and Hilton, 1985) appear to be part of the C1 group of adrenaline-containing neurons (Goodchild, Moon, Dampney and Howe, 1984; Hökfelt, Fuxe, Goldstein and Johansson, 1974; Ross, Ruggiero, Joh, Park and Reis, 1984a). Many of these RVLM neurons are tonically active and have discharge which is correlated with the cardiac-related component of basal sympathetic nerve discharge (Barman and Gebber, 1983; 1985) and inhibited by the stimulation of baroreceptors (Barman and Gebber, 1985; Brown and Guyenet, 1984; McAllen, 1986a). Recently, pacemaker-like potentials have been recorded from RVLM neurons *in vitro*, and *in situ* after blocking excitatory afferent inputs to these cells (Sun, Hackett and Guyenet, 1988). Histological investigations have demonstrated projections from these neurons to the thoracic spinal cord (Amendt, Czachurski, Dembowsky and Seller, 1979; Caverson and Ciriello, 1987; Dampney, Czachurski, Dembowsky, Goodchild and Seller, 1987; Ross *et al*. 1984a). Electrophysiological experiments have shown such projections to descend in the dorsolateral funiculus and terminate in the intermediolateral cell column (Barman and Gebber, 1987; Goodchild *et al*. 1984; Lebedev, Krasyukov and Nikitin, 1986; Lovick, 1985; McAllen, 1986a) and central autonomic nucleus (Caverson *et al*. 1983)

of the spinal cord, regions known to contain sympathetic preganglionic perikariya (Dembowsky, Czachurski and Seller, 1985a).

Recent interest in the RVLM a source of vasomotor tone was kindled by Schläfke and Loeschke (1967) who demonstrated that bilateral localized cooling of a 2-mm^2 area on the ventral surface of the brainstem caused significant decreases in blood pressure. Feldberg and Guertzenstein (1972) reported that bilateral application of pentobarbital to the ventral surface of the brain stem caused pronounced depressor effects. Subsequently, Guertzenstein (1973) and Guertzenstein and Silver (1974) localized a site on the ventral surface of the medulla (the "glycine-sensitive area") where bilateral electrolytic lesions or topical application of the inhibitory amino acid glycine produced a fall in arterial blood pressure comparable to that observed after cervical spinal cord transection. These studies, using glycine, provided the first evidence that tonic activity of cells with somata located within the RVLM was crucial for the maintenance of resting levels of arterial pressure. Glycine is known to inhibit the discharge of cell bodies, but not of fibers (Werman, Davidoff, and Aprison, 1968) by increasing the chloride conductance of the neuronal membrane (Betz, 1987). These results have been confirmed by a number of workers who have shown that discrete lesions of the RVLM or topical application of glycine, or the inhbitory amino acid

gamma-aminobutyric acid (GABA) to the ventral surface of the medulla causes decreases in ongoing sympathetic nerve activity (Dean and Coote, 1986; Granata, Numao, Kumada and Reis, 1986), and hypotension, bradycardia and peripheral vasodilation (Bennaroch et al. 1986; Hilton et al. 1983; Marshall, 1986). Similar responses have been elicited by microinjections of inhibitory amino acids (Pilowsky et al. 1985; Ross et al. 1984b; Willette, Punnen-Grandy, Krieger and Sapru, 1987) into the region. In contrast, stimulation of neurons, but not fibers (Goodchild, Dampney and Blander, 1982), in the RVLM by microinjections of the excitatory amino acids, glutamate or homocysteate, or topical applications of glutamate or kainate, produces excitation of sympathetic discharge, pressor responses, tachycardia and peripheral vasoconstriction (Bennaroch et al. 1986; Dampney, Goodchild, Robertson and Montgomery, 1982; Dampney and McAllen, 1988; Gatti, Da Silva, Hamosh and Gillis, 1985; Gatti, Norman, Da Silva and Gillis, 1986; Goodchild et al. 1984; Lovick, 1985; 1987; Lovick and Hilton, 1985; McAllen, 1986b; 1986c; McAllen, Neil and Loewy, 1982; Pilowsky et al. 1985; Ross et al. 1984b; Willette, et al. 1987).

This evidence leads to the conclusion that neurons in the RVLM provide tonic excitatory drive to regulate the activity of sympathetic preganglionic vasomotor neurons. The neurotransmitter released by the spinally projecting RVLM neurons is unknown. Reis (Reis, Ruggiero and Granata, 1986)

has insisted that the neurons providing tonic vasomotor drive are part of the C1 group of adrenalin-containing cells. However, iontophoretic application of epinephrine onto sympathetic preganglionic neurons inhibits their discharge (Coote, Macleod, Fleetwood-Walker and Gilbey, 1981a; Guyenet and Cabot, 1981), suggesting that the C1-spinal projection is inhibitory, rather than excitatory. It is possible that epinephrine is co-localized in C1 neurons with other excitatory neurotransmitters (e.g. substance P or glutamate, see below) or that other, non-adrenergic cells may be involved in mediating responses from the RVLM. Indeed, only half of RVLM cells with spinal projections are immunoreactive for the adrenalin synthesizing enzyme phenylethenolamine-N-methyl transferase, indicating that a substantial part of this projection is non-adrenergic (Tucker, Saper, Ruggiero and Reis, 1987). Loewy and co-workers have proposed that substance P may transmit vasomotor responses following application of kainic acid to the ventral surface of the medulla (Loewy and Sawyer, 1982; Takano, Martin, Leeman and Loewy, 1984). Many cells in the RVLM which stain for substance P-like immunoreactivity (Lorenz, Saper, Wong, Ciaranello and Loewy, 1985; Pilowsky, Minson, Hodgson, Howe and Chalmers, 1986) project to the intermediolateral cell column of the spinal cord (Helke, Neil, Massari and Loewy, 1982). Iontophoretic application of substance P to preganglionic neurons increases their discharge (Gilbey,

McKenna and Schramm, 1983). Many other transmitters may potentially mediate tonic or reflex excitatory medullary inputs to sympathetic preganglionic neurons. These include neuropeptide Y, serotonin, vasopressin and oxytocin (Calaresu and Yardley, 1988; McCall, 1988). Recently, Morrison and Reis (1987) reported that sympathetic preganglionic excitatory responses evoked by electrical stimulation of the RVLM were abolished by iontophoretic application of the glutamate antagonist kynurenic acid, suggesting glutamate as a possible transmitter of these neurons.

Although the RVLM apparently plays a crucial role in regulating preganglionic vasomotor outflow, recent work by Barman and Gebber (1987) suggests that vasomotor tone is not generated within this region; rather, it is generated by neurons antecedent to those in the RVLM, perhaps in the dorsal medulla. A dorsal pressor area was first described by Wang and Ranson (1939) and Alexander (1946). In the early 1970's Gootman and Cohen (1971) and Nathan (1972) evoked excitatory responses in the splanchnic nerve by electrical stimulation of points in the dorsal medulla in cats. Although large lesions in the dorsal pressor region resulted in only small decreases in arterial blood pressure in the cat (Chai and Wang, 1968, Manning, 1965), lesions in this region in the rabbit produced severe hypotension, suggesting that this region functioned "as the so-called tonic vasomotor center of the brainstem" (Kumada, Dampney and Reis, 1979).

However, subsequent experiments indicated that such lesions destroyed fiber pathways originating in the RVLM which coursed dorsally before projecting to the spinal cord (Dampney and Moon, 1980). Nonetheless, Barman and Gebber have recently published a series of papers implying that neurons in the dorsal medulla of the cat may be a source of tonic vasomotor activity (Barman and Gebber, 1983; 1987; Gebber and Barman, 1985). They demonstrated that dorsal medullary neurons fired 35 ms earlier than neurons in the RVLM with respect to the cardiac-related bursts in sympathetic nerve activity. Firing of these dorsal medullary neurons is inhibited by baroreceptor activation, suggesting a sympatho-excitatory function, and they send projections to the RVLM and not to the spinal cord.

Specificity of Descending Excitatory Projections

A number of recent investigations have demonstrated functional specificity among neurons within the RVLM. McAllen (1986c) reported that chemical stimulation of neurons within the RVLM by microinjections of excitatory amino acid evokes pressor responses, peripheral vasoconstriction and increased mass discharge of a variety of sympathetic nerves, without affecting nictitating membrane, pilomotor or sudomotor activity, or intestinal motility. These results suggest that the RVLM-spinal projection selectively excites

vasomotor neurons. Barman et al. (1984) found that firing of some neurons in the RVLM was more strongly correlated with that of the external carotid than the renal nerve, whereas firing of others displayed the opposite relationship. Moreover, they showed that whereas some RVLM neurons could be antidromically activated by stimulation in widely separated spinal cord segments (T2, T6 and T11), others could only be activated by stimulation in T2, but not in T5, T6 or T11 (Barman and Gebber, 1985). These data indicate that some RVLM-spinal projections are limited to upper thoracic spinal segments. The RVLM appears to be topographically organized. Lovick (1987) and Dampney and McAllen (1988) reported that the contribution of various vascular beds to the increase in peripheral resistance caused by microinjection of excitatory amino acids into the RVLM varies according to the exact location of the injection site. The neuronal pools which affect sympathetic outflow to the heart, kidney and adrenal medulla appear to be located in the anterior end of the RVLM, whereas those affecting mesenteric and hindlimb vaso-constrictor activity appear to be found in the posterior portion of the region (Lovick, 1987). Within the posterior portion of the RVLM, neurons affecting hindlimb muscle vasoconstrictor activity have been found lateral to those affecting cutaneous vasoconstrictor discharge (Dampney and McAllen, 1988).

The investigations described above found evidence for specificity among neurons within the RVLM after activating them with excitatory amino acids. It is possible that the neurons within the RVLM which were activated in those studies were not tonically active. No one has determined if tonic activity of neurons within the RVLM is distributed selectively to certain components of sympathetic outflow by inhibiting their firing with inhibitory amino acids. Yet, there is evidence suggesting that not all components of sympathetic outflow depend upon excitatory drive from the medulla for the maintenance of ongoing discharge. Meckler and Weaver (1985) found that spinal cord transection in cats significantly decreased firing of cardiac and renal nerves, but splenic nerve activity was not significantly affected. Ninomiya and Irisawa (1975) reported that whereas discharge of renal and spenic nerves depended upon supraspinal sources of excitation, approximately half of the activity of mesenteric nerves could be generated at spinal levels. It has also been reported that ongoing activity of some (supposedly motility-regulating) neurons innervating the pelvic viscera is not affected by thoracic spinal cord transection, whereas that of other (supposedly vaso-constrictor) neurons is abolished (Bartel, Blumberg and Jänig, 1986). Transection of the spinal cord in rats actually increases the activity of some abdominal sympathetic

nerves, whereas lumbar sympathetic nerve activity is significantly reduced (Taylor and Schramm, 1987; see below).

Descending Inhibitory Projections

Descending inhibitory projections impinge on sympathetic preganglionic neurons. Tonic sympathoinhibition was first observed by Alexander (1946), who demonstrated that ongoing cardiac nerve discharge, which had been completely abolished by transection of the neuraxis at the level of the obex, resumed partially, following a low bulbar transection in cats. Cold blockade of the spinal cord in cats significantly increases the amplitude and duration, and significantly decreases the latency of the spinal component of a somatosympathetic reflex, suggesting that descending bulbospinal projections tonically inhibit this component of the reflex (Dembowsky, Czachurski, Amendt and Seller, 1980). More recently, Schramm and co-workers have reported that transection of the cervical spinal cord in rats significantly increases renal and spleno-gastric nerve activity, suggesting that spinal systems generating these sympathetic outflows are tonically inhibited by supraspinal inputs (Osborn, Livingstone and Schramm, 1987; Taylor and Schramm, 1987). Descending sympathoinhibitory pathways have been found in all four quadrants of the spinal cord (Barman, 1984). Neurons located in the medial depressor area, first described by

Ranson and Billingsley (1916) and Wang and Ranson (1939), may be a source of tonically active sympathoinhibitory pathways. Projections from the caudal raphe nuclei to the intermediolateral cell column of the spinal cord have been demonstrated histologically (Loewy, 1981). Electrophysiological studies demonstrated that this region of the medulla contained sympathoexcitatory, as well as sympathoinhibitory neurons, but only the sympathoinhibitory neurons projected to the intermediolateral cell column (Morrison and Gebber, 1984). Electrical stimulation of the midline medulla inhibited evoked responses in T_3 and T_{10} white rami (Coote and Macleod, 1975; Gilbey, Coote, Macleod and Peterson, 1981). Moreover, Cabot, Wild and Cohen (1979) reported that electrical stimulation of raphe nuclei completely inhibited the ongoing discharge of single sympathetic preganglionic neurons. The role of the raphe in tonic sympathoinhibition was clearly demonstrated by two studies in which electrolytic lesions of the caudal raphe nuclei caused significant increases in renal (Barman and Gebber, 1978) and cardiac nerve activity (McCall and Harris, 1987). The finding that IPSP's rarely can be recorded from sympathetic preganglionic neurons argues against the existence of a tonically active sympathoinhibitory projection synapsing on these cells (Dembowsky et al. 1985b). Such a projection may synapse on neurons within the spinal cord, or within the medulla, which are antecedent to the preganglionic neurons. Thus, the decrease

in sympathetic outflow observed following stimulation of the midline medulla would be attributed to disfacilitation, rather than direct inhibition, of sympathetic preganglionic discharge. Spinal interneurons which are engaged in inhibitory autonomic reflexes have been documented (Gebber, Taylor and Weaver, 1973; McCall, Gebber and Barman, 1977). McCall (1987) has suggested that neurons in the midline medulla tonically inhibit sympatho-excitatory neurons in the RVLM. This inhibition is independent of baroreceptor inputs.

Another source of tonic sympathoinhibition is a region in the caudal ventrolateral medulla (CVLM), containing the A1 norepinephrine cell group (See Calaresu and Yardley, 1988 for review). Electrical or chemical stimulation of this region decreased arterial blood pressure (Blessing and Reis, 1982; Willette et al. 1987) and decreased splanchnic nerve activity (Willette et al. 1987). Conversely, electrolytic or chemical lesions of this region (Blessing, West and Chalmers, 1981; Granata et al. 1986) or inhibition of tonic activity of neurons within the CVLM by microinjections of the GABA agonist muscimol (Willette, Punnen, Kreiger and Sapru, 1984; Willette et al. 1987) produced large increases in blood pressure and significant increases in renal nerve discharge (Granata et al. 1986). The cardiovascular responses were abolished by microinjection of tetrototoxin (Granata et al. 1986) or muscimol (Willette et al. 1984) into the RVLM, suggesting that elements within the CVLM tonically inhibit

sympatho-excitatory neurons located in the RVLM. In addition, anatomical investigations have shown that the CVLM sends projections to the spinal cord (Hudson, Fuxe, Goldstein and Kalia, 1986; McKellar and Loewy, 1982).

REFLEX ACTIVITY OF SYMPATHETIC NERVES

Arterial Pressoreceptors

Receptors and Pathways

Arterial pressoreceptors (baroreceptors), in the carotid sinus and aortic arch contribute to cardiovascular homeostasis by regulating arterial blood pressure (Heymans and Neil, 1958; Kirchheim, 1976; Spyer, 1981). These receptors, located largely in the adventitia of the vessel wall, respond to distortion of their receptor regions by producing action potentials that are transmitted to the central nervous system (Heymans and Neil, 1958). Afferent fibers from carotid sinus baroreceptors course through the carotid sinus nerve, a branch of the glossopharyngeal nerve. Afferent fibers from aortic arch baroreceptors travel in the aortic depressor nerve, often found in a sheath with the vagus nerve. Both nerves contain myelinated and unmyelinated fibers (Abboud and Thames, 1983).

In most species, baroreceptor afferent fibers are not spontaneously active at pressures below 40-70 mm Hg (Abboud and Thames, 1983; Kirchheim, 1976). When the pressure in an isolated carotid sinus or aortic arch is increased from approximately 70 mmHg to 150 mmHg discharge rates of baroreceptor afferent nerves increase linearly. At pressures of approximately 175 mmHg to 200 mmHg the afferent nerves reach their maximal firing frequency. Although some differences exist, in general, carotid sinus and aortic arch baroreceptors respond similarly to fluctuations in blood pressure, leading to similar reflex effects (Abboud and Thames, 1983; Kirchheim, 1976).

Initial studies of the projections of carotid sinus and aortic baroreceptors involved sectioning the ninth and tenth cranial nerves and subsequently examining the medulla for sites of degeneration. These studies indicated that fibers from both nerves terminated densely in the intermediate area of the nucleus tractus solitarius (NTS; see Spyer, 1981 for review). Central projections of baroreceptor afferent nerves have been studied most extensively in cats and rabbits. Anatomical (Ciriello and Calaresu, 1981; Davies and Kalia, 1981; Kalia and Welles, 1980) and electrophysiological investigations (Ciriello and Calaresu, 1981; Jordan and Spyer, 1977) have shown that fibers in the carotid sinus and aortic depressor nerves terminate bilaterally, with predominant ipsilateral projections, in the medial,

dorsomedial, dorsolateral and commissural subnuclei of the NTS. Projections to other subnuclei also have been described (Ciriello and Calaresu, 1981). Spyer and co-workers (1984) have antidromically activated cell bodies of baroreceptor afferent nerves in the ipsilateral nodose and petrosal ganglia from the lateral, ventrolateral, medial and dorsomedial subnuclei of the NTS. The functional role of the NTS was demonstrated by Miura and Reis (1972) who reported that small bilateral lesions in the nucleus within 1 mm rostral to the obex abolished all reflex blood pressure and heart rate changes to electrical stimulation of carotid sinus baroreceptor afferent fibers.

Baroreceptor-induced inhibition of sympathetic nerve activity has been shown to be mediated at supraspinal (Biscoe and Samson, 1970; Coote and Macleod, 1974), as well as spinal (Coote, Macleod, Fleetwood-Walker and Gilbey, 1981b; Gebber et al. 1973; McCall et al. 1977; McLachlan and Hirst, 1981) sites. However, the central pathways engaged in the baroreceptor reflex arc are unknown. Projections from the NTS terminate in various regions including the parabrachial and Kölliker-Fuse nuclei, the paraventricular nucleus of the hypothalamus, the nucleus ambiguus, the RVLM and CVLM and the intermediolateral cell column of the spinal cord (Loewy and Burton, 1978). Granata et al. (1985) suggested that baroreceptor reflexes are mediated by the RVLM in the rat. They reported that combined lesions of the right NTS and left

RVLM did not alter resting arterial pressure, but did abolish the reflex depressor response and bradycardia elicited by baroreceptor stimulation. As the RVLM is thought to receive a GABAergic inhibitory input from the CVLM (Granata et al. 1986) the involvement of CVLM GABAergic neurons in baroreceptor reflexes has been proposed (Willette et al. 1984). Two recent reports support this hypothesis. Sun and Guyenet (1987) have demonstrated that bilateral microinjections into the RVLM, of the GABA receptor antagonist bicuculline, abolished the sympathoinhibition produced by baroreceptor stimulation. Gordon (1987) has reported that selective blockade of N-methyl-D-aspartate acid receptors in the CVLM abolished depressor responses evoked by aortic nerve stimulation. Taken together, the data support the notion that baroreceptor reflexes are mediated by an excitatory glutaminergic projection to the CVLM, and an inhibitory GABAergic projection from the CVLM to the RVLM.

Reflex Effects of Baroreceptors

Activation of baroreceptors by increasing pressure in an isolated carotid sinus or aortic arch decreases heart rate, blood pressure and resistance in most vascular beds. Unloading baroreceptors produces the opposite effects; tachycardia, pressor responses and peripheral vaso-constriction (Kirchheim, 1976). Adrian et al. (1932) were

among the first investigators to describe the sympatho-inhibition produced by activation of baroreceptors, a phenomenon which has been studied by many investigators (Kirchheim, 1976).

The first investigations of baroreceptor effects on different vascular beds were those of Löfving (1961) and Folkow et al. (1961). These investigators reported that occlusion of the carotid artery in cats caused marked decreases in skeletal muscle blood flow, moderate decreases in intestinal blood flow and virtually no change in cutaneous or renal blood flow. Similar results have been reported by others (Hadjiminas and Öberg; 1968; Kendrick et al. 1972). Electrophysiological studies also have demonstrated that different components of sympathetic outflow are not uniformly effected by barorecptor inputs. Jänig and colleagues demonstrated that sympathetic nerve discharge to skeletal muscle blood vessels was strongly influenced, whereas sympathetic activity to cutaneous vessels was only weakly influenced by baroreceptor inputs (Blumberg et al. 1980; Jänig, 1985). Others have reported similar weak effects of baroreceptors on cutaneous vasoconstrictor nerves (Iriki, Kozawa, Korner and Dorward, 1979; Ninomiya, Irisawa and Nisimaru, 1975). Ninomiya and Irisawa (1975) reported that stimulation of baroreceptors by increasing blood pressure caused large decreases in the discharge of splenic and renal nerves, while firing of mesenteric nerves was not affected

significantly. In a similar series of experiments, increases in blood pressure caused greater decreases in renal than in gastric nerve activity (Nisimaru, 1971). Irisawa et al. (1973) actually observed increases in the activity of some mesenteric nerves during large increases in blood pressure caused by intravenous injections of norepinephrine. Tobey and Weaver (1987) recently have reported greater decreases in renal than splenic nerve discharge in response to norepinephrine-induced increases in blood pressure. Obviously, as baroreceptor reflex effects on different sympathetic nerves may be quantitatively or qualitatively different, responses of individual components of sympathetic outflow should not be interpretted as representative of the sympathetic nervous system as a whole.

Viscero-sympathetic Reflexes

Autonomic reflexes originating from organs in the thoracic, abdominal and pelvic cavities have been investigated extensively. The sensory innervation of of the viscera and visceral-autonomic reflexes have been recently reviewed (Abboud and Thames, 1983; Cervero and Morrison, 1986; Jänig and McLachlan, 1987). This literature review will concentrate on sympathetic reflexes originating from the heart and the intestine.

Reflexes Originating in the Heart

Cardiac Receptors with Vagal Afferent Fibers

Myelinated and unmyelinated afferent fibers project centrally in the vagal nerves and terminate in the intermediate and caudal regions of the NTS (Donald and Shepherd, 1978). Myelinated cardiac vagal afferent fibers innervate mechanoreceptors in the walls of all four chambers of the heart and the venoatrial junctions. Receptors located at the venoatrial junctions respond to increases in cardiac volume. Receptors located in the atria are activated mainly during atrial systole and increase their discharge during positive ionotropic interventions. The ventricular mechanoreceptors fire during ventricular systole and increase their discharge in response to distension. Non-myelinated vagal fibers (C fibers) also innervate mechanosensitive receptors in all four chambers of the heart and the coronary vasculature (Abboud and Thames, 1983; Donald and Shepherd, 1978). Many small myelinated and all unmyelinated vagal afferents can be activated by various irritant chemical substances (Coleridge and Coleridge, 1980).

Reflex Effects of Cardiac Vagal Afferent Nerves

Electrical stimulation of cardiac vagal afferent fibers increases heart rate and causes peripheral vasodilation

(Öberg and White, 1970b). Activation of these nerves by distension of balloons at the pulmonary vein-atrial junction produced reflex tachycardia, pressor responses (Ledsome and Linden, 1964), a diuresis and naturesis (Wennergren, Henriksson, Weiss and Öberg, 1976) and non-uniform responses in the discharge of different sympathetic nerves (Karim, Kidd, Malpus and Penna, 1972). Sympathetic nerve activity to the heart was increased, sympathetic nerve activity to the kidney was decreased and sympathetic outflow to the spleen and to hindlimb skeletal muscle was not affected. In contrast, stimulation of vagal receptors by injections of veratridine into the left atrium produced significant decreases in splenic nerve activity. Still, the inhibition of discharge in splenic nerves was significantly less than that of renal nerves (Weaver, Fry and Meckler, 1984).

Tonic influences of vagal afferent nerves on vasomotor activity have been demonstrated by vagal cold-block and vagotomy and by hemorrhage in sino-aortic denervated animals. Vagotomy and cold-block caused tachycardia and constriction in skeletal muscle, mesenteric and renal vascular beds (Öberg and White, 1970a; Mancia, Donald and Shepherd, 1973; Pelletier et al. 1971) and constriction in the splanchnic venous bed (Pelletier et al. 1971). Tonic inhibitory effects appeared to be preferentially distributed to the heart, kidney and splanchnic circulation; sympathetic outflow to skeletal muscle and skin was minimally influenced by tonic

vagal nerve inputs (Öberg and White, 1970b; Pelletier et al. 1971). This pattern of sympathoinhibition is qualitatively different from that originating from arterial baroreceptors. As described previously, arterial baroreceptors appear to influence preferentially, sympathetic outflow to skeletal muscle. Cardiac receptors with vagal afferent axons also reflexly regulate renin secretion and vasopressin secretion (Abboud and Thames, 1983).

Cardiac Receptors with Fibers in Sympathetic Nerves

Afferent fibers coursing through the cardiac sympathetic nerves innervate mechanosensitive receptors in all four chambers of the heart, the coronary vessels and the aorta. The axons may be myelinated or unmyelinated. The cell bodies of these "cardiac sympathetic afferent fibers" (Malliani, 1982) are located in the dorsal root ganglia of the upper thoracic segments of the spinal cord. Within the spinal cord, these fibers project rostrocaudally through Lissauer's tract and terminate largely in Rexed's laminae I, V and VII (DeGroat, 1986). In general, these fibers fire in a temporal relation to the cardiac cycle and their discharge rates are increased during increases in pressure. Ventricular receptors with non-myelinated axons do not appear to fire in synchrony with ventricular systole, although they are responsive to both ventricular contraction and distension

(Malliani, 1982). The receptors of sympathetic afferent fibers also are chemosensitive; their firing is increased by chemical substances such as bradykinin, potassium or veratridine applied topically to the epicardial surface (Coleridge and Coleridge, 1980) or administered by an intra-coronary route (Malliani, 1982; Malliani, Lombardi and Pagani, 1986).

Reflex Effects of Cardiac Sympathetic Afferent Nerves

Electrical stimulation of cardiac sympathetic afferent nerves in vagotomized cats produced pressor responses which were abolished by alpha-receptor blockade (Peterson and Brown, 1971). Small pressor responses were elicited by selective activation of A-delta fibers. Increasing the stimulus current to activate C fibers caused greater increases in blood pressure. The neural circuits mediating this reflex were shown to be complete within the spinal cord. Electrical stimulation of these nerves also increases myocardial contractility and heart rate, decreases the discharge of vagal efferent nerves and increases that of cardiac sympathetic nerves (Malliani, 1982). Activation of cardiac sympathetic afferent nerves by mechanical or chemical stimulation, or by myocardial ischemia causes sympathetic excitation leading to tachycardia, peripheral vasoconstriction and pressor responses (Malliani, 1982 for

review). Epicardial superfusion of bradykinin has been shown to cause pressor responses and widespread neural excitation in vagotomized, baroreceptor-denervated cats (Weaver, Meckler, Fry and Donoghue, 1983). Thus, stimulation of cardiac receptors with sympathetic afferent fibers causes reflex responses opposite to those elicited by vagal afferent nerve stimulation. When total cardiac innervation is intact sympathoinhibitory reflexes initiated by vagal afferent nerves often are predominant (Donald and Shepherd, 1978).

Reflexes Originating in the Intestine

Receptors and Sensory Innervation

Afferent nerve fibers from the small intestine are found in the vagus and the splanchnic nerves. The splanchnic nerves also contain afferent fibers from the lower esophagus, stomach, gall bladder, liver, spleen and pancreas, and represent the afferent arm of many viscero-autonomic reflexes (Jänig and Morrison, 1986). Central projections of the greater splanchnic nerve in cats have been studied extensively by Kuo and co-workers. Following application of HRP to the central cut end of the greater splanchnic nerve, peroxidase-labeled somata were found in ipsilateral dorsal root ganglia, from T1 to T13, with a peak distribution in ganglia T5 - T11 (Kuo and DeGroat, 1985). A similar distribution has been demonstrated in the rabbit (Torigoe

et al. 1985). The majority of splanchnic afferent neurons were small to medium size (<40μm), however some larger cells (45-80μm) were observed. These data are consistent with the results of an electron microscopy study which indicated that splanchnic afferent nerves contain many thin C fibers and A-delta fibers, but few larger A-beta fibers (Kuo, Yang, Yamasaki and Krauthamer, 1982; DeGroat, 1986). Electrophysiological investigations also have demonstrated a paucity of large diameter afferent fibers in the thoracic (Floyd and Morrison, 1974; Ranieri, Mei and Crousillat, 1973) and lumbar (Bahns, Ernsberger, Jänig and Nelke, 1986) splanchnic nerves.

Kuo and DeGroat (1985) reported that within the spinal cord splanchnic afferent fibers projected rostrocaudally through Lissauer's tract, sent collaterals along the lateral margin of the dorsal horn and terminated in Rexed's laminae I, V and VII. The predominant distribution was ipsilateral, but some fibers did terminate contralaterally, in laminae I and V. As these laminae contain cell bodies of the spinoreticular and spinothalamic tracts (Foreman and Blair, 1988) these splanchnic afferent fiber projections may be contributing to supraspinal sympathetic reflexes, as well as sensory perception. In addition, the projection to lamina VII was in the vicinity of the intermediolateral cell column, suggesting a monosynaptic connection with sympathetic preganglionic neurons. Splanchnic afferent fibers also formed a prominent supraspinal projection in the medial

dorsal columns which terminated in the caudal gracile nucleus. As discussed by Kuo and DeGroat (1985), this finding is consistent with those of electrophysiological studies. Similar spinal and supraspinal projections have been described for afferent fibers in the renal, cardiac, hypogastric and lumbar splanchnic nerves (DeGroat, 1986).

Splanchnic afferent fibers are spontaneously active. Their endings are mechanosensitive and chemosensitive (Jänig and Morrison, 1986). Large diameter, rapidly conducting fibers are thought to innervate pacinian corpuscles associated with the arteries supplying the intestine. Gammon and Bronk (1935) first described such receptors in the mesentery of the cat; their axons disharged during the systolic phase of the cardiac cycle, suggesting that the receptors subserved a baroreceptor function. However, the effective stimulus for increasing the discharge of these fibers was distension of the mesenteric vessels, rather than increased arterial pressure, indicating that they innervated blood volume receptors, rather than pressoreceptors. This finding has been recently confirmed (Martin and Longhurst, 1986). Thin myelinated and unmyelinated afferent fibers are thought to innervate slowly adapting mechanoreceptors, which respond to distension and contraction of the gastrointestinal tract, traction on the mesenteries, and punctate stimulation (Bessou and Perl, 1966; Floyd and Morrison, 1974; Gernandt and Zotterman, 1946; Longhurst, Kaufman, Ordway and Musch,

1984a; Ranieri et al. 1973). Longhurst and co-workers (Longhurst et al. 1984a; Lew and Longhurst, 1986) proposed that endings of C fibers are mechanically-insensitive and that chemical stimuli are most effective in activating these receptors. Many workers have documented that receptors of both A-delta and C fibers are sensitive to chemical stimuli. The algogenic substances bradykinin and capsaicin frequently have been used to activate these receptors and study their reflex effects (Jänig and Morrison, 1986).

Central Neurotransmitters of Visceral Afferent Fibers

Investigations using Capsaicin

The central neurotransmitters of visceral afferent fibers are unknown, but there is a wealth of evidence indicating that small myelinated and unmyelinated visceral afferent neurons contain a number of biologically active peptides, including substance P, vasoactive intestinal polypeptide, leucine enkephaline, cholecystokinin and somatostatin (Dockray and Sharkey, 1986). Extensive deposits of these substances are found in cells in dorsal root ganglia, in dorsal roots and in nerve terminals in the dorsal horn of the spinal cord in rats and cats (DeGroat, 1986: Dockray and Sharkey, 1986; Massari, Tizabi, Park, Moody, Helke and O'Donohue, 1983). Substance P has been proposed to be a transmitter of small unmyelinated sensory neurons that

relay information from nociceptors (see Pernow, 1983 for review). The iontophoretic application of substance P to cells in the dorsal horn of the cat spinal cord selectively increases the discharge of neurons which are also activated by noxious thermal or mechanical stimuli, whereas activity of neurons which respond to light pressure or hair movement is unaffected or depressed (Henry, 1976; Sastry, 1979). The release of substance P-like immunoreactivity from the spinal cord following stimulation of unmyelinated and thin myelinated afferent fibers of the sciatic nerve of cats has been reported. This release was inhibited by intrathecal infusions of morphine (Yaksh, Jessel, Gamse, Mudge and Leeman, 1980). In conscious human subjects stimulation of these fiber groups evokes painful sensations (Turebjörk and Hallin, 1973).

The neurotoxin capsaicin (8-methyl-n-vanillyl-6 nonamide), the irritant substance in hot peppers, has proved to be a valuable tool in studying the organization and function of small unmyelinated afferent fibers. When given to neonatal rats it causes selective, permanent degeneration of these afferent fibers (Fitzgerald, 1983). Chronic systemic administration to adult rats results in the depletion of substance P-like immunoreactivity in small sensory neurons and in their terminals in the dorsal horn of the spinal cord (Jessell, Iversen and Cuello, 1978; Burks, Buck and Miller, 1985). The extent of the depletion is

greatest in laminae I - III (Fitzgerald, 1983). No depletion of substance P-like immunoreactivity occurs in the ventral horn, indicating that capsaicin selectively affects primary afferent neurons.

Substance P or other capsaicin-sensitive peptides appear to be involved in transmitting nociceptive information from the abdominal viscera. Capsaicin pretreatment attenuates writhing responses in rodents induced by intraperitoneal injections of acetylcholine or phenylquinone (Cervero and McRitchie, 1982; Gamse, 1982). This treatment also attenuates viscero-visceral excitatory sympathetic reflexes caused by intraperitoneal injections of bradykinin (Cervero and McRitchie, 1982) or iodine (Holzer, Schluet, Lippe and Sametz, 1987), and cardiovascular reflexes induced by intestinal distension (Lembeck and Skofitsch, 1982). In addition, substance P has been proposed to be a spinal cord transmitter of hepatic afferent neurons which may be stimulated by bradykinin to elicit activation of the hypothalamo-hypophysial system in rats (Stoppini, Barja, Mathison and Baertschi, 1984).

Reflex Effects of Intestinal Afferent Nerves

Electrical stimulation of visceral (Franz, Evans and Perl, 1966) or mixed (visceral and somatic; Beacham and Perl, 1964) afferent nerves in cats evokes excitatory responses in

the firing of sympathetic preganglionic neurons. These investigators were unable to demonstrate temporal or spatial facilitation of reflex responses, suggesting that the evoked responses engaged only a limited number of preganglionic neurons. Electrical activation of intestinal afferent nerves in cats produced non-uniform vascular responses; pronounced constriction of the renal vascular bed, moderate constriction in the intestinal bed and dilation in the hindlimb vasculature (Johansson and Langston, 1964).

Receptors with mechanosensitive endings have been activated by passive distension or contraction of the hollow viscera, producing increases in arterial blood pressure (Floyd et al. 1982; Longhurst, Spilker and Ordway, 1981; Weaver, 1985) and sympathetic nerve discharge (Floyd et al. 1982; Weaver, 1985). Ninomiya and co-workers have preferentially excited sympathetic efferent outflow to the intestine by distending a segment of the viscus with physiological saline. Renal and splenic efferent nerves were not engaged in this excitatory reflex, nor was arterial pressure affected; thus it appeared that this reflex response was selectively directed to neurons regulating intestinal motility (Ninomiya and Irisawa, 1975; Ninomiya et al. 1974). Jänig and co-workers also have described motility-regulating neurons which are selectively affected by visceral afferent inputs (Jänig, 1986).

Congestion of the mesenteric vasculature by obstruction of the portal vein or by infusion of blood caused increased efferent nerve discharge to the intestine (Andrews, Andrews and Orbach, 1972). Similarly, congestion of the splenic vasculature increases blood pressure, heart rate and greater excitation of splenic than of renal efferent nerve activity (Tobey and Weaver, 1987). The receptors activated by vascular congestion apparently are analogous to the volume receptors described by Gammon and Bronk (1935). Tuttle and McCleary (1979) elicited excitatory reflex responses by occlusion of the superior mesenteric artery, a maneuver which should have inactivated such volume receptors. However, in their investigation, the possibility exists that chemical stimuli caused the reflex responses.

Many investigators have produced excitatory sympathetic reflexes following stimulation of visceral receptors with the algogenic substances capsaicin and bradykinin (Baraz, Khyutin and Molnar, 1968; Calaresu et al. 1984; Floyd et al. 1977; Guzman, Braun and Lim, 1962; Haupt, Jänig and Kohler, 1983; Lew and Longhurst, 1986; Longhurst et al. 1984b; Meckler and Weaver, 1988; Reimann and Weaver, 1980). These substances may activate mechanosensitive receptors, chemically-sensitive receptors or polymodal receptors (Jänig and Morrison, 1986). Guzman et al. (1962) evoked pseudaffective responses (vocalization, hyperpnea, hypertension) in dogs and cats following intra-arterial injections of bradykinin in

abdominal organs and proposed that the peptide was stimulating nociceptors. Intra-arterial injections of capsaicin or potassium into the superior mesenteric artery in cats caused systemic pressor responses and increased renal nerve activity (Baraz et al. 1968; Khayutin, Mitsányi, Sonina and Erdélyi, 1969). Similarly, applications of bradykinin and capsaicin to the gastric serosa in cats increased blood pressure, heart rate, contractility and systemic vascular resistance (Longhurst, 1984b).

The neural circuits mediating many viscero-sympathetic reflexes appear to be complete within the spinal cord. Excitatory responses in firing of preganglionic nerves evoked by electrical stimulation of visceral (Franz et al. 1966) or mixed (Beacham and Perl, 1964) nerves have been observed in acutely spinalized cats. Beacham and Kunze (1969) stimulated mechanosensitive receptors in the kidney by increasing ureteral pressure and elicited reflex discharges in efferent renal nerves and vasomotor responses in spinal cats. Increases in blood pressure (Downman and McSwiney, 1946) and efferent mesenteric nerve activity (Andrews et al. 1972) have been produced by stimulation of intestinal receptors in spinal cats. Excitatory sympathetic reflexes, mediated by exclusively spinal neural circuits, also have been elicited by chemical stimulation of cardiac (Weaver, Fry, Meckler and Oehl, 1983) and splenic (Meckler and Weaver, 1988) receptors.

Weaver and co-workers have documented that stimulation of visceral receptors causes reflex pressor responses, tachycardia and excitation of splenic and renal nerve discharge. Depending on the origin of the stimulus these reflex effects may or may not be uniformly distributed to these two nerves. Chemical stimulation of splenic receptors by intra-arterial injections of bradykinin or capsaicin into a vascularly isolated spleen caused significantly greater excitation of efferent splenic than renal nerve activity (Calaresu, Tobey, Heidemann and Weaver, 1984). In contrast, stimulation of intestinal receptors by serosal application of bradykinin evoked greater renal than splenic excitation (Meckler and Weaver, 1988), and stimulation of urinary bladder mechanoreceptors caused equivalent excitation of these two components of sympathetic discharge (Weaver, 1985). Responses of splenic nerves exceeded those of renal nerves only when receptors in the spleen, and not when other abdominal viscera were stimulated, suggesting that viscero-sympathetic reflexes may be organized according to Sherrington's "local sign" principle (1906), to provide the largest output to the organ from which the reflex originates. This organization has been proposed to regulate the pattern of reflex responses caused by stimulation of receptors in several organs including the heart (Malliani, 1982), urinary bladder (Floyd et al. 1982) and small intestine (Ninomiya et al. 1974).

Heterogeneous responses to visceral stimulation among individual fibers within efferent sympathetic nerve bundles have been demonstrated. Jänig and co-workers described subgroups of neurons in the lumbar splanchnic and hypogastric nerves which responded to stimulation of visceral afferent nerves, but not not to stimulation of arterial baroreceptors or chemoreceptors. These investigators proposed that such neurons regulated visceral motility functions. A second subgroup of fibers, which responded to baroreceptor and chemoreceptor inputs, but not to visceral afferent inputs, were classified as visceral vasoconstrictor neurons (Jänig, 1986; Jänig and McLachlan, 1987). Meckler and Weaver (1988) found that only half of the fibers in splenic nerves were engaged in excitatory reflexes caused by chemical stimulation of intestinal receptors. Conversely, firing of virtually all splenic fibers was increased following stimulation of splenic receptors. Rogenes (1982) observed heterogeneous responses among renal neurons elicited by chemical stimulation of renal afferent nerves. These findings demonstrate that it is inappropriate to regard whole sympathetic nerves as homogeneous populations of axons with identical synaptic inputs. Although recordings of ongoing and reflex activity from whole sympathetic nerves may provide some information about the organization of the sympathetic nervous system, single fiber recordings are necessary to accurately determine the degree of specificity within sympathetic outflow.

METHODS

GENERAL METHODS

Preparation of animals-cats. Forty nine cats of either sex (1.8-5.0 kg) were anesthetized with intravenously administered alpha-chloralose (60-80 mg/kg; Sigma Chemical Co., St. Louis, MO.). Supplementary doses (8-20 mg/kg) were given as needed. Body temperature was monitored with an esophageal or a rectal probe and maintained at 37° C with a circulating hot water pad. A catheter (PE-90 or PE-160; Clay Adams, Parsippany, NJ) was passed through the urethra to allow continuous emptying of the urinary bladder. Cannulae were passed into the abdominal aorta via the femoral arteries (PE-160) and into the inferior vena cava through a femoral vein (PE-90) for monitoring systemic arterial pressure and withdrawing blood for analyses of arterial gas pressures and for administration of fluids or drugs, respectively. A tracheostomy tube was inserted and cats were artificially respired with room air (Harvard Apparatus 665 animal repirator, Millis, MA). Gallamine tiethiodide (4-5 mg/kg, Davis-Geck, Pearl River, NY; Rhone-Poulenc, Montreal, Quebec) was given to induce muscle relaxation during surgery. Additional gallamine (2-5 mg/kg) was administered as needed during the experiment after assessment of the animal's plane of anesthesia. This assessment was based on the cat's

palpebral and paw pinch reflexes, the stability of the blood pressure, and the size of the pupils. End tidal CO_2 was continuously monitored (CO_2 analyzer, model 901-MKZ, P.K. Morgan, UK, or model LB-2, Sensormedics, Anaheim, CA) and maintained at 3.0%-4.5%. Arterial blood samples were periodically withdrawn to measure pH and blood gas pressures (Corning blood gas analyzer, model 165, Medfield, MA). Acceptable values were pH: 7.35-7.45, $PaCO_2$: 25-40 mmHg, and PaO_2: > 80 mmHg. Acid/base disorders were corrected by adjusting the respiratory volume and/or rate, or by infusing an appropriate volume of a 1.0 M sodium bicarbonate solution.

The renal and mesenteric arteries were exposed by a left retroperitonial approach. In some cats, the thirteenth rib was removed and the diaphraghm was reattached to the intercostal muscles of the twelfth rib to obtain adequate access to the mesenteric artery. Renal and mesenteric nerves were identified on their respective arteries and small bundles were carefully dissected free from surrounding tissue, severed and desheathed. Exposed nerves were covered with warm mineral oil and all exposed tissue was coated with petroleum jelly to prevent dehydration. A pneumothorax was made to eliminate artifacts in the neural recordings caused by movement associated with ventilation.

Data aquisition. Systemic arterial pressure was monitored using Gould-Statham P-23 ID transducers (Gould Inc, Oxnard CA). Heart rate was computed from the arterial pressure pulse using a Grass Instruments tachograph (model 7P4H, Grass Instruments, Quincy, MA). Neural activity was recorded using bipolar platinum-iridium electrodes connected to a high-impedence input stage (model H1P511, Grass Instruments) and amplified at bandwidths of 30 Hz - 3 kHz or 1 Hz - 3 kHz (Grass P511 preamplifiers). All neural activity was monitored on an oscilloscope (Tektronix 5113 and 5223) and audio amplifier (Grass AM 8 audio monitor). The neural signals were full-wave rectified and cummulatively integrated over 10 s intervals (Grass Instruments 7P10 integrators), and displayed along with arterial pressure and heart rate on a Grass Instruments polygraph. All physiological signals were recorded on electromagnetic tape (Vetter model D, Rebersburg, PA; Racal, model 7DS, UK) for subsequent off-line analyses.

SPECIFIC METHODS - RESPONSES OF MESENTERIC AND RENAL NERVES TO THE STIMULATION OF INTESTINAL RECEPTORS AND PRESSORECEPTORS.

Multifiber recordings

Preparation of animals (13 cats). In these experiments, intestinal receptors were stimulated by the injection of bradykinin into the vascularly isolated intestinal circulation. The small intestine was approached by a retroperitoneal incision and vascularly isolated as shown in Figure 1. Ligatures were tightened around the pyloric sphincter and the ileo-colonic junction to prevent collateral circulation from the adjacent viscera. The superior mesenteric artery and vein were snared with polyethylene tubing (PE-10) distal to the colonic artery and vein without damaging the mesenteric nerves. When the mesenteric snares were tightened, the small intestinal vasculature was isolated without interrupting blood flow to the other viscera. One branch of the superior mesenteric artery distal to the snares was cannulated with PE-50 tubing for subsequent injection of bradykinin. At the end of each experiment, the vessels were occluded and polygraph ink was injected into the superior mesenteric artery to verify that the small intestinal vasculature was indeed isolated. In 9 cats, a dorsal laminectomy at the first cervical segment exposed the spinal

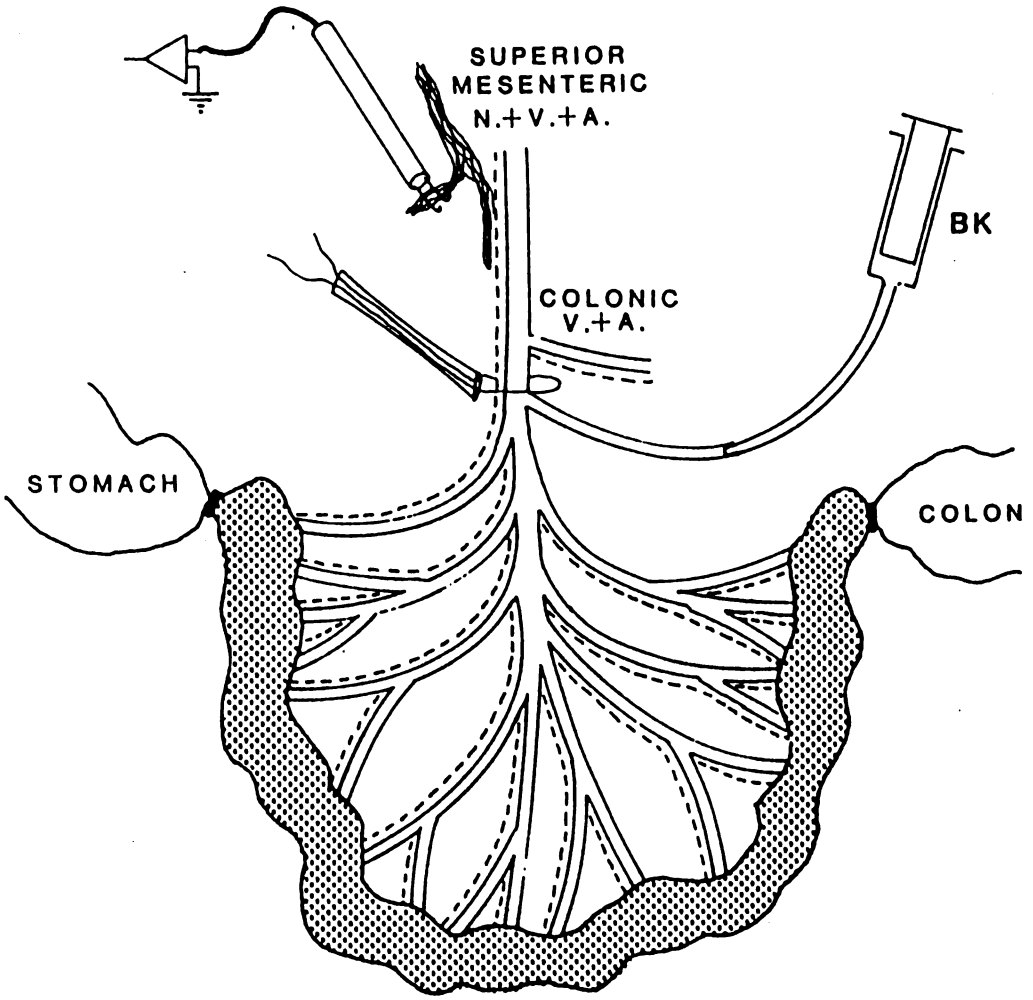

Figure 1. Diagram of experimental preparation of the small intestine.

cord for subsequent transection. In 5 cats, the vagi were severed bilaterally, without damaging the aortic depressor nerves, to remove vagal afferent innervation of the intestine.

Experimental protocol. To establish stable control conditions, blood pressure and nerve activity were monitored for at least 30 min before beginning stimulation protocols. Control values were obtained for 1-2 min before each procedure. Arterial and cardiopulmonary pressoreceptors were stimulated by intravenous infusions of norepinephrine (0.1-1.0 μg/kg; Levophed, Winthrop Laboratories, New York, NY) or phenylephrine (1.0-10.0 μg/kg; Neo-Synephrine, Winthrop Laboratories) and unloaded by hemorrhage (10-30 ml blood). To stimulate intestinal receptors, the superior mesenteric artery and vein were occluded, and 1-5 μg bradykinin triacetate (Sigma Chemical Company) in 0.1 ml physiological saline was flushed into the intestinal circulation with 0.5 ml saline. Equal volumes of saline were injected as controls. Neural and cardiovascular responses were monitored for 2 min; the snares were released 1 min after the injection and a 1-min recovery period began approximately 5 min after the injection. In 9 cats, this procedure was repeated 1 hr after transection of the spinal cord at the first cervical segment. The completeness of the transection was verified postmortem. After transection, mean arterial pressure was

maintained at, or above, 100 mmHg by an intravenous infusion of phenylephrine (5.0-10.0 $\mu g\ kg^{-1}\ min^{-1}$). This stimulus was repeated only once per hour, to prevent tachyphylaxis to bradykinin from developing. In 5 cats, bradykinin was injected into the isolated intestinal vasculature following surgical denervation of the intestine. Intestinal denervation was accomplished by severing all nerves and associated connective tissue adjacent to the mesenteric artery and vein. To verify that mesenteric and renal nerves could be reflexly excited after denervation of the intestine, 10 μg bradykinin was injected into the abdominal aorta to stimulate receptors in other abdominal viscera and lumbar skeletal muscle (Weaver et al. 1984).

Data aquisition and quantitation. Multifiber activity of mesenteric and renal nerves was recorded simultaneously, as described in GENERAL METHODS. Neural activity was digitized, full-wave rectified, and integrated over 10-s intervals by a PDP 11/23 computer, and expressed as $\mu V \cdot s/10$ s. Because voltage was recorded extracellularly and varied according to nerve bundle size and contact with the electrode, the units of quantification were considered arbitrary, and relative changes from baseline were assessed. Background electrical noise levels in the neural recordings were determined after ganglionic blockade with the short acting blocker, trimethaphan camsylate (0.2 mg/kg, i.v.,

Arfonad; Hoffman-LaRoche) during the course of an experiment, or with hexamethonium (5 mg/kg, i.v.; Mann Research Laboratories, New York, NY) at the end of an experiment. This background noise was subtracted from the neural signal when the data were quantified.

Data analysis. Changes from control in multifiber nerve activity and mean arterial pressure [1/3 (systolic pressure - diastolic pressure) + diastolic pressure] were tested with completely blocked analyses of variance. Mean values were compared with a test of least significant differences. Differences were considered significant when the probability value was less than 5 percent, and variability was expressed as pooled standard error of the mean derived from the analyses of variance (Sokol and Rohlf, 1969). The non-parametric Friedman test was used to compare magnitudes of multifiber mesenteric nerve responses to magnitudes of simultaneously recorded multifiber renal nerve responses (Sokol and Rohlf, 1969).

Single fiber recordings.

Preparation of animals (18 cats). In this group of experiments, the central cut ends of mesenteric or renal nerve bundles were progressively teased apart until firing of individual fibers could be recorded. Because of the extended

length of these experiments (15-20 hr), the preparation of the intestine was simplified to increase the probability of maintaining the animals in good condition for the duration of the experiments. The small intestine was externalized via a midline laparotomy and placed in a saline-filled plastic dish. All vascular and neural connections between the intestine and central structures remained intact. The dish was covered with plastic wrap to prevent cooling and dehydration of the intestine. Intestinal receptors were stimulated by superfusing bradykinin over the serosal surface. In all cats, a dorsal laminectomy was performed at the first cervical segment for subsequent spinal cord transection.

Experimental protocol. After unit activity and blood pressure were stable for approximately 30 min, ongoing neuronal discharge and arterial pressure were tape recorded for 5 to 20 min. Parameters recorded during this period were used to generate the interspike-interval and time-interval histograms described below. Control values were obtained for 2 min before each procedure. Systemic pressoreceptors were stimulated and unloaded by graded changes in arterial pressure produced by bolus infusions of phenylephrine and sodium nitroprusside (1.0-10.0 μg/kg, i.v.; Sigma Chemical Company). To stimulate intestinal receptors, saline was removed from the intestinal container and 10 μg bradykinin in

25 ml warm physiological saline was superfused over the serosal surface of the exteriorized small intestine. After 2 min, the bradykinin was withdrawn from the intestinal dish and the intestine was thoroughly rinsed with approximately 300 ml warm physiological saline. A 2-min recovery period of unit discharge and arterial pressure was recorded 10 to 15 min after each procedure. In some cats, this stimulus was repeated 1 hour after high cevical spinal cord transection, as described above. When spontaneous or reflex discharge of mesenteric or renal units ceased after spinal cord transection, 1-2 ml of a saturated KCl solution was given intravenously at the end of the experiment to cause depolarization of neurons and demonstrate that these cells were able to discharge. Fast-sweep oscillographic records of the KCl-evoked action potentials were compared to those of spontaneously occuring action potentials which were recorded prior to transection of the spinal cord to verify that the same fiber was firing. Units were identified on the basis of waveform and peak-to-peak amplitude. If activity of a fiber could not be elicited by the KCl injection, it was assumed that the electrode had moved during spinal cord transection; therefore, data obtained after transection of the spinal cord were not used in statistical analyses.

Data aquisition and quantitation. The central ends of mesenteric (14 cats) or renal (4 cats) nerve bundles were placed on a small black plastic platform and teased apart progressively until discharge of individual neurons could be recorded. In many of these experiments, neuronal discharge was recorded at a bandwidth of 100 Hz - 3 kHz to increase the signal/noise ratio. Activity of single fibers was discriminated from that of other active fibers by identifying action potentials with distinctive waveforms and amplitude, and discharge frequency (spikes/sec) of spontaneously active single fibers was determined by counting the output pulses of a slope/height window discriminator (Federick Haer, Brunswick, ME) with a PDP 11/23 computer (Digital Equipment Corporation, Maynard, MA.) The ganglionic blocker trimethaphan camsylate was administered during the course of an experiment, or hexamethonium was given at the end of an experiment to verify that activity was being recorded from postganglionic fibers. Interspike-interval histograms were generated using a Nicolet 1070 (Madison, WI) or an IBM AT computer (RC Electronics computerscope software, Santa Barbara, CA) to determine whether fibers were firing at constant or irregular intervals (window lengths = 0.4-5.0 s). Since the shortest interspike interval reported for postganglionic sympathetic fibers is 28 ms (Hallin and Torebjörk, 1974), these histograms provided additional criteria that activity was being recorded from a single

fiber, rather than from multiple fibers with similar waveforms and amplitudes. Window discriminator thresholds were considered acceptable if more than 95% of interspike intervals were greater than 20 ms (see e.g. Figure 6).

Correlation of spontaneous neuronal activity with the arterial pressure pulse was also tested with the Nicolet 1070 and IBM AT computers (RC Electronics). The sweep of the computer was triggered by peak systolic pressure; a time-interval histogram of unitary discharge was generated, and the corresponding arterial pulse wave was averaged (window length = 500 ms). These histograms were compared with those triggered by output pulses of a stimulator (Grass Instruments), with a stimulation frequency approximating that of the real event triggers. Unit activity was considered to be correlated with the arterial pressure pulse if oscillations in the arterial pressure-triggered histogram were three times as large as those in the stimulator-triggered histograms. All histograms and averages were displayed in analog form on an oscilloscope and recorder (X-Y Recorder, model 7015A, Hewlett-Packard, USA; Proprinter, IBM, USA).

Data Analysis. To assess changes from control in the firing frequency of single fibers, 95% confidence limits were calculated from 12 periods of control discharge rates (10 s/period; confidence limits = sample mean ±

$t_{[0.025]}$ · S.E.). Responses were considered excitatory if the discharge rates exceeded the upper limits confidence intervals and inhibitory if the discharge rates were less than the lower limits of the confidence intervals. Mean responses within groups of fibers were tested with a least-significant-differences test after a completely blocked analysis of variance. Responses (percent of control) between groups of fibers were compared with the non-parametric Wilcoxon two sample test for unpaired data and the non-parametric Friedman test for paired data (Sokol and Rohlf, 1969).

SPECIFIC METHODS - ROLE OF SUBSTANCE P OR OTHER CAPSAICIN-SENSITIVE PEPTIDES IN THE CENTRAL TRANSMISSION OF INTESTINO-SYMPATHETIC REFLEXES.

Capsaicin treatment. Fifteen adult male rats (200-400 g) were treated for 5 days with capsaicin (s.c.) dissolved in an ethanol vehicle. The rats received 50 mg/kg on the first day and 100, 200, 200 and 400 mg/kg on subsequent days. The cumulative dose of capsaicin was 950 mg/kg. Rats were used in this study because capsaicin has been shown to cause depletion of substance P in rodents. Chronic subcutaneous administration of capsaicin causes depletion of substance P in prevertebral ganglia of guinea pigs (Wilken, Fagre, Jew and Williams, 1983) and dorsal root

ganglia and sensory nerve terminals in the dorsal horn of the spinal cord of rats (Jessel et al. 1978; Burks, et al. 1985). Nine additional rats received injections of the ethanol vehicle (1 ml/kg s.c.) for 5 days. Nine of the capsaicin-treated rats and the nine vehicle-treated rats were killed 5 days after termination of treatment, and the spinal cords (segments T_3-L_3) and dorsal root ganglia (T_3-L_3) were excised and frozen on dry ice. These tissues were assayed for substance P-like immunoreactivity. Six of the capsaicin-treated rats served as subjects for the electrophysiological studies.

Radioimmunoassay. Spinal cords from the vehicle- and capsaicin-treated rats were sectioned transversly with a cryostat into 1-mm sections and the dorsal horns punched out using an 18-gauge stainless steel cannula. The dorsal root ganglia (9-15/rat) were pooled, the microdissected dorsal horns were pooled, and then both samples were homogenized in an appropriate volume of 90% methanol in water and centrifuged at 3000 g for 20 min. Aliquots of the supernatants were analyzed for substance P-like immunoreactivity using a double antibody radioimmunoassay, and the pellets were analyzed for protein as described by Lowry and co-workers (Lowry, Rosebrough, Farr,and Randall, 1951). Substance P antiserum was obtained from Peninsula Laboratories (Catalogue No. RAS7451, Belmont CA). [^{125}I]

substance P was obtained from Amersham Corporation (Arlington Heights, IL). Tissue concentrations of substance P are expressed as substance P-like immunoreactivity/mg protein.

Electrophysiological experiments. Preparation of animals. Capsaicin-treated rats (n=6) used in the electrophysiological experiments received subcutaneous injections of capsaicin over a 5 day period as described above. Vehicle was not administered to the control rats. The rats were anesthetized with intraperitoneal injections of chloralose (50 mg/kg) and urethane (500 mg/kg). Supplementary doses of chloralose (i.v.) were given as needed. The level of anesthesia was assessed by evaluating the rats' paw pinch and palpebral reflexes. Body temperature was monitored via a rectal probe and maintained at 37° C with a heating pad. A tracheostomy tube was inserted, and a femoral artery and jugular vein were cannulated to measure arterial pressure and for the administration of fluids or drugs, respectively. To compensate for fluid losses, Normosol (Abbott Laboratories, North Chicago, IL) was infused intravenously at a rate of 3 ml/hr. The small intestine was externalized via a midline laparotomy and placed in a saline-filled plastic pouch. Mesenteric and renal nerves were dissected free from surrounding tissue and multifiber nerve activity was recorded and quantified as described above.

Experimental protocol. Intestinal receptors were stimulated by the application of 0.5-1.0 μg bradykinin in 1.0 ml physiological saline to the serosal surface of the small intestine. Neural and cardiovascular responses were monitored for 2 min; the intestine was then rinsed with 10-20 ml warm physiological saline. Neural responses to the stimulation of arterial and cardiopulmonary pressoreceptors also were evaluated. Pressoreceptors were stimulated by infusing phenylephrine (5-10 μg/kg, i.v.) to increase systemic arterial pressure.

Data Analysis. Changes from control in nerve activity and blood pressure were evaluated as previously described. For comparisons of magnitudes of neural and cardiovascular responses between the capsaicin-treated and untreated rats, the responses were converted to percentages, normalized by square root transformation, and tested with a completely random analysis of variance (Sokal and Rohlf, 1969). Differences in substance P-like immunoreactivity between capsaicin-treated and vehicle-treated rats also were assessed with a completely random analysis of variance. Differences were considered different when $P < 0.05$ and variability was expressed by a coefficient of variation or standard error of the mean.

SPECIFIC METHODS - INFLUENCE OF ROSTRAL VENTROLATERAL MEDULLA ON MAGNITUDE AND RHYTHM OF DISCHARGE OF MESENTERIC AND RENAL NERVES.

Preparation of animals. Experiments were done on 18 chloralose-anesthetized, artificially respired cats. General surgical procedures have been described in the GENERAL METHODS section. Mesenteric and renal nerves were exposed and prepared for recording mass activity as described above.

The ventral surface of the brainstem was exposed as described by Guertzenstein (1973). Cats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), in a supine position. The esophagus and larynx were severed and reflected rostrally through the mouth. Following lateral retraction of overlying muscles, the basioccipital bone was carefully removed with rongeurs as close to the tympanic bulla as possible without damaging the nerves coursing through the jugular foramen. The dura and arachnoid were left intact until immediately before the application of glycine. At this time they were cut longitudinally and retracted laterally to expose the ventral surface of the medulla. In 4 cats, sino-aortic denervation and vagotomy were performed by severing the vagus and glossopharyngeal nerves as thy passed into the jugular foramen. In all cats, the first cervical segment of the spinal cord was exposed

ventrally by removing the vertebral arch of the atlas and the odontoid process of the axis with rongeurs.

Experimental protocol. The inhibitory amino acid glycine (BDH Chemicals, Toronto, Ontario) was dissolved in normal saline at a concentration of 200 mg/ml; the pH was adjusted to 7.35-7.45 and the solution was warmed to 37° C. Small cotton pledgets (1-2 mm diameter) soaked with the glycine solution were topically applied to both sides of the ventral medulla, 2-4 mm lateral to the midline and 1-2 mm caudal to the trapazoid bodies. This corresponds to the "glycine-sensitive area" described by Guertzenstein and Silver (1974). Activity of renal and mesenteric nerves was simultaneously recorded to determine if excitatory drive from the RVLM selectively influences the magnitude or frequency characteristics of ongoing discharge of these nerves. In 4 cats, responses to the application of glycine were evaluated while supporting arterial pressure with intravenous infusions of phenylephrine (2.0-10.0 $\mu g\ kg^{-1}\ min^{-1}$; Neo-Synephrine, Sterling, Aurora, Ontario), and, after denervation of systemic pressoreceptors, to ensure that the observed changes in the periodicity of nerve firing were not secondary to decreases in arterial blood pressure. Cotton pledgets soaked with warm physiological saline were applied as controls. Pledgets were removed after 5 min and the ventral surface of the medulla was rinsed with approximately 30 ml warm

physiological saline. After recovery of neural discharge and blood pressure to control levels the application of glycine was repeated. During the nadir of the decrease in nerve discharge following this second application of glycine, the spinal cord was transected at the first cervical segment to determine if sympathoinhibition contributed to the decrease in nerve activty. In 13 of 18 cats, conduction in the cervical spinal cord was blocked prior to the transection by the injection of 0.3 ml of 2% lidocaine (Rugby Laboratories, Rockville Center, NY) directly into the spinal cord. The completeness of the transection was verified postmortum. After spinal cord transection, mean arterial blood pressure was maintained at, or above, 85 mmHg by intravenous infusions of phenylephrine (5.0-10.0 $\mu g\ kg^{-1}\ min^{-1}$).

Data aquisition. Systemic arterial pressure, heart rate and nerve discharge were monitored and recorded as described previously. In 9 experiments, neural activity was amplified at a bandwidth of 30 Hz - 3 kHz. In 9 other experiments, activity was amplified at a bandwidth of 1 Hz - 3 kHz to reveal the low frequency components of the signal. At this bandwidth bursts of nerve discharge appear as slow oscillations in voltage (see Figures 24 and 25).

Data analysis. Nerve activity was integrated as described above. Neural signals that had been recorded at the wide bandwidth of 1 Hz - 3 kHz were filtered at a bandwidth of 100 Hz - 2 kHz (Differential amplifier, Frederick Haer, Brunswick, ME) before integration. Statistical methods used to analyze the data included analyses of variance, least-significance-difference tests and Friedman tests as described previously.

Power density spectra of the neural signals recorded at the bandwidth of 1 Hz - 3 kHz were constructed by an RC Electronics Computerscope system. The signals were filtered with a low-pass frequency of 50 Hz and sampled at a rate of 1 kHz. This procedure prevented aliasing of the high frequency components of the signal (Malmstadt, Enke & Crouch, 1981). The spectra of each nerve were normalized by expressing relative power density in arbitrary units proportional to watts, and total power in the 1-6 Hz frequency range was computed. Changes in total power in this frequency range were used as indices of changes in the slow rhythm in nerve firing. Comparisons between percent changes in total power of paired renal and mesenteric nerve activities were made using the Friedman test. This test also was used to compare percent changes in total power of the density spectra to percent changes in integrated nerve activity (Sokal & Rohlf, 1969).

MULTI- AND SINGLE FIBER MESENTERIC AND RENAL NERVE RESPONSES TO THE STIMULATION OF INTESTINAL RECEPTORS AND PRESSORECEPTORS

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Multifiber Recordings

RATIONALE

Most reports of fractionated sympathetic discharge have stressed differences between visceral, skeletal muscle and cutaneous sympathetic activity. The viscera generally have been considered as a collective unit, and, therefore, ongoing and reflex activity patterns of different components of abdominal visceral sympathetic outflow rarely have been compared. The present investigation was designed to provide information about the organization of sympathetic outflow to the small intestine and kidney. Multifiber mesenteric and renal nerve responses to the stimulation of systemic pressoreceptors and intestinal receptors were characterized in cats with intact neuraxes and after high cervical spinal cord transection. The following hypotheses were tested.

HYPOTHESES

1. Stimulation of systemic pressoreceptors causes greater inhibition of renal than mesenteric nerve discharge.

2. Chemical stimulation of intestinal receptors causes greater excitation of multifiber mesenteric than renal nerve activity.

3. Spontaneous activity of mesenteric, but not renal, nerves can be generated by spinal systems immediately following a high cervical spinal cord transection. Excitatory sympathetic responses elicited by intestinal receptor stimulation can be mediated by exclusively spinal circuits.

RESULTS

Neural responses to stimulation or unloading of arterial and cardiopumonary pressoreceptors. Activity of all multifiber nerves was tested for pressoreceptor sensitivity at the beginning of each experiment. An increase in mean arterial pressure from 105 $\pm$ 6 mmHg to 164 $\pm$ 7 mmHg, caused by bolus intravenous injections of phenylephrine (1.0-10.0 μg/kg) or norepinephrine (0.1-1.0 ug/kg), significantly inhibited ongoing activity of mesenteric and renal nerves by 32.5 $\pm$ 5.5% and 84.7 $\pm$ 5.2%, respectively (Figure 2). The magnitude of inhibition of activity of renal nerves was

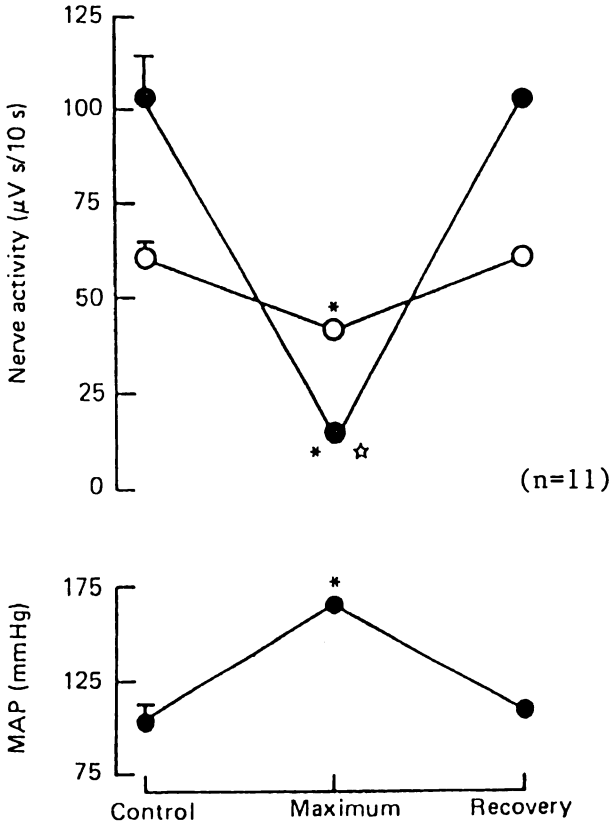

Figure 2. Responses of whole mesenteric and renal nerves and blood pressure to stimulation of pressoreceptors by intravenous infusions of noradrenaline (0.1-1.0 μg/kg) or phenylephrine (1.0-10.0 μg/kg).

The ordinate of the top panel indicates activity of mesenteric (open circles) and renal (closed circles) nerves that was integrated in 10-s intervals. The ordinate of the bottom panel indicates mean arterial pressure (SAP) in mmHg. The control periods (C) and recovery periods (R) represent average nerve discharge and mean arterial pressure accumulated during 1 min; the response periods (MAX) represent average nerve discharge and blood pressure accumulated during the 10 s of maximum sympathoinhibition. Population sizes are noted in parentheses. Variability is expressed as pooled standard error. Asterisks indicate significant differences from control. The pressor responses caused greater inhibition of renal than mesenteric nerve activity, as indicated by the star.

significantly greater than that of mesenteric nerves. The modest inhibition of mesenteric nerve activity is consistent with another report (Irisawa et al. 1973). In 2 cats, hemorrhage decreased blood pressure from 83 to 45 mmHg and from 108 to 50 mmHg, increased mesenteric nerve activity by 39% and by 69%, and increased renal nerve discharge by 89% and by 60%, respectively. All nerve bundles used in this study may have contained vasomotor fibers, as their activity was either inhibited by stimulation, or excited by unloading arterial and cardiopulmonary pressoreceptors. Fifteen mesenteric nerve bundles were not used in this investigation, because their activity appeared to be insensitive to pressoreceptor influences. Activity of many of these bundles clearly was increased during phenylephrine-induced pressor responses. Ninomiya and co-workers also have described diverse responses among multifiber mesenteric nerves following intravenous injections of norepinephrine (Irisawa et al. 1973).

Stimulation of intestinal receptors with bradykinin. The responses of pressoreceptor sensitive nerves to the chemical stimulation of intestinal receptors were investigated. Injection of 1-5 μg bradykinin into the arterial circulation of the vascularly isolated small intestine increased the discharge of mesenteric and renal nerves and caused small increases in systemic arterial

Figure 3. Response of one cat to chemical stimulation of intestinal receptors.

Integrated discharge of mesenteric and renal nerves is shown in the top two panels. Activity was integrated over 10-s intervals and is expressed as $\mu V \cdot s/10$ s. Systemic arterial pressure (SAP) is illustrated in the lower panel. Time in seconds and injection of 1.0 μg bradykinin (arrow) into the isolated intestinal vasculature are indicated beneath these panels. The superior mesenteric artery and vein were occluded immediately before the time period of the illustrated samples of nerve activity and arterial pressure. The recovery values of these variables 4 min after bradykinin injection and 1 min after release of the occluded blood vessels are illustrated in the group of panels on the right.

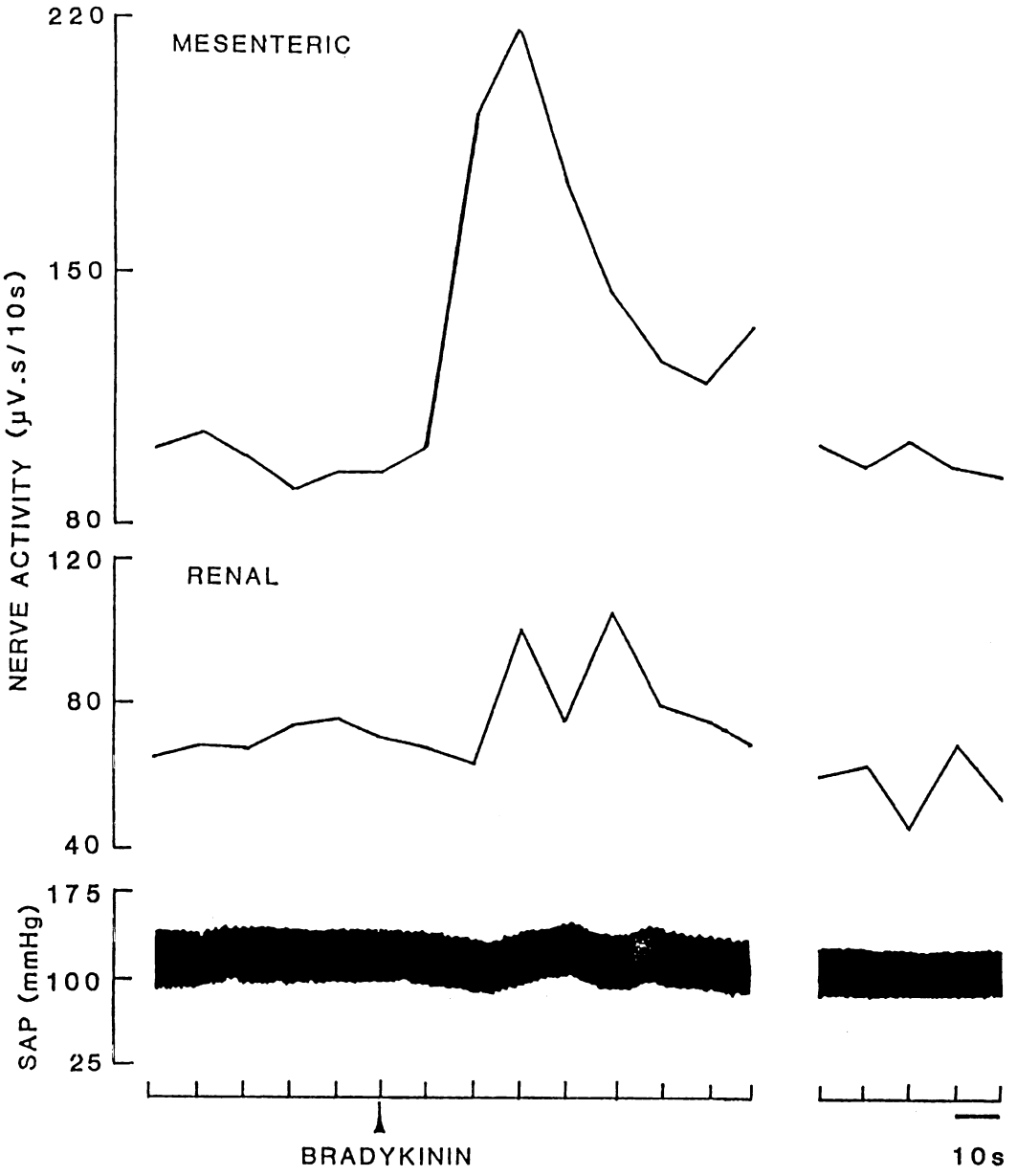
220
MESENTERIC
150
80
NERVE ACTIVITY (μV.s/10s)
120
RENAL
80
40
175
100
25
SAP (mmHg)
BRADYKININ
10s

Figure 3.

pressure (Figure 3). The excitatory response usually began 20-30 s after the injection of bradykinin, and mesenteric excitation typically lasted 1 min. The transient decrease in renal nerve activity during the excitatory response shown in Figure 3 probably was elicited by pressoreceptors, due to the small increase in blood pressure. This non-uniform pattern of excitation was observed consistently in all of the cats tested. Mean increases in activity of mesenteric and renal nerves were 218 $\pm$ 61% and 41 $\pm$ 16%, respectively (Figure 4), and were accompanied by a mean increase in systemic arterial pressure of 6.8 $\pm$ 2.2 mmHg. Occlusion of the superior mesenteric artery and vein for 2 min or injection of 0.6 ml physiological saline into the isolated intestinal circulation had no effect on spontaneous nerve discharge or systemic arterial pressure. In 5 experiments the vagi were severed bilaterally to determine if vagal afferent nerves were engaged in the reflex response to chemical stimulation of intestinal receptors. The response patterns of this group were not different from those with intact vagi, and, therefore, responses of both groups were pooled. The sympatho-excitation was shown to be reflexly mediated via mesenteric afferent nerves in 5 cats. Following surgical denervation of the small intestine, the injection of up to 10 μg bradykinin into the intestinal circulation failed to evoke increases in nerve activity or systemic arterial pressure (Figure 4). After intestinal denervation, 10 μg bradykinin

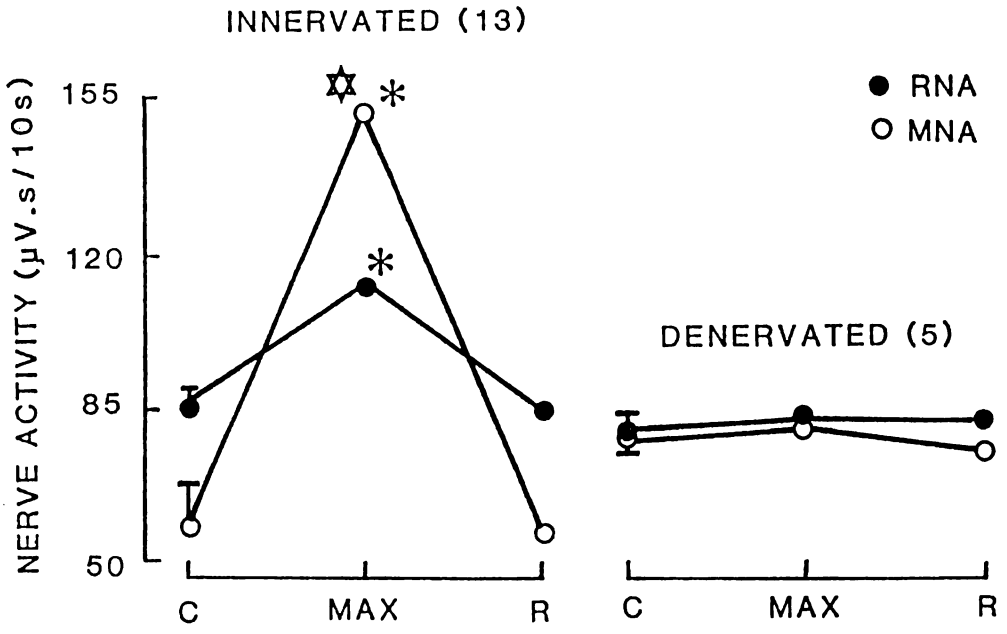

Figure 4. Mean responses of sympathetic activity to stimulation of intestinal receptors with bradykinin (1.0 μg) in cats with intact intestinal innervation and after surgical denervation of the small intestine.

The ordinate indicates activity of mesenteric (open circles) and renal (closed circles) nerves that was integrated in 10-s intervals. The control periods (C) and recovery periods (R) represent average nerve discharge accumulated during 1 min, and the periods of maximum response after bradykinin injection (MAX) represent average nerve discharge accumulated during 10 s. Population sizes are noted in parentheses. Format and abbreviations as in Figure 2. In cats with intact intestinal innervation (left panel), stimulation of intestinal receptors caused significant excitation of mesenteric and renal activity; excitation of discharge of mesenteric nerves was significnatly greater than that of renal nerves, as indicated by the star. Denervation of the intestine abolished these responses (right panels).

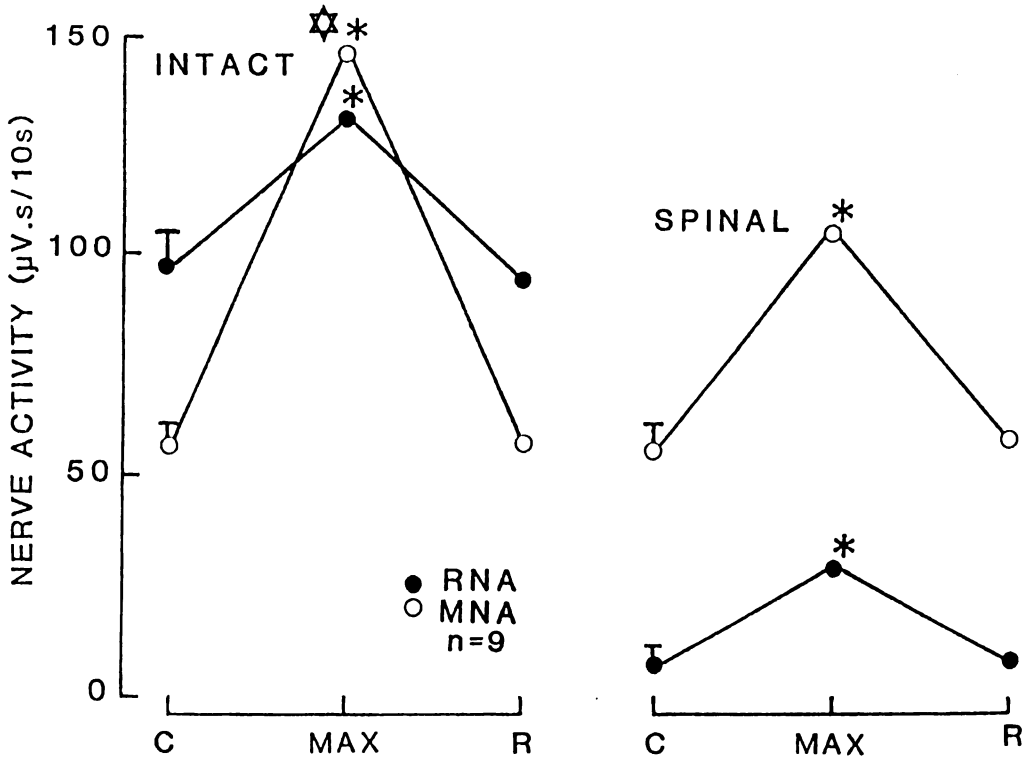

Figure 5. Mean responses of mesenteric and renal sympathetic activity to stimulation of intestinal receptors with bradykinin before, and 1 hr following high cervical spinal cord transection in 9 cats.

Format and abbreviations as in Figure 4. Chemical stimulation of intestinal receptors caused significant increases in activity of mesenteric and renal nerves before (Intact) and after (Spinal) transection. Excitation of activity of mesenteric nerves was significantly greater than that of renal nerves in the intact, but not in the spinal state.

was injected into the abdominal aorta to stimulate receptors in other viscera and skeletal muscle (Weaver et al. 1984). This procedure always caused excitation of mesenteric and renal efferent nerve activity, indicating that these nerves remained intact during denervation of the small intestine.

Responses to intestinal stimulation in spinal cats. The extent to which these excitatory reflexes depend upon supraspinal pathways was assessed in 9 cats by injecting bradykinin into the isolated small intestinal vasculature before and 1 hr after spinal cord transection at the first cervical segment. Before spinal transection mass activity of both mesenteric and renal nerves appeared to have a continuous component and a phasic component which was synchronous with the cardiac cycle. The phasic component was more prominent in renal than mesenteric activity, as described previously by Ninomiya and Irisawa (1975). Following spinal cord transection the spontaneous discharge of renal nerves was significantly decreased , while that of mesenteric nerves was not affected (Figure 5). Only the continuous component, and not the phasic component, of nerve activity was evident after transecting the spinal cord. Stimulation of intestinal receptors with bradykinin still caused significant increases in mesenteric and renal nerve activities, indicating that a component of this reflex is complete within the spinal cord (Figure 5). The absolute

increases (in $\mu V \cdot s/10$ s) in mesenteric nerve activity and renal nerve activity in the spinal cats were not significantly different from those observed when the neuraxis was intact. However, because of the low tonic levels of renal nerve discharge after spinal transection, the increases in renal nerve activity relative to basal nerve discharge were greater after spinal transection, and, thus, the percentage change in renal nerve activity was substantially greater in the spinal cats than in the cats with intact neuraxes. Consequently, excitation of activity of mesenteric nerves was not significantly greater than that of renal nerves in the spinal cats.

Single Fiber Recordings

RATIONALE

A non-uniform multifiber response pattern may reflect greater magnitudes of excitation of all neurons in one nerve relative to those in another. Alternatively, differential reflexes may result from the summation of heterogeneous responses among subpopulations of neurons comprising one or both nerves. Therefore, the bases of the multifiber responses described above were determined by recording discharge from individual fibers within mesenteric and renal nerves. The following hypotheses were tested.

HYPOTHESES

1. More renal than mesenteric nerve fibers are engaged in reflexes originating from systemic pressoreceptors.

2. More mesenteric than renal nerve fibers have discharge which is excited by chemical stimulation of intestinal receptors.

3. More mesenteric than renal nerve fibers discharge spontaneously after high cervical spinal cord transection.

RESULTS

Ongoing activity. Mesenteric and renal nerve fibers had similar resting discharge rates that ranged from 0.1 to 3.8 (mean = 1.2 $\pm$ 0.2) and 0.1 to 6.4 (mean = 1.3 $\pm$ 0.3) spikes/s, respectively. Most fibers fired at lower rates within these ranges as indicated by the median discharge rates of 0.7 and 0.6 spikes/s. These ranges of discharge are similar to the previously reported discharge rates of fibers in the renal nerve (Dorward et al. 1987; Kidd, Linden and Scott, 1981; Rogenes, 1982), the splenic nerve (Meckler and Weaver, 1987), the deep peroneal nerve (Jänig, 1985), cardiac and vertebral nerves (Kollai and Koizumi, 1980a), and the hypogastric nerve (Floyd, Hick and Morrison, 1982).

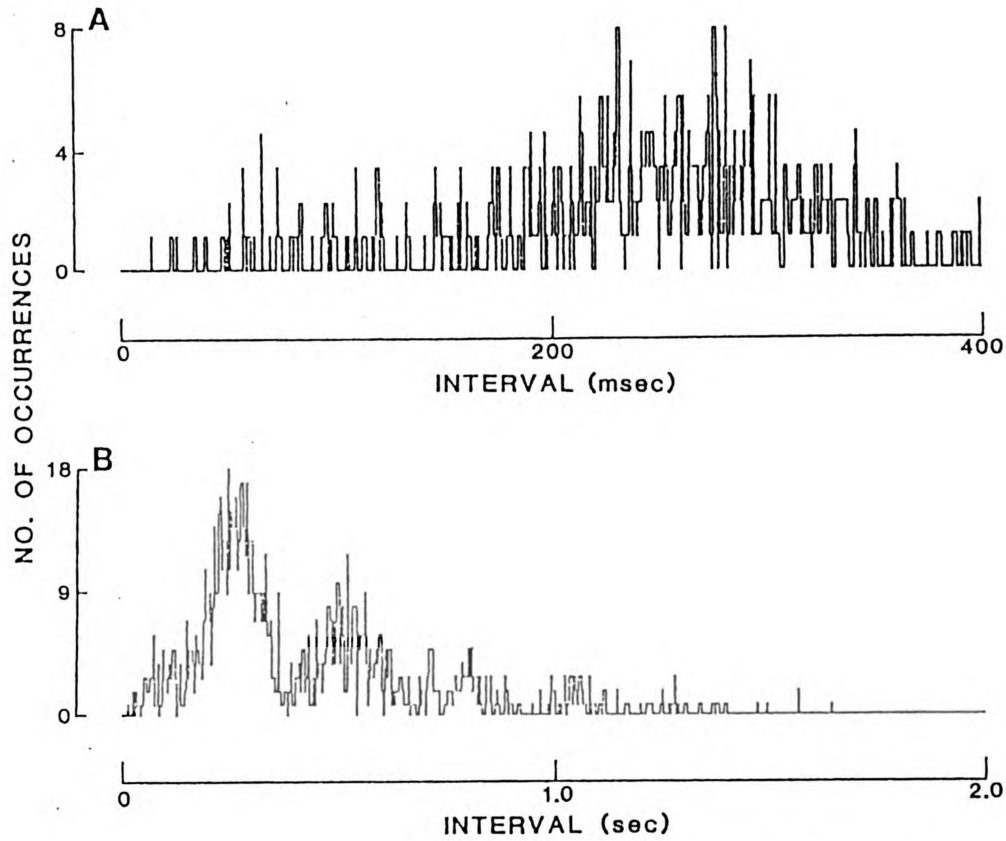

Figure 6. Inter-spike interval histograms of firing of a single renal nerve fiber.

Histograms constructed from 946 consecutive action potentials. Abscissa (Interval) indicates the time between two consecutive action potentials. Ordinate indicates the number of occurences of each inter-spike interval. Panel A; window = 400 ms; histogram consists of 531 samples. Shortest inter-spike interval = 14 ms. Panel B; window = 2 s; histogram consists of 945 samples. The most frequent interval (250 ms) was that of the cardiac cycle. The three peak intervals occured at 250 ms, 560 ms and 875 ms.

The inter-spike interval histograms of unitary discharge had either no peaks (9 mesenteric nerve fibers, 1 renal nerve fiber), very broad assymetrical peaks (8 mesenteric fibers, 3 renal fibers), or multiple peaks (4 mesenteric fibers, 4 renal fibers; Figure 6), indicating that none of the units discharged with regularity. Although neurons typically had discharge rates of less than 1 spike/s, action potentials often occured in pairs or in bursts, and instantaneous frequencies as high as 100 Hz occasionally were observed. The minimum inter-spike interval ranged from 10 to 240 ms (mean = 50 $\pm$ 16 ms), and over 95% of the inter-spike intervals on each histogram were greater than 20 ms. Although discharge of different fibers could be distinguished with the use of a window discriminator, it is possible that some of the shorter inter-spike intervals (< 20 ms) resulted from simultaneous recordings from two different units with similar waveforms and amplitude. In the histogram of renal unit activity illustrated in Figure 6 (top panel), the shortest inter-spike interval was 14 ms (corresponding to an instantaneous frequency of 71 Hz). Peaks in the histograms revealed the most frequent intervals between consecutive action potentials; 12 of 21 mesenteric nerve fibers and 7 of 8 renal nerve fibers fired with a high probability of inter-spike intervals in the range of 150 - 1000 ms. In the histogram of renal unit activity illustrated in Figure 6 (lower panel), the most common intervals were 250 ms, 560 ms

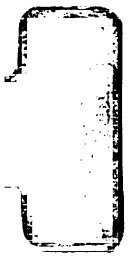

Figure 7. Relationship between firing of a renal postganglionic fiber and arterial blood pressure.

Neurogram of neuronal firing is displayed above a simultaneously sampled tracing of systemic arterial pressure, illustrating that the fiber fired during diastole.

Figure 8. Relationship between activity of another renal fiber and systemic arterial pressure.

A post-stimulus histogram of unit activity (spikes) is displayed above a simultaneously triggered average of the arterial pulse wave (SAP). The horizontal axis indicates time in ms. The stimulus to trigger sampling of unit discharge and arterial pressure was peak systolic arterial pressure. Forty one hundred and thirty three samples (window length = 500 ms) were used to generate this histogram and average. Unit activity was correlated with the arterial pressure pulse.

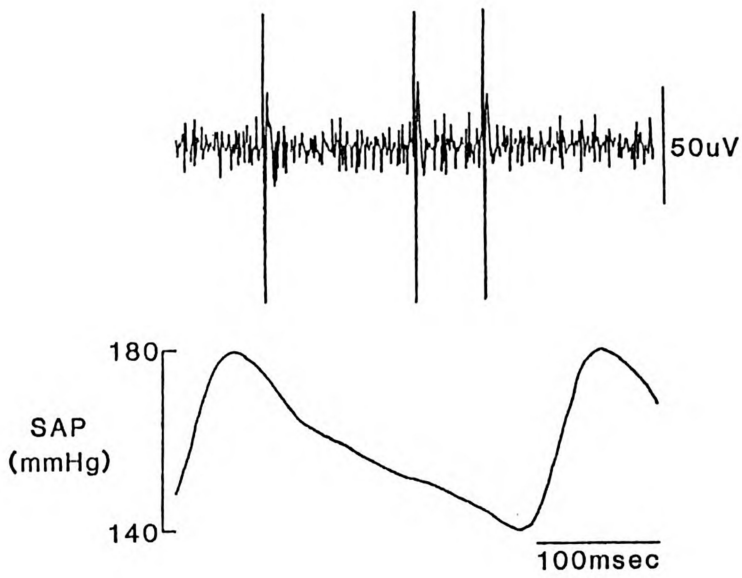

Figure 7.

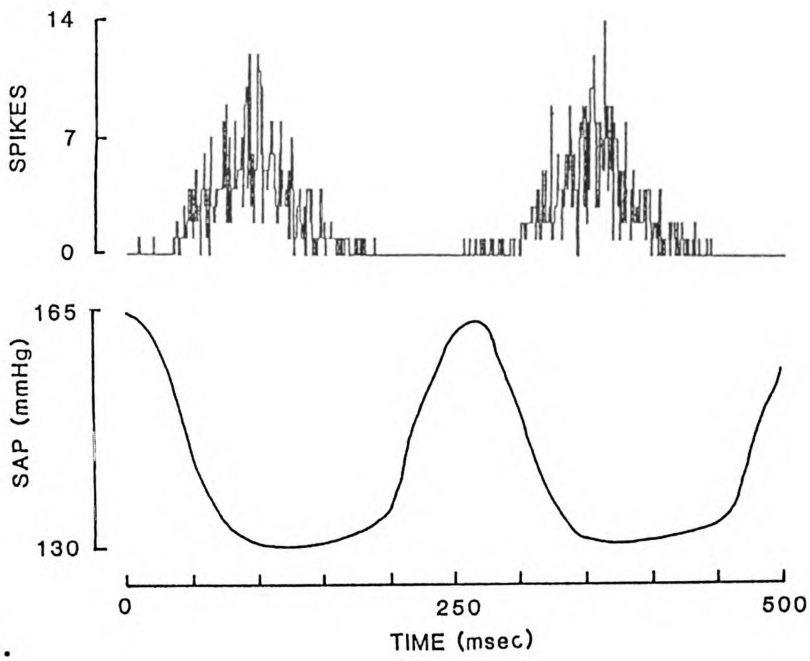

Figure 8.

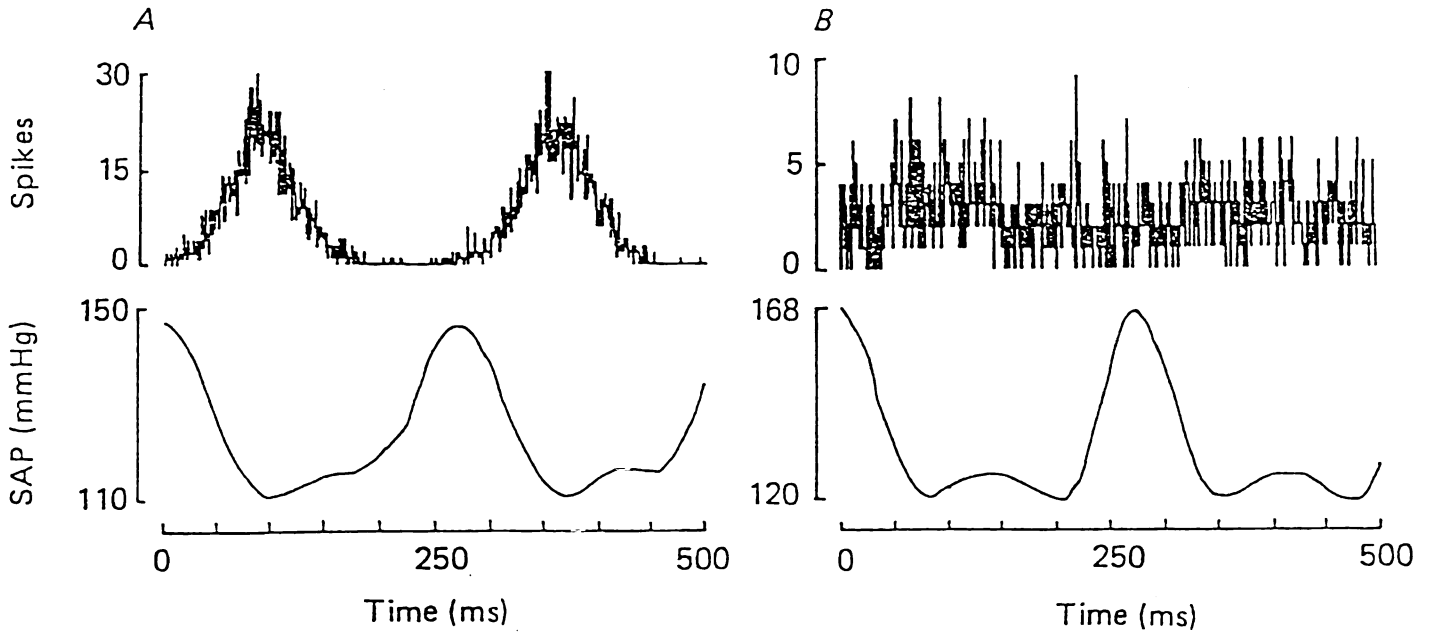

Figure 9. Relationships between activity of 2 mesenteric fibers and systemic arterial pressure.

Format and abbreviations as in Figure 8. Window length = 500 ms. Top panel shows histogram of activity of a mesenteric neuron that was correlated with the arterial pulse wave (2613 samples). Bottom panel shows histogram of activity of a mesenteric neuron that was not clearly correlated with the arterial pressure pulse (2425 samples).

and 875 ms, corresponding to instantaneous frequencies of 4.0, 1.8, and 1.1 Hz, respectively. The discharge of this fiber appeared to be synchronized with the cardiac cycle, as the highest peak of the histogram occurs at an interval of 250 ms, the length of the cardiac cycle in this cat. Such cardiac rhythmicity was a consistent feature of the discharge of single renal nerve fibers, as demonstrated by the neurogram of activity and the post-stimulus time histogram of discharge of two other renal fibers in Figures 7 and 8. All 7 renal postganglionic fibers tested had activity which was correlated with the arterial pressure pulse, discharging primarily during the diastolic phase of the cardiac cycle (Figures 7 and 8). In contrast, spontaneous activity of only 11 of 19 mesenteric fibers was correlated with the arterial pulse wave (Figure 9A), whereas discharge of 8 other mesenteric fibers bore no relationship to the pressure pulse (Figure 9B).

Neuronal responses to unloading and stimulation of pressoreceptors. In addition to testing activity of fibers for correlation with the arterial pressure pulse, reflex responses of 15 mesenteric fibers and 7 renal fibers to graded decreases and increases in arterial pressure were investigated. Blood pressure was decreased to unload arterial and cardiopulmonary pressoreceptors by intravenous injection of nitroprusside (1.0-10.0 μg/kg) and increased to

Figure 10. Responses of mesenteric nerve fibers to stimulation or unloading systemic pressoreceptors.

Bars represent mean discharge rates of mesenteric fibers expressed as spikes/s (UNIT ACTIVITY) and corresponding changes in mean arterial pressure (Δ MAP) during 2-min control periods (C), during 10-s depressor responses caused by intravenous injections of nitroprusside (NP), and during 10-s phenylephrine-induced pressor responses (PE). As described in the text, the mesenteric population could be classified into two subgroups with respect to these stimuli; fibers with pressoreceptor-sensitive (left panel) and pressoreceptor-insensitive (right panel) discharge. Control mean systemic arterial pressures were 142 ± 2 mmHg (pressoreceptor-sensitive) and 127 ± 3 mmHg (pressoreceptor-insensitive). Number of fibers is noted in parentheses. Variability is indicated by pooled standard error. Asterisks indicate significant differences from control. Mean discharge of pressoreceptor-sensitive mesenteric units was significantly excited by decreases and inhibited by increases in arterial pressure, whereas mean discharge of pressoreceptor-insensitive fibers was not significantly affected by these stimuli.

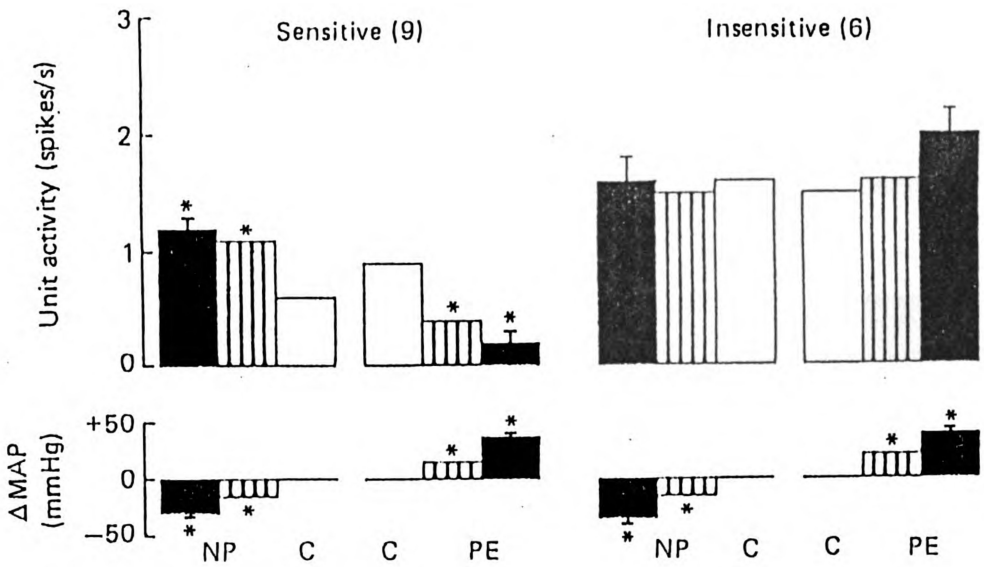
Sensitive (9)
Insensitive (6)
Unit activity (spikes/s)
3
2
1
0
ΔMAP (mmHg)
+50
0
−50
NP
C
C
PE
NP
C
C
PE

Figure 10.

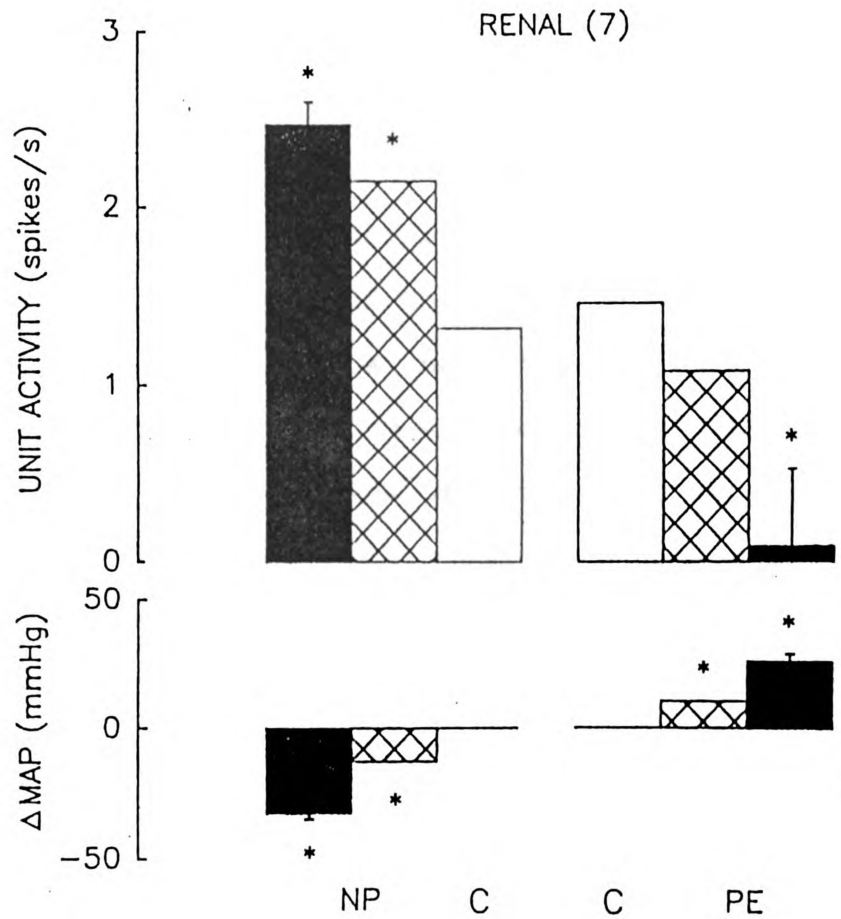

Figure 11. Responses of renal nerve fibers to stimulation or unloading systemic pressoreceptors.

Bars represent mean discharge rates of renal fibers expressed as spikes/s (UNIT ACTIVITY) and corresponding changes in mean arterial pressure (Δ MAP) during control periods (C), during depressor responses caused by intravenous injections of nitroprusside (NP) and during phenylephrine-induced pressor responses (PE). Format as in Figure 10. Control mean systemic arterial pressure was 160 ± 6 mmHg. Mean discharge of renal neurons was significantly excited by decreases, and inhibited by the largest increase in arterial blood pressure.

stimulate pressoreceptors by intravenous injections of phenylephrine (1.0-10.0 μg/kg). According to confidence interval tests, responses of mesenteric fibers could be divided into two subgroups. Nine of fifteen mesenteric fibers were engaged in pressoreceptor reflexes (Figure 10, left panel). Firing of 7 of these fibers was both excited by unloading pressoreceptors and inhibited by stimulation of pressoreceptors. One fiber was affected only by decreases in blood pressure; one fiber was affected only by increases in blood pressure. Unloading of pressoreceptors caused maximum increases in neuronal discharge which ranged from 35 to 400% (mean ± S.D. = 141 ± 111%). Stimulation of pressoreceptors caused cessation of firing of five fibers in this group, and a 70 ± 8% (mean ± S.D.) decrease in the discharge of three other fibers. Activity of 7 of these 9 pressoreceptor-sensitive mesenteric fibers was correlated with the arterial pulse wave. The second subgroup of mesenteric fibers consisted of 6 units which appeared to be insensitive to pressoreceptor influences (Figure 10, right panel). This group included 3 units that had excitatory responses to increases in blood pressure, 2 units that had inhibitory responses to decreases in blood pressure, and 1 unit that did not respond significantly to any change in blood pressure. Two of 4 of these pressoreceptor insensitive fibers, which could be tested for correlation with the arterial pressure pulse, had pulse synchronous discharge. In contrast to the

diverse mesenteric neuronal responses, renal responses were homogeneous; activity of all 7 renal units was excited when blood pressure was decreased by nitroprusside, and inhibited during the phenylephrine-induced pressor responses (Figure 11). These results are similar to those of Dorward et al. (1987) who found that virtually all renal neurons investigated in rabbits were sensitive to pressoreceptor influences. As a group, responses of pressoreceptor-sensitive mesenteric fibers were similar in magnitude to those of renal fibers. These experiments demonstrated the differential effects of stimulation and unloading of systemic pressoreceptors on activity of different visceral sympathetic neurons, as all renal, but only 56% of mesenteric neurons were engaged in pressoreceptor reflexes.

Stimulation of intestinal receptors with bradykinin. Stimulation of intestinal receptors also evoked unequal responses of mesenteric and renal neurons. Discharge of a mesenteric fiber that increased from 0.5 to 4.2 spikes/s is shown in the left group of panels of Figure 12. Firing of this fiber had been shown to be inhibited by stimulation of pressoreceptors, as described above. The right group of panels of Figure 12 displays responses of two renal units; the discharge rate of the larger spike increased from 1.3 to 3.2 spikes/s, and that of the smaller spike increased from 0.5 to 1.9 spikes/s after administration of bradykinin.

Figure 12. Representative oscillographic records showing responses of 1 mesenteric nerve fiber and 2 renal nerve fibers to the stimulation of intestinal receptors with bradykinin.

Top tracings show unit activity during control period. Middle tracings show the 10-s maximum response after application of bradykinin. Unit discharge during recovery period (14 min after washout of bradykinin) is displayed in the bottom tracings. Horizontal and vertical calibrations are indicated. Stimulation of intestinal receptors had a greater effect on firing of the mesenteric fiber than on firing of the renal fibers.

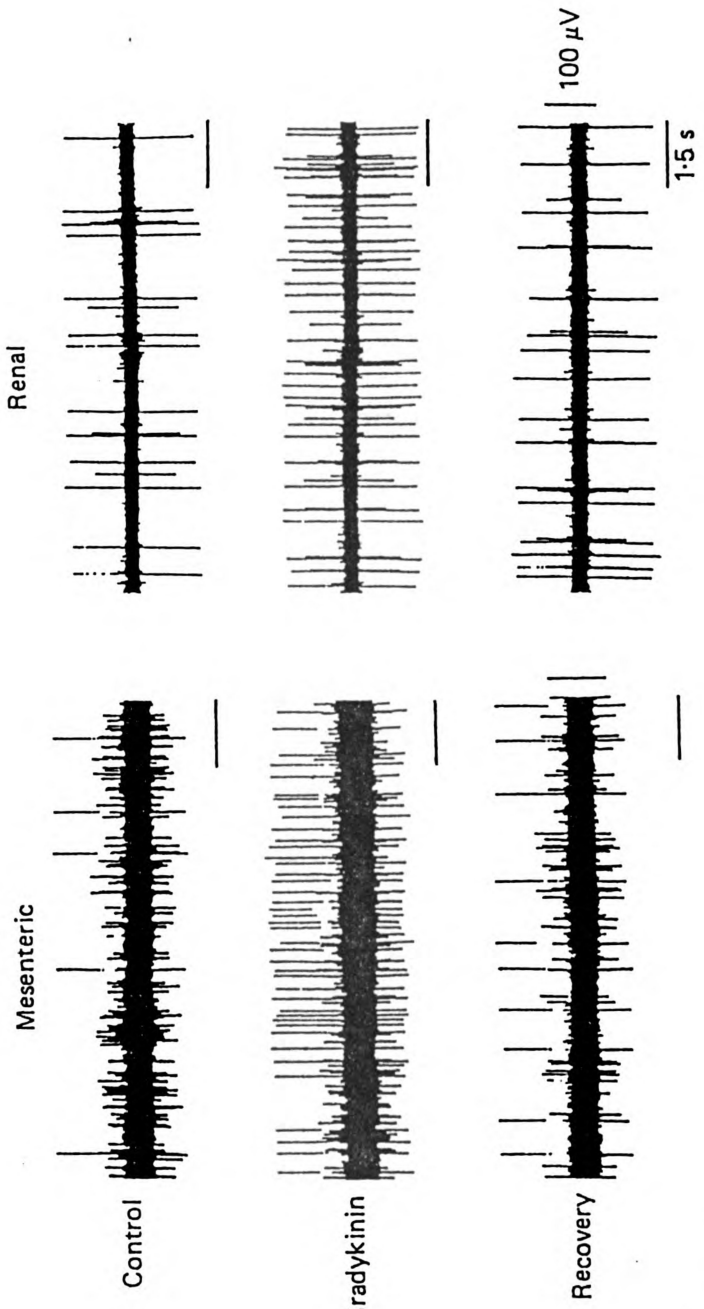

Figure 12.

According to confidence interval tests, discharge rates of most of the 18 mesenteric and 7 renal postganglionic fibers tested increased following application of 10 μg bradykinin to the serosal surface of the small intestine. Bradykinin increased activity of 15 mesenteric fibers, decreased activity of 1 mesenteric fiber, and caused no change in the activity of 2 mesenteric fibers. The magnitude of excitation among the 15 mesenteric fibers was variable, ranging from a 40% increase to a 17.4-fold increase (mean ± S.D. = 5.6 ± 5.4-fold) in neuronal discharge. Responses of individual fibers were distributed randomly throughout this range; and, therefore, mesenteric units could not easily be classified into subgroups based on the magnitude of their response to bradykinin. Activity of 5 renal fibers was excited by this stimulus, and activity of 2 renal fibers was not affected. The magnitude of excitation of the 5 renal nerve fibers ranged from a 23% to a 3.8-fold (mean ± S.D. = 1.5 ± 1.4-fold) increase in firing frequency. This range of excitation of renal fibers was similar to that reported by Meckler (1987). In that study, the renal nerve fibers appeared to comprise a homogeneous population; no subgroups within this range of renal excitatory responses could be detected. Magnitudes of responses of the 15 mesenteric and the 5 renal fibers that were excited by bradykinin were compared (Figure 13). Activity of mesenteric and renal fibers increased by 560 ± 140% and 151 ± 64% (mean ± S.E.), respectively.

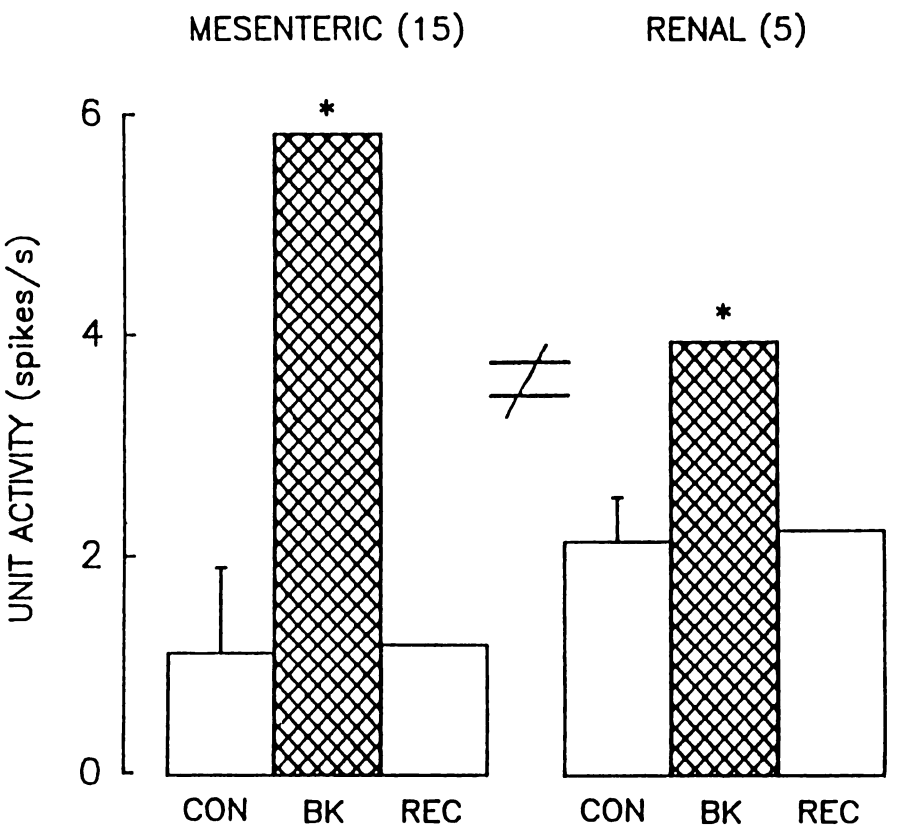

Figure 13. Responses in activity of mesenteric and renal neurons caused by stimulation of intestinal receptors with bradykinin.

Bars represent mean rates of discharge expressed as spikes/s. The 2-min control period (CON), the 10-s period of maximum response (BK) and the 2-min recovery period (REC) are indicated. Numbers of fibers are noted in parentheses. Variability is indicated by pooled standard error. Asterisks indicate significant difference from control. Excitation of activity of mesenteric neurons was significantly greater in magnitude than that of renal neurons ($\neq$).

Although this stimulus excited approximately the same proportion of fibers within both nerves, the increase in activity of mesenteric neurons was significantly greater than that of renal neurons. The cardiovascular responses caused by serosal applications of bradykinin were markedly different from those which had been elicited by injecting the peptide into the isolated intestinal vasculature. A 6.8 ± 2.2 mmHg increase in mean arterial pressure had been elicited by intra-arterial injections of bradykinin into the isolated intestinal circulation. When the intestinal vessels were not excluded from the systemic circulation, and bradykinin was applied to the serosal surface, mean arterial pressure increased by 24.0 ± 4.9 mm Hg. Responses usually began within 10 s, and the maximum increases in unit activity and blood pressure occurred 24.3 ± 3.0 and 47.0 ± 4.4 s, respectively, after serosal application of bradykinin.

Since stimulation of intestinal receptors caused increases in systemic arterial pressure, and, since a major portion of the activity of renal, but not mesenteric, nerves is sensitive to pressoreceptor inputs (Figures 2,10 and 11), the activation of arterial and cardiopulmonary pressoreceptors may have contributed to the differential distribution of reflex responses to these two nerves. To investigate this possibility mesenteric units were classified according to their responses to the stimulation and unloading of pressoreceptors and to the correlation of their activity

with the arterial pressure pulse. Magnitudes of responses of these subgroups to intestinal stimulation were compared. The 6 mesenteric neurons which were not affected by stimulation or unloading of pressoreceptors (Figure 10) clearly had the greatest responses to the stimulation of intestinal receptors. Activity of one such fiber that increased from 3.8 spikes/s to 18.0 spikes/s is illustrated in Figure 14. The decrease in amplitude of the action potentials seen during excitatory response is similar to effects which have been reported by other investigators (Polosa, 1968; Brown and Guyenet, 1984). Mean discharge rates of these 6 pressoreceptor-insensitive units increased from 1.5 $\pm$ 0.5 to 10.4 $\pm$ 2.6 spikes/s after serosal application of bradykinin. In contrast, activity of fibers which was inhibited by stimulation and/or excited by unloading of pressoreceptors was increased more moderately, from 0.7 $\pm$ 0.2 to 2.2 $\pm$ 0.4 spikes/s. Stimulation of intestinal receptors evoked equivalent responses among neurons which exhibited activity related to the cardiac cycle (510 $\pm$ 230% increase), and neurons which did not appear to have activity correlated with the arterial pressure pulse (540 $\pm$ 230% increase). The excitation of activity among any subgroup of mesenteric fibers was significantly greater in magnitude than that of renal fibers.

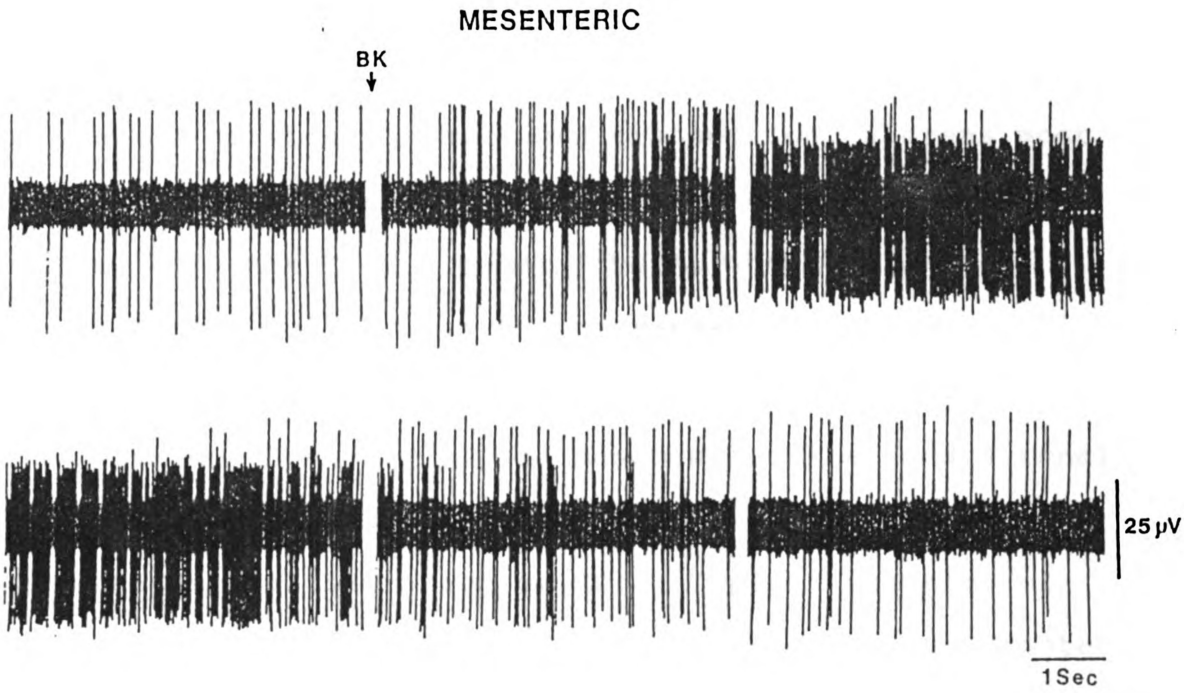

Figure 14. Oscillographic records showing response of one "pressoreceptor-insensitive" mesenteric unit to the stimulation of intestinal receptors with bradykinin.

Record illustrates approximately 35 s of unit activity. Breaks in record, between successive panels, are approximately 1 s in duration. Application of bradykinin (BK) is indicated after a 5 s control period. The unit's firing increased within 5 s, reaching maximal discharge rates at 10-15 s after application of bradykinin. By 30 s, the discharge rate returned to control levels. Horizontal and vertical calibrations are indicated. The decrease in amplitude of the action potentials during rapid firing is consistent with a previous report (Polosa, 1968).

Ongoing and reflex single-fiber activity in spinal cats. Electrical recordings from 9 mesenteric and 4 renal fibers were maintained throughout spinal transection and up to 1 hr afterward. The majority of mesenteric neurons continued to fire after cervical spinal cord transection, whereas most renal neurons became quiescent. Eight of 9 mesenteric fibers, but only 1 of 4 renal fibers, which were spontaneously active when the neuraxis was intact, continued to spontaneously discharge 1 hr after spinal cord transection. Activity of one mesenteric fiber and 3 renal fibers could be elicited in the spinal state only by intravenous administration of KCl. Typical examples of ongoing activity of a mesenteric unit which was unaffected by spinal cord transection, and a renal unit which ceased firing after severing the spinal cord, are illustrated in Figure 15. After transection of the spinal cord, the renal fiber was induced to fire by administration of KCl. As a group, the spontaneous firing rate of mesenteric neurons was unaltered by spinal transection. Before transection, the mean discharge rate of mesenteric nerve fibers was 1.1 $\pm$ 0.2 spikes/s. After severing the spinal cord, the mean discharge rate of these fibers was 0.9 $\pm$ 0.3 spikes/s. The one renal fiber that maintained discharge after spinal transection, fired at a rate of 0.2 spikes/s in the spinal state, considerably lower than it's firing rate of 2.2 spikes/s prior to severing the spinal cord. Following spinal cord

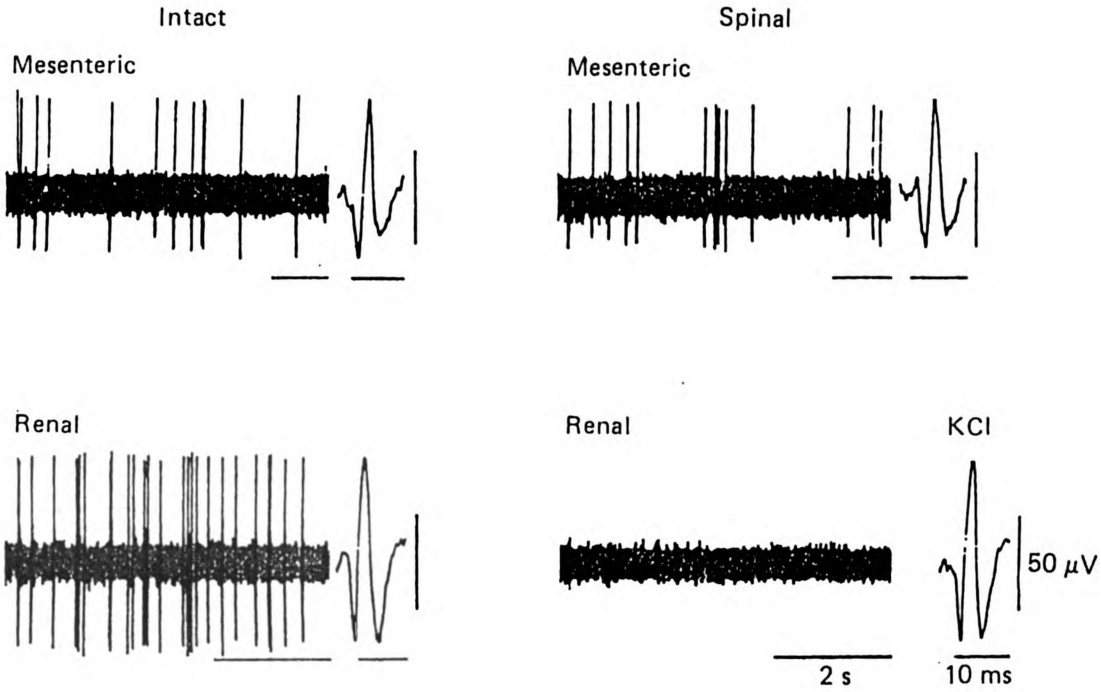

Figure 15. Oscillographic records of ongoing mesenteric and renal unit activity in 2 cats before, and 1 hr after high cervical spinal transection.

Unit discharge in cats with intact neuraxes is shown in the left panels, and 1 hr after spinal cord transection, in the right panels. Each panel displays a tracing of neuronal activity at a slow time base (horizontal calibration, 2 s), followed by a tracing of activity at a faster time base (horizontal calibration, 10 ms), showing the contour of the action potential. Whereas transecting the spinal cord did not affect the discharge rate of mesenteric fiber, the renal unit ceased firing. The renal unit was induced to fire in the spinal state by intravenous injection of saturated KCl.

transection, discharge of mesenteric and renal nerve fibers was no longer correlated with the cardiac cycle even though some of these fibers did have pulse-related activity when the neuraxis was intact. Activity of an additional 2 mesenteric units and 1 renal unit not studied in the intact state could be discriminated and analyzed for responses to intestinal receptor stimulation after the spinal cord was transected.

Stimulation of intestinal receptors 1 hr after spinal transection evoked excitatory responses among all 10 mesenteric units. Discharge of 1 renal unit was excited by this stimulus and that of another was inhibited. All of these units were spontaneously active and responded to the afferent stimulation. No recruitment of 3 silent renal fibers or 1 silent mesenteric fiber was observed. Mesenteric units could be classified into two subgroups, based on their responses to bradykinin. Discharge of four mesenteric fibers increased less than 100%, whereas discharge of 5 fibers increased more than 3-fold. Three pressoreceptor-insensitive mesenteric fibers that had the most intense responses to bradykinin when the neuraxis was intact also had the greatest responses after spinal cord transection. Responses of 6 mesenteric units could be compared before and after spinal cord transection. Before transection, stimulation with bradykinin increased activity of the mesenteric units by 6.3 ± 2.7 spikes/s, and after transection, the response of these units was significantly attenuated to an increase of

3.3 $\pm$ 1.6 spikes/s. The reflex increases in firing of these mesenteric fibers remained significant after transection. Thus, exclusively spinal pathways may contribute to the responses observed in the intact cats, although the intestino-intestinal reflex does appear to contain a significant supraspinal component.

DISCUSSION

Chemical stimulation of intestinal receptors evoked a greater increase in the discharge of mesenteric efferent nerves than renal efferent nerves, supporting the hypothesis that viscero-sympathetic reflexes may be centrally organized according to Sherrington's local sign principle (1906), to cause preferential excitation of neural activity directed to the organ from which the reflex originates. This organization has been proposed to regulate the pattern of reflex responses caused by stimulation of receptors in a variety of organs, including the spleen (Calaresu et al. 1984), urinary bladder (Floyd et al. 1982), heart (Malliani, 1982) and small intestine (Ninomiya et al. 1974). The functional significance of the non-uniform neural response pattern to chemical stimulation of intestinal receptors has recently been investigated (Weaver et al. 1987), and the reflex has been shown to cause greater vasoconstriction in mesenteric than in renal vascular beds. The current results are similar to those of Ninomiya et al. (1974), who reported that the stimulation of intestinal mechanoreceptors excites mesenteric nerve activity more than renal nerve activity. However, the reflex increase in mesenteric nerve activity described by Ninomiya et al. (1974) occured in the absence of cardiovascular effects, and, thus, it was notably different from that observed in the present study.

Intra-arterial injections or topical application of the algogenic substances bradykinin and capsaicin have been used by a number of investigators to stimulate receptors in a variety of viscera (Calaresu et al. 1984; Guzman et al. 1962; Haupt et al. 1983; Lew and Longhurst, 1986; Longhust et al. 1984b; Malliani, 1982; Meckler and Weaver, 1988; Ohno, Yajima, Urano and Nakamura, 1984; Tobey and Weaver, 1987; Weaver et al. 1987), causing excitation of A-delta and C fiber afferent nerve activity (Longhurst et al. 1984a; Mense and Schmidt, 1974). In the present study, the afferent neurons stimulated by injections of bradykinin into the superior mesenteric artery, or by serosal application of the peptide, may comprise a homogeneous population. Injection of bradykinin into the inferior mesenteric artery and the topical application of bradykinin to the wall of the colon have been shown to activate the same group of afferent fibers (Haupt et al. 1983). Indeed, both methods of administration were equally effective in evoking excitation of sympathetic outflow.

Although neural response patterns caused by intra-arterial injections and serosal applications of bradykinin were similar, cardiovascular responses were markedly different. A 6.8 ± 2.2 mmHg increase in mean arterial pressure had been elicited by intra-arterial injections of bradykinin into the isolated intestinal circulation. When the intestinal vessels were not excluded from the systemic

circulation, and bradykinin was applied to the serosal surface, mean arterial pressure increased by 24.0 ± 4.9 mmHg. These results suggest that the intestino-sympathetic excitatory reflex is preferentially directed to mesenteric vasomotor neurons, and is not distributed widely to other components of sympathetic vasomotor outflow. Weaver et al. (1987) have shown that this reflex causes greater vasoconstriction in the mesenteric than renal vascular beds. Constriction in the mesenteric circulation appears to make an important contribution to the pressor responses initiated by stimulation of intestinal receptors.

Non-uniform responses in activity of whole nerves may reflect greater magnitudes of excitation of discharge of all fibers in one nerve relative to those of the other. Alternatively, differential reflexes may result from summation of heterogeneous responses among subpopulations of neurons comprising one or both nerves. For example, Meckler and Weaver (1988) reported that intestinal receptor stimulation caused greater responses of whole renal than whole splenic nerve activity because firing of most renal neurons but only half the population of splenic neurons was excited by this input. The bases of the multifiber response patterns were determined by evaluating responses of individual mesenteric and renal nerve fibers to the stimulation of intestinal receptors and pressoreceptors. Few studies have characterized patterns of activity of visceral

postganglionic fibers, and activity of individual fibers within mesenteric nerves has not previously been described. Chemical stimulation of intestinal receptors caused increases in discharge of 84% of mesenteric and 71% of renal nerve fibers. Meckler and Weaver (1988) reported that activity of 87% of renal postganglionic fibers was excited by this stimulus. Although this stimulus excited the same proportion of fibers in both nerves, the increase in firing of mesenteric fibers was significantly greater than that of renal fibers (Figure 13). Therefore, the non-uniform multifiber response pattern must reflect greater magnitudes of excitation of mesenteric than renal neurons, rather than no response in a subpopulation of renal neurons. Conversely, because only 60% of mesenteric fibers (Figures 9 and 10), but all renal fibers (Figures 8 and 11), were engaged in pressoreceptor reflexes, the differential multifiber response patterns caused by stimulation of pressoreceptors probably involved inhibitory responses among only a subpopulation of mesenteric fibers.

The finding that activity of some mesenteric units is excited, and that activity of other mesenteric units is inhibited following injections of phenylephrine and nitroprusside, respectively, is puzzling, because these response patterns are opposite to those initiated by arterial and cardiopulmonary pressoreceptors. However, only one unit responded "inappropriately" to injections of both

phenylephrine and nitroprusside. Discharge of 6 of 7 units which was excited or not affected during the phenylephrine-induced pressor responses was excited during depressor responses to nitroprusside, and firing of 4 of 5 five fibers which was inhibited or not affected when blood pressure was lowered by nitroprusside infusions was inhibited during pressor responses to phenylephrine. Therefore, activity of these fibers was not insensitive to all pressoreceptor inputs. These neuronal responses may reflect an interaction of opposing reflexes. The changes in blood pressure caused by injections of phenylephrine and nitroprusside may have altered mesenteric vascular volume to affect the activity of mesenteric baroreceptors, to elicit a reflex opposite to that caused by stimulation of arterial and cardiopulmonary pressoreceptors. Andrews et al. (1972), have described one such group of receptors, which, when activated by distension within the mesenteric venous bed, evoke a reflex excitation of efferent mesenteric nerve activity.

Mesenteric nerves contain elements which innervate blood vessels, myenteric ganglia and the intestinal mucosa (Costa and Furness, 1984). Mesenteric fibers in the present study can be classified into two distinct subgroups, based on their sensitivity to pressoreceptor influences. Since arterial and cardiopulmonary pressoreceptors affect intestinal blood flow (Brooksby and Donald, 1971; Greenway and Lister, 1974), but only weakly affect intestinal motility via sympathetic

mechanisms (see e.g. Fig. 5 of McAllen, 1986c), it is conceivable that the pressoreceptor-sensitive fibers subserve a vasomotor (or venomotor) function, whereas the pressoreceptor-insensitive fibers regulate motility and secretion. Jänig (1986) has suggested that postganglionic fibers with discharge affected by pressoreceptors and chemoreceptors are vasoconstrictor in function, whereas fibers with discharge affected by visceral afferent stimulation control non-vascular visceral functions. However, these characteristics could not be used to classify fibers in the present study, as 5 of 6 of the pressoreceptor-sensitive mesenteric nerve fibers which could be tested, had excitatory responses to intestinal afferent nerve stimulation. In addition, Weaver et al. (1987) have demonstrated that reflex mesenteric vasoconstriction caused by stimulation of intestinal receptors is unaffected by denervation of systemic pressoreceptors. This implies that pressoreceptor-insensitive, as well as pressoreceptor-sensitive, fibers may subserve vasomotor functions.

Since stimulation of intestinal receptors caused pressor responses, activation of baroreceptors may have contributed to the differential pattern of the neural reflexes by suppressing renal nerve responses. Other workers have shown that excitatory reflex responses of renal nerves to chemical stimulation of intestinal receptors with KCl are augmented after vagotomy and carotid occlusion (Khayutin et al. 1969).

Moreover, differences between splenic and renal nerve responses to stimulation of splenic receptors in sino-aortic denervated cats are exaggerated when carotid sinus and aortic pressoreceptor afferent nerves are left intact (Tobey and Weaver, 1987). Zanchetti and co-workers also have reported that sino-aortic reflexes contribute significantly to unequal hemodynamic response patterns during defense behavior in the cat (Baccelli et al. 1981). In the present study, the 6 mesenteric fibers which exhibited pressoreceptor-independent activity had the most intense responses to the chemical stimulation of intestinal receptors, suggesting that the stimulation of systemic pressoreceptors may have contributed to the unequal distribution of reflex responses to mesenteric and renal nerves. However, excitation of firing of pressoreceptor-sensitive mesenteric nerve fibers was still greater than that of renal nerve fibers. In addition, the three pressoreceptor-insensitive fibers that had the greatest responses to bradykinin when the neuraxis was intact continued to respond with the greatest intensity after spinal cord transection. Furthermore, although renal whole-nerve responses to stimulation of intestinal receptors are augmented after sino-aortic denervation and vagotomy, these responses are still smaller than those of mesenteric nerves (Weaver et al. 1987). Therefore, although activation of arterial and cardiopulmonary pressoreceptors may contribute to the unequal mesenteric and renal nerve responses elicited

by stimulation of intestinal receptors, pressoreceptor inputs are not crucial to the genesis of this non-uniform neural response pattern.

The central organization of the reflex responses to stimulation of intestinal receptors was investigated further by studying reflex responses of mesenteric and renal nerves to the stimulation of intestinal receptors both before, and 1 hr after high cervical spinal cord transection. Whereas spontaneous multifiber activity of mesenteric nerves was not affected by spinal transection, that of renal nerves was significantly depressed. Recordings of single-unit firing revealed that spinal cord transection caused the cessation of discharge of 75% of renal, but only 11% of mesenteric neurons. However, as recordings were made from only 4 renal nerve fibers after transection of the spinal cord, it was not possible to characterize the discharge pattern of the population accurately. Meckler (1987), using surgical procedures and techniques similar to those used in the present study, was able to maintain recordings from 13 renal nerve fibers throughout high cervical transection and for up to 1 hr afterward. The combined data from the present study and that of Meckler (1987) shows that transection of the spinal cord caused cessation of activity of 9 of 17 renal units. The discharge rate of the 8 renal units that continued to fire in the spinal state was 1.4 ± 0.5 spikes/s, and was not different from their mean spontaneous firing rate

of 1.5 ± 0.4 spikes/s prior to severing the spinal cord. This indicates that the profound decrease in multifiber renal nerve activity following spinal cord transection was due to the cessation of activity of a subpopulation of renal neurons, rather than a general decrease in the spontaneous discharge of all neurons. These results (the present study; Meckler, 1987), and those of other investigations (Meckler and Weaver, 1985; Ninomiya and Irisawa, 1975), suggest that ongoing sympathetic outflow to the splanchnic capacitive circulation is less dependent upon supraspinal drive than is that directed to the kidney.

The functional significance of maintained mesenteric and splenic (Meckler and Weaver, 1985; 1988) nerve activity in the spinal state remains to be determined. Since phenylephrine infusions were necessary to support systemic arterial blood pressure, sustained sympathetic influences on these capacitive beds may not have been sufficient to support splanchnic vascular tone. Failure of this discharge to cause vasoconstriction could occur because nerve impulses which are delivered phasically produce greater vascular tone than continuous impulses (Andersson 1983; Nilsson et al. 1985), and spinal transection did eliminate the phasic, irregular component of mesenteric nerve activity (see Figure 25). The significance of the maintained mesenteric nerve discharge in the spinal animals will be discussed in more detail in a subsequent section.

Results obtained from recording multifiber nerve activity in spinal cats suggested that the intestino-sympathetic excitatory reflex does contain a significant spinal component. However, because of the extreme differences in basal multifiber activity of mesenteric and renal nerves in the spinal cats, responses of these nerves to stimulation of intestinal receptors could not be appropriately compared. Are the unequal mesenteric and renal response patterns organized within the spinal cord? A comparison of the responses of single units within mesenteric and renal nerves would answer this question. Meckler (1987) reported excitation of discharge of 7 renal nerve fibers following the chemical stimulation of intestinal receptors in spinal cats. His data are combined with data from the present study in Figure 16. The magnitude of excitation of renal fibers ranged from a 50% increase to a 3.3-fold increase (mean ± S.D. = 1.2 ± 1.0-fold) in neuronal discharge. The excitation of mesenteric neuronal activity was significantly greater than that of renal neuronal activity indicating that exclusively spinal pathways may contribute greatly to the responses observed in cats with intact neuraxes. Still, the intestino-intestinal reflex appeared to contain a significant supraspinal component, as the magnitude of excitation of mesenteric fibers was attenuated after spinal transection. Horeyseck and Jänig (1974) have suggested that sympathetic reflexes are

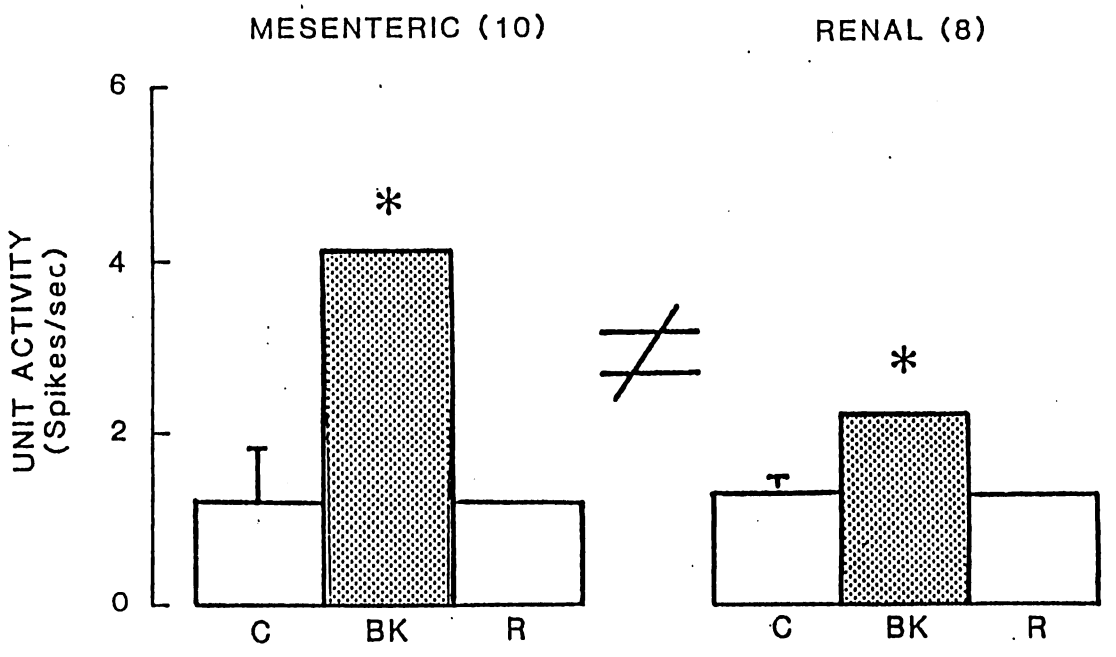

Figure 16. Responses in activity of mesenteric and renal neurons caused by stimulation of intestinal receptors 1 hr after high cervical spinal cord transection.

Format and abbreviations as in Figure 13. Figure includes data from Meckler (1987). Data only from those neurons that were spontaneously firng are shown. Excitation of activity of mesenteric neurons was significantly greater in magnitude than that of renal neurons (≠).

diminished after spinal cord transection, due to decreased preganglionic excitability caused by withdrawl of tonic descending drive. However, mesenteric reflexes were not likely to be diminished for this reason, as ongoing mesenteric activity was not significantly decreased by spinal cord transection.

In conclusion, chemical stimulation of intestinal receptors causes greater excitation of mesenteric than of renal multifiber efferent nerve activity. Neural circuits complete within the spinal cord are sufficient for the expression of these unequal response patterns. Since this stimulus excited the same proportion of fibers in both nerves, the differential multifiber response pattern must reflect greater magnitudes of excitation of mesenteric than renal neurons, rather than no response in a subpopulation of renal neurons. Although concomitant stimulation of pressoreceptors may contribute to the differential distribution of reflex responses to these two nerves, stimulation of pressoreceptors did not appear to be crucial to the genesis of the non-uniform response pattern. The results support the hypothesis that viscero-sympathetic reflexes may be organized to cause preferential excitation of neural activity directed to the organ from which the reflex originates. Alternatively, mesenteric nerve discharge may be particularly sensitive to any visceral afferent influence.

CAPSAICIN TREATMENT ATTENUATES THE REFLEX EXCITATION OF SYMPATHETIC ACTIVITY CAUSED BY CHEMICAL STIMULATION OF INTESTINAL AFFERENT NERVES

Brain Research, 397: 145-151, 1986

RATIONALE

The experiments described above demonstrated that stimulation of intestinal afferent nerves with bradykinin produces significant excitation of mesenteric and renal efferent nerve activity. As it is known that bradykinin stimulates nociceptors (Guzman et al. 1962), the possibility that substance P or other capsaicin-sensitive peptides play a role in the neural transmission of this reflex was investigated. Substance P and other capsaicin-sensitive peptides have been implicated as transmitters of primary sensory neurons involved in nociceptive reflexes (Holzer et al. 1987; Pernow, 1983). Rats were used in this study because capsaicin has been shown to cause depletion of substance P from small sensory neurons in rodents.

HYPOTHESES

1. Chemical stimulation of intestinal receptors with bradykinin evokes a pattern of mesenteric and renal sympathetic nerve excitation in rats similar to that seen in cats (i.e. greater excitation of mesenteric than renal nerve discharge).

2. Chronic subcutaneous administration of capsaicin causes a depletion of substance P-like immunoreactivity in dorsal root ganglia and in the dorsal horn of the spinal cord. Capsaicin pretreatment attenuates the intestino-sympathetic excitatory responses, implying a role for substance P or other capsaicin-sensitive peptides in the central transmission of this reflex. Capsaicin treatment does not effect baroreceptor reflexes.

RESULTS

Capsaicin treatment-general effects and effect on substance P-like immunoreactivity. Chronic capsaicin administration did not appear to affect the behavioral state of the rats. The capsaicin-treated rats were not debilitated; their body weights were stable for the duration of the treatment, they consumed normal quantities of food and water, and they responded to various sensory stimuli (e.g. noise, handling) similarly to the vehicle-treated group.

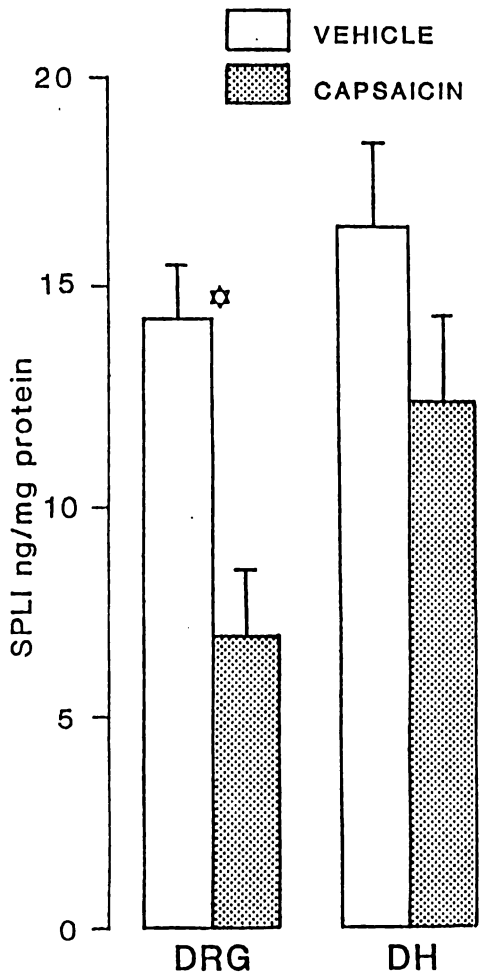

Figure 17. Magnitudes of substance P-like immunoreactivity in capsaicin- and vehicle-treated rats.

Bars represent magnitudes of substance P-like immunoreactivity (SPLI) from radioimmunoassay of homogenized dorsal root ganglia (DRG) of spinal segments T_3-L_3 and of dorsal horns (DH) of the same segments in capsaicin-treated (stippled bars, n = 9) and vehicle-treated rats (open bars, n = 9). Variability indicated by standard error bars. Capsaicin-treated rats were given increasing doses of capsaicin in an ethanol vehicle for 5 days (see text). The cumulative dose of capsaicin was 950 mg/kg. The star indicates significant difference in substance P-like immunoreactivity between capsaicin- and vehicle-treated rats.

Mean arterial pressure of the capsaicin-treated rats (86 ± 8 mmHg) was not different from that of the untreated group (98 ± 11 mmHg). Radioimmunoassay data from capsaicin- and vehicle-treated rats are shown in Figure 17. Capsaicin treatment significantly decreased dorsal root ganglia substance P-like immunoreactivity by 52%. Dorsal horn substance P-like immunoreactivity was not significantly decreased by this treatment.

Stimulation of intestinal receptors. The application of 0.5 - 1.0 μg of bradykinin to the serosal surface of the intestine in the untreated rats caused significant increases in efferent mesenteric and renal nerve activities. Mean mesenteric and renal nerve responses during the first min of stimulation are shown in Table 1 and Figures 18 and 19. In the untreated rats, mesenteric and renal nerve activities were significantly excited 20 s after the application of bradykinin and remained increased for the duration of the stimulus. The average percent changes in activity of mesenteric and renal nerves during the first min of bradykinin stimulation were 100 ± 21% and 33 ± 9%, respectively. Nerve activity remained excited during the second minute of stimulation and slowly returned to control values after the washout of bradykinin. Pretreatment with capsaicin caused a significant attenuation of the mesenteric nerve responses and suppression of the renal nerve

TABLE 1. Neural responses to administration of 0.5–1.0 µg bradykinin to the serosal surface of the small intestine in untreated rats and in rats pretreated with capsaicin.

Experimental Group	Parameter	n	Stimulation period (seconds)								
			Control	10	20	30	40	50	60	Recovery	CV
UNTREATED	MNA	5	47.5	64.2	105.6*	113.2*	92.7*	79.4*	71.4*	48.2	0.22
	RNA	5	18.1	18.8	24.0*	27.7*	25.3*	22.5*	21.8*	20.9	0.11
CAPSAICIN-TREATED	MNA	6	26.4	40.1*	48.3*	45.8*	40.3*	38.2*	38.1*	26.1	0.30
	RNA	5	28.0	31.3	32.2	30.5	26.4	26.3	26.2	29.0	0.16

MNA, mesenteric nerve activity; RNA, renal nerve activity; n, sample size; CV, coefficient of variation; control, 1 min of nerve activity prior to administration of bradykinin; recovery, 1 min of nerve activity 7.9 $\pm$ 1 min after washout of bradykinin; nerve activity is expressed as µV·sec/10 sec; *, significantly different from control; $P < 0.05$.

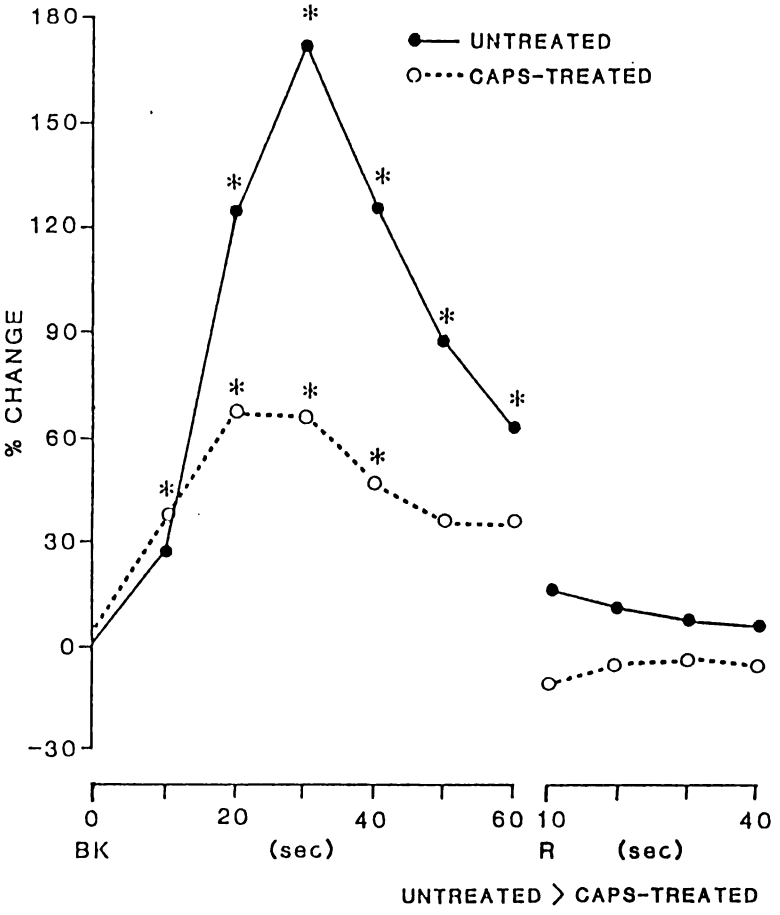

Figure 18. Responses of mesenteric nerves to stimulation of intestinal receptors in untreated and capsaicin-treated rats.

Mean reponses (expressed as percent change from control) of mesenteric nerve activity during the first min of stimulation of intestinal receptors by the application of bradykinin (BK, 0.5-1.0 μg) to the serosal surface of the small intestine in untreated (solid line, n = 5) and capsaicin-treated (dotted line, n = 6) rats. Nerve activity is represented on the abscissa as percent change; the ordinate indicates time in s. Bradykinin was administered at the time indicated, and neural responses were monitored for 60 s. The recovery period (R) began 7.9 ± 1.0 min after the washout of bradykinin. Asterisks indicate significant changes from control. The increase in mesenteric nerve activity in the untreated rats was significantly greater than that in the capsaicin-treated rats.

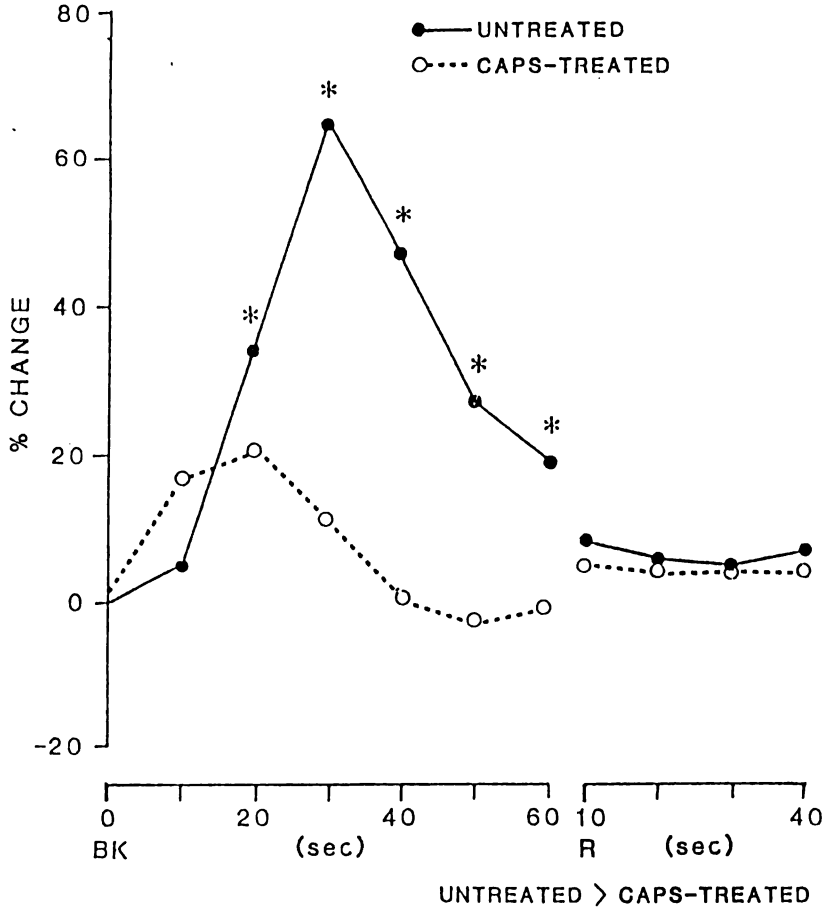

Figure 19. Responses of renal nerves to stimulation of intestinal receptors in untreated and capsaicin-treated rats.

Mean responses (expressed as percent change from control) of renal nerve activity during the first min of stimulation of intestinal receptors by the application of bradykinin (BK, 0.5-1.0 μg) to the serosal suface of the small intestine in untreated (solid lines, n = 5) and capsaicin-treated (dotted lines, n = 5) rats. Format and abbreviations as in Figure 18. The increase in renal nerve activity in the untreated rats was significantly attenuated by capsaicin treatment.

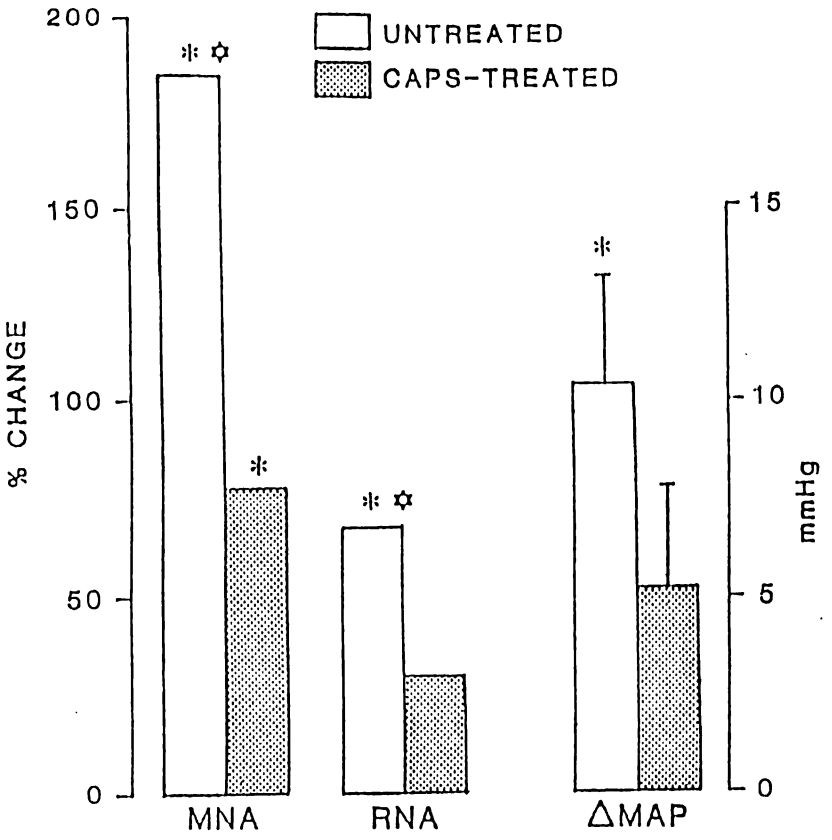

Figure 20. Maximum responses of sympathetic activity and mean arterial pressure to stimulation of intestinal receptors in untreated and capsaicin-treated rats.

Bars represent maximum changes from control (% change) in activity of mesenteric (MNA) and renal (RNA) nerves, and in mean arterial pressure (ΔMAP, expressed as mmHg), in untreated (open bars, n = 5) and capsaicin-treated (stipled bars, n = 6) rats. Asterisks indicate significant difference from control. Stars indicate significant difference between responses in the untreated and capsaicin-treated rats. Coefficients of variation in the nerve responses: MNA, untreated = 0.3; MNA, capsaicin-treated = 0.46; RNA, untreated = 0.16; RNA, capsaicin-treated = 0.1. Variability in blood pressure responses is indicated by standard error. Capsaicin treatment attenuated the increase in mesenteric nerve activity and abolished increases in renal nerve activity and mean arterial pressure.

responses. The increase in mesenteric nerve activity during the first min of intestinal stimulation in the capsaicin-treated rats was 48 ± 6%. Capsaicin treatment had no preferential effect on responses of either nerve as the mesenteric nerve responses were significantly greater than those of renal nerves in both the untreated and the capsaicin-treated groups.

In the untreated rats maximum increases in discharge of mesenteric and renal nerves of 184 ± 41% and 67 ± 16%, respectively, occured 20-30 s after application of bradykinin. These neural responses were accompanied by a 10 ± 3 mmHg increase in arterial blood pressure (Figure 20). In the capsaicin-treated rats the maximum mesenteric nerve response following bradykinin was significantly attenuated to an increase in nerve discharge of 79 ± 39%. Bradykinin did not significantly affect renal nerve activity or blood pressure in the capsaicin-treated rats.

Stimulation of pressoreceptors. Neural responses to the stimulation of pressoreceptors in the untreated rats were not significantly different from those in the capsaicin-treated group. Pressoreceptors were stimulated by injecting 5-10 μg phenylephrine (i.v.) to increase blood pressure (Table 2). In the untreated rats, a 57 ± 9 mmHg increase in mean arterial pressure caused significant inhibition of mesenteric and renal nerve activities, by 21 ± 6% and 66 ± 11%,

TABLE 2. Neural and cardiovascular responses to the infusion of 10 µg phenylephrine (i.v.) in untreated rats and in rats pretreated with capsaicin.

Experimental Group	ΔMAP (mmHg)	Parameter	n	Control	Response	Recovery	CV
UNTREATED	57 ± 9	MNA	6	92.2	68.9*	91.7	0.18
		RNA	5	24.5	11.2*	26.1	0.19
CAPSAICIN-TREATED	51 ± 13	MNA	5	27.8	20.8*	27.5	0.09
		RNA	4	34.4	8.2*	37.3	0.52

MNA, mesenteric nerve activity; RNA, renal nerve activity; ΔMAP, increase in mean arterial pressure caused by phenylephrine; n, sample size; CV, coefficient of variation; control, 1 min of nerve activity prior to infusion of phenylephrine; response, 10 s of nerve activity during maximum increase in blood pressure; recovery, 1 min of nerve activity 1.8 ± 0.3 min after return of arterial pressure to control; nerve activity is expressed as µV·sec/10 sec; *, significantly different from control; $P < 0.05$.

respectively. In the capsaicin-treated rats, a 51 ± 13 mmHg increase in blood pressure inhibited activity of mesenteric nerves by 32 ± 11% and renal nerves by 69 ± 10%. The modest decreases in mesenteric nerve activity caused by pressoreceptor stimulation are consistent with those observed in cats (Figure 2).

DISCUSSION

Stimulation of intestinal afferent nerves by serosal applications of bradykinin produced significant excitation of mesenteric and renal efferent nerve activity and increases in mean arterial pressure in rats. Capsaicin treatment caused significant depletion of substance P from the dorsal root ganglia and significant attenuation of the reflex, implying a role for substance P or other capsaicin-sensitive peptides in the central transmission of this reflex.

Peripheral ganglionic intestino-intestinal reflexes, possibly mediated or modulated by substance P or other peptides, have been demonstrated (Szurszewski, 1981). This suggests that substance P could act to excite preferentially mesenteric nerve activity through the peripheral nervous system. Chronic capsaicin treatment depletes substance P-like immunoreactivity in both prevertebral (Wilken et al. 1983) and dorsal root ganglia (Jessel et al. 1978). In the present study, substance P did not apparently have preferential involvement in the mesenteric component of the reflex, since the pattern of neural responses observed in the untreated rats (greater excitation of mesenteric than renal nerve discharge) was also present in the rats treated with capsaicin. Therefore, ganglionic reflexes mediated by substance P or other capsaicin-sensitive peptides did not contribute to the differential nature of the reflex.

The neural and cardiovascular responses to stimulation of bradykinin-sensitive intestinal receptors were similar to those observed in cats (Figure 3). The nature of the visceral receptors initiating these reflexes has been debated. Longhurst and colleagues suggest that bradykinin causes cardiovascular reflexes primarily through stimulation of chemically-sensitive or polymodal receptors (Longhurst et al. 1984a). Other workers propose that the activation of mechanosensitive afferent nerves by bradykinin (either directly or indirectly via smooth or cardiac muscle contraction) produces reflex changes in cardiovascular function (Floyd et al. 1982; Haupt et al. 1983; Malliani, 1982). Guzman et al. (1962) evoked pseudaffective responses in dogs and cats following intra-arterial injections of bradykinin and proposed that the peptide was stimulating nociceptors. These results differ from those of Malliani and co-workers, who have reported that intracoronary injections of bradykinin caused cardiovascular reflexes without eliciting signs of pain in conscious dogs (Pagani, Pizzinelli, Furlan, Guzzetti, Rimoldi, Sandrone and Malliani, 1985).

The results of the present study support the notion that bradykinin elicits cardiovascular reflexes by stimulating nociceptors. Capsaicin administration significantly decreased substance P-like immunoreactivity in dorsal root ganglia and depressed the bradykinin-induced excitation of

mesenteric and renal nerve firing and pressor responses, implying a role for substance P in the transmission of the reflex. Numerous reports indicate that substance P is a transmitter of sensory neurons that relay information from nociceptors (see Pernow, 1983 for review). Large concentrations of substance P have been found in the dorsal roots and dorsal horn of the spinal cord (DeGroat, 1986; Massari et al. 1983). The iontophoretic application of substance P to cells in the dorsal horn of the cat spinal cord selectively increases the discharge of neurons which are also activated by noxious thermal or mechanical stimuli, whereas activity of neurons which respond to light pressure or hair movement is unaffected or depressed (Henry, 1976; Sastry, 1979). The release of substance P-like immunoreactivity from the spinal cord following stimulation of A-delta and C afferent fibers of the sciatic nerve of cats has been reported. This release was inhibited by intrathecal infusions of morphine (Yaksh et al. 1980). In conscious human subjects stimulation of these fiber groups evokes painful sensations (Turebjörk and Hallin, 1973).

Although the neural and cardiovascular responses to the stimulation of intestinal receptors were markedly attenuated, this stimulus did produce significant excitation of mesenteric nerve activity in the capsaicin-treated rats. Therefore, it is probable that a component of the reflex employs a neurotransmitter which is not sensitive to

capsaicin and remains functional after capsaicin treatment. This hypothesis is tenable because bradykinin can stimulate non-noxious as well as nociceptive afferent nerves (Floyd et al. 1982; Guzman et al. 1962; Longhurst et al. 1984a; Malliani, 1982; Pagani et al. 1985), and capsaicin treatment is thought to affect only the nociceptive components of the reflex (Fitzgerald, 1983). Alternatively, the reflex may be mediated solely by substance P and other capsaicin-sensitive peptides, and the degree to which the reflex is attenuated relates to the extent of depletion of these transmitters. Immunohistochemical studies have shown decreases in substance P, somatostatin, VIP and cholecystokinin-like immunoreactivity in the spinal cord following treatment of neonatal rats with capsaicin (Gamse, Leeman, Holzer and Lembeck, 1981; Jancsó,Hökfelt, Lundberg, Kiraly, Halász, Nilsson, Terenius, Rehfeld, Steinbusch, Verhofstad, Elde, Said and Brown, 1981). Since the neurochemical effects of capsaicin administration are not completely selective for substance P, it is possible that other peptides which are depleted by capsaicin act as neurotransmitters or neuromodulators of this reflex. The results of the present study suggest that the reflex may be mediated in part by substance P, since the extent of depression of the reflex in the capsaicin-treated rats appeared to correlate with the depletion of substance P. Capsaicin treatment caused a 52% decrease in substance P-like immunoreactivity in the dorsal

root ganglia, and the maximum reflex increases in discharge of mesenteric and renal nerves were attenuated by 57% and 62%, respectively.

Other studies have suggested that capsaicin sensitive peptides are involved in the central transmission of reflexes originating in the gastrointestinal tract. Capsaicin pretreatment in rats attenuates viscero-visceral excitatory sympathetic reflexes caused by intraperitoneal injections of bradykinin (Cervero and McRitchie, 1982) or iodine (Holzer et al. 1987) and cardiovascular reflexes induced by intestinal distension (Lembeck and Skofitsch, 1982). Substance P also has been proposed to be a spinal cord transmitter of hepatic afferent neurons which may be stimulated by bradykinin to elicit activation of the hypothalamo-hypophysial system in rats (Stoppini et al. 1984). In addition, Kaufman and co-workers (Kaufman, Kozlowski, and Rybicki, 1985) have shown that substance P may act as a neurotransmitter or neuromodulator of the reflex pressor response to exercise in cats.

Systemic treatment with capsaicin appears preferentially to deplete substance P in CNS regions receiving input from peripheral nociceptors (see Fitzgerald, 1983 for review). Substance P levels in non-sensory regions or nuclei receiving non-noxious sensory input (e.g. nucleus tractus solitarius) are not affected by capsaicin treatment (Helke, DiMicco, Jacobowitz and Kopin, 1981). Furthermore, chronic capsaicin

treatment does not affect the baroreceptor reflex in conscious guinea pigs (Furness, Elliot, Murphy, Costa and Chalmers, 1982). Therefore, in the present study neural responses to the stimulation of baroreceptors, a non-noxious stimulus, were evaluated to test the specificity of the actions of capsaicin. Capsaicin treatment did not appear to have generalized effects on sympathetic reflexes, as activities of mesenteric and renal sympathetic nerves were inhibited by pressoreceptor activation similarly in the untreated and in the capsaicin-treated rats.

In summary, capsaicin treatment led to significant depletion of substance P-like immunoreactivity in the dorsal root ganglia. In addition, the reflex excitation of mesenteric and renal nerves caused by stimulation of intestinal receptors with bradykinin was significantly attenuated. These results suggest that the central transmission of this reflex response to intestinal receptor stimulation is mediated in part by substance P or other capsaicin-sensitive peptides.

VENTROLATERAL MEDULLARY NEURONS: EFFECTS ON MAGNITUDE AND RHYTHM OF DISCHARGE OF MESENTERIC AND RENAL NERVES IN CATS

Submitted for publication in The Journal of Physiology

RATIONALE

The rostral portion of the ventrolateral medulla (RVLM) contains neurons thought to provide tonic drive to vasomotor and cardiac sympathetic nerves (Calaresu and Yardley, 1988). The finding of sustained firing of mesenteric, but not of renal nerves, in spinal cats (see above) suggests that projections from the RVLM provide tonic drive to renal, but not to mesenteric nerves. However, inputs from the RVLM may affect the periodicity of discharge of both nerves similarly. The role of the RVLM in the maintenance and periodicity of ongoing discharge of mesenteric and renal nerves was assessed by testing the following hypotheses.

HYPOTHESES

1. Inhibition of tonic activity of neurons within the RVLM (blockade) by bilateral application of the inhibitory amino acid glycine causes greater reductions in overall discharge of renal nerves than that of mesenteric nerves.

2. RVLM blockade reduces the 2-6 Hz rhythm in firing of both mesenteric and renal nerves similarly.

3. Blockade of the RVLM unmasks tonically active sympathoinhibitory systems which may contribute to the decreases in nerve discharge. This hypothesis was tested by comparing magnitudes of nerve discharge observed after spinal cord transection to those seen following RVLM blockade.

RESULTS

Stimulation of systemic pressoreceptors. In 17 of 18 cats, nerve activity was tested for pressoreceptor sensitivity by evaluating responses to increases in arterial pressure, elicited by intravenous infusions of phenylephrine (5-20 μg/kg). All renal nerves, and 16 of 17 mesenteric nerves tested had discharge inhibited by stimulation of pressoreceptors, and, thus, these nerves probably contained vasomotor fibers. An increase in mean arterial pressure of 43 ± 13 mm Hg significantly reduced the discharge of renal and mesenteric nerves from 41 to 4 μV·s/10 s (pooled S.E. = 6.5) and from 26 to 13 μV·s/10 s (pooled S.E. =1.9), respectively.

Glycine application to the RVLM. Effects on magnitude of renal and mesenteric nerve discharge. Bilateral application of glycine-soaked cotton pledgets to the ventral surface of the rostral medulla in 14 cats elicited decreases in nerve discharge, blood pressure and heart rate which began within seconds, and reached minimum levels within 2-6 min. In the example shown in Figure 21, firing of the renal nerve decreased more than that of the mesenteric nerve; this relationship was consistently observed in every cat tested. Mean discharge of renal and of mesenteric nerves decreased from 32 to 7 μV·s/10 s (pooled S.E. = 3.5) and from 32 to 21 μV·s/10 s (pooled S.E. = 1.7), respectively (Figure 22). The decrease in firing of renal nerves was significantly greater than that of mesenteric nerves. These neural responses were accompanied by significant reductions in mean arterial pressure, from 126 to 68 mmHg (pooled S.E. = 3.4, Figure 22) and heart rate, from 250 to 222 beats/min (pooled S.E. = 3.2). The durations of these responses were variable. Discharge of mesenteric nerves returned to control within 19 $\pm$ 5 min (mean $\pm$ S.E.) after removal of the pledgets. However, renal nerve activity, arterial blood pressure and heart rate remained decreased for 33 $\pm$ 5 min, 36 $\pm$ 6 min and 32 $\pm$ 6 min, respectively, after removal of the pledgets. As a control, cotton pledgets soaked with warm physiological saline were applied to the medulla in 7 cats.

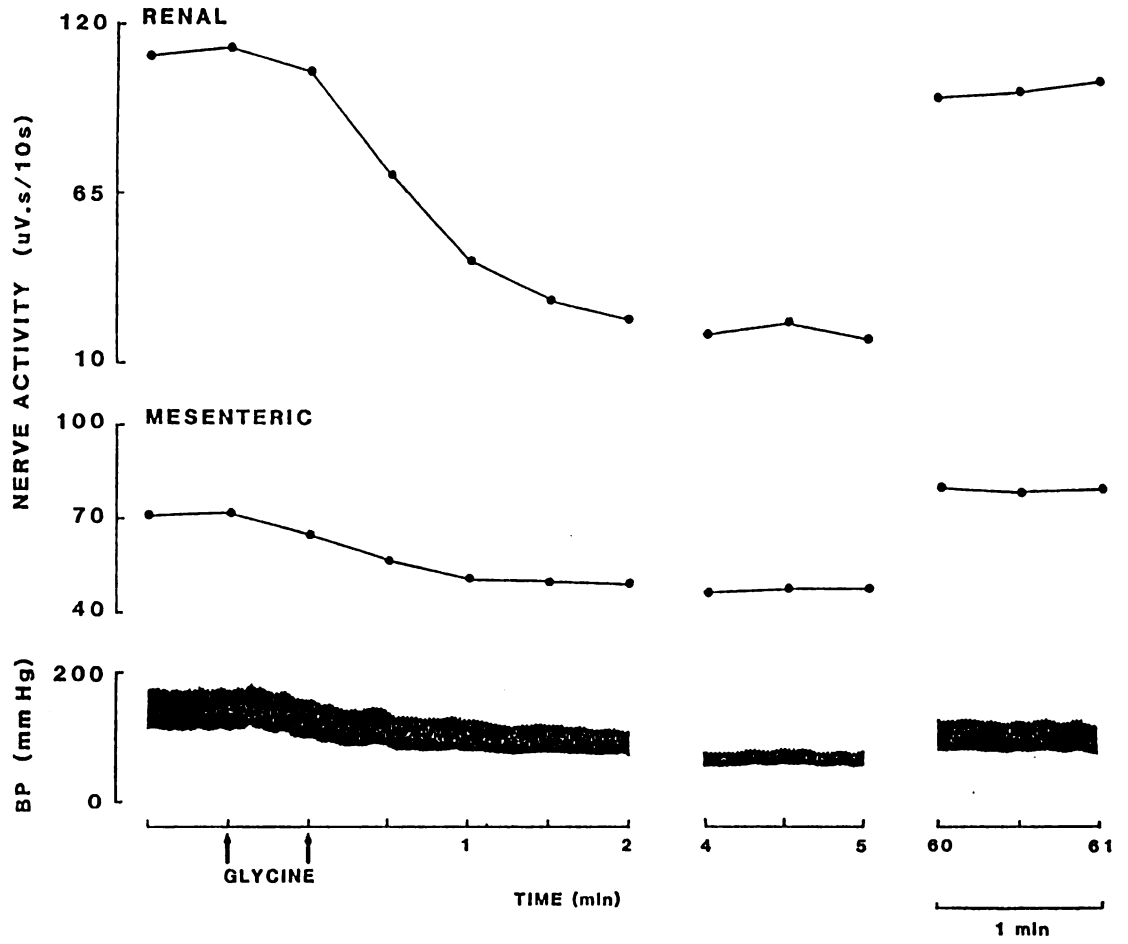

Figure 21. Representative neural and cardiovascular responses to application of glycine to the rostral ventrolateral medulla.

Response of one cat to bilateral application of cotton pledgets soaked in glycine (200 mg/ml, arrows) to the ventral surface of the rostral medulla. Integrated activity (expressed as $\mu V \cdot s/10$ s) of renal and mesenteric nerves is illustrated in the top two panels. Systemic arterial pressure (BP) is illustrated in the bottom panel. Time in minutes is indicated beneath these panels. The pledgets were removed after 5 min. Recovery values 60 min after application of glycine are shown on the far right.

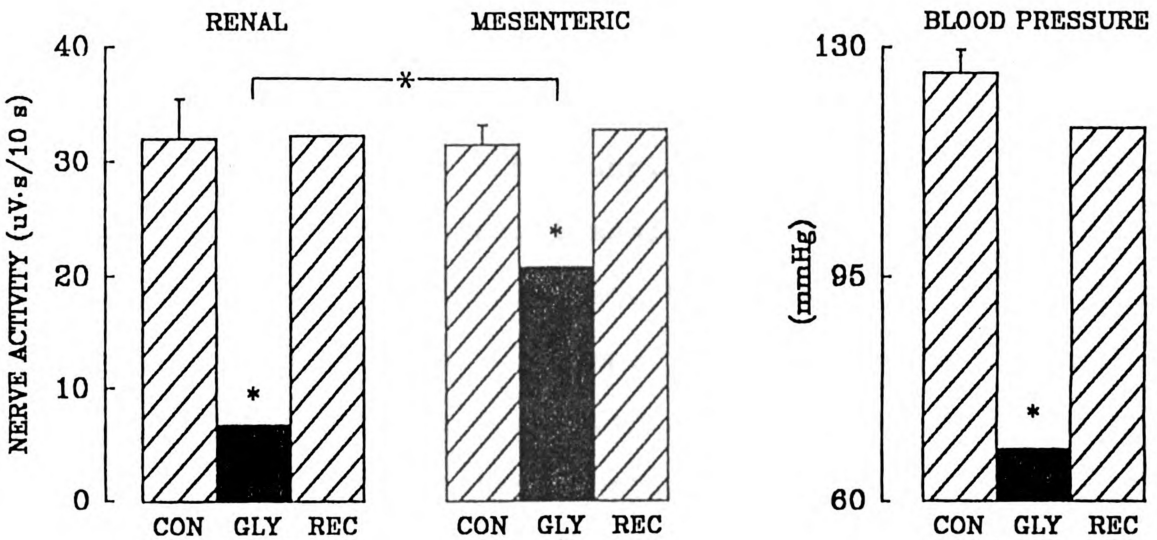

Figure 22. Responses of sympathetic activity and mean arterial pressure to bilateral application of glycine to the ventral surface of the rostral medulla in 14 cats.

Bars represent averages of integrated discharge of renal and mesenteric nerves (expressed as μV·s/10 s) and mean arterial pressure (mm Hg) during 1 min control periods (CON), during 1 min of maximum change from control after application of glycine (GLY) and during 1 min recovery periods (REC). Variability is indicated by pooled standard error. Maximum changes from control occured 4-6 min after application of glycine. Recovery values were recorded 1 hr after application of glycine. Small asterisks indicate significant difference from control. The reduction in renal nerve activity was significantly greater than that of mesenteric nerve activity as indicated by the large asterisk.

This procedure did not affect nerve activity, blood pressure or heart rate.

To determine if decreases in nerve activity observed during RVLM blockade could be attributed to descending sympathoinhibitory systems the application of glycine was repeated, and the spinal cord was severed at the first cervical segment at the nadir of the decrease in nerve activity (3.5 $\pm$ 0.3 min after application of glycine). An increase in nerve discharge immediately after transection of the spinal cord would demonstrate the presence of tonically active sympathoinhibitory systems. The magnitude of renal nerve discharge after transection of the spinal cord was not significantly different from that during blockade of the RVLM (Figure 23), indicating that the reduction in renal nerve firing after application of glycine to the RVLM was not due to tonic sympathoinhibition. In contrast, firing of mesenteric nerves increased after transection of the spinal cord in 10 of 14 cats. The mean magnitude of mesenteric nerve discharge recorded at 5, 30 and 60 min after transection was not significantly different from that recorded during the control period (Figure 23), suggesting that sympathoinhibitory systems may have contributed to the diminished mesenteric nerve firing during RVLM blockade.

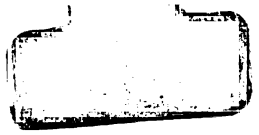

Figure 23. Ongoing discharge of sympathetic nerves after bilateral application of glycine to the ventral surface of the rostral medulla and after high cervical spinal cord transection in 14 cats.

Bars represent mean integrated discharge of renal and mesenteric nerves during 1 min control periods (CON), during 1 min of maximum change from control after application of glycine (GLY), and 5, 30 and 60 min following spinal cord transection at the first cervical segment (C1X). The spinal cord was transected 3.5 $\pm$ 0.3 min after application of glycine. Variability is indicated by pooled standard error. Asterisks indicate significant difference from control. Star indicates significant difference from activity 60 min following C1X. Following C1X renal nerve discharge remained depressed but mesenteric nerve activity increased, returning to control levels.

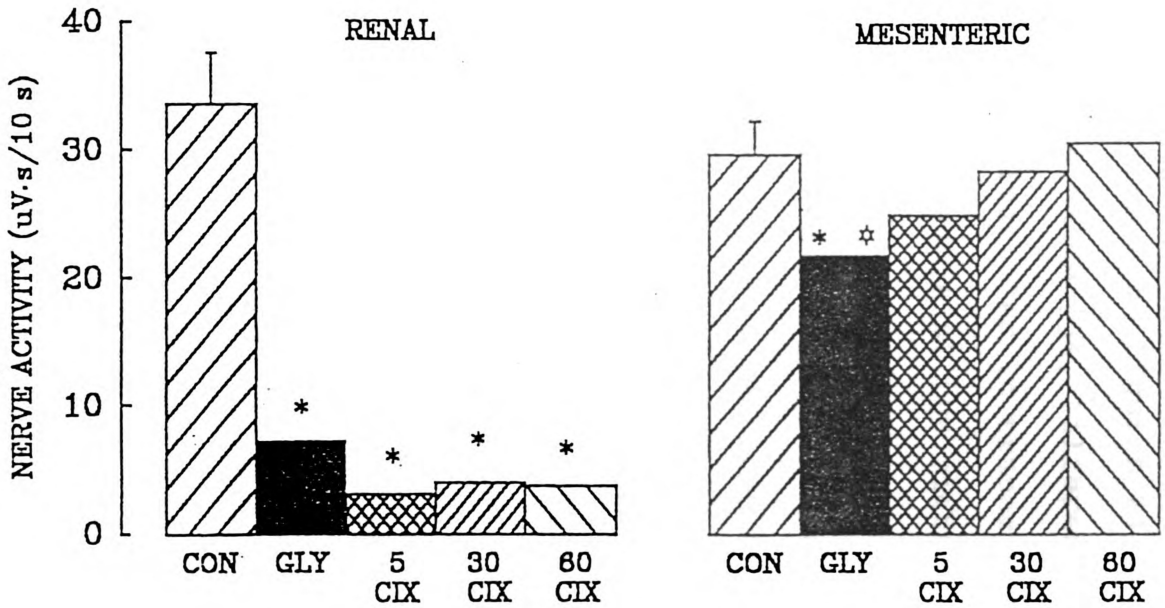
RENAL
MESENTERIC
NERVE ACTIVITY (uV.s/10 s)
40
30
20
10
0
CON
GLY
5 CIX
30 CIX
60 CIX
*
☼

Figure 23.

Periodicities in sympathetic nerve discharge. Slow frequency periodicities in the discharge of renal and mesenteric nerves could be observed when activity was recorded at a bandwidth of 1 Hz to 3 kHz in 9 cats. Neurograms of activity recorded at such a bandwidth are illustrated in traces A of Figures 24 and 25. The same intervals of nerve discharge, filtered at a bandwidth of 100 Hz to 2 kHz are illustrated in traces B, showing only the high frequency components of the signal. Both renal and mesenteric nerves discharged in bursts (Figures 24 and 25, CONTROL, traces B) which appeared as slow oscillations in discharge when activity was recorded at the wide bandwidth (traces A). Power spectral analyses revealed that the major frequencies of the oscillations in nerve discharge ranged from 1-6 Hz (Figures 24 and 25, CONTROL, traces C). As reported by others (Barman and Gebber, 1980), the power density spectra usually contained a peak at, or near, the heart rate. This peak was not a pulse artifact as it was no longer present after transection of the spinal cord. Moreover, this peak disappeared following denervation of the buffer nerves in 4 cats. Occasionally, slower periodicities, at, or near, the respiratory rate were observed, but, as it was not clear if these periodicities were artifacts caused by movement associated with ventilation, they were not analyzed further. Faster periodicities of 10 Hz which have been

Figure 24. Neurograms of ongoing discharge and corresponding power density spectra of one renal nerve during a control period, during the maximum change from control following application of glycine to the ventral surface of the rostral medulla and 1 hr following high cervical spinal cord transection.

Trace A of each panel illustrates a 1.5 s neurogram of nerve activity amplified at a bandwidth of 1 Hz to 3 kHz. The same interval of nerve discharge, filtered at a bandwidth of 100 Hz to 2 kHz is shown in trace B of each panel. Background noise level in the nerve recording after ganglionic blockade with hexamethonium (HEX) is shown at the bottom of the figure. Vertical and horizontal calibrations are indicated. Trace C of each panel illustrates power density spectra of the 1 Hz - 3 kHz discharge. Density spectra are normalized so that relative power (proportional to watts) is plotted against frequency. Note differences in scale of the density spectra. Application of glycine (GLYCINE) and transection of the spinal cord (SPINAL) reduced filtered nerve discharge to 18% and 15% of control, respectively (traces B), and reduced total power in the 1-6 Hz frequency band of the spectra to 1% and 0% of control, respectively (traces C).

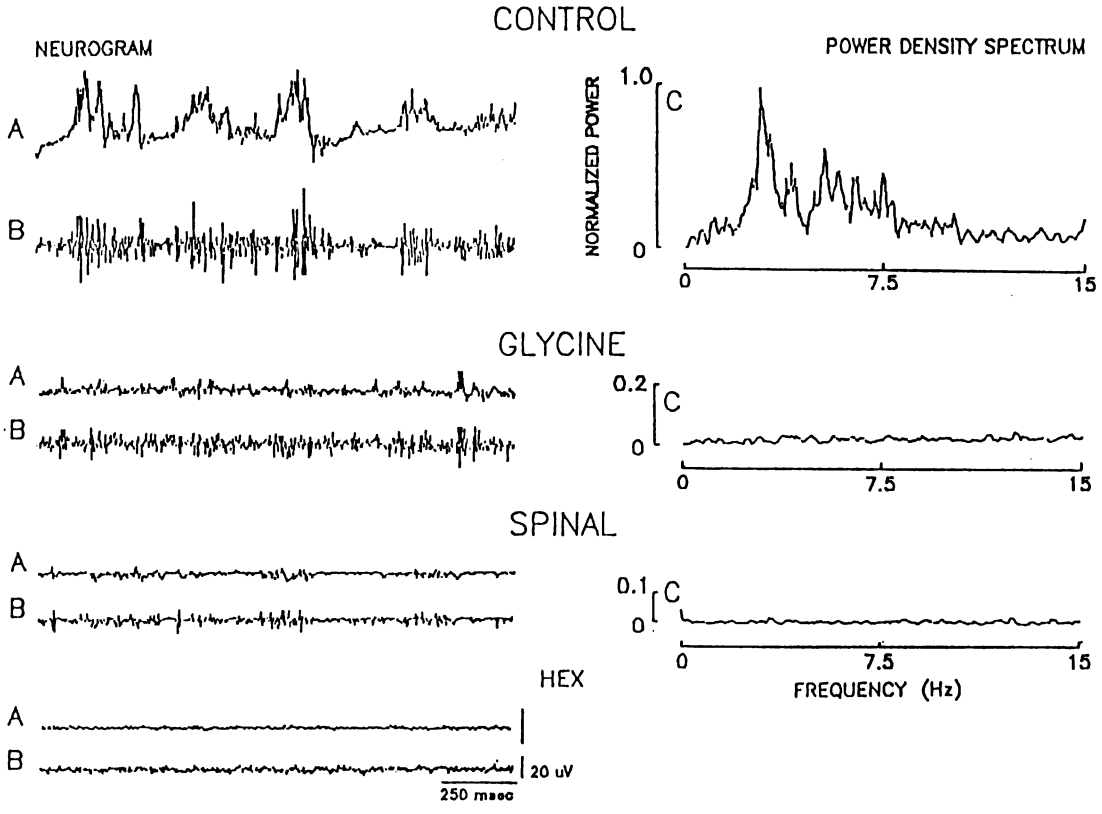
CONTROL
NEUROGRAM
POWER DENSITY SPECTRUM
A
B
NORMALIZED POWER
1.0
C
0
0
7.5
15
GLYCINE
A
B
0.2
C
0
0
7.5
15
SPINAL
A
B
0.1
C
0
0
7.5
15
FREQUENCY (Hz)
HEX
A
B
20 uV
250 msec

Figure 24.

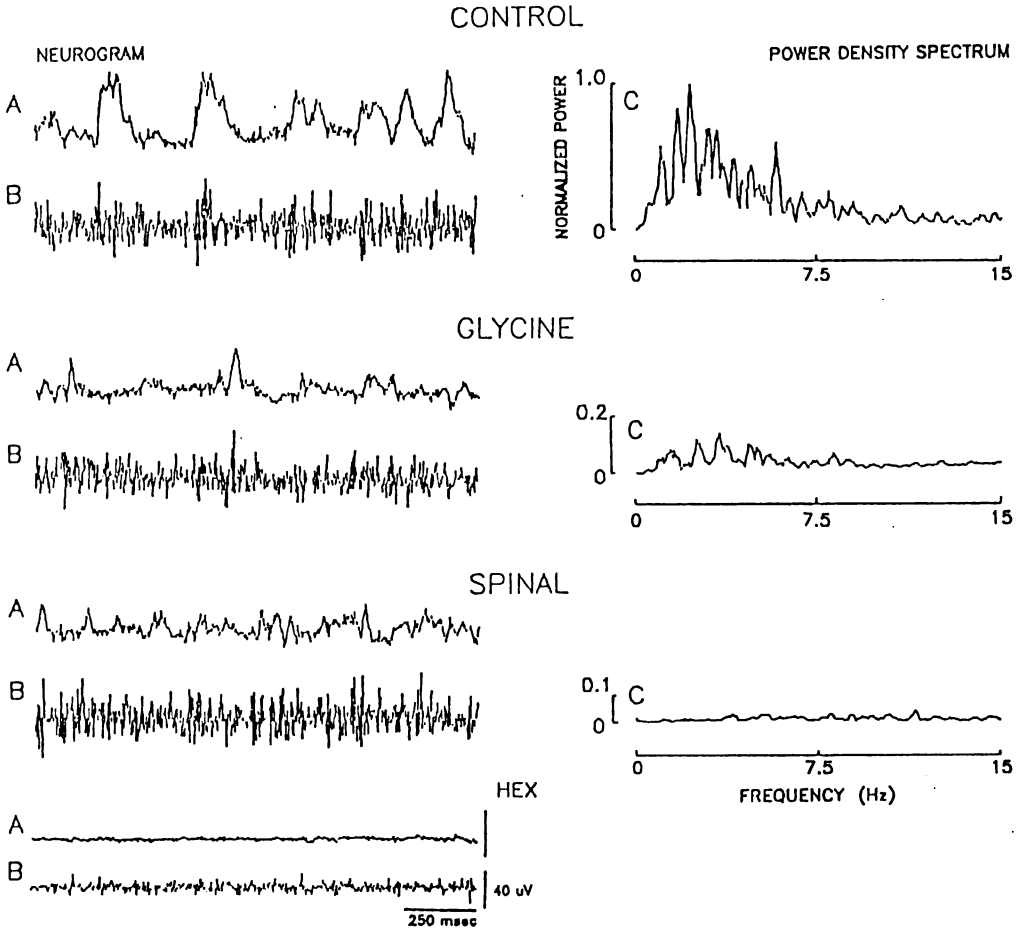

Figure 25. Neurograms of ongoing discharge and corresponding power density spectra of one mesenteric nerve during a control period, during the maximum change from control following application of glycine to the ventral surface of the rostral medulla and 1 hr following high cervical spinal cord transection.

Activity was recorded in the same cat as the renal nerve discharge shown in Figure 4. Format as in Figure 4. Application of glycine and transection of the spinal cord reduced filtered nerve discharge to 73% and 95% of control, respectively (traces B), and reduced total power in the 1-6 Hz frequency band of the density spectra to 24% and 2% of control, respectively (traces C).

described by other investigators (Gootman and Cohen, 1981) were never observed in the present study.

Glycine application to the RVLM. Effect on rhythm of discharge of renal and mesenteric nerves. Application of glycine to the RVLM consistently elicited decreases in the slow rhythm in nerve firing (Figures 24 and 25, GLYCINE, traces A). Changes in total power of the density spectra in the 1-6 Hz frequency range were used as quantitative indices of changes in the slow rhythm in nerve firing. Changes in overall nerve discharge were assessed by quantifying changes in integrated discharge of the filtered (100 Hz - 2 kHz) neural signals. In the density spectrum shown in Figure 24 (GLYCINE, trace C), total power of the renal nerve discharge in the 1-6 Hz frequency band was reduced to 1% of control. Integration of the filtered neural signal revealed that activity of this renal nerve was reduced to 18% of control during the RVLM blockade (Figure 24, GLYCINE, trace B). Similarly, total power in the 1-6 Hz band of the density spectrum of mesenteric nerve discharge shown in Figure 25 (GLYCINE, trace C) was reduced to 24% of control, whereas integrated filtered nerve discharge was only decreased to 73% of control following application of glycine to the medulla (GLYCINE, trace B). Non-parametric statistics indicated that the percentage decreases in total power in the 1-6 Hz frequency range of the renal and mesenteric nerve density

Figure 26. Effects of application of glycine to the ventral surface of the rostral medulla and of high cervical spinal transection on ongoing discharge of renal and mesenteric nerves in 9 cats.

Activity of renal and mesenteric nerves was recorded simultaneously in cats 1-7. In cat 8 only renal, and in cat 9, only mesenteric nerve activity was recorded. Bars represent total power of the density spectra in the 1-6 Hz frequency band (Filled Bars) and integrated nerve activity (Cross Hatched Bars) expressed as percent of control (prior to application of glycine). Non-parametric statistics indicated the following: After glycine application; Δ renal power > Δ renal activity; Δ mesenteric power > Δ mesenteric activity; Δ renal activity > Δ mesenteric activity; Δ renal power = Δ mesenteric power. After spinal transection; Δ renal activity = Δ renal power; Δ mesenteric power > Δ mesenteric activity; Δ renal activity > Δ mesenteric activity; Δ renal power > Δ mesenteric power.

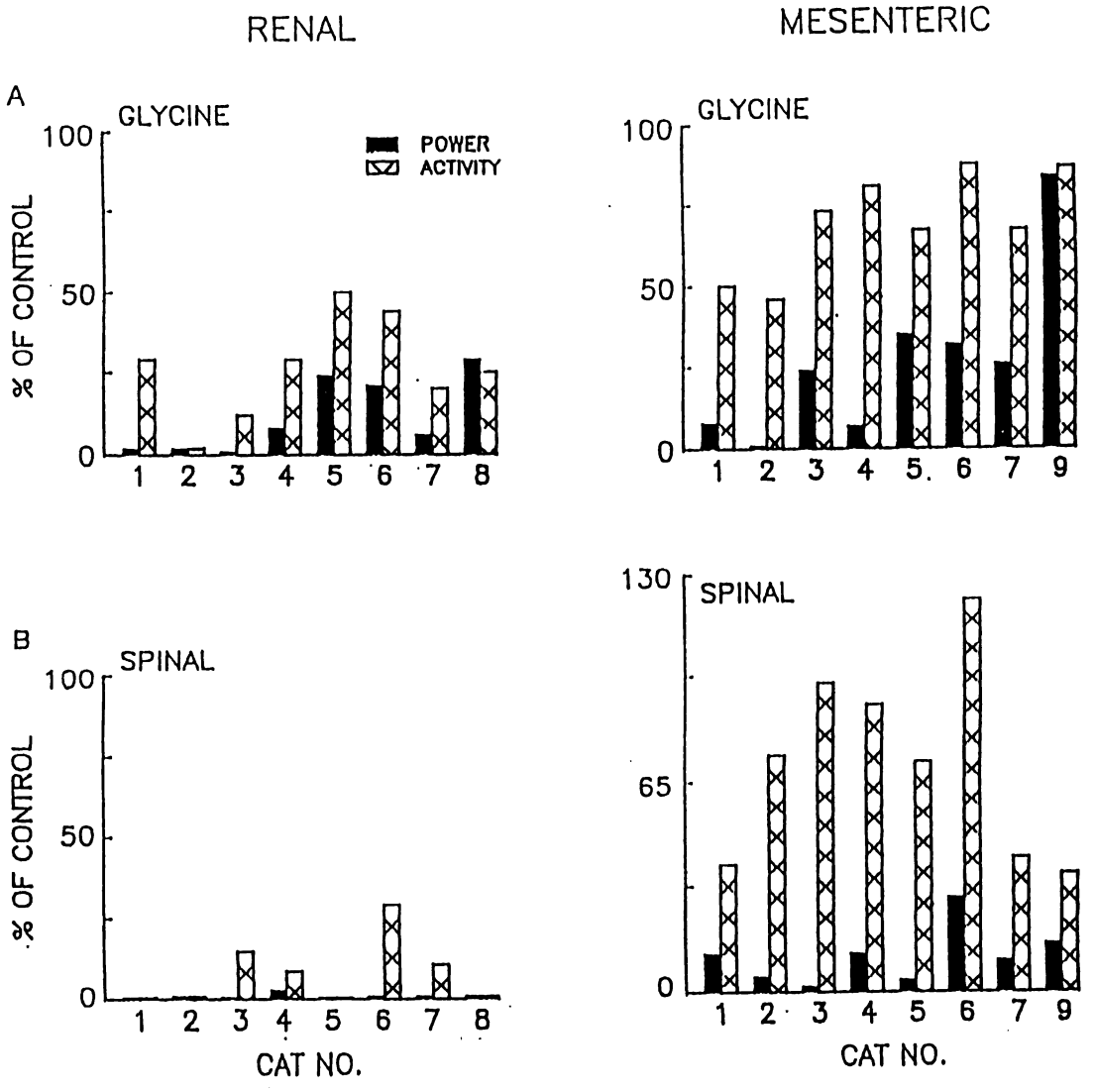
RENAL
MESENTERIC
A
GLYCINE
POWER
ACTIVITY
% OF CONTROL
100
50
0
1 2 3 4 5 6 7 8
GLYCINE
100
50
0
1 2 3 4 5 6 7 9
B
SPINAL
% OF CONTROL
100
50
0
1 2 3 4 5 6 7 8
CAT NO.
130
SPINAL
65
0
1 2 3 4 5 6 7 9
CAT NO.

Figure 26.

Table 3. Changes in total power of the density spectra (slow rhythm in nerve firing) and in mean arterial pressure following topical application of glycine to the RVLM, bilaterally.

		BP Decreased		BP Supported		Denervated	
Nerve	Cat No.	%Δ Power	ΔMAP	%Δ Power	ΔMAP	%Δ Power	ΔMAP
REN	1	-79	-33	-79	-5	-84	-55
	2	-94	-55	-92	-2	-92	-18
	3	-71	-12	-89	-1	-82	-74
MES	1	-68	-33	-73	-5	-46	-55
	2	-74	-55	-73	-2	-87	-18
	4	-16	-12	-16	-7	-69	-78

REN, renal nerve; MES, mesenteric nerve; %Δ power, percentage change from control in total power of the density spectra in the 1-6 Hz frequency range; ΔMAP, change in mean arterial pressure in mmHg; BP Decreased, arterial pressure was allowed to decrease following application of glycine; BP supported, arterial pressure was supported by intravenous infusion of phenylephrine (5-10 $\mu g\ kg^{-1}\ min^{-1}$); denervated, 60 min after sinoaortic denervation and vagotomy.

spectra (i.e. slow rhythm in nerve firing) were significantly greater than the decreases in integrated nerve discharge (Figure 26A). Although integrated discharge of renal nerves was diminished more than that of mesenteric nerves the reductions in total power of the renal and mesenteric nerve spectra were not significantly different from each other.

As the inhibition of activity of neurons in the RVLM caused pronounced decreases in arterial blood pressure, the reductions in the slow rhythm in nerve firing may have been secondary to the unloading of pressoreceptors. This possibility was assessed in four cats by evaluating neural responses to RVLM blockade while infusing phenylephrine to maintain arterial blood pressure within 10 mmHg of control levels, and also, after sinoaortic denervation and vagotomy (Table 3). Changes in total power in the 1-6 Hz frequency range of the density spectra seen during responses to glycine, in which arterial pressure was allowed to decrease, were not different from those seen during the support of arterial blood pressure. Moreover, reductions in total power seen after denervation of systemic pressoreceptors were similar to, or greater than, those seen in cats with intact buffer nerves. Therefore, decreases in pressoreceptor inputs during the responses to glycine probably did not significantly contribute to the loss of rhythmicity in sympathetic nerve discharge.

Sympathetic rhythms following spinal cord transection. Following transection of the spinal cord at the first cervical segment the slow rhythm in firing of most nerves was virtually abolished. In the density spectrum shown in Figure 24, total power of renal discharge in the 1-6 Hz frequency range was abolished (SPINAL, trace C). Integrated discharge of the filtered renal nerve signal was reduced to 15% of control after the spinal cord was severed (SPINAL, trace B). Similarly, total power of the spectrum of mesenteric nerve activity (1-6 Hz frequency band) illustrated in Figure 25 was reduced to 2% of control after spinal cord transection (SPINAL, trace C). However, integrated discharge of the mesenteric nerve was maintained at 95% of control (Figure 25, SPINAL, trace B). Nearly all mesenteric nerve discharge which persisted after severing the spinal cord ceased after ganglionic blockade with hexamethonium (Figure 25), indicating that the majority of this activity is not generated peripherally (Szurszewski, 1981); rather, it is transmitted to mesenteric nerves via the preganglionic splanchnic nerve. Non-parametric statistics indicated that the percentage decreases in the slow rhythm (i.e. power) in renal nerve discharge were not significantly different from the percentage decreases in integrated renal nerve activity (Figure 26B). In contrast, although the slow rhythm in firing of mesenteric nerves was significantly reduced,

integrated activity of the filtered mesenteric nerve signal was not significantly affected by spinal cord transection (Figure 26B).

DISCUSSION

The sympathetic nervous system can produce distinct patterns of nerve discharge to control selectively the functions of various effector organs. In contrast to Cannon's (1930) concept of a system which is characterized by "general diffuse action", it is now widely recognized that different elements within the sympathetic nervous system can display different patterns of discharge, and that descending projections may selectively influence certain sympathetic outflows (Baccelli et al. 1981; Barman et al. 1984; Dampney and McAllen, 1988; Jänig, 1985; Kollai and Koizumi, 1980b; Meckler and Weaver, 1985; 1988).

The results of the present investigation support the contention that tonic excitatory drive from the RVLM is not essential for the maintenance of ongoing discharge of all components of sympathetic outflow. Whereas firing of renal nerves appears to be contingent upon such drive, activity of mesenteric nerves can be generated within spinal cord systems. The RVLM has been shown to be topographically organized (Lovick, 1987). The neuronal pool which affects sympathetic outflow to the kidney appears to be located in the anterior end of the RVLM, whereas those neurons affecting mesenteric vasoconstrictor activity appear to be found in the posterior portion of the region (Lovick, 1987). As the goal of this study was to abolish all excitatory drive from this

region, glycine was topically applied using cotton pledgets large enough to cover, yet small enough to be restricted to the region of the RVLM (1-2 mm diameter). Therefore, it is unlikely that differences in renal and mesenteric responses observed after the application of glycine resulted from unequal distribution of glycine to renal and mesenteric neuronal pools within the RVLM.

The unequal effects of RVLM blockade on the discharge of renal and mesenteric sympathetic outflows may reflect differences in the passive electrophysiological properties of their preganglionic neurons. Sympathetic preganglionic neurons have been classified based on differences in their electrophysiological properties, but it is not known whether such differences relate to functional specificity (Dembowsky, Czachurski and Seller, 1986). If sympathetic preganglionic neurons presynaptic to mesenteric neurons have lower thresholds for activation, or lower resting membrane potentials than do those impinging on renal neurons, fewer synaptic inputs would be required to activate mesenteric neurons, and, thus, their discharge would be more likely to withstand the removal of an excitatory input than would that of renal neurons. In addition, it is conceivable that preganglionic neurons presynaptic to mesenteric neurons may not receive as many inputs from the RVLM as those innervating renal neurons. A recent anatomical study has shown that projections from the RVLM are not uniformly distributed

throughout the rostro-caudal extent of the thoraco-lumbar spinal cord (Caverson and Ciriello, 1987).

Although inhibition of tonic activity of neurons within the RVLM elicited greater decreases in the discharge of renal than mesenteric nerves, mesenteric nerve discharge was significantly reduced by 30%. Two other studies also have provided evidence that discharge of mesenteric nerves or their preganglionic splanchnic nerves is influenced strongly by RVLM inputs (Dean and Coote, 1986; Hilton et al, 1983). Dean and Coote (1986) reported that the tonic sympathetic vasoconstrictor activity of splanchnic, renal and skeletal muscle fibers is virtually abolished by bilateral application of glycine to the ventral surface of the medulla. Hilton et al. (1983) observed that the application of glycine produced greater increases in mesenteric than renal or femoral vascular conductance, and hypothesized that "the ventral medulla contributes significantly to the maintenance of arterial blood pressure, particularly through its constrictor effect on the splancnic resistance vessels." These results, and those of the present study, contrast with the finding that firing of mesenteric nerves remains unabated following high cervical spinal cord transection (Figures 4 and 15). This discrepancy may be explained if spinal systems are capable of driving mesenteric, but not renal, nerve discharge when all supraspinal input is removed, and, if mesenteric and renal spinal neurons receive inhibitory, as well as

excitatory bulbospinal inputs when the neuraxis is intact. Such tonic sympathoinhibition, independent of baroreceptor inputs, has been demonstrated (Barman and Gebber, 1978; Dembowsky et al. 1980; McCall and Harris, 1987). Selective removal of the excitatory input from the RVLM would unmask the sympathoinhibitory influence, resulting in a decrease in mesenteric nerve firing. Transection of the spinal cord would sever both the excitatory and inhibitory pathways, and, thus, ongoing mesenteric nerve activity would be sustained. In contrast, if the origin of ongoing renal nerve activity is supraspinal, both application of glycine to the ventral medulla and transection of the spinal cord would diminish renal nerve activity. This hypothesis was tested by transecting the spinal cord at the first cervical level during the response to glycine. Recovery of nerve activity after blocking spinal cord conduction would have suggested that the applications of glycine to the ventral surface of the medulla unmasked tonically active bulbospinal sympathoinhibitory pathways. A similar protocol was used by Alexander (1946), who demonstrated that ongoing cardiac nerve discharge, which had been completely abolished by transection of the neuraxis at the level of the obex, resumed partially, following a low bulbar transection.

Indeed, activity of mesenteric nerves did increase following spinal cord transection in 10 of 14 cats, suggesting that the diminished mesenteric nerve activity

during application of glycine to the ventral medulla may have been caused, in part, by descending sympathoinhibitory inputs. Alternatively, following removal of supraspinal influences latent excitatory synaptic inputs may be expressed (Dostrovsky, Millar and Wall, 1976), and the recovery of mesenteric nerve firing after spinal cord transection may reflect the plasticity of spinal structures controlling a portion of the mesenteric nerve outflow (Taylor and Schramm, 1987). However, such latent synaptic influences should also have been expressed following removal of excitatory drive from the RVLM. As this was not the case, the hypothesis is tenable that the recovery of mesenteric nerve discharge following spinal cord transection may have resulted from a decline of sympathoinhibitory effects.

The slow oscillations in sympathetic nerve discharge which were evident when activity was amplified at a wide bandwidth have been analyzed in detail by Barman and Gebber (1980) and Gebber and Barman (1980). Topical application of glycine to the medulla consistently caused greater percentage decreases in the slow rhythm in nerve firing (i.e. total power of the density spectra in the 1-6 Hz frequency range) than in integrated nerve activity (Figures 24, 25 and 26). The reductions in the slow rhythm in firing of renal and mesenteric nerves were similar. Transection of the spinal cord resulted in greater reductions in the slow rhythm in discharge of both nerves. Therefore, although renal and

mesenteric nerves differ in their dependence on supraspinal inputs for the maintenance of ongoing discharge, such inputs are necessary to synchronize firing of both groups of nerves into bursts of action potentials. In the absence of this descending input firing of both renal and mesenteric nerves is asynchronous.

At least two hypotheses may account for the loss of rhythmicity in the discharge of mesenteric nerves following removal of descending inputs. First, two sub-groups of mesenteric neurons may exist. One sub-group may depend upon drive from the RVLM for the maintenance of ongoing activity and discharge with a 1-6 Hz periodicity. A second sub-group may have asynchronous discharge which is generated within the spinal cord. After removal of descending influences, only the second group of neurons which have asynchronous discharge patterns would remain active. Alternatively, the majority of mesenteric nerve activity may be generated within the spinal cord, and descending inputs from the RVLM may synchronize this discharge into slow waves. In the absence of the descending inputs, nerve activity would become asynchronous. This second possibility is likely, as single mesenteric fibers which fire with cardiac rhythmicity when the neuraxis is intact continue to discharge without such rhythmicity after transection of the spinal cord.

The functional significance of the mesenteric nerve activity that persists after inhibition of activity of

neurons within the RVLM, or after transection of the spinal cord remains to be determined. Although McAllen (1986b) reports that projections from the RVLM appear to impinge selectively on vasomotor neurons, this does not imply that every vasomotor neuron depends upon excitatory drive from the RVLM for the maintenance of ongoing discharge. Indeed, the decreases in mesenteric and renal nerve activity caused by application of glycine to the medulla (34% and 78%, respectively) were rarely as great in magnitude as those elicited by pressoreceptor stimulation (50% and 90%, respectively), suggesting that some vasomotor neurons remained active after RVLM blockade. Moreover, some mesenteric fibers, as well as renal fibers and some splenic fibers (Meckler and Weaver, 1988) which continue to fire after severing the spinal cord had activity which was sensitive to pressoreceptor influences when the neuraxis was intact; supposedly these neurons subserve vasomotor functions (Jänig, 1985). Still, following blockade of the RVLM mesenteric vascular conductance is significantly increased (Hilton et al. 1983; Marshall, 1986; Willette et al. 1987) and arterial pressure falls to spinal levels (the present study; Benarroch et al. 1986; Guertzenstein and Silver, 1974; Hilton et al. 1983; Marshall, 1986; Ross et al. 1984b). Failure of sustained mesenteric nerve activity to support vascular tone could occur because nerve impulses which are delivered asynchronously produce less vascular tone than do

phasically delivered impulses (Andersson, 1983; Nilsson et al. 1985), and RVLM blockade and spinal cord transection did cause mesenteric nerve activity to become asynchronous. However, even if the sustained nerve activity was sufficient to cause mesenteric vasoconstriction, such vasoconstriction may not be capable of supporting arterial pressure, as spinal transection causes profound decreases in sympathetic outflow to other vascular beds (Meckler and Weaver, 1985; Taylor and Schramm, 1987) and significant decreases in cardiac output (Fitzsimons and Weaver, 1988).

In conclusion, excitatory drive from the RVLM is not essential for the maintenance of ongoing activity of all sympathetic nerves. Whereas discharge of renal nerves is strongly dependent upon such drive, discharge of mesenteric nerves can be generated within the spinal cord. However, the RVLM does equally influence the frequency characteristics of the discharge of both nerves. Normally, mass activity of both renal and mesenteric nerves is synchronized into bursts of action potentials with periodicities of 1-6 Hz. In the absence of input from the RVLM nerve discharge becomes asynchronous. The functional significance of the persistent mesenteric nerve firing seen after blockade of the RVLM or transection of the spinal cord awaits further investigation.

SUMMARY AND CONCLUSIONS

1. Chemical stimulation of intestinal receptors with bradykinin consistently caused greater reflex excitation of mesenteric (159% increase) than renal (36% increase) efferent multifiber nerve activity and significant pressor responses. Neural circuits complete within the spinal cord are sufficient to produce these reflex responses.

2. Mass discharge of renal nerves was inhibited (85% decrease) more than that of mesenteric nerves (23% decrease) following stimulation of systemic pressoreceptors.

3. Spinal transection caused significant decreases in tonic multifiber renal nerve activity without altering the ongoing discharge rate of mesenteric nerves.

4. Chemical stimulation of intestinal receptors caused increases in activity of 84% of mesenteric and 71% of renal nerve fibers. The magnitude of excitation of mesenteric unit activity (5.6 fold) was significantly greater than that of renal unit activity (1.0 fold).

5. Ongoing discharge of only 58% of mesenteric fibers and of all renal fibers was correlated with the arterial pressure pulse. Similarly, 60% of mesenteric fibers and all renal fibers had activity which was affected by stimulation and/or unloading of systemic pressoreceptors.

6. Severing the spinal cord caused the cessation of discarge of 75% of renal, but only 11% of mesenteric nerve fibers.

7. Chronic capsaicin treatment in rats caused significant depletion of substance P-like immunoreactivity in dorsal root ganglia and significant attenuation of the excitatory reflex responses elicited by chemical stimulation of intestinal receptors.

8. Inhibition of neurons in the rostral ventrolateral medulla by bilateral application of glycine caused an 80% reduction in firing of whole renal nerves, but only a 30% reduction in the mass discharge of mesenteric nerves.

9. Mass discharge of both nerves oscillated with a 1-6 Hz periodicity which disappeared after blocking the firing of neurons within the rostral ventrolateral medulla with glycine.

10. Transection of the spinal cord during the glycine response caused an even greater decrease in the level of renal nerve activity. In contrast, mesenteric nerve discharge increased following spinal cord transection, returning to control levels.

11. The 1-6 Hz rhythm in firing of both nerves was abolished after spinal cord transection.

These investigations indicated that the discharge of mesenteric and renal sympathetic outflow may respond

differentially to supraspinal and visceral afferent influences. Activity of renal nerves is influenced more than that of mesenteric nerves by supraspinal inputs. Whereas mesenteric nerve activity can be generated within the spinal cord, tonic discharge of renal nerves appears to depend upon excitatory drive from the rostral ventrolateral medulla. Moreover, reflexes involving supraspinal neural circuits (e.g. pressoreceptor reflexes) appear to have greater influences on firing of renal than mesenteric nerves. In contrast, the intestino-sympathetic excitatory reflex, which can be mediated by exclusively spinal pathways, is preferentially directed to the mesenteric sympathetic outflow. The unequal response patterns of these two groups of nerves probably reflect the dissimilar functions of the organs which they innervate.

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