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SYMPATHETIC RESPONSES TO STIMULATION OF SPLENIC AFFERENT AND PRESSORECEPTOR NERVES

By

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ABSTRACT

SYMPATHETIC RESPONSES TO STIMULATION OF SPLENIC AFFERENT AND PRESSORECEPTOR NERVES

By

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Reflex control of abdominal circulation is a sympathetic nervous system function which is important in cardiovascular homeostasis. This study utilized electrophysiological techniques to investigate reflex responses of splenic, renal, and mesenteric nerves to stimulation of splenic receptors and activation of vascular pressoreceptors. The inceptive observation in this series of investigations was that stimulation of splenic receptors by capsaicin, bradykinin, or congestion produced greater splenic than renal responses in sino-aortic denervated, vagotomized cats. Next, intensity-response curves of sympathetic nerve activity to stimulation of splenic receptors were studied because it was postulated that intensity of receptor stimulation determines the magnitude of reflex responses and thus determines unequal neural responses. Intense splenic receptor activation exaggerated differences in splenic and renal responses. Potential spinal components of reflexes initiated from the spleen were investigated to determine if responses to congestion and chemical stimulation utilized the same central pathways. Spleno-splenic and spleno-renal reflexes

caused by stimulation with chemicals or congestion were shown to include a major spinal component. Characteristics of afferent fibers initiating these responses were studied. Mechanical and chemical stimuli both activated one group of splenic afferent fibers as well as separate groups to produce reflex responses. Mechanical stimulation produced a pattern of afferent responses different from that produced by chemical stimulation. Excitatory splenic and renal responses to splenic receptor stimulation were not suppressed equally by pressoreceptor activation; renal reflex responses were suppressed while splenic were not. Vascular pressoreceptors also had greater inhibitory influences on tonic renal than splenic nerve activity. Responses of another sympathetic nerve innervating a capacitive bed might be similar to those of splenic nerves. Mesenteric and splenic responses were similar. These findings have revealed characteristics of a reflex pathway which may make significant contributions to cardiovascular control.

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Don't you know, I'm still standing
Better than I ever did
Lookin' like a true survivor
Feelin' like a little kid
I'm still standing
After all this time

Elton John and Bernie Taupin

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

INTRODUCTION

Reflex control of abdominal circulation is a function of the sympathetic nervous system which is important in cardiovascular homeostasis. The splanchnic (splenic and mesenteric) and renal beds are major components of the abdominal circulation. The splanchnic circulation is considered to be primarily a capacitive bed (with resistive capabilities), and the renal circulation is primarily a resistive bed. The large capacitance of the splanchnic circulation, as well as the large magnitude of blood volume and blood flow, enables the splanchnic bed to take up and release blood (Brooksby & Donald, 1972; Greenway, 1983; Lundgren, 1983). The compartments (spleen, intestines, and liver) contribute to varying degrees in response to situations such as hemorrhage (Cohen et al., 1970), exercise (Guntheroth & Mullins, 1963; Guntheroth et al., 1967; Vatner et al., 1974), and anxiety or stress situations (i.e., fright, pain, and shock avoidance; Guntheroth & Mullins, 1963; Guntheroth et al., 1967). The redistribution of blood flow during these situations is under the influence of the sympathetic nervous system and results from specific unequal vascular responses in different target organs. For example, studies comparing the responses of the splenic, intestinal, and hepatic resistance beds to hemorrhage demonstrated that splenic and intestinal vasoconstriction is more intense than hepatic

vasoconstriction and that the hepatic vessels may even dilate (Cohen et al., 1970). Because the kidneys receive 20% of the cardiac output, increases or decreases in renal vascular resistance can affect the total systemic vascular resistance and systemic blood pressure (Shepherd & Vanhoutte, 1980). Hemorrhage and anxiety can cause renal vasoconstriction (Gross & Kircheim, 1980; Pelletier et al., 1971) whereas exercise can cause renal vasoconstriction after splenectomy in dogs (Vatner et al., 1974).

Different responses of various vascular beds may result from differences in sympathetic outflow to the beds. A differential or nonuniform aspect of blood flow which implied sympathetic control of the circulation initially was shown by the experiments of Folkow and collaborators (Folkow et al., 1961). These investigators demonstrated that muscle blood flow was decreased more than renal blood flow during baroreceptor unloading by occlusion of the carotid arteries. Although few investigators have focused on comparisons within the abdominal circulation, hemodynamic studies have demonstrated patterned blood flow responses to various stimuli (Mancia et al., 1975b; Wennergren, 1975). In addition to hemodynamic studies, investigators have directly recorded electrical activity from sympathetic nerves in attempts to determine the underlying mechanisms of nonuniform hemodynamic changes. Ninomiya and coinvestigators (Ninomiya et al., 1971; Ninomiya & Irisawa, 1975) studied the effects of baroreceptors on the activity of sympathetic nerves of the spleen, kidney, heart, and intestine. These investigators concluded that arterial baroreceptors

inhibited splenic nerve activity more than cardiac, renal, or intestinal nerve activity.

One way in which reflex control of the vascular components of the abdominal circulation can be accomplished is by activation of viscero-sympathetic reflexes. These reflexes can be initiated by cardiovascular receptors in the heart and great vessels as well as from a variety of receptors within other viscera. Stimulation of receptors in the abdominal viscera and splanchnic circulation can evoke reflex responses which result in changes in cardiovascular dynamics and sympathetic outflow (see Section B.1.c.). Few investigators have examined responses of more than one component of sympathetic outflow to abdominal visceral stimulation to discern nonuniform patterns. Stimuli, such as chemical activation of cardiac receptors and activation of sodium- and angiotensin-sensitive central receptors (Tobey et al., 1983; Weaver et al., 1983a, 1983b, 1984), are capable of producing a nonuniform pattern of abdominal sympathetic outflow. Thus, it is relevant to determine if stimulation of visceral receptors in the abdominal region is capable of producing differential responses of sympathetic nerves innervating various abdominal organs. Simultaneous recordings of different sympathetic nerves are necessary to distinguish between differential sympathetic outflow and target organ responsiveness.

Very little work has been done to describe the contribution of afferent nerves originating from the spleen to the regulation of the cardiovascular system. Herman et al. (1982) illustrated that

electrical stimulation of splenic afferent nerves caused reflex increases in renal and cardiopulmonary sympathetic efferent activity, heart rate, ventricular contractile force, and systemic blood pressure. Calaresu et al. (1984) stimulated splenic afferent nerves with increases in intrasplenic pressure and chemicals, such as bradykinin and capsaicin, and produced reflex excitation of splenic and renal nerves and increases in systemic blood pressure. The splenic excitation was greater than renal. In addition, Herman et al. (1982) showed that mechanical compression of the spleen or increases in splenic venous pressure increased the discharge rate of splenic afferent nerves and concluded that splenic receptors were low-pressure baroreceptors.

The present study was designed to investigate further the reflex influences of splenic afferent and baroreceptor afferent nerves on abdominal splenic, renal, and mesenteric sympathetic outflow. The research plan consisted of seven sections: (a) reflexes initiated from the spleen in vagotomized, sino-aortic denervated animal were characterized further; (b) intensity-response characteristics of reflexes initiated from the spleen were determined; (c) spinal components of sympathetic reflexes initiated from the spleen were revealed; (d) afferent nerve responses to stimulation of splenic receptors were characterized; (e) influence of cardiovascular pressoreceptors on reflex sympathetic responses initiated from the spleen were determined; (f) influence of cardiovascular pressoreceptors on tonic splenic and renal sympathetic activity were compared; and

(g) splenic and mesenteric reflex responses to splenic receptor activation were compared. The rationale for each of these experiments is presented in the following literature review.

LITERATURE REVIEW

Sympathetic Influences on Capacitance and Resistance Vascular Beds

Vascular beds with a capacitive function, such as the spleen and intestine, may receive different sympathetic neural influences than beds with a resistive function, such as the kidney. Because these vascular beds have different functions, activation of reflex pathways may cause a differential pattern of neural responses to the different beds, or equivalent sympathetic outflow may cause different responses of the target organs. Either mechanism could result in nonuniform hemodynamic responses for capacitance and resistance portions of the abdominal circulation.

Sympathetic Influences on the Spleen

The third to tenth thoracic spinal segments provide sympathetic outflow to the spleen, a major capacitive vascular bed (Kuo et al., 1980). Splenic postganglionic nerves arise from the celiac and paravertebral ganglia (Kuo & Krauthamer, 1981; Meckler & Weaver, 1984). The sympathetic efferent innervation of the spleen is distributed to the capsule, arterioles, trabeculae, and venous channels (Fillenz, 1970; Utterback, 1944). Activation of the sympathetic adrenergic innervation of the spleen results in contraction of vascular, trabecular, and capsular smooth muscle to cause expulsion of blood from the

spleen. Changes in volume of the spleen can be achieved by changes in the discharge rate of splenic efferent nerves as well as by circulating vasoactive substances, e.g., epinephrine and angiotensin (Hertting & Suko, 1966). Early investigations showed that maximal electrical stimulation of the splenic nerves of the cat resulted in expulsion of up to 5 ml blood/kg body weight or 75% of control splenic volume (Celander, 1954; Greenway et al., 1968). During the electrical stimulation, arterial inflow decreased, venous outflow and pressure increased, and the hematocrit of expelled splenic venous blood increased to 70-80%. This is an active process because mechanical occlusion of the splenic artery alone caused little change in splenic volume and small changes in venous hematocrit (less than 10%). The maximal contraction was obtained at 2-3 Hz.

Reflexly mediated changes in splenic blood volume during graded receptor stimulation have not been studied extensively. In studies using dogs, Carneiro and Donald (1977) showed that withdrawal of arterial baroreceptor influence by carotid occlusion resulted in splenic contraction, a small increase in splenic venous pressure, and a decrease in splenic volume. Pelletier et al. (1971) showed that splenic blood volume also was influenced by vagally innervated cardiopulmonary receptors. Withdrawal of cardiopulmonary receptor influence resulted in an increase in venous pressure in the occluded spleen. Neurophysiological evidence has shown that sympathetic outflow to the spleen (and thus splenic volume) is highly responsive to changes in the activity of aortic and carotid sinus baroreceptors. Studies by

Ninomiya and colleagues (Ninomiya et al., 1971; Ninomiya & Irisawa, 1975) have described a cardiac-related rhythm in splenic nerve activity of cats. The splenic nerve activity was inhibited when aortic pressure was increased. Also they showed that either electrical stimulation of the aortic nerve or increased pressure within an isolated carotid sinus inhibited splenic nerve activity.

Studies have been done in conscious animals to evaluate the contribution of the spleen to "stress" situations. In a classical study, Barcroft and Stephens (1927) observed that the spleen contracted in response to running, swimming, and hemorrhage. Guntheroth and co-workers (Guntheroth & Mullins, 1963; Guntheroth et al., 1967) reported that exercise less consistently caused splenic contraction but that hemorrhage, hypoxia, and fright consistently caused contraction. Vatner et al. (1974) exercised dogs before and after splenectomy and concluded that release of red blood cells from the spleen was an important part of an integrated cardiovascular response to severe exercise. It was postulated that release of red blood cells from the spleen increased oxygen delivery adequately so that reduction and redistribution of mesenteric and renal blood flow was not necessary.

The spleen acts as a reservoir of hemoconcentrated blood and is a major part of the capacitive circulation in dogs and cats. A three-compartment model of the spleen was developed by Song and Groom (1971a, 1971b). The fast compartment received 90% of the total splenic blood flow, had a hematocrit of 37%, and contained 33% of the

splenic red blood cells. It made up a rapid direct pathway (t 1/2 of 30 sec) through the spleen and was the usual arteriovenous channel through the spleen. The intermediate compartment received 9.8% of the total splenic blood flow, had a hematocrit of 75%, and contained 56% of the splenic red cells. This compartment was identified as the splenic red pulp and was the storage site for red cells in the spleen. The transit time was 8 minutes. The slow compartment received 0.2% of the total splenic blood flow, contained 11% of the splenic red cells, and had a t 1/2 of more than one hour. This compartment was part of the red pulp and was thought to trap eosinophils for maturation.

Sympathetic Influences on the Mesenteric Circulation

Another component of the abdominal capacitive circulation is the mesenteric bed. The spinal segments which provide sympathetic outflow to the intestines are the third to thirteenth thoracic segments (Kuo et al., 1980). Mesenteric postganglionic fibers travel from the celiac, superior mesenteric, and paravertebral ganglia. The sympathetic innervation of the intestines is distributed to vascular smooth muscle, intestinal smooth muscle, and the enteric plexus.

Folkow et al. (1963, 1964) showed that electrical activation of mesenteric nerves produced a frequency-dependent decrease in intestinal blood flow and volume. The initial decrease in blood flow returned toward control values despite continued stimulation and was termed the "autoregulatory escape phenomenon." During submaximal (4 Hz) stimulation of mesenteric nerves, the distribution of blood flow

to the intestinal wall was not altered, and the mucosal blood flow was much greater than the smooth muscle blood flow (Greenway et al., 1976). Electrical stimulation at 4-6 Hz resulted in maximal steady-state increases of vascular resistance from 50-100% above control values. The blood volume of the intestines began to decrease at 1 Hz and was maximally reduced at 4-6 Hz which mobilized 30-40% of the intestinal blood volume. The decrease in blood volume was maintained during the stimulation period.

Using plethysmography to measure ileal blood flow in cats, Greenway and Lister (1974) produced a hemorrhage of 15% of blood volume and calculated that the gastrointestinal tract contributed 22% of the volume removed. During an infusion to increase blood volume by 34%, they calculated that the gastrointestinal tract pooled 40% of the volume added. Removal of carotid baroreceptor influence by carotid artery occlusion was shown to cause increased resistance of intestinal vessels (Hadjiminas & Oberg, 1963; Kendrick et al., 1972). These investigators interpreted the vasoconstriction of intestinal vessels to result from an increase in intestinal vasoconstrictor fiber discharge. In an earlier study (Oberg & White, 1970b), stimulation of carotid baroreceptors resulted in a decrease in resistance to flow and an increase in volume. The same response occurred with stimulation of cardiac vagal afferent nerves. Studies describing the hemodynamic changes during emotion, fighting, and treadmill exercise in the cat have been done (Adams et al., 1969, 1971). Mesenteric conductance decreased (i.e., resistance increased) more during the muscular

activity of fighting and walking on the treadmill than during nonsupportive fighting (i.e., emotion).

The mesenteric vasculature exhibits escape from sympathetic adrenergic vasoconstriction. It appears to involve a mechanism inherent in vascular smooth muscle and involves a relaxation of the vessels (Baker & Mendel, 1967; Dresel & Wallentin, 1966; Greenway et al., 1976; Lautt & Graham, 1977; Ross, 1971). Also, the intestinal circulation exhibits autoregulation (Johnson, 1960). In addition to functioning as a capacitive bed, the mesenteric circulation is responsible for the uptake of products of digestion.

Sympathetic Influences on the Kidney

The renal circulation is a resistance bed with a limited capacitive capability. The sympathetic outflow which provides innervation to the kidney is located in the fifth thoracic to third lumbar spinal segments (Takeuchi et al., 1964). The postganglionic fibers innervating the kidney have cell bodies in the celiac, superior mesenteric, and renal ganglia, as well as in the paravertebral ganglia of the lumbar sympathetic chain (Kuo et al., 1982; Meckler & Weaver, 1984). Sympathetic postganglionic fibers innervate vascular structures, renal tubules, and juxtaglomerular apparatus of the kidney (Barajas, 1978; Barajas & Wang, 1979; DiBona, 1977).

Early studies demonstrated that electrical stimulation of renal nerves at relatively high frequencies (2-5 Hz) causes marked decreases in renal blood flow (Block et al., 1952a, 1952b; Houck, 1951). Later investigators used more modest levels of stimulation and still showed

decreased renal blood flow (Johns et al., 1976). In dogs, hemorrhage of 10% of the blood volume produced renal vasoconstriction (Pelletier et al., 1971); the vasoconstrictor responses when the vagi were intact were similar to those when the carotid baroreceptors were intact. Oberg and Thoren (1973a) demonstrated that electrical stimulation of myelinated afferent fibers in the vagal cardiac nerve of cats induced a vasoconstriction in the kidney. Stimulation of unmyelinated fibers in the cardiac nerve caused a renal vasodilation. Their interpretation was that the moderate constrictor responses were due to activation of atrial receptor fibers, and the marked dilator responses were due to activation of ventricular receptor fibers. Stimulation of visceral receptors by increased hepatic portal pressure produced renal vasodilation (Niijima, 1976). Renal vasodilation was produced also by stimulation of left atrial cardiopulmonary receptors in the dog (Lloyd & Friedman, 1977; Mason & Ledsome, 1974). Karim and Kappagoda (1980) demonstrated that the renal vasodilation caused by left atrial receptor stimulation was greatest during withdrawal of the inhibitory influence of carotid baroreceptors. Purtock et al. (1977) stimulated thoracic sympathetic afferent nerves at a low frequency (3 Hz) and a high frequency (30 Hz) during systemic hypotension in dogs. Low-frequency stimulation produced renal vasodilation, decreased renal vascular resistance, and increased renal blood flow. In contrast, high-frequency stimulation produced renal vasoconstriction and decreased renal blood flow. While the above studies in anesthetized animals indicate that reflex responses of the kidney are prominent,

studies in conscious animals have shown that the influences of arterial baroreceptors and cardiopulmonary receptors on renal blood flow and renal vascular resistance are quite small (Gross et al., 1979; Kaczmarczyk et al., 1978; Vatner et al., 1974). In contrast, Gross and Kirchheim (1980) demonstrated that auditory stimuli such as the sound of a gun shot could decrease renal blood flow by 40%.

Although renal hemodynamic responses appear to be less easily produced by afferent input than responses of other vascular beds (Folkow et al., 1961; Gorman et al., 1985; Kendrick et al., 1972a), reflexes can produce changes in efferent sympathetic nerve activity to change nonhemodynamic aspects of renal function (DiBona, 1982). Direct electrical stimulation of renal nerves at low frequencies (0.5-2 Hz) can produce decreased urinary sodium excretion (LaGrange et al., 1973; Slick et al., 1975) and increased renin release (Taher et al., 1976; Zambraski & DiBona, 1977) without causing changes in renal blood flow or glomerular filtration rate. In addition, reflex activation of renal efferent nerves can result in increased sodium reabsorption and renin release without changes in renal blood flow or glomerular filtration rate (DiBona, 1982).

Sympathetic Reflexes Initiated by Visceral Afferent Nerves

Control of the circulation can be effected by activation of peripheral receptors such as those in skin, vascular system, viscera, or from central emotion centers. Differences in regional distribution of blood flow may be due to greater sympathetic outflow to one

vascular bed than to another. Activation of visceral receptors could be important in the regulation of the cardiovascular system because initiation of viscero-sympathetic reflexes could cause nonuniform sympathetic responses.

Viscero-Visceral Reflexes

Historical Background

Coote and Downman (1966) stimulated spinal sensory nerves at the third and tenth thoracic segments and recorded reflex discharges from postganglionic inferior cardiac and renal nerves. They demonstrated that the reflex pathway involved a supraspinal and a spinal component. However, they stimulated dorsal roots which contained somatic as well as visceral afferent nerves, and thus they were eliciting somatosympathetic as well as viscero-sympathetic responses. Previous investigators had also stimulated visceral afferent nerves but reported changes in arterial pressure or pupil size as an indication of sympathetic responses (Bain et al., 1935; Downman, 1952; Gammon & Bronk, 1935; Irving et al., 1937; Sherrington, 1899). Since then, many investigators have selectively stimulated visceral afferent nerves and observed responses of organs or changes in nerve activity.

Reflex Responses to Cardiac and Thoracic Afferent Stimulation

Karim et al. (1972) showed that activation of left atrial receptors by balloon distension at the pulmonary vein-atrial junctions produced an increase in efferent cardiac sympathetic nerve activity, a

decrease in renal nerve activity, no change in splenic or lumbar sympathetic nerve activity, and an increase in heart rate. Malliani and colleagues investigated aspects of sympathetic afferent cardiac nerve stimulation. Activation of cardiac receptors by coronary occlusion, increased coronary flow, or myocardial ischemia induced reflex discharges of preganglionic and postganglionic fibers to the heart (Brown & Malliani, 1971; Malliani et al., 1969). Electrical or chemical stimulation of sympathetic cardiac afferent fibers produced pressor responses, tachycardia, and increases in myocardial contractility (Malliani et al., 1972, 1973; Peterson & Brown, 1971). Weaver and co-workers used various stimuli to activate sympathetic and vagally innervated receptors in the thoracic region. Electrical stimulation of sympathetic cardiopulmonary afferent nerves caused both excitatory and inhibitory renal nerve responses (Weaver, 1977). Stimulation of these afferent nerves by intravascular volume expansion produced an inhibition of renal nerve activity, no change in lumbar sympathetic nerve activity, and an increase in mean arterial pressure. Activation of sympathetic afferent nerves by stretch of the ventricles caused an increase in renal nerve activity, and stretch of the aorta also produced an increase in renal nerve activity as well as a pressor response. Volume expansion to stimulate pulmonary vascular receptors caused an inhibitory renal nerve response (Weaver et al., 1979). Stimulation of cardiac sympathetic receptors by epicardial bradykinin resulted in excitatory responses of renal, cardiac, splenic, gastrohepatic, and adrenal nerves, and pressor responses (Reimann & Weaver,

1980; Weaver et al., 1983b). Excitatory renal nerve responses also were produced by epicardial or intracoronary potassium chloride (Reimann & Weaver, 1980) and by intracoronary or atrial bradykinin (Reimann & Weaver, 1980; Weaver et al., 1984). Stimulation of vagal cardiac receptors by bradykinin or veratridine produced inhibitory renal and splenic nerve responses and depressor responses (Reimann & Weaver, 1980; Weaver et al., 1984).

Reflex Responses to Abdominal Visceral Stimulation

Longhurst and coinvestigators have examined the cardiovascular reflexes that can be initiated from the stomach. Passive gastric distension produced an increase in myocardial contractility, systemic vasoconstriction, and a pressor response (Longhurst et al., 1981). Longhurst and Ibarra (1984) demonstrated that gastric distension produced regional vascular responses. Vasoconstriction of the kidney, large intestine, and spleen occurred with no change in the liver. These vascular responses were shown to be caused by stimulation of alpha adrenergic receptors. Application of the chemicals capsaicin and bradykinin to the serosal surface of the stomach caused cardiovascular reflexes similar to those caused by distension (Longhurst et al., 1984b). These reflexes were mediated by splanchnic afferent and efferent nerves. Kostreva et al. (1980) stimulated hepatic pressure receptors by occlusion of the thoracic vena cava. The resultant increase in hepatic venous pressure caused a reflex increase in renal and cardiopulmonary sympathetic nerve activity. Ashton et al. (1982)

injected capsaicin into the portal circulation to stimulate hepatic receptors and caused reflex decreases in mean arterial pressure, heart rate, and renal vascular resistance. This reflex also utilized a splanchnic nerve pathway.

Distension of the gallbladder has been reported to cause pressor responses in cats (Newman, 1974) and in ferrets (Cervero, 1982). Moreover, Ordway and Longhurst (1983) showed that chemical stimulation of the serosal surface of the gallbladder by capsaicin and bradykinin causes increased heart rate, myocardial contractility, systemic vascular resistance, and mean arterial pressure. However, these investigators could not produce reflex responses with distension of the gallbladder. Ordway et al. (1983) demonstrated that the pancreas also is a reflexogenic area as application of capsaicin and bradykinin to the serosa of the pancreas produced reflex excitatory cardiac and systemic vascular responses.

Andrews et al. (1972) showed that increased afferent intestinal nerve discharge caused by mesenteric venous congestion elicited a reflex sympathetic nerve response back to the same organ. The increase in mesenteric venous pressure was produced by obstruction of the portal vein. Distension of a segment of small intestine resulted in an increase in efferent intestinal nerve activity and a small increase in renal nerve activity (Ninomiya et al., 1974). Later Ninomiya and Irisawa (1975) demonstrated that distension produced similar small splenic and renal nerve responses in contrast to a much larger increase in efferent intestinal nerve activity. Occlusion of

the portal vein to increase pressure in mesenteric veins resulted in an inhibition of renal and adrenal nerve activity (Niijima, 1976). An increase in mesenteric venous pressure from 3 to 7.5 mmHg caused inhibition of renal and adrenal nerve activity while a change from 3 to 15 mmHg produced no response. Low-intensity electrical stimulation of the mesenteric nerve produced decreased discharges in renal and adrenal nerves. Khayutin and co-workers demonstrated that stimulation of intestinal vascular receptors with capsaicin or potassium chloride produced pressor responses (Baraz et al., 1968), vasoconstriction in the kidney, and increased renal nerve discharge (Khayutin et al., 1969). The reflex responses were shown to be dose dependent. Ordway et al. (1984) applied capsaicin and bradykinin to the serosal surface of the jejunum to activate receptors and observed increased myocardial contractility, systemic vascular resistance, and systemic arterial pressure. Activation of intestinal receptors by topical bradykinin produced excitatory mesenteric, renal, and splenic nerve responses in addition to pressor responses (Meckler, unpublished results; Stein et al., 1986; Stein, unpublished results).

Electrical stimulation of splenic nerves produced increases in efferent cardiopulmonary sympathetic and renal nerve activity, ventricular contractile force, heart rate, and blood pressure (Herman et al., 1982). Stimulation of splenic receptors with capsaicin, bradykinin, or congestion produced increases in splenic and renal nerve activity, systemic arterial pressure, and heart rate (Calaresu et al., 1984). Further characterization of reflexes initiated from the

spleen was needed to determine if congestion also could produce unequal neural reflexes which could be produced by capsaicin and bradykinin.

A number of conflicting reports exist regarding reflex actions of renal afferent nerves. The differences may be due to species, anesthetic, method of stimulating afferent nerves, or type of afferent nerve stimulated. Ueda et al. (1967) activated renal afferent nerves by compression of the kidney, renal vein occlusion, and renal artery occlusion. They found that compression and renal vein occlusion caused a reflex decrease in efferent renal nerve activity and systemic depressor responses, while renal artery occlusion caused a reflex increase in efferent activity and systemic pressor responses. Electrical stimulation of renal afferent nerves caused inhibitory responses of efferent renal nerves and systemic pressure. Beacham and Kunze (1969) reported that activation of renal afferent nerves by increased renal vein or ureteral pressure reflexly excited renal efferent activity. Electrical stimulation of renal nerves caused an inhibition of ipsilateral efferent nerve activity and a depressor response in rabbits (Aars & Akre, 1970). An increase in stimulation frequency caused a further inhibition of efferent renal nerve activity and a larger depressor response. Calaresu et al. (1976) electrically stimulated renal afferent nerves in cats and produced increases in arterial pressure and heart rate, and mesenteric vasoconstriction, but no change in renin release in the contralateral kidney. In later investigations, Calaresu et al. (1978) demonstrated that two

contralateral renorenal reflex responses could be produced by selective stimulation of renal afferent A and C fibers. Because two different types of afferent fibers were activated, the responses were distinguished by differences in latency of onset. Renal vein occlusion to increase intrarenal pressure reflexly inhibited contralateral efferent renal and efferent cardiopulmonary sympathetic activity to cause changes in renal vascular resistance and ventricular contractile force with no alteration of heart rate (Kostreva et al., 1981). Stimulation of renal chemosensitive receptors caused excitatory ipsilateral and contralateral reflex responses of renal nerves and caused only small changes in arterial pressure and heart rate (Recordati et al., 1982). Bradykinin infused into the arterial circulation of kidneys of conscious rats produced mesenteric vasoconstriction, ipsilateral and contralateral renal vasoconstriction, pressor responses, and tachycardia (Smits & Brody, 1982). Kopp and co-workers found that stimulation of renal mechanoreceptors in the dog resulted in contralateral excitatory renorenal reflexes (Kopp et al., 1985).

Pathways of Abdominal Visceral Reflexes

Afferent Pathways

The splanchnic nerve is the major pathway for many abdominal visceral afferent fibers as well as for efferent sympathetic nerves which can be involved in viscero-visceral reflexes. Kuo et al. (1982b) examined the major splanchnic nerve components by means of a low-power, wide-field electron microscopic technique. The nerve components were determined by a selective nerve degeneration technique.

The ventral roots of the third thoracic to first lumbar segments were sectioned to cause degeneration of preganglionic fibers, spinal nerves from the third thoracic to first lumbar segments were sectioned to eliminate the sensory and preganglionic fibers (to study postganglionic fibers), and the data for sensory fibers were obtained by subtracting the preganglionic and postganglionic components from normal nerve specimens. They found that 10% of all myelinated fibers were sensory fibers which tended to be of a diameter of 1-14 microns. Unmyelinated sensory fibers comprised less than 20% of all of the unmyelinated fibers. Thus, visceral afferent fibers consist of comparatively few large myelinated fibers and a larger number of small myelinated and unmyelinated fibers (Kuo et al., 1982b).

Labeling of the sensory fibers in the splanchnic nerve with horseradish peroxidase demonstrated cell bodies in the ipsilateral dorsal root ganglia located from the third thoracic to thirteenth thoracic spinal segments (Kuo et al., 1981). The cell bodies were oval to round in shape, had diameters ranging from 22 to 45 microns, and were found throughout the ganglion (Cervero et al., 1984; Kuo et al., 1981). The visceral afferent fibers in the major splanchnic nerve entered Lissauer's tract and gave off collaterals which projected ventrally along the lateral and medial margins of the dorsal horn (Cervero & Connell, 1984; Kuo & deGroat, 1985). The lateral bundle of fibers projected to lamina I, V, and VII; some of these afferent fibers projected to the dorsal grey commissure and to the vicinity of the central canal (Kuo & deGroat, 1985). A few of the

horseradish peroxidase-labeled fibers were found in Lissauer's tract and lamina I and V of the contralateral spinal cord. Small groups of splanchnic afferent fibers were followed to the area of sympathetic preganglionic neurons in the intermediolateral column and seen to pass through dorsal dendritic arbor of preganglionic neurons. The medial bundle of splanchnic afferent fibers was less prominent than the lateral bundle. Most fibers projected to the ipsilateral dorsal column, and some projected to the dorsal grey commissure. The splanchnic afferent fibers in the dorsal column traveled in the fasciculus gracilis and terminated mainly in the nucleus gracilis, and a few of the fibers terminated in the cuneate nucleus. Kuo and deGroat (1985) also showed that splanchnic afferent fibers sent collaterals rostral and caudal to terminate in lamina I, V, and VII of adjacent spinal segments.

Many of the early electrophysiological investigations of central projections of splanchnic afferent nerves used electrical stimulation of the splanchnic nerve and activated only the myelinated fibers, A-beta and A-delta. It was found that the large fast-conducting afferent fibers (A-beta) of the splanchnic nerve were located mainly in the dorsal columns, and the more slowly conducting fibers (A-delta) were mainly in the ventrolateral columns of the spinal cord (Amassian, 1951; Downman & Evans, 1957). The fast-conducting fibers projected as far rostral as the cortex (Amassian, 1951; Downman, 1951). Later investigations showed that the A-beta and A-delta fibers projected to the brainstem and thalamus (Aidar et al., 1952; McLeod, 1958;

Rigamonti et al., 1978). Electrical activation of A-delta and C fibers in the splanchnic nerve excited spinal neurons located in lamina I and V-IX (Cervero, 1983a, 1983b; Pomeranz et al., 1968). The ascending projections were found in the contralateral ventrolateral funiculus and ipsilateral dorsolateral funiculus (Cervero, 1983a, 1983b) which indicated spinothalamic and spinoreticular tracts. The A-beta fibers of the splanchnic nerve did not excite spinal neurons (Cervero, 1983a; Pomeranz et al., 1968).

Although afferent projections from specific organs innervated by splanchnic nerves have not been investigated, information is available regarding central projections of renal afferent nerves. Neuroanatomical studies indicate that renal afferent fibers terminate in lower thoracic levels of the spinal cord, and the patterns of termination are similar to those of splanchnic afferent nerve fibers (Ciriello & Calaresu, 1983; Kuo et al., 1983). Anatomical and electrophysiological studies also have revealed supraspinal terminations of renal afferent fibers. Wyss and Donovan (1984) utilized a fluorescent dye technique to demonstrate monosynaptic renal afferent projections to the brainstem. Schramm and coinvestigators used electrophysiological techniques to describe myelinated renal afferent fibers from mechanoreceptors which travel in the fasciculus gracilis and terminate in the nucleus gracilis and nucleus solitarius (Knuepfer & Schramm, 1985; Simon & Schramm, 1984).

Efferent Pathways

The central efferent pathway for viscero-visceral reflexes ends on the sympathetic preganglionic neurons (SPN). The cell bodies of the neurons are found within the spinal cord in the vicinity of the lateral funiculus, the lateral horn, the intermediate zone, and the central canal (Chung et al., 1975; Petras & Cummings, 1972). The preganglionic neurons are distributed along the spinal cord in groups, and the axons exit from the spinal segment in which the cell body is located (Oldfield & McLachlan, 1980; Rubin & Purves, 1980). SPNs are spherical or spindle shaped, and the diameters range from 7 to 40 microns and lengths range from 25 to 70 microns. Four to eight prominent dendrites are found on each cell and can extend up to 1300 microns away from the cell body (Dembowsky et al., 1985a). Investigations of the third thoracic segment of the spinal cord (Dembowsky et al., 1985a) revealed SPNs in the intermediolateral cell column (IML) and central autonomic area (CA) which had dendrites with longitudinal orientation. In contrast, other investigators (Barber et al., 1984; Deuchl & Illert, 1981) found that at thoracic and lumbar levels of the cord, dendrites of some SPNs had mediolateral orientations. These SPNs were in the lateral funiculus, intermediate zone, and central canal areas. Axons originate from the soma or proximal dendrites (Dembowsky et al., 1985a; Rethelyi, 1972). Axons of neurons in the lateral funiculus and IML run ventrally along the lateral border of the ventral horn and leave the spinal cord with the lateral portion of the ventral root. Axons of CA neurons run laterally and then turn

ventral near the IML column (Hancock & Peveto, 1979). As many as 40% of the axons of SPNs may be unmyelinated in the cat (Coggeshall et al., 1976). Anatomical evidence for axon collaterals has been demonstrated in rats (Forehand, unpublished observations). Dembowsky et al. (1985a) described spines on axons which might represent synapses at nodes of Ranvier.

Most of the electrophysiological investigations of SPNs have utilized extracellular recordings although several investigators have been successful in recording intracellularly (Coote & Westbury, 1979; Dembowsky et al., 1985b; McLachlan & Hirst, 1980; Yoshimura & Nishi, 1982; Yoshimura et al., 1986). Only 10-30% of SPNs are spontaneously active, and these neurons have a low discharge rate of 0.1-6 Hz. Action potentials have a prolonged duration of 3-12 msec (Dembowsky et al., 1985b; McLachlan & Hirst, 1980). SPNs also exhibit a prolonged after spike hyperpolarization of 25-500 msec up to 3 sec (Dembowsky et al., 1985b; McLachlan & Hirst, 1980; Yoshimura et al., 1986). None of the investigations have demonstrated evidence of "pacemaker" potentials or ongoing depolarizations (Coote & Westbury, 1979; Dembowsky et al., 1985b; McLachlan & Hirst, 1980; Yoshimura & Nishi, 1982). Intracellular recordings of SPNs revealed predominantly excitatory postsynaptic potentials (EPSPs) and occasionally inhibitory postsynaptic potentials (IPSPs). Single-step EPSPs were recorded as well as summation EPSPs (up to 20 mv) of 4 to 7 steps (Dembowsky et al., 1985b). Threshold for discharge of SPNs ranged from 3 to 10 mv on different SPNs (Coote & Westbury, 1979; McLachlan & Hirst, 1980). The rare

occurrence of IPSPs could have been due to masking by the preponderance of EPSPs or could indicate that inhibition of SPN discharge is mediated by disfacilitation (Dembowsky et al., 1985b). Electrical stimulation of somatic and visceral afferent fibers and of descending pathways demonstrated that EPSPs could be evoked in SPNs through spinal and supraspinal pathways (Dembowsky et al., 1985b; McLachlan & Hirst, 1980).

Investigations of the efferent fibers in the major splanchnic nerve (Kuo et al., 1982b) revealed that 90% of all myelinated axons were preganglionic fibers (2-4 microns). Unmyelinated preganglionic fibers in the major splanchnic nerve outnumbered the myelinated preganglionic fibers by 2:1. Postganglionic unmyelinated fibers were found also.

The cell bodies of the postganglionic fibers which innervate the abdominal viscera can be found in the sympathetic chain ganglia, and celiac and superior mesenteric ganglia (Kuo & Krauthamer, 1981; Kuo et al., 1982a; Meckler & Weaver, 1984). Splenic and renal postganglionic cell bodies were found in the thoracic chain from the stellate ganglion to the thirteenth thoracic ganglion, in the lumbar chain from the first to third lumbar ganglia, and in the superior mesenteric and celiac ganglia. Different distributions of renal and splenic postganglionic cell bodies were found (Meckler & Weaver, 1984).

Central Pathways

In contrast to a large number of studies of the central pathways of somatosympathetic reflexes, few have investigated the central pathways of viscero-visceral reflexes in detail. Coote and Downman (1966) found that sensory nerve stimulation (dorsal roots) produced an early small amplitude response in inferior cardiac and renal nerves which was mediated via a spinal pathway and a later large amplitude response which was mediated via a supraspinal pathway. They hypothesized that the supraspinal component of the reflex predominated because of suppression of sympathetic spinal reflexes by descending tonic inhibition (Illert & Gabriel, 1972). Dembowsky et al. (1980) demonstrated that the spinal component of somatosympathetic reflexes is indeed suppressed by descending tonic inhibition from the medulla. Others have shown that viscero-sympathetic reflexes evoked by electrical stimulation have a small spinal component and a large supraspinal component (Coote, 1982; Coote et al., 1969). Electrical stimulation of visceral afferent fibers produced spinal and supraspinal components of reflexes during intracellular recordings of SPNs (Dembrowsky et al., 1985b), and those investigators speculated that there is a suprapontine as well as a supramedullary component to the reflex. However, activation of visceral afferent fibers with chemical or natural stimulation (Schondorf et al., 1983; Weaver et al., 1983a) produced spinal reflexes whose magnitude was similar to that of supraspinal reflexes. In addition, studies of single postganglionic neurons demonstrated that reflex changes in discharge rates in response to visceral

afferent activation were similar in spinal and neuraxis intact states (Weaver et al., 1986b). The difference between conclusions obtained with electrical stimulation and chemical or natural stimulation of visceral afferent nerves may occur because electrical stimulation activated only a portion of afferent fibers and did not provide sufficient spatial summation to the sympathetic preganglionic neurons in the spinal state or because patterns of electrical stimulation do not provide adequate temporal summation to these neurons in the spinal state.

There are a number of descending pathways to the sympathetic preganglionic neurons which could be involved in mediating or modifying viscero-visceral reflexes. Cell groups in the brainstem and hypothalamus are known to send projections to the vicinity of autonomic neurons (Amendt et al., 1979; Dampney, 1981; Hudson & Kalia, 1986; Kalia et al., 1986; Loewy, 1981). Excitatory descending pathways travel in the dorsolateral funiculus (Coote & Macleod, 1975; Dembowsky et al., 1980; Foreman & Wurster, 1973; Illert & Gabriel, 1972), while inhibitory descending pathways travel in the dorsolateral funiculus, ventrolateral funiculus, ventral funiculus, and dorsal funiculus (Barman & Wurster, 1978; Coote & Macleod, 1974a, 1974b; Dembowsky et al., 1980; Illert & Gabriel, 1972; Illert & Seller, 1969).

Gebber and collaborators (Barman & Gebber, 1985; Morrison and Gebber, 1985) have found differences in axonal branching patterns of sympathoexcitatory neurons from the brainstem. One group of neurons

projected only to the level of the first thoracic segment, while the other group had a widely separated branching pattern which included branches to the second, sixth, and eleventh thoracic segments. Although much of the evidence for interneurons is implied, Gebber and colleagues (Barman & Gebber, 1984; Gebber & McCall, 1976; McCall et al., 1977) have presented electrophysiological evidence for interneurons in pathways mediating sympathetic excitation and inhibition. A number of investigators have presented evidence for intersegmental pathways in the spinal cord mediating excitation and inhibition (Faden et al., 1979; Kirchner et al., 1975b; Schondorf et al., 1983; Weaver et al., 1983a).

Although some anatomical studies of the spinal cord have tended to reject the possibility of monosynaptic connections of visceral afferent fibers with SPNs (Petras & Cummings, 1972), Kuo and deGroat (1985) have shown in an anatomical study direct projections of splanchnic afferent fibers to spinal autonomic centers. Electrophysiological investigations have not provided evidence for a monosynaptic connection (Beacham & Perl, 1964a; Dembowsky et al., 1985b). A detailed understanding of viscero-sympathetic reflexes is lacking as no investigations of pathways of viscero-sympathetic reflexes have been conducted except to define the reflexes as spinal or supraspinal.

Spinal Component of Viscero-Sympathetic Reflexes

Many viscero-visceral reflexes have a spinal component (Coote & Downman, 1966; Coote et al., 1969; Dembowsky et al., 1985b; Schondorf et al., 1983; Weaver et al., 1983a). Although the exact pathway of

spinal reflexes is not known, Kuo and deGroat (1985) have shown that visceral afferent fibers project to spinal autonomic centers. Evidence that sympathetic activity is present in spinal animals has been shown by a number of investigators (Dembowsky et al., 1985b; Meckler & Weaver, 1985; Polosa, 1968), and most researchers concur that the total ongoing activity is decreased (compared to that in neuraxis-intact animals). However, Meckler and Weaver (1985) found variation in the amount of ongoing activity between individual animals and difference in the ongoing activity among various postganglionic nerves. Splenic nerve activity after high cervical spinal transection did not decrease significantly while renal and cardiac nerve activity decreased more than 50% (Meckler & Weaver, 1985). The discharge pattern in the spinal animal is also less synchronized (Mannard & Polosa, 1973; Meckler & Weaver, 1985). The mechanism for the generation of the ongoing sympathetic activity is not known, but the activity appears not to be due to anoxia or hypercapnia (Meckler & Weaver, 1985) nor to pacemaker potentials in the SPN (Coote & Westbury, 1979; Dembowsky et al., 1985b; McLachlan & Hirst, 1980; Yoshimura & Nishi, 1982). The ongoing activity may be related to the amount or complexity of input to the SPN; i.e., the greater the amount of input or the more complex the input, the greater the level of ongoing activity (Dembowsky et al., 1985b; Mannard & Polosa, 1973; Polosa, 1968). For example, isolation of a spinal cord section after spinal cord transection reduced the discharge rate of SPNs (Mannard & Polosa, 1973). Because spinal afferent nerves may inhibit as well as excite

sympathetic activity (Kirchner et al., 1975a, 1975b; Wyszogrodski & Polosa, 1973), the effect of dorsal rhizotomy could be a decrease in the discharge rate (Polosa, 1968) or no change (Meckler & Weaver, 1985).

Historically a number of investigators have demonstrated cardiovascular reflexes in spinal animals (Alexander, 1945; Brooks, 1933, 1935; Downman & McSwiney, 1946; Langley, 1924; Murkherjee, 1957; Sherrington, 1906). Beacham and Perl (1964a, 1964b) conducted the first modern electrophysiological investigation of spinal sympathetic reflexes. They recorded preganglionic activity of upper thoracic and upper lumbar SPNs, and stimulated myelinated fibers of spinal sensory nerves at the first to fourth thoracic segments and at the first to fourth lumbar spinal segments. They found that the reflex responses were not strictly segmental, excitatory and inhibitory responses were evoked, and some spontaneously active preganglionic fibers did not participate in the reflexes. Based on the reflex latency, variation in reflex latency, and not strictly segmental distribution of reflex responses compared to usual segmental organization for skeletal motoneurons, Beacham and Perl (1964a) concluded that there was no evidence for monosynaptic connections. Fernandez de Molina and Perl (1965) recorded from postganglionic nerves and stimulated spinal sensory nerves in spinal animals. The reflex discharges appeared in only certain postganglionic branches of the stellate and upper lumbar ganglia. The stimuli also caused pressor responses and vasoconstriction in femoral, inferior mesenteric, and brachial arteries. Because

dorsal roots and spinal sensory nerves were stimulated in the above investigations, there was no way to evaluate the input of visceral afferent nerves apart from that of somatic afferent nerves. Franz et al. (1966) stimulated the splanchnic nerve and demonstrated that stimulation of visceral afferent fibers could initiate spinal sympathetic reflexes. Activation of myelinated visceral fibers produced reflex responses that were similar to those produced by excitation of somatic or mixed (somatic and visceral) nerves. In addition, activation of nonmyelinated visceral fibers produced prolonged after discharge of SPNs and reflexly activated additional SPNs. Other investigators (Coote & Downman, 1966; Coote et al., 1969) also have described characteristics of spinal sympathetic reflexes. None of these electrophysiological investigations (Beacham & Perl, 1964a; Coote et al., 1969; Franz et al., 1966) found evidence for monosynaptic connections in spinal sympathetic reflexes.

A number of studies have described spinal sympathetic reflexes directed back to the organ of origin. Beacham and Kunze (1969) stimulated renal mechanoreceptors and reflexly activated renal efferent nerve activity. Recordati et al. (1982) showed that activation of renal chemosensitive receptors produced excitatory renal efferent nerve responses. Andrews et al. (1972) stimulated intestinal mechanoreceptors and recorded increased efferent intestinal nerve activity in the spinal animal. Malliani and colleagues (Brown & Malliani, 1971; Malliani et al., 1972; Pagani et al., 1974) have investigated many aspects of cardio-cardiac spinal reflexes. Stimulation of cardiac

receptors by increased coronary artery pressure, myocardial ischemia, and coronary sinus occlusion reflexly activated SPNs at the third thoracic segment and inferior cardiac nerves. Functional consequences of spinal cardio-cardiac reflexes were shown when electrical or chemical stimulation of afferent cardiac sympathetic nerves increased myocardial contractility and heart rate (Malliani et al., 1972, 1973). No one has investigated the spinal component of reflexes initiated from the spleen and directed to components of abdominal sympathetic outflow.

Spinal sympathetic reflexes can also extend many spinal segments away from the entrance of the afferent input. Activation of urinary bladder afferent nerves by electrical stimulation or distension of the urinary bladder produced reflex responses (excitatory and inhibitory) of SPNs in the cervical sympathetic trunk (Laskey et al., 1979; Schondorf et al., 1983). Weaver (1981) demonstrated an excitatory cardiorenal reflex when cardiac receptors were activated by epicardial application of bradykinin. Other evidence for intersegmental pathways of spinal reflexes was presented by Weaver et al. (1983a).

Investigators have noted that with electrical stimulation more afferent input is needed to produce a spinal reflex response the same size as one produced with an intact neuraxis (Laskey et al., 1979). The spinal component of a cardio-cardiac reflex is sometimes difficult to detect with the neuraxis intact (Coote, 1984; Malliani, 1982), and evidence implies that the spinal component of the cardio-cardiac reflex is normally suppressed by an inhibitory bulbospinal system

(Coote, 1984). In contrast, chemical or natural activation of visceral afferent fibers of the heart (Weaver et al., 1983a) or urinary bladder (Schondorf et al., 1983) produced spinal reflexes readily. Also, reflex changes in discharge rates of single postganglionic neurons during activation of mesenteric visceral afferent fibers were similar before and after spinal cord transection (Weaver et al., 1986b), implying little, if any, suppression of the spinal component of reflexes initiated from mesenteric receptors.

Intensity-Response Characteristics of Reflex Responses

Few investigators have described intensity- or concentration-dependent effects on the magnitudes of viscero-sympathetic reflexes. Khayutin and collaborators have described concentration-response curves after activating mesenteric receptors and afferent fibers (Baraz et al., 1968; Khayutin et al., 1969, 1976) to distinguish between non-nociceptive and nociceptive activation of afferent fibers. They used a number of different substances (e.g., capsaicin, bradykinin, potassium ions) to initiate reflex responses of systemic arterial pressure, renal vascular resistance, renal nerve activity, and cardiac contractility. The dose-response curves described were composed of two parts; they concluded that the first portion of the curve represented stimulation of receptors (non-nociceptive activation) and the second portion represented either stimulation of another type of receptor with a higher threshold or the direct excitation of afferent fibers (nociceptive activation). Although Khayutin and

colleagues described the qualitative changes in renal nerve activity in response to increasing concentrations of potassium ions, they did not quantify the nerve activity. Ferreira et al. (1973) demonstrated that intra-arterial injections of bradykinin into the spleen of dogs produced dose-dependent reflex increases of blood pressure. Staszewska-Barczak and co-workers (Staszewska-Barczak et al., 1976; Staszewska-Barczak & Dusting, 1977) activated receptors in the left ventricle with a range of doses of bradykinin. They described dose-related pressor responses and tachycardia after the threshold dose of bradykinin (10-20 ng) had been administered. No investigator has generated intensity-response curves of sympathetic nerve activity to stimulation of abdominal visceral receptors to compare the curves of two sympathetic postganglionic nerves.

Characteristics of Abdominal Visceral Afferent Nerves

Stimulation of abdominal visceral afferent nerves can reflexly activate the cardiovascular system and sympathetic outflow (see section B.1.a.). Studies indicate that the splanchnic nerves form the predominant pathway for afferent nerves that transmit the reflexes (see section B.2.a.). Many different researchers have examined the afferent innervation of the various viscera in the abdominal area in order to understand the input which can produce reflex responses.

Andrews and Palmer (1967) described four types of afferent responses in the liver. Afferent fibers which increased their discharge rate in response to anoxia, to increased biliary pressure,

and to increased hepatic venous congestion were found. They also described a group of afferent fibers which were spontaneously active but did not respond to any of the applied stimuli. Hepatic afferent fibers which responded to increased portal venous pressure were further investigated by Niijima (1977) and Kostreva et al. (1980). The afferent fibers were sensitive to increases in portal venous pressure of a few mmHg, were determined to be small myelinated fibers, and had receptive fields distributed throughout the liver and portal venous wall. Cervero (1982) studied the afferent fibers of the biliary system. Using increased biliary pressure as the stimulus, he described a group of low-threshold afferent fibers which were not spontaneously active, increased their discharge rate as biliary pressure increased, and reached a maximal firing rate at biliary pressures of 20-30 mmHg. He classified another group as high-threshold afferent fibers and these also were not spontaneously active but increased their discharge rate only when biliary pressure exceeded 20 mmHg. Both rapidly and slowly adapting fibers were found in the two groups.

Gernandt and Zotterman (1946) examined intestinal afferent fibers using light tough and other mechanical stimuli. They concluded that the wall of the small intestine was supplied by C, A-delta, and a few A-beta fibers. The A-beta fibers responded to light touch and innervated Pacinian corpuscles. The smaller fibers responded only to more prolonged or stronger mechanical stimuli and were activated by peristaltic waves or induced intestinal contractions. Bessou and Perl

(1966) described a mechanoreceptor of the small intestine which exhibited spontaneous discharge related to peristaltic activity. The small myelinated fibers responded to localized pressure and distension of the small intestine. In response to distension, the frequency of discharge was correlated with the rate of inflation and end pressure, and the increased discharge adapted with sustained pressure. Andrews et al. (1972) investigated the responses of intestinal afferent nerves and reflex responses of intestinal efferent nerves to mesenteric venous congestion. The spontaneously active afferent fibers increased their frequency of discharge roughly in proportion to the increase in mesenteric venous pressure, did not adapt readily, and decreased their discharge frequency after release of the portal vein (decrease in venous pressure). They concluded that congestion of the mesenteric venous bed produced afferent impulses which reflexly elicited a sympathetic efferent response. Mechanoreceptors distributed throughout the mesentery and innervated by splanchnic fibers were investigated by Morrison (1973) and Ranieri et al. (1973). Morrison (1973) described the punctate nature of the receptive field which occurred at branch points of arterial blood vessels. The fibers exhibited slowly adapting responses to localized stretch or external pressure and were A-delta and C fibers. The receptive fields were found in the mesentery of the small intestine, pancreas, spleen, gallbladder, portal vein, and colon. He confirmed observations by Bessou and Perl (1966) concerning distension of the gut and observations by Andrews et al. (1972) concerning portal vein occlusion.

Ranieri et al. (1973) described slowly adapting fibers which had conduction velocities in the A-delta and C fiber range, and "on-off" fibers which had conduction velocities in the A-beta range and were thought to be innervating Pacinian corpuscles. They found that the majority of the cell bodies of afferent nerves were located in the eighth to eleventh thoracic dorsal root ganglia.

In contrast to other viscera, only two reports appear in the literature regarding splenic afferent nerves. In 1964, Lim et al. recorded activity of splanchnic afferent fibers during injections of bradykinin, potassium chloride, or aceteylcholine into the spleen. All three substances produced increased discharge of the splanchnic afferent nerves. They did not quantify the nerve activity. Herman et al. (1982) examined some characteristics of splenic afferent nerves. They described a linear relationship between increases in splenic venous pressure and discharge rate of the afferent fibers. The afferent responses were slowly adapting or nonadapting. Tonic activity was not observed, and responses were found only in C fibers. They also concluded that the receptors were located in the venous vasculature of the spleen. However, they did not correlate the responses of splenic afferent nerves with the responses of efferent sympathetic nerves during increases in splenic venous pressure.

Renal mechanoreceptors have been described by Beacham and Kunze (1969). Renal afferent fibers responded to increases in renal vein and ureteral pressure. The changes in pressure reflexly activated renal efferent activity. The small-to-medium myelinated afferent

fibers entered the spinal cord between the eighth thoracic and fourth lumbar segments; the majority entered between the first and third lumbar segments. Recordati and colleagues investigated renal chemosensitive receptors (Recordati et al., 1978, 1980, 1981). They classified two groups of chemosensitive receptors as R1 and R2. R1 receptors were specifically activated by renal ischemia and were not tonically active, while the R2 chemosensitive receptors were activated by backflow of nondiuretic urine into the renal pelvis and were tonically active. The R2 receptors responded to the chemical composition of the urine and not to the increase in pelvic pressure or pelvic distension. R2 receptors also were activated by renal ischemia. Activation of renal chemosensitive receptors produced excitatory efferent renal nerve responses (Recordati et al., 1982).

Characteristics of afferent nerves from the colon have been studied by Janig and collaborators (Blumberg et al., 1983; Haupt et al., 1983). Sixty-five percent of the units tested were activated by distension of the colon. Most of these mechanosensitive units were spontaneously active at low frequencies (less than 1 impulse/sec) and had unmyelinated or small myelinated fibers. Eighty percent of the units had steady-state responses, 33% had dynamic and steady-state responses, and less than 20% had transient responses. Most of the mechanosensitive units had thresholds for response at distension pressures of less than 25 mmHg. Mechanosensitive units also responded to chemical stimulation by bradykinin and potassium chloride. Ischemia of the colon elicited responses from these units also.

Longhurst and coinvestigators explored abdominal viscera for receptors which respond to chemicals such as capsaicin and bradykinin (Lew & Longhurst, 1986; Longhurst et al., 1984a). They stimulated receptors located in the stomach, pylorus, duodenum, liver, gallbladder, porta hepatis, mesentery, pancreas, jejunum, and ileum. All of the A fibers were sensitive to mechanical stimulation such as light touch and exhibited either rapidly or slowly adapting responses. Capsaicin activated 38% of the A fibers while bradykinin activated 58%. The C fibers were sensitive to more intense mechanical stimulation such as pinching. Capsaicin activated 100% of the C fibers and bradykinin activated 73%. They concluded that C fibers innervating abdominal viscera were chemosensitive, while the A fibers were polymodal, sensitive to both mechanical and chemical stimulation.

Influence of Cardiovascular Receptors

Sympathetic reflexes may be altered or modified by input from cardiovascular receptors. Many viscero-sympathetic reflexes produce pressor responses which could activate cardiovascular pressoreceptors. Two major groups of cardiovascular pressoreceptors are the high-pressure sinoaortic baroreceptors and the low-pressure cardiopulmonary receptors.

Receptors and Afferent Pathways

Receptors of the sinoaortic baroreceptors are located in the carotid sinus and in the aortic arch (Brown, 1980; Kirchheim, 1976). The receptors respond to pressure-induced deformations which determine

the discharge frequency. Afferent fibers from the carotid sinus and aortic arch receptors include myelinated and unmyelinated fibers. The myelinated fibers have a threshold of 40 to 70 mmHg, increase their discharge rate linearly between 75 and 150 mmHg, and fire at maximum frequency at approximately 175 to 200 mmHg (Abboud & Thames, 1983). The pressure stimulus to the arterial baroreceptors has both a static and dynamic component which can influence the discharge rate. The unmyelinated fibers have higher threshold and saturation pressures as well as lower sensitivities and maximum discharge rates than the myelinated fibers. The total baroreceptor input can be increased by an increase in discharge rate of the fibers and by the recruitment of silent fibers as the arterial pressure exceeds their threshold.

The carotid sinus afferent fibers travel centrally in the carotid sinus nerve (CSN) which is a branch of the glossopharyngeal nerve. The aortic arch afferent fibers (aortic depressor nerve in the cat; ADN) travel with the vagal and cervical sympathetic nerves. The afferent nerves have their cell bodies in the petrosal ganglion and terminate in the nucleus tractus solitarius (NTS; Abboud & Thames, 1983; Dampney, 1981; Spyer, 1981). The baroreceptor afferent fibers terminate in the dorsomedial and commissural regions of the NTS (Ciriello et al., 1981a, 1981b; Davies & Kalia, 1981; Kalia & Welles, 1980; Panneton & Loewy, 1980).

Low-pressure baroreceptors include cardiopulmonary receptors with vagal afferent fibers. The receptors are located in the atria, ventricles, and pulmonary vasculature (Abboud & Thames, 1983; Donald &

Shepherd, 1978). The cardiac mechanoreceptors respond to changes in cardiac volume and contractility while the pulmonary receptors have a discharge pattern similar to that of arterial baroreceptors. Myelinated and unmyelinated afferent fibers travel in the vagal nerves and have cell bodies in the nodosal ganglion. The vagal afferent fibers terminate in the NTS in the same general regions as the sinoaortic baroreceptor fibers (Abboud & Thames, 1983; Bishop et al., 1983; Dampney, 1981; Kalia & Mesulam, 1980a, 1980b).

Central Reflex Pathways

Although projections from the NTS to other portions of the NTS, medullary reticular formation, nucleus ambiguus, pontine parabrachial nucleus, hypothalamus, and autonomic areas of the spinal cord have been described, the localization of the second synapse and further course of the central pathways of the baroreceptor reflexes are unknown (Dampney, 1981; Spyer, 1981). Recently a group of investigators (Granata et al., 1983; Reis et al., 1984) has hypothesized that neurons originating in the cardiovascular region of the NTS synapse in or project through the rostral ventrolateral medulla where adrenergic neurons are located and mediate the vasodepressor responses from arterial baroreceptors and cardiopulmonary receptors.

Baroreceptor inhibition of sympathetic activity is mediated at spinal (Barman & Wurster, 1978; Coote & Macleod, 1974b; Coote et al., 1981; Gebber, 1976; McCall et al., 1977) and supraspinal levels (Coote & Macleod, 1974b; Coote et al., 1981; Gebber et al., 1973;

Kirchner et al., 1971; Koizumi et al., 1971; Taylor & Gebber, 1975). A controversy exists as to whether the spinal site of baroreceptor influence is mediated directly onto the SPN (Coote et al., 1981; Loewy & Burton, 1978) or via spinal interneurons (McCall et al., 1977). The supraspinal site for baroreceptor inhibition is unknown, but within the medulla such inhibitory actions may occur at sites within the ventrolateral reticular formation (Dampney, 1981). Other possible areas are the hypothalamus, pontine parabrachial nucleus, and rostral ventrolateral medulla. Baroreceptor inhibition of sympathetic activity is mediated via a descending pathway in the dorsolateral funiculus of the spinal cord (Spyer, 1981). Spinal sites of cardiopulmonary receptor influence have not been investigated.

Reflex Effects of Baroreceptors

Green and Heffron (1968) were among the first investigators to describe the inverse relationship between baroreceptor activity and changes in postganglionic nerve activity. Kezdi and Geller (1968) showed that pulsatile pressure in the carotid sinus was more effective in inhibiting sympathetic discharge than was static pressure. Also they showed that the combined effect of left and right carotid sinus input was greater than that of either sinus alone. Much of the work describing the influence of arterial baroreceptors in various vascular beds utilized an indirect method of estimating sympathetic activity (Brender & Webb-Peploe, 1969; Hadjimanas & Oberg, 1968; Kendrick et al., 1972a, 1972b). These investigators described nonuniform influence on various vascular beds and concluded that tonic influence of

arterial baroreceptors was greater on skeletal muscle resistance beds than on capacitance beds, that intestinal vasculature was not strongly engaged in baroreceptor reflexes, and that the renal vascular bed was less sensitive to carotid baroreceptors than were muscle or intestinal beds. The response of the splenic vascular bed to baroreceptor input was similar to that of the hindlimb capacitance bed. Ninomiya and colleagues recorded changes in sympathetic nerve activity during increases in arterial pressure (Irisawa et al., 1973; Ninomiya & Irisawa, 1969, 1975; Ninomiya et al., 1971; Nisimaru, 1971). They reported that sympathetic nerve activity to the heart, kidney, spleen, stomach, and intestine exhibited grouped discharge patterns synchronous with the cardiac cycle and was inhibited by baroreceptor inputs. The magnitudes of response of these nerves to baroreceptor inputs were quantitatively nonuniform. It was suggested that baroreceptor reflex effects were minimal to organs of the gastrointestinal system and maximal to the spleen. Also the investigators concluded that phasic nerve activity which was synchronous with the cardiac cycle originated at or above the medullary level (Ninomiya & Irisawa, 1975). Thames and coinvestigators studied baroreceptor reflex influences in rabbits (Brown & Thames, 1982; Guo et al., 1982; Thames & Ballon, 1984). They reported that aortic and carotid baroreceptor reflex influences on renal nerve activity add by occlusive summation. They also showed that hindlimb vascular responses to arterial and cardiopulmonary baroreceptor inputs add by occlusive summation. Thames and co-workers found greater inhibition of renal nerve activity than of lumbar

sympathetic nerve activity during increases in arterial pressure, and they found that the responses were in part mediated by sinoaortic baroreceptors.

In contrast to the arterial baroreceptors which are relatively localized, the cardiopulmonary receptors with vagal afferent fibers are widely distributed in the heart and lungs. As a result, the information concerning the influence of these receptors comes from investigations utilizing either the withdrawal or interruption of vagal influence (Mancia & Donald, 1975; Mancia et al., 1973; Oberg & White, 1970a; Pelletier et al., 1971) or activation of one restricted group of cardiopulmonary receptors (Karim et al., 1972; Oberg & Thoren, 1973a, 1973b; Oberg & White, 1970a). Activation of a restricted group of cardiac receptors produced a nonuniform effect on sympathetic outflow. Activation of left atrial receptors resulted in increased cardiac sympathetic nerve activity, decreased renal nerve activity, and no change in splenic or lumbar sympathetic nerve activity (Karim et al., 1972). Stimulation of vagal cardiac nerves resulted in depressor responses, and vasodilation in skeletal muscle, renal, and intestinal vascular beds (Oberg & Thoren, 1973a, 1973b; Oberg & White, 1970a). Most of the sympathoinhibitory responses from cardiac nerve stimulation were thought to be due to activation of ventricular unmyelinated afferent fibers (Oberg & Thoren, 1973a, 1973b). Evidence for tonic vasomotor inhibition from cardiopulmonary receptors has been presented using vagal blockade. Investigators have shown that interruption of the vagi resulted in increased heart rate,

systemic arterial pressure, vasoconstriction of muscle, renal and mesenteric vascular beds, and decreased splanchnic capacitance (Mancia et al., 1973; Oberg & White, 1970; Thoren et al., 1976). Vagally innervated receptors from the heart and lungs contribute to the tonic inhibition (Mancia et al., 1975b). During a cardiovascular disturbance such as hemorrhage, the cardiopulmonary receptors were shown to exert their main influence on splanchnic resistance and capacitance vessels, and on renal circulation (Pelletier et al., 1971). The inhibitory influence of cardiopulmonary receptors, which can be increased by volume expansion or pressor responses, acts on the kidney to cause changes in blood flow, nerve activity, and renin secretion (Mancia et al., 1975a; Thames et al., 1978). A generalized activation of cardiopulmonary receptors by volume expansion produced a decrease in renal nerve activity (Clement et al., 1972). In another investigation, activation of cardiopulmonary receptors produced vasodilation in skeletal muscle, kidney, and large intestine, but no significant response in small intestine, liver, or adrenal gland (Lloyd & Friedman, 1977). Thames and coinvestigators (Brown & Thames, 1972; Guo et al., 1982) found that cardiopulmonary baroreceptors mediate in part the inhibitory reflex responses of renal and lumbar nerves to increases in arterial pressure.

Because of central interactions between influences of cardiopulmonary receptors and carotid sinus baroreceptors, the level of tonic inhibition exerted by the cardiopulmonary receptors varies inversely with that of carotid sinus baroreceptors (Mancia et al., 1976). Even

so, the cardiopulmonary receptors can exert an inhibitory influence in the presence of normally functioning carotid baroreceptors (Thames et al., 1978). The influence of the cardiopulmonary receptors appears to be directed preferentially toward the kidney (Little et al., 1975; Mancia et al., 1975b; Oberg & Thoren, 1973b; Oberg & White, 1970a).

Interaction of Baroreceptor Reflexes With Other Reflexes

Mukherjee (1957) noted that pressor responses and renal vasoconstriction in response to bladder distension were greater after vagotomy and carotid sinus denervation. He attributed this to the "buffering" action of the carotid sinus and aortic baroreceptors. Another viscero-visceral reflex which was attenuated by baroreceptors was described by Khayutin et al. (1969). Activation of mesenteric receptors caused renal vasoconstriction, increased renal nerve activity, and pressor responses. The magnitude of these responses was enhanced and the duration of the sympathetic discharge extended after vagotomy and bilateral carotid occlusion. A large number of researchers have studied somatosympathetic reflexes and have observed interactions between those reflexes and baroreceptor reflexes. Coote and Macleod (1974b) described somato-cardiac and somato-renal reflexes which were inhibited during activation of carotid sinus receptors. Both spinal and supraspinal components of the reflexes were inhibited. Barman and Wurster (1978) showed that baroreceptor-inhibition of a somatosympathetic reflex affected both the spinal and supraspinal components although the supraspinal component was more susceptible to

inhibition. Thames and colleagues have investigated the effects of activation of carotid baroreceptors and vagally innervated cardiopulmonary receptors on somatosympathetic reflexes (Abboud et al., 1981; Thames & Abboud, 1979). The reflex was initiated by electrical stimulation of the sciatic nerve. Activation of vagal afferent nerves by pressor responses produced during the reflex inhibited sympathetic vasoconstriction to the kidney. The renal responses to somatic stimulation were attenuated by volume expansion and augmented by vagotomy. Maximal carotid sinus activation blocked the renal response to somatic stimulation. Also it was shown that carotid sinus input could inhibit reflex vasoconstrictor responses in skeletal muscle. They concluded that sinoaortic and cardiopulmonary baroreceptors serve to limit sympathetic discharge to the kidney and skeletal muscle during activation of somatic afferent nerves. Investigations examining the interaction between vascular pressoreceptor reflexes and other visceral reflexes are scarce. Also there are few investigations examining the relative contributions of sino-aortic baroreceptor and cardiopulmonary receptor influence to the reflex interactions.

METHODS

Surgical Preparations

Seventy-five cats of either sex (2.0-6.0 kg) were anesthetized with intravenously administered alpha-chloralose (80 mg/kg; Sigma Chemical Co., St. Louis, MO). Additional doses of 20 mg/kg were administered when necessary. A femoral artery and vein were cannulated (PE-160; Clay-Adams, Parsippany, NY), and a tracheotomy tube was placed in the trachea. Systemic arterial blood pressure was monitored from a cannula placed in the aorta (via the femoral artery) which was attached to a pressure transducer (Statham P23; Gould Inc., Oxnard, CA). Gallamine triethiodide (4 mg/kg i.v.; Davis-Geck, Pearl River, NY) was administered to provide muscle relaxation during the surgical preparations and during experimental procedures. Additional gallamine was administered only when the animal exhibited skeletal muscle contractions in response to afferent stimulation. Before additional gallamine (1-2 mg/kg) was administered, the pupil size, ear twitch, and nociceptive reflexes were assessed to determine the animal's plane of anesthesia. After administration of gallamine, all animals were artificially respired with a Harvard 665 Animal Respirator (Harvard Apparatus, South Natick, MA). Body temperature measured in the esophagus was maintained at 37 degrees Centigrade by a circulating hot water heating pad. End-tidal CO_2 concentration was

monitored with a Morgan 901-MK2 CO_2 analyzer (PK Morgan, Chatham, Kent, England) and maintained at 3.5-4.0%. Animals were respired with 50% O_2 in air. Arterial blood samples were analyzed periodically (Corning 165 Blood Gas Analyzer; Corning Medical, Medfield, MA) and acceptable values were: pH, 7.35-7.45; pCO_2, 25-35 mmHg; and pO_2 not less than 80 mmHg. Any deviations from these values were corrected with changes in respiratory volume and/or rate or with i.v. administration of sodium bicarbonate (appropriate volume of 1.0M solution to correct base deficit).

Vagotomy and sino-aortic denervation (SAD/VX) were accomplished by sectioning the vagus and glossopharyngeal nerves as they passed into the jugular foramen. Sino-aortic denervated (SAD) animals had vagal afferent nerves intact and sino-aortic nerves sectioned. The aortic depressor nerves were identified and sectioned at their junction with the superior laryngeal nerves. The carotid sinus nerves were identified at their junction with the glossopharyngeal nerves and sectioned; in addition, all nerves in the region of the carotid sinus were sectioned. The absence of an increase in mean arterial pressure during occlusion of the common carotid arteries demonstrated that carotid sinuses had been denervated. In the vagotomized (VX) animals, carotid sinus and aortic depressor nerves were intact and both vagi were sectioned. Vagotomy was done to eliminate the influence of all vagally innervated vascular pressoreceptors. The most significant consequence of vagotomy probably was denervation of cardiopulmonary

receptors. The aortic nerves were identified at their junction with the superior laryngeal nerves, and only the vagi were sectioned.

The spleen was approached through a left flank incision. To obtain maximum access to the spleen, the last two ribs were resected, and the diaphragm was attached to intercostal muscles of the eleventh rib. Later, a pneumothorax was created by inserting a short length of tygon tubing between the reattached diaphragm muscles and the rib to provide stability in neural recordings. The vascular isolation of the spleen was accomplished by ligation and section of all vascular connections to other organs while leaving the splenic artery, vein and nerves intact (Figure 1). The splenic artery was cannulated (PE-50) via the left gastric artery for injections into the spleen, and the splenic vein was cannulated (PE-50) via a gastroepiploic vein to monitor splenic venous pressure (Statham P23 pressure transducer). Snares were placed around the splenic artery and vein proximal to the cannulation site, taking care not to damage the splenic nerves. To ensure that vascular isolation of the spleen was complete, Evans blue dye (1-2 ml of a 10 mg/ml solution) was injected into the spleen during occlusion of both splenic artery and vein. After injection of the Evans blue dye, the spleen was examined for any vascular leaks, and other organs were examined for any evidence of remaining vascular connections from the spleen.

Postganglionic splenic fibers were identified next to the splenic artery, a small nerve bundle was sectioned, and the connective tissue surrounding the nerve was carefully removed in preparation for

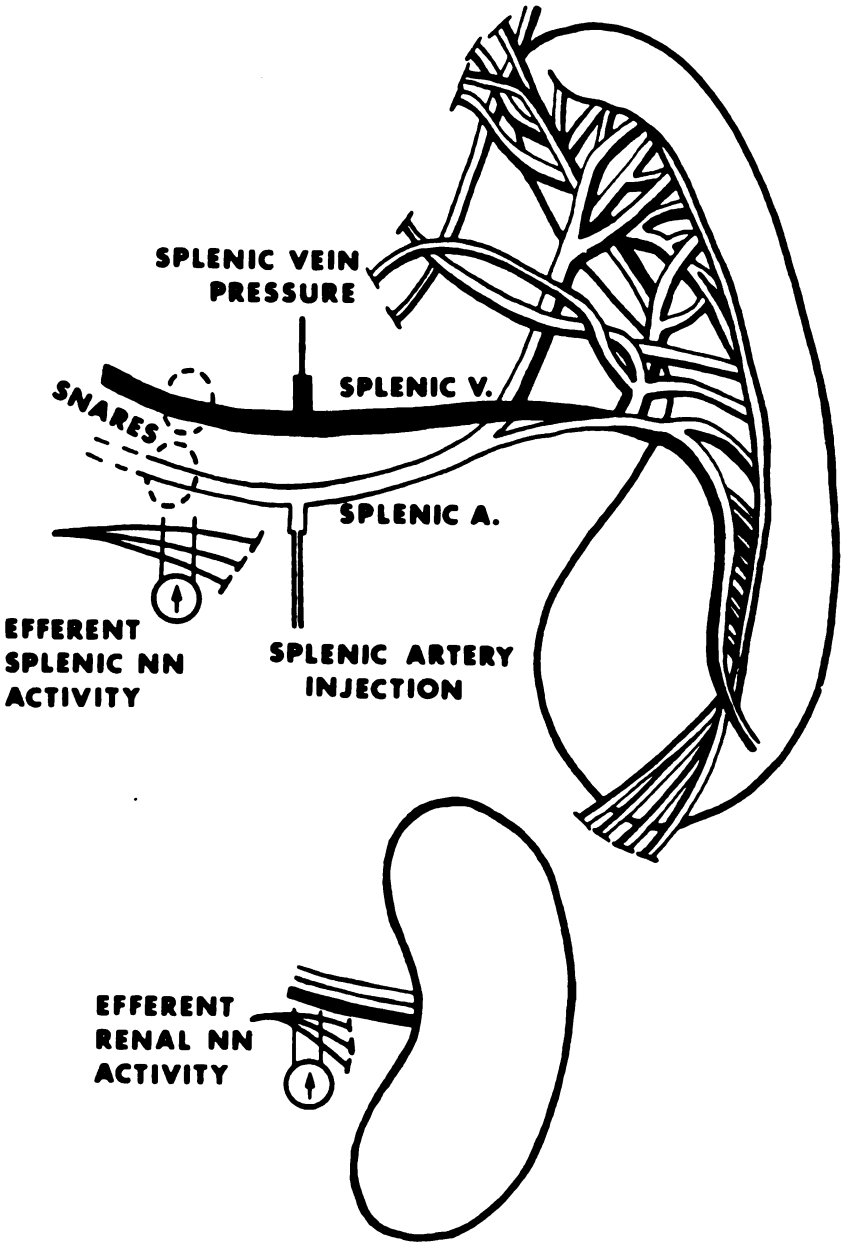

Figure 1. Diagram of experimental preparation of spleen and kidney.

recording efferent nerve activity. The smallest nerve bundle with adequate length (10-15 mm; to place across electrode hooks) was selected to maintain maximal afferent and efferent innervation of the spleen. Postganglionic renal nerves were identified at the renal pelvis and sectioned; the central cut end was dissected in preparation for recording efferent nerve activity. Renal nerves were selected which coursed from the superior pole of the solar plexus to the renal pelvis. Nerves from the paravertebral ganglia were never selected. Postganglionic mesenteric nerves were identified and prepared for recording efferent nerve activity. Mesenteric nerves were selected from nerve bundles which closely surrounded the superior mesenteric artery and could be traced to the solar plexus.

Some splenic nerves were identified and prepared for recording afferent electrical activity. Splenic nerve bundles were isolated distal to the branching of the primary splenic artery (Figure 2). A bipolar recording electrode was positioned near the isolated bundles. A bipolar stimulating electrode was placed 10-20 mm distal to the recording electrode. Individual nerve bundles were sectioned and placed on the recording electrode immediately prior to testing their responses to mechanical and chemical stimulation. Exposed nerves were immersed in warmed mineral oil (37 degrees Centigrade) and surrounding tissue was coated with petroleum jelly to prevent tissue dehydration. In 12 animals a dorsal laminectory of the first cervical vertebra was performed to expose the spinal cord for later transection.

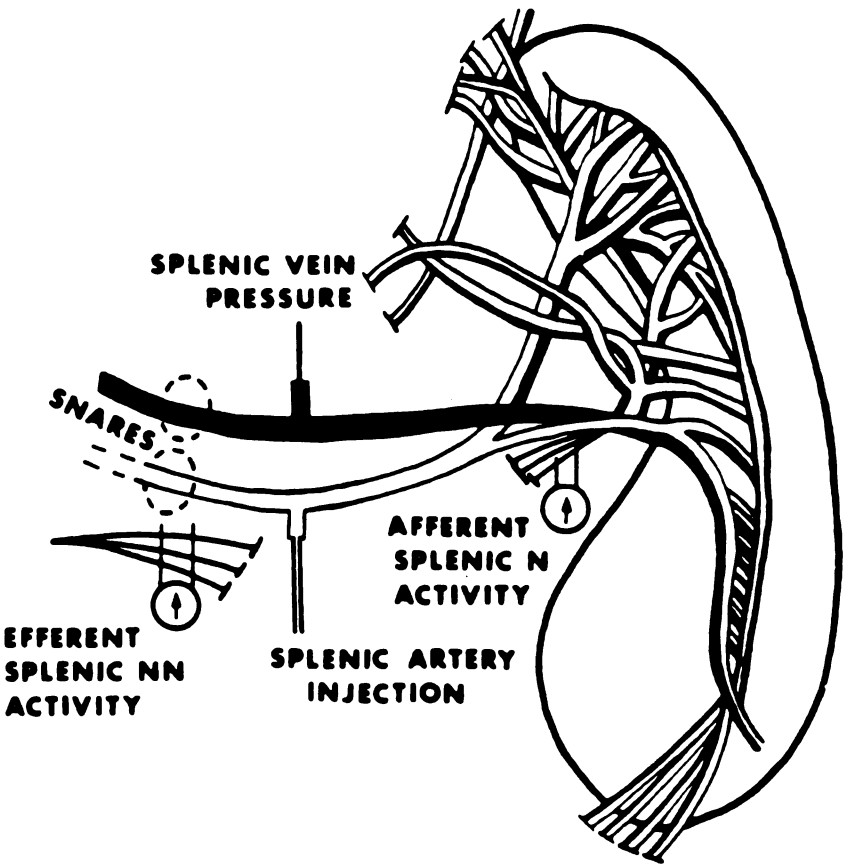

Figure 2. Diagram of experimental preparation for recording splenic afferent nerve activity.

Data Acquisition

Multifiber activity of postganglionic splenic, renal, and mesenteric nerves was recorded using bipolar platinum-iridium electrodes. The recording electrodes were connected to a high-impedance Grass probe (model HIP511; Grass Instruments, Quincy, MA). The signal was amplified with a Grass P511 A.C. preamplifier with the low-frequency cutoff set at 30 Hz and the high-frequency cutoff set at 3000 Hz. The amplified output was audible from a Grass audio amplifier (model AM8B) and visible on a Tektronix dual beam storage oscilloscope (model D13; Tektronix, Inc., Beaverton, OR). The output of the amplifier also was directed to a Vetter 8-track tape recorder (model D; A. R. Vetter Co., Rebersburg, PA) to be recorded on magnetic tape, and to a Grass 7D polygraph. The electrical activity displayed by the polygraph was full-wave rectified and integrated cumulatively during 10-s periods by Grass 7P10B integrators and displayed to provide initial estimates of neural responses. Background electrical noise level was assessed during the experiments by administering trimethaphan camsylate (0.2 mg/kg; Hoffman-LaRoche, Inc., Nutley, NJ), or after the experiments by administering hexamethonium (10 mg/kg; Mann Research Laboratories, New York, NY), or by crushing the nerve bundle central to the recording electrodes. After the experiment, neural activity stored on magnetic tape was digitized, full-wave rectified, and integrated during 10-s intervals by a PDP 11-23 microprocessor (Digital Equipment Corporation). The integrated noise values were subtracted from the total integrated signal to obtain the values for integrated neural

activity in microvolt-seconds per 10-s intervals. Because the voltage was recorded extracellularly and varied according to bundle size and contact with the electrode, these units were considered to be arbitrary, and relative changes from baseline were assessed. In some experiments with few-fiber preparations, neural activity was analyzed using a spike counting program for the PDP 11-23 microprocessor. Spikes with amplitudes greater than background noise were counted during 10-s intervals and reported as spikes/second (sp/s). Efferent splenic nerve activity which was recorded during studies of afferent nerve responses was analyzed using 5-s intervals.

Single- or few-unit activity of afferent splenic nerves was recorded using bipolar platinum electrodes and amplified with the band pass set at 100-1000 Hz. After the experiment, neural activity stored on magnetic tape was spike-counted during 5-s intervals by a spike-sorting program for the PDP 11-23 microprocessor. The amplitude limits or "window" was set to count only the identifiable spike.

Conduction velocities of afferent fibers were determined by measuring the conduction time for the evoked potential between the stimulating electrode and the recording electrode, and dividing the distance between these electrodes by the conduction time. The stimulating electrode was placed 10-20 mm distal to the recording electrode (Figure 2). From the consistent amplitude and smooth contour of the evoked A-delta spike, it could be determined with certainty that the evoked A-delta spike was single. The evoked A-delta spike was compared with the spontaneous A-delta spike to identify it for

determination of conduction velocity. Splenic nerves contain comparatively few myelinated fibers compared to unmyelinated fibers (see Calaresu et al., 1984). The spontaneously active C spikes had small amplitudes, and their size was clearly different from that of A-delta spikes. When more than one C spike was seen in the evoked potential, either the C spike that matched the one in the spontaneous activity was chosen or the average conduction velocity of the evoked C potentials was used as the index for estimation of conduction velocity. Afferent fibers were classified as A-delta fibers if they had conduction velocity greater than 5 m/s and as C fibers if they had conduction velocity less than 2 m/s.

Experimental Preparations and Protocol

General Preparations

Capsaicin was prepared by mixing 5.0 mg of capsaicin (Sigma Chemical Co., St. Louis, MO), 4.5 ml of normal saline, 0.5 ml of ethanol, and a drop of Tween 80 (Sigma Chemical Co.) to produce an initial concentration of 1.0 mg/ml and heating the solution to 55 degrees Centigrade and gently stirring until dissolved. Further dilutions were made with normal saline at room temperature. Bradykinin triacetate (Sigma Chemical Co.) was dissolved in saline at an initial concentration of 100 µg/ml and further dilutions were made with normal saline. Capsaicin and bradykinin were injected in volumes of 0.1 to 0.5 ml and flushed through the cannula with 1.0 ml saline (cannula had volume of approx. 0.3 ml). Injections of similar volume of vehicle did not produce reflex responses nor activation of afferent

fibers. Blood pressure and mean discharge rate of the nerves were monitored for 15 min before an experiment was begun to establish that stable control conditions existed.

Specific Protocols

Reflexes Initiated From the Spleen in Vagotomized, Sino-Aortic Denervated Animals

Receptors of the vascularly isolated spleen were stimulated, and responses of splenic and renal nerves were recorded in vagotomized, sino-aortic denervated animals (n=26). The protocol consisted of a 1-min control period, occlusion of the splenic artery, occlusion of the splenic vein, and injection into the splenic artery of: (a) warm, physiological saline (7-50 ml) to increase intrasplenic pressure; (b) capsaicin (5.0 μg in 0.25 ml of physiological saline); or (c) bradykinin (10.0 μg in 0.1 ml of physiological saline). All drugs were flushed into the spleen with 1.0 ml of physiological saline. The responses were monitored for 2 min, the occlusions were released approximately 3 min after injection of drugs or 1 min after completion of saline infusion, and a 1-min recovery period was sampled 3 min after release of the occlusions. Neither occlusion of the splenic artery and vein for 2 min nor vehicle injection produced reflex responses. Animals were allowed to recover for 15 to 30 min between experimental protocols. The substances were administered in random order. Widespread visceral and somatic receptor stimulation produced by injection of 20 μg of capsaicin into the descending aorta (via the femoral

artery cannula) was used to examine the pattern of sympathetic neural responses produced by this afferent input, and to demonstrate that renal nerve responses to splenic receptor simulation were not maximal responses of those individual nerves.

Intensity-Response Characteristics of Reflexes Initiated From the Spleen

Various doses of capsaicin and different degrees of splenic congestion were used to stimulate receptors of the vascularly isolated spleen, and splenic and renal nerve responses were recorded in vagotomized, sino-aortic denervated animals (n=25). After the control period and occlusion of the splenic artery and vein, warm physiological saline or capsaicin was injected into the splenic artery. The monitoring of responses, release of vessels, and recovery period were similar to those described above. The splenic congestion achieved splenic venous pressures of (a) 15 mmHg (4-16 ml of saline), (b) 25 mmHg (10-30 ml of saline), (c) 40 mmHg (20-50 ml of saline), and (d) 50 mmHg (30-65 ml of saline). The doses of capsaicin used were: 10, 100, 250, 500 ng, 1, 2, 5, 10, 20, 50, 100, 200, and 300 μg in 0.1 to 1.0 ml of saline.

Spinal Sympathetic Reflexes Initiated From the Spleen

Receptors of the vascularly isolated spleen were stimulated, and splenic and renal efferent nerve responses were recorded in vagotomized, sino-aortic denervated animals (n=12) before and one hour after spinal cord transection at the first cervical segment. The

protocols for administration of capsaicin, bradykinin, and congestion were the same as those described above and were conducted first in animals with intact neuraxes. After the three protocols were completed, gallamine (4 mg/kg) was administered and the spinal cord was transected at the level of the first cervical vertebra, and the animals continued to be artificially respired as they had been throughout the experiments. If necessary, infusion of phenylephrine (0.12 μg/ml; Winthrop Laboratories, New York, NY) in saline was administered (0.04-0.3 cc/min; Harvard 975 infusion pump; Harvard Apparatus, South Natick, MA) to maintain mean arterial pressure (MAP=[1/3(systolic pressure-diastolic pressure)] + diastolic pressure) above 70 mmHg. A one-hour stabilization period was allowed before repeating the above protocols. Injection of capsaicin (20 μg) into the descending aorta to cause widespread visceral and somatic receptor stimulation was done after the other protocols.

Afferent Nerve Responses to
Stimulation of Splenic Receptors

Receptors of the vascularly isolated spleen were stimulated, and afferent and efferent splenic nerve responses were recorded in vagotomized, sino-aortic denervated animals. To prepare for recording afferent activity, a small nerve bundle was sectioned distal to the branching of the primary splenic artery. The distal cut end was subdivided if possible and placed on the recording electrode. Only fibers exhibiting spontaneous activity were tested. If more than two distinguishable units were present, the fiber was subdivided and a

portion of the fiber was replaced on the recording electrode. The afferent activity was monitored for 15 min before starting experimental protocols. The protocol consisted of a 1-min control period, occlusion of the splenic artery and vein, and injection into the splenic artery of: (a) warm, physiological saline (9-27 ml) to increase intrasplenic pressure; (b) capsaicin (1.0 μg or 2.0 μg in 0.5 ml of saline); (c) bradykinin (10 μg in 0.1 ml of saline); or (d) norepinephrine (1.0 μg in 0.1 ml saline) to induce contraction of the spleen. The response was monitored for 2 min, and the occlusions were released approximately 3 min after injection of drugs or 1 min after completion of saline infusion. After the fiber was tested with the experimental protocols, it was orthodromically activated to obtain an estimate of conduction velocity. Each of the preceding steps was repeated for each fiber bundle.

Influence of Cardiovascular Pressoreceptors on Reflex Sympathetic Responses

Receptors of the vascularly isolated spleen were stimulated, and splenic and renal efferent nerve responses were recorded in groups of animals subjected to different degrees of pressoreceptor denervation (Intact, n=8; Vagotomized, VX, n=6; Sino-aortic denervated, SAD, n=6; SAD/VX, n=26). The protocol for these experiments was similar to that described above for capsaicin, bradykinin, and congestion. The volume of saline used to increase intrasplenic pressure was 15-30 ml.

Influence of Cardiovascular Pressoreceptors on Tonic Sympathetic Activity

Responses to increased systemic arterial pressure were evaluated in Intact (n=10), Vagotomized (VX, n=10), Sino-aortic denervated (SAD, n=13), and SAD/VX (n=9) groups. Efferent activity of splenic and renal nerves was recorded. The protocol consisted of a 1-min control period followed by injection into the femoral vein catheter of either: (a) 3 ml of 6% Dextran 75 in physiological saline (Abbott Laboratories, North Chicago, IL) or norepinephrine (0.05 μg or 1.0 μg in 0.1 ml of physiological saline; Breon Laboratories, Inc., New York, NY). The responses were monitored for 2 min. A recovery period was sampled when nerve activity and blood pressure had returned to control levels (approximately 4-1/2 min after the response). Dextran or 0.05 μg of norepinephrine was used to produce small increases in blood pressure.

Comparisons of Splenic and Mesenteric Reflex Responses to Splenic Receptor Activation

Receptors of the vascularly isolated spleen were stimulated, and splenic and mesenteric nerve responses were recorded in animals with all pressoreceptors intact (n=8). Only mesenteric nerve activity which could be excited by unloading pressoreceptors and inhibited by pressoreceptor activation was studied to ensure that sympathetic responses were recorded from mesenteric nerves with vasomotor components. Pressoreceptors were unloaded by hemorrhage which caused a decrease in MAP of 70 mmHg below control MAP. Pressoreceptors were

activated by reinfusion of blood to increase MAP to 25 mmHg above initial control values. In three experiments, reinfusion of blood did not increase arterial pressure above initial control so a bolus of phenylephrine (10 μg in 5 ml saline) was injected into the femoral venous catheter to increase arterial pressure. The protocol for these experiments was similar to that described above for capsaicin, bradykinin, and congestion. The volume of saline used to increase intrasplenic pressure was 6-24 ml.

Data Analyses

In all experiments changes from control values of efferent nerve activity, mean arterial pressure, heart rate, and splenic venous pressure caused by experimental manipulations were tested with an analysis of variance using a complete block design. Mean treatment values were compared to control values with a test of Least Significant Differences (Sokal & Rohlf, 1969). Differences were considered significant when $P < 0.05$ and variability was expressed by a coefficient of variability or standard error of the mean.

A nonparametric Friedman test (Sokal & Rohlf, 1969) was used to compare the magnitudes of neural responses between nerves. Integrated voltages or spike counted averages of splenic, renal, and mesenteric nerve activity were expressed as ratios (splenic/renal or splenic/mesenteric) during control periods and during periods of maximum reflex response. Maximum responses consisted of the 10-s interval of maximum or minimum activity after application of the stimulus. When

the responses were multiphasic, the greatest change from control was used. The Friedman test was used to compare the ratios of control activity to those of activity during the maximum response. Changes in these ratios between control and maximum response documented unequal magnitudes of responses of the two nerves. Differences detected by the Friedman test were considered significant when $P < 0.05$.

Integrated voltage values and spike counted averages were used for statistical analysis, but data are expressed as mean percentage of control in some of the figures. The duration of a response was defined as the interval from the first 10-s interval of significant change in neural activity until the return to control value. The level of activity during control period was very stable.

Additional Analyses Used in Specific Sections

Reflexes Initiated From the Spleen in Vagotomized, Sino-Aortic Denervated Animals

The magnitudes of reflex excitation of splenic or renal nerve activity in response to capsaicin stimulation of splenic receptors were compared across the four states of pressoreceptor innervation using a one-way analysis of variance. Data were expressed as percentage of control and were normalized by square root transformation before performing the analysis of variance. Mean values were compared with a Student-Newman-Kuels test (Sokal & Rohlf, 1969), and differences were considered significant when $P < 0.05$.

Spinal Sympathetic Reflexes
Initiated From the Spleen

The nonparametric Friedman test was used to compare the magnitude of neural responses before and after spinal cord transection. Integrated voltages and spike counted averages of splenic nerve activity before (neuraxis intact) and after (spinalized) spinal cord transection were expressed as ratios (neuraxis intact/spinalized) during control period and during periods of maximum reflex response. The Friedman test was used to compare the ratios of control activity to those of activity during the maximum response. Changes in these ratios between control and maximum response documented different magnitudes of splenic nerve responses in the two experimental conditions, i.e., neuraxis intact and spinalized. Differences detected by the Friedman test were considered significant when $P < 0.05$. This analysis was also used to compare renal reflex responses before and after spinal cord transection.

Analysis of variance using a complete block design was used to test the absolute change of quantified (integrated voltages and spike counted) splenic nerve activity during reflex responses before and after spinal cord transection. The control activity was subtracted from the maximum response to obtain a value for absolute change in the neuraxis intact and spinalized states. Mean values were compared with a test of Least Significant Differences. Differences were considered significant when $P < 0.05$. This analysis was also used for the renal nerve activity.

Changes in baseline nerve activity caused by spinal transection were tested. Comparison of control activities was done using a one-way analysis of variance. Control activity of each nerve in animals with intact neuraxis and spinalized states was expressed as a ratio (neuraxis intact/spinalized). The ratios were normalized by square root transformation before performing the analysis of variance to compare the ratios of splenic to renal control activity. The ratios were constructed for integrated voltages and for spike counted averages. Mean values were compared with a test of Least Significant Differences. Differences were considered significant when $P < 0.05$.

Afferent Nerve Responses to Stimulation of Splenic Receptors

If a single spike could not be discriminated throughout the response to an experimental protocol, the fiber was classified as multiunit. During the experimental protocols, control rates of discharge were averaged during the 60-s period prior to the occlusions of the splenic artery and vein. Maximum rates of discharge were averaged during a 5-s interval and rates were expressed as spikes per second (sp/s). A unit was considered to respond to a stimulus if there was an increase in spike activity (during 5-s time interval) greater than activity during any 5-s interval in the control period. The threshold pressure was determined as the splenic venous pressure at which the unit initially increased its firing rate. The stimulus duration was the length of time (in 5-s intervals) that the splenic venous pressure was above threshold pressure. Afferent activity,

stimulus duration, and duration of response are expressed as mean ± standard error of the mean. The unit responses to congestion were categorized as adapting or nonadapting. Adapting responses were those in which the unit returned to control discharge rate in spite of maintained splenic venous pressure above threshold. Nonadapting responses were those in which the unit maintained an increased discharge rate as long as the splenic venous pressure was above threshold.

Influence of Cardiovascular Pressoreceptors on Tonic Sympathetic Activity

The magnitudes of pressoreceptor-induced inhibition of tonic splenic or renal nerve activity were compared across the four states of pressoreceptor innervation using a one-way analysis of variance. Data were expressed as percentage of control and normalized by square root transformation before performing the analysis of variance. Mean values were compared with a Student-Newman-Kuels test (Sokal & Rohlf, 1969), and differences were considered significant when $P < 0.05$.

RESULTS

Reflexes Initiated From the Spleen in Vagotomized, Sino-Aortic Denervated Animals

Activation of splenic receptors can produce excitatory sympathetic reflexes (Calaresu et al., 1984). Does the character of the stimulus (chemical versus mechanical) determine the pattern of unequal splenic and renal reflex responses? Or can congestion and capsaicin be two ways of producing the same reflex? In the present investigation, injection of capsaicin into the spleen caused excitation of splenic and renal nerves and increases in systemic arterial pressure, splenic venous pressure, and heart rate (Table 1). A typical response is illustrated in Figure 3. Increased activity in both nerves occurred approximately 5 s after injection and lasted 10-80 s. The increase in splenic and renal nerve activity is illustrated in Figure 4. Mean responses to this stimulation are shown in Figure 5 and Table 1. The significant excitation of splenic nerves was greater than that of renal nerves when compared using the nonparametric Friedman test. Pressor responses, tachycardia, and contraction of the spleen accompanied the splenic reflexes (Figure 5, Table 1). The changes in splenic venous pressure were caused in part by direct action of capsaicin (Donnerer & Lembeck, 1982).

Stimulation of splenic receptors with bradykinin produced neural responses similar to those produced by capsaicin (Figure 5, Table 1).

Table 1. Mean responses to stimulation of splenic receptors in sino-aortic denervated, vagotomized animals.

	Splenic NA (μV·s/10s)		Renal NA (μV·s/10s)	SAP (mmHg)	HR (bpm)
Capsaicin					
Control	28		115	115	237
Maximum	41*	†	136*	146*	241*
Recovery	28		117	113	237
CV	0.17		0.10	0.10	0.01
n = 26					
Bradykinin					
Control	28		112	104	247
Maximum	38*	†	126*	121*	250*
Recovery	28		114	105	249
CV	0.17		0.07	0.08	0.01
n = 15					
Congestion					
Control	25		127	111	237
Maximum	31*	†	144*	130*	239
Recovery	24		133	110	234
CV	0.16		0.17	0.10	0.02
n = 20					

These data are integrated voltage values statistically analyzed for the illustrations in Figure 5. The statistical methods used to compare these values are described in Methods.

CV, coefficient of variability; NA, nerve activity in μV·s/10s; *, significantly different from control; †, significant change in splenic/renal nerve activity ratio determined by Friedman test; SAP, mean systemic arterial pressure in mmHg; HR, heart rate in beats/min; n, number of animals.

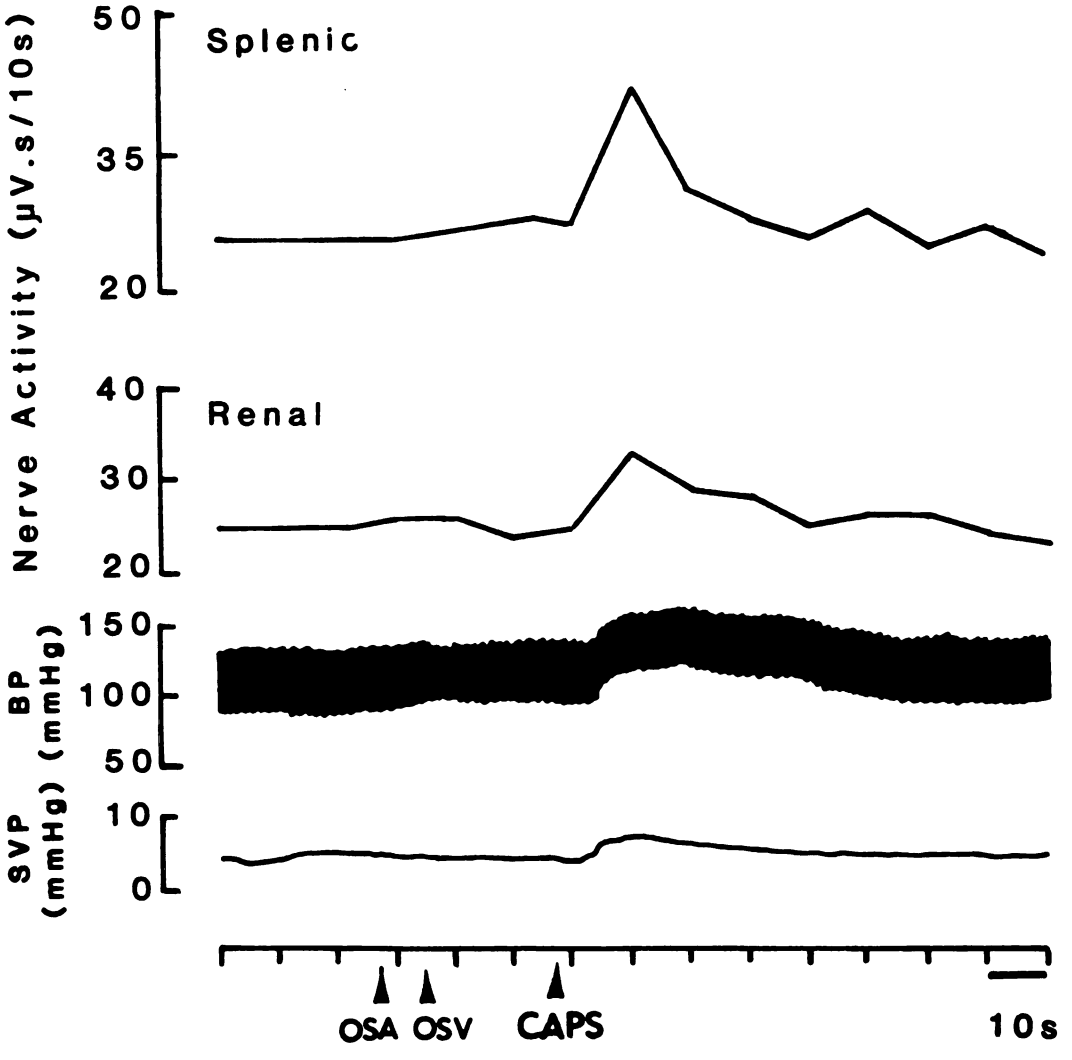

Figure 3. Responses of a sino-aortic denervated, vagotomized animal to intrasplenic **capsaicin.**

Effects of injection of 5 μg capsaicin into splenic artery of sino-aortic denervated, vagotomized cat on integrated splenic and renal sympathetic nerve activity, blood pressure (BP), and splenic venous pressure (SVP). Sympathetic activity is expressed as μV•s/10s interval. The time-base marks indicate occlusion of splenic artery (OSA), occlusion of splenic vein (OSV), and injection of capsaicin (CAPS).

Figure 4. Nerve responses to stimulation of splenic receptors with capsaicin.

Effects of stimulation of splenic receptors by capsaicin on splenic and renal nerve activity. Vertical lines at far right represents calibration for splenic (top) and renal (bottom) nerve activity. The horizontal line represents 0.5 second.

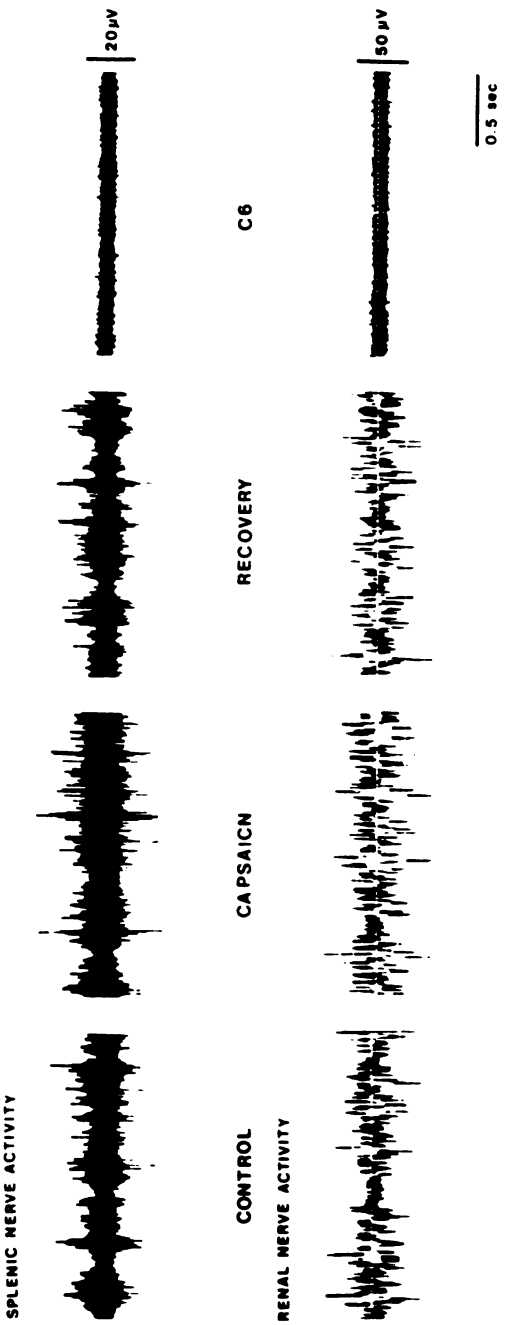
SPLENIC NERVE ACTIVITY
RENAL NERVE ACTIVITY
CONTROL
CAPSAICN
RECOVERY
C6
20 µV
50 µV
0.5 sec

Figure 4.

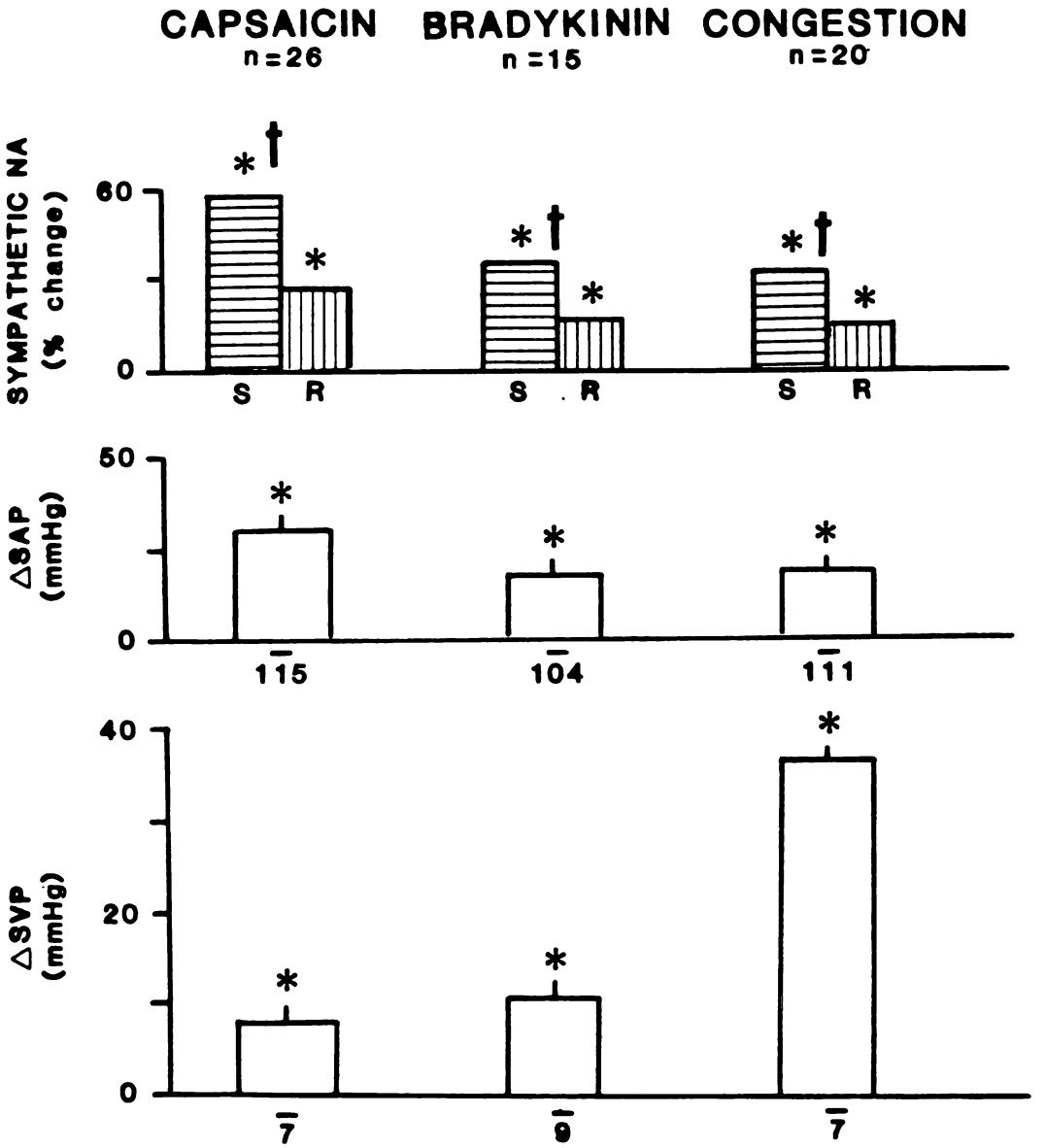

Figure 5. Mean responses to stimulation of splenic receptors in sino-aortic denervated, vagotomized animals.

Mean changes in sympathetic nerve activity (NA), systemic arterial pressure (SAP), and splenic venous pressure (SVP) caused by stimulation of splenic receptors of sino-aortic denervated, vagotomized animals by capsaicin, bradykinin and congestion. Percent changes are calculated from the data in Table 1. S, splenic nerve activity; R, renal nerve activity; *, mean values significantly different from control ($P < 0.05$); †, neural responses significantly different from each other using Friedman test; SAP and SVP are mean changes $\pm$ standard error; n, number of animals. Numbers at bottom of ΔSAP and ΔSVP bars are mean control values for the group.

Stimulation of receptors by bradykinin caused excitation of splenic and renal nerves, and splenic nerve excitation was greater than renal when compared using the Friedman test (Table 1). The neural responses occurred after a latency of 10 to 30 s and lasted 10 to 50 s. Pressor responses, tachycardia, and contractions of the spleen accompanied the splenic reflexes (Figure 5, Table 1). Part of the change in splenic venous pressure probably was due to the direct action of bradykinin (Gilman et al., 1980).

Increases in intrasplenic pressure to cause splenic congestion produced excitatory neural and cardiovascular responses. Splenic congestion also caused greater splenic than renal sympathetic responses (Figure 5, Table 1). The neural responses lasted 10 to 100 s. Congestion produced significant pressor responses and increases in splenic venous pressure. Significant heart rate changes did not occur.

In a survey of all experiments in sino-aortic denervated, vagotomized animals, reflex neural responses to capsaicin stimulation of splenic receptors were detected in all 28 animals whereas reflex responses to congestion were detected in 14 of the 28 animals. Thus, the probability of observing a reflex neural response to capsaicin was greater than to congestion.

Injections of capsaicin into the aorta to cause widespread visceral and somatic receptor stimulation were used to test responsiveness of splenic and renal nerves to other visceral afferent input and to determine if an unequal pattern of neural responses was produced by

this excitatory input. The responses of splenic nerves were larger than those of renal (137% increase and 60% increase, respectively), and these excitatory splenic and renal responses were generally larger than those produced by splenic receptor stimulation.

Because the mean control voltage values of the two nerves differed, procedures in which splenic and renal nerve control voltage values were similar were grouped (Table 2a) to determine if the smaller renal sympathetic responses correlated with the higher basal voltage of renal nerves. The splenic nerve response to capsaicin stimulation of splenic receptors in this subgroup was still greater than the renal response. The reflex responses expressed as percentage change in this subgroup of splenic and renal nerves were similar to that of the entire group. The splenic nerve response of the subgroup was an increase of 41% compared to an increase of 57% of the entire group, and the renal nerve response of the subgroup was an increase of 21% compared to an increase of 25% of the larger group. Responses of systemic arterial pressure and splenic venous pressure in this subgroup also were similar to those of the entire group (Table 2a).

In some experiments with few-fiber preparation, another method of quantifying neural data was used. With the spike counting method, capsaicin stimulation of splenic receptors also caused greater excitation of splenic than renal nerves (Table 2b).

In summary, activation of splenic receptors by capsaicin, bradykinin, or congestion could produce greater splenic than renal nerve

Table 2. a. Comparison of responses with similar control values;
b. Comparison of responses quantified by spike counting.

Responses to stimulation of splenic receptors by capsaicin in procedures with similar control values of splenic and renal nerve activity and in procedures with spike counted nerve activity.

a. Voltage integrated responses of splenic and renal nerve activity to capsaicin in procedures with similar control values.

	Splenic NA (μV·s/10s)		Renal NA (μV·s/10s)	SAP (mmHg)	HR (bpm)	SVP (mmHg)
Control	46		44	106	251	7
Maximum	65*	†	52*	131*	254*	14*
Recovery	45		41	99	252	7
CV	0.12		0.08	0.07	0.01	0.35
n = 7						

b. Spike counted responses of splenic and renal nerve activity to capsaicin.

	Splenic NA (sp/s)		Renal NA (sp/s)
Control	67		129
Maximum	105*	†	158*
Recovery	66		125
CV	0.17		0.12
n=12			

CV, coefficient of variability; NA, nerve activity; sp/s, spikes per second; n, number of animals; *, significantly different from control; †, significant change in splenic/renal nerve activity ratio determined by the Friedman test; SAP, mean systemic arterial pressure; HR, heart rate; SVP, splenic venous pressure; bpm, beats per minute. The statistical methods used to compare these values are described in Methods.

responses. The unequal pattern of reflex neural responses did not depend on unequal initial baseline values of nerve activity.

Intensity-Response Characteristics of Reflexes Initiated From the Spleen

Although one could expect to observe a dose- or intensity-response relationship in the responses of each nerve to stimulation by capsaicin or congestion, it is possible that intensity-response relation of splenic nerves could differ from that of renal nerves. The first stimulus-response curves to be considered are responses to capsaicin. The threshold for detectable reflex neural responses was 1.0 μg of capsaicin (Figure 6). Splenic and renal nerves responded in a consistent pattern to increasing doses of capsaicin (Table 3); the splenic nerve responses tended to be greater than the renal responses. The renal response curve reached a plateau at the highest doses of capsaicin (Figure 6). Significant pressor responses were observed with capsaicin doses as low as 0.1 μg (Figure 6). Neural and pressor responses to increasing concentrations of capsaicin were not linear. Neural response curves increased rapidly from 1.0 to 10 μg, declined, and then again increased at doses of 100 μg and higher. The pressor response curve increased slowly from 0.1 to 1.0 μg, showed a more rapid rise from 1.0 to 20 μg, then a decline from 50 to 300 μg (Figure 6); the mean control MAP was 116 $\pm$ 3 mmHg. The tachycardia produced by capsaicin stimulation of splenic receptors also exhibited a dose-response relationship. The mean heart rate was 236 $\pm$ 2 bpm and the heart rate increases ranged from 1-9 bpm. The splenic venous pressure

Figure 6. Dose-response curves produced by **capsaicin.**

Mean changes in splenic and renal nerve activity (NA) and systemic arterial pressure (SAP) caused by stimulation of splenic receptors with increasing doses of capsaicin. Responses of splenic (closed circles) and renal (closed triangles) are expressed as % change and are calculated from data in Table 3. ΔSAP are mean changes from control (open squares). *, mean values significantly different from control ($P < 0.05$). Number of animals is shown in Table 3.

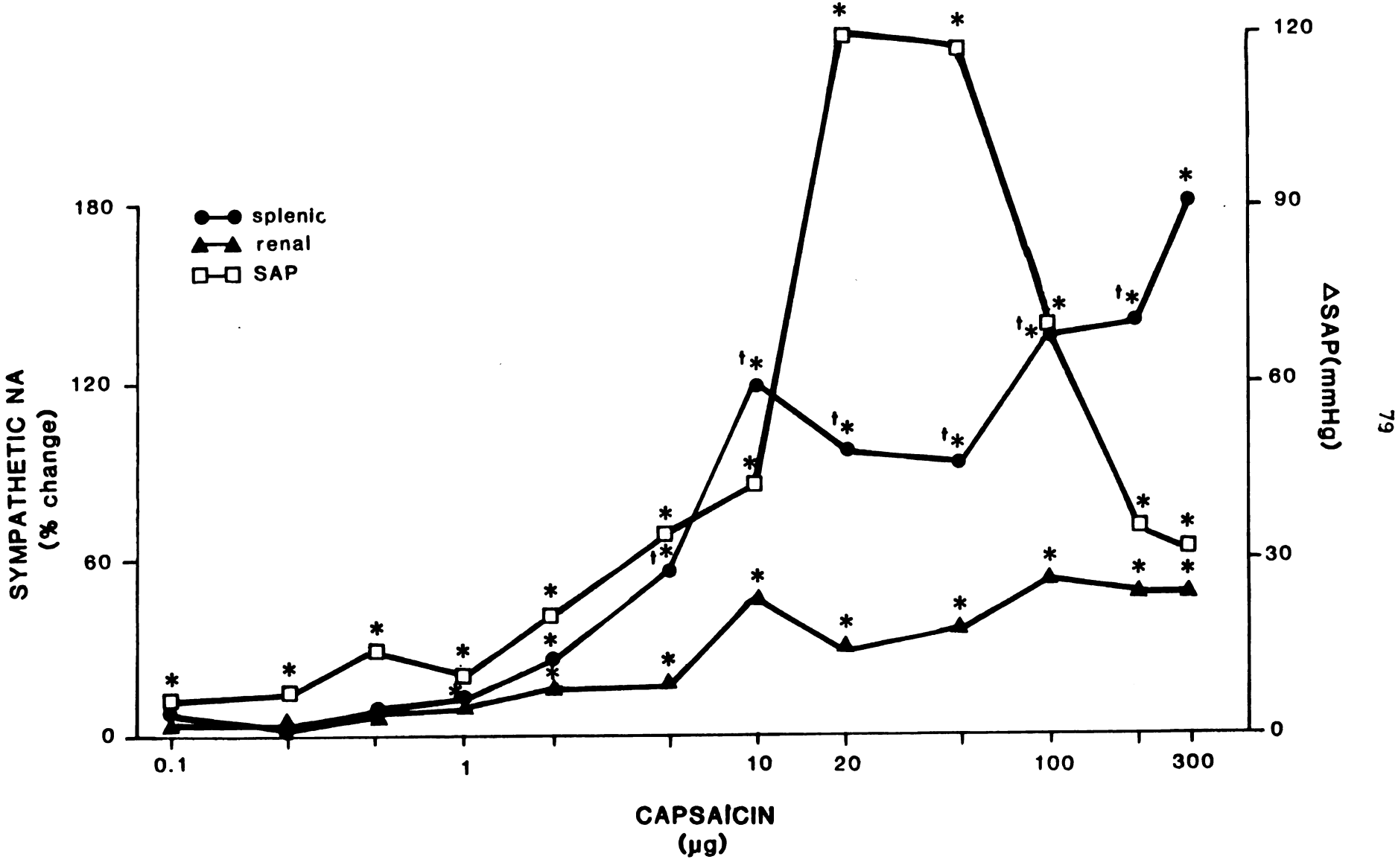
splenic
renal
SAP
SYMPATHETIC NA
(% change)
180
120
60
0
ΔSAP(mmHg)
120
90
60
30
0
0.1
1
10
20
100
300
CAPSAICIN
(μg)

Figure 6.

Table 3. Splenic and renal nerve responses to increasing doses of capsaicin.

	Splenic NA (μV•s/10s)					Renal NA (μV•s/10s)				
Dose of capsaicin	C	M	R	CV		C	M	R	CV	n
100 ng	33	36	33	0.08		124	129	125	0.03	6
250 ng	32	34	32	0.08		125	130	126	0.03	6
500 ng	34	39	34	0.15	NS	119	127*	122	0.03	7
1 μg	32	37	34	0.15	NS	141	151*	140	0.05	8
2 μg	33	41*	33	0.16	NS	143	159*	143	0.07	8
5 μg	30	43*	30	0.20	†	153	180*	160	0.10	10
10 μg	18	32*	18	0.27	†	117	162*	113	0.19	11
20 μg	17	31*	18	0.23	†	129	159*	126	0.15	9
50 μg	15	27*	17	0.29	†	131	169*	137	0.14	11
100 μg	20	39*	21	0.43	†	143	207*	140	0.25	11
200 μg	21	36*	22	0.26	†	153	216*	160	0.17	4
300 μg	10	27	11			174	249	192		3

These data are integrated voltage values statistically analyzed for the illustration in Figure 6. The statistical methods used to compare these values are described in Methods. Format is similar to that of Table 1. C, control; M, maximum; R, recovery; NS, nonsignificant change in splenic/renal ratio determined by Friedman test.

did not show a dose-response relationship to capsaicin. The splenic venous pressure consistently increased by 4-9 mmHg at each dose of capsaicin.

Increases in intrasplenic pressure to 15 and 25 mmHg produced no reflex neural effects. Splenic congestion to 40 mmHg produced reflex excitation of splenic and renal nerves and pressor responses (Figure 7, Table 4). Extreme congestion to 50 mmHg also produced splenic and renal nerve excitation and the splenic response was greater than the renal response. The more intense stimulation of splenic receptors by this congestion appeared to exaggerate the differences between the responses of the two nerves (Figure 7). The pressor responses tended to increase as the stimulation intensity increased. There were no significant heart rate changes with congestion of the spleen.

In summary, stimulation of splenic receptors by capsaicin produced dose-response relationships in which renal responses reached a plateau before splenic responses. Intense activation of splenic receptors by congestion exaggerated the unequal neural responses.

Spinal Sympathetic Reflexes Initiated From the Spleen

Do reflexes initiated by chemical and mechanical stimulation of splenic receptors have spinal components? Neural responses to splenic receptor stimulation by capsaicin were still present after high cervical spinal cord transection (Figure 8, Table 5). Stimulation of splenic receptors by bradykinin and congestion also produced spinal reflexes (Figures 9 and 10, Table 5). In Figure 8 the magnitude of

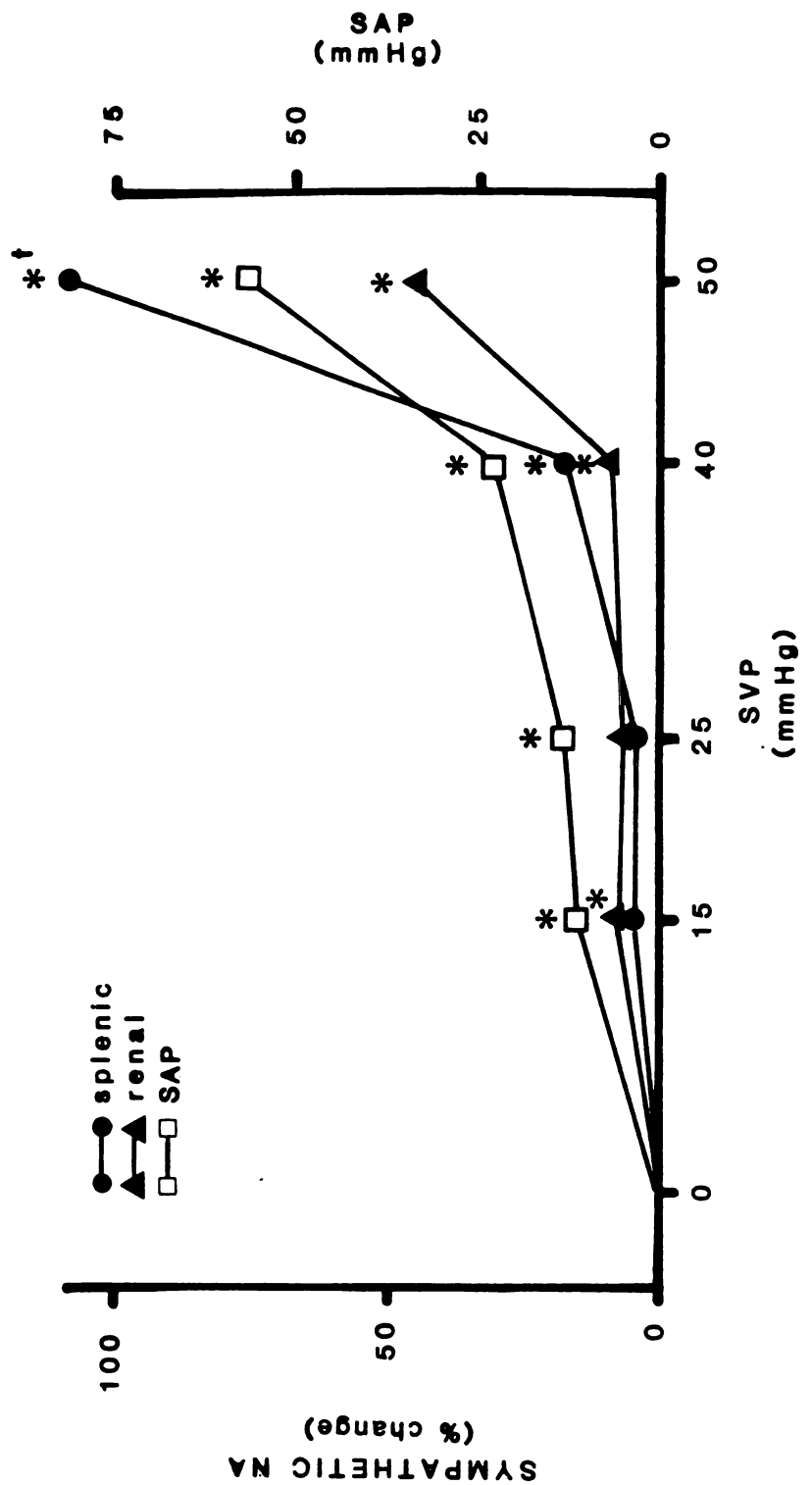

Figure 7. Dose-response curves produced by splenic congestion.

Mean changes in splenic and renal nerve activity and systemic arterial pressure caused by stimulation of splenic receptors with increases in splenic venous pressure (SVP). Percent changes are calculated from the data in Table 4. Format is similar to that of Figure 5.

Table 4. Responses to increases of intrasplenic pressure (congestion).

		Splenic NA (μV·s/10s)		Renal NA (μV·s/10s)	SAP (mm/Hg)	SVP (mmHg)
Congestion to 15 mmHg n = 6	Control	33		250	110	7
	Maximum	34	NS	273*	121	17*
	Recovery	30		248	110	7
	CV	0.07		0.05	0.04	0.22
Congestion to 25 mmHg n = 6	Control	33		255	110	7
	Maximum	34		267	124*	27*
	Recovery	33		156	119	8
	CV	0.09		0.67	0.07	0.11
Congestion to 40 mmHg n = 5	Control	35		159	110	7
	Maximum	41*	NS	171*	132*	42
	Recovery	34		156	112	8
	CV	0.07		0.03	0.09	0.07
Congestion to 50 mmHg n = 6	Control	31		94	95	7
	Maximum	50*	†	140*	152*	53*
	Recovery	39		96	112	8
	CV	0.22		0.23	0.17	0.27

These data are integrated voltage values statistically analyzed for the illustration in Figure 7. The format is similar to that of Table 1. NS, nonsignificant change in splenic/renal ratio determined by Friedman test; SVP, splenic venous pressure.

Figure 8. Sympathetic responses to **capsaicin** before and after spinal cord transection.

Mean responses to stimulation of splenic receptors with capsaicin before (closed symbols) and after (open symbols) spinal cord transection at C1. The axis indicates integrated nerve activity in μV•s/10s. The ordinate indicates activity of splenic (circles) and renal (triangles) nerves that was integrated in 10s intervals. C, control; M, maximum; R, recovery; n, number of animals.

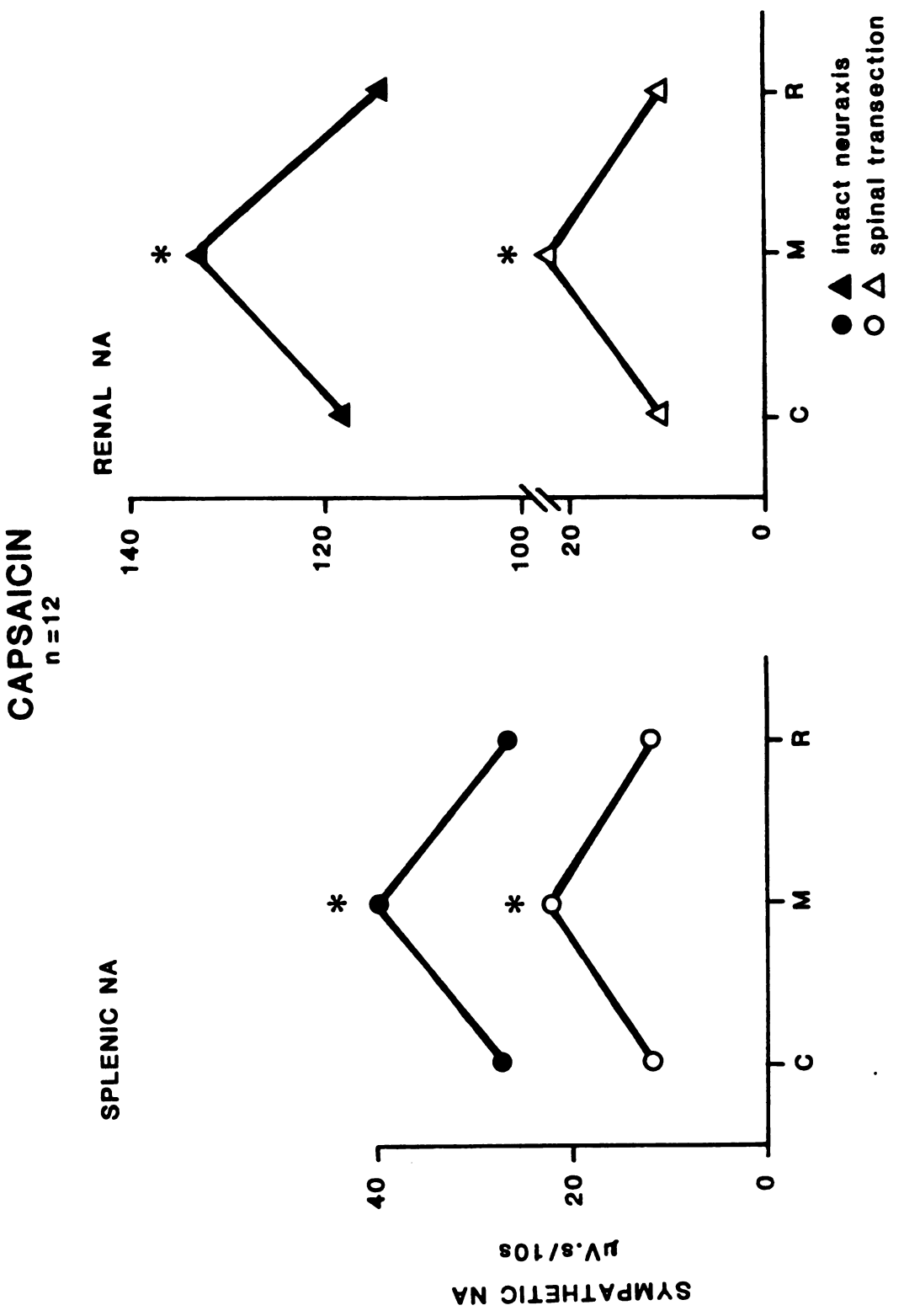
CAPSAICIN
n=12
SPLENIC NA
RENAL NA
SYMPATHETIC NA
μV.s/10s
40
20
0
140
120
100
20
0
C
M
R
intact neuraxis
spinal transection

Figure 8.

Table 5. Responses to stimulation of splenic receptors before and after spinal cord transection.

	NEURAXIS INTACT					SPINALIZED				
	Splenic NA		Renal NA	SAP	SVP	Splenic NA		Renal NA	SAP	SVP
Capsaicin										
Control	27		118	104	8	12		11	86	9
Maximum	40*	†	133*	131*	18	22	NS	23*	95*	19*
Recovery	27		114	100	7	11		11	87	9
CV	0.17		0.05	0.01	0.34	0.25		0.40	0.06	0.34
n = 12										
Bradykinin										
Control	27		115	104	10	10		11	83	10
Maximum	38*	†	128*	120*	21*	16*	NS	18*	89*	20*
Recovery	27		116	106	11	10		11	83	11
CV	0.19		0.07	0.08	0.36	0.33		0.36	0.04	0.30
n = 12										
Congestion										
Control	26		112	107	7	10		13	90	9
Maximum	33*	†	126*	120*	46*	14*	NS	17*	95	47*
Recovery	24		109	109	9	10		13	96	12
CV	0.18		0.14	0.10	0.16	0.15		0.17	0.09	0.25
n = 11										

These data are integrated voltage values statistically analyzed for the illustrations in Figures 8, 9 and 10. Format is similar to that of Table 1. NS, nonsignificant change in splenic/renal ratio determined by Friedman test,; SVP, splenic venous pressure in mmHg.

Figure 9. Sympathetic responses to **bradykinin** before and after spinal cord transection.

Mean responses to stimulation of splenic receptors with bradykinin before and after spinal cord transection. Format is identical to that of Figure 8.

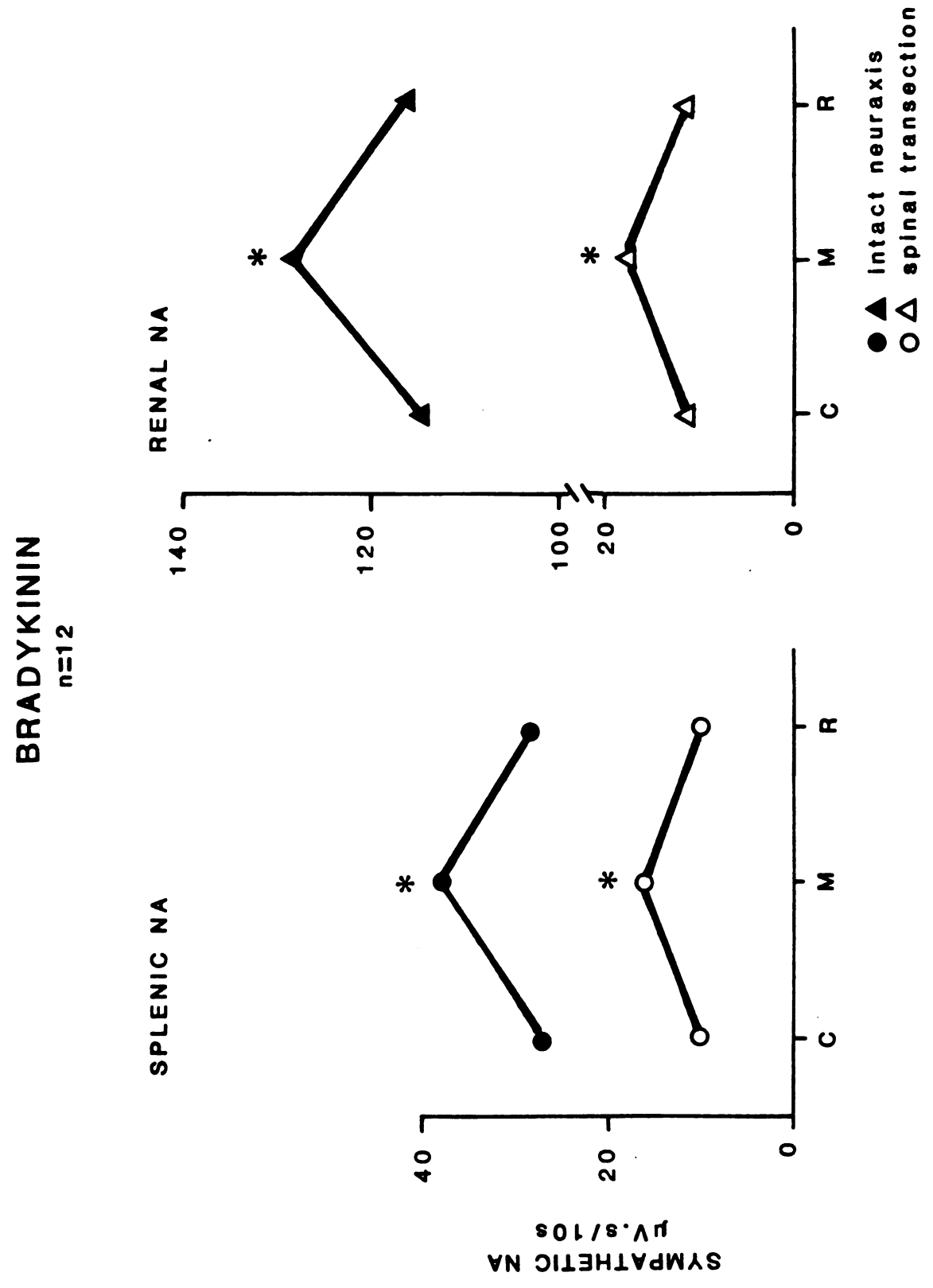
BRADYKININ
n=12
SPLENIC NA
RENAL NA
SYMPATHETIC NA
μV.s/10s
40
20
0
140
120
100
20
0
C
M
R
C
M
R
*
*
*
*
● ▲ Intact neuraxis
○ △ spinal transection

Figure 9.

Figure 10. Sympathetic responses to congestion before and after spinal cord transection.

Mean responses to stimulation of splenic receptors with congestion before and after spinal cord transection. Format is identical to that of Figure 8.

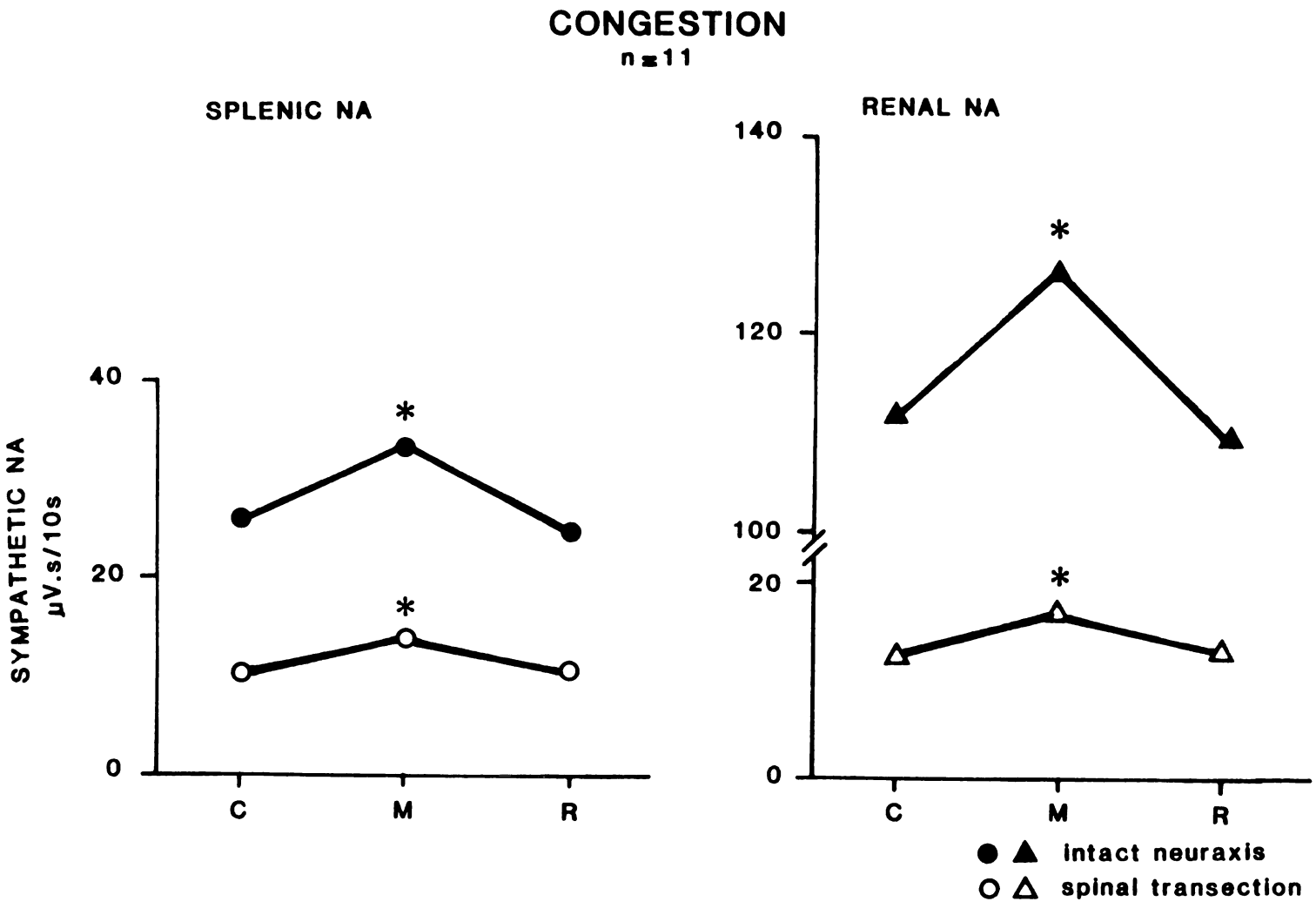
CONGESTION
n = 11
SPLENIC NA
RENAL NA
SYMPATHETIC NA
μV.s/10s
40
20
0
140
120
100
20
0
C
M
R
intact neuraxis
spinal transection

Figure 10.

the reflex responses of either splenic or renal nerves appeared to be similar before and after spinal cord transection. However, control activity of both sympathetic nerves decreased significantly after spinal cord transection and renal activity decreased more than splenic activity (Table 8). The absolute changes of nerve activity during reflex responses before and after cord transection were compared (Table 7a). Stimulation of splenic receptors with capsaicin caused equivalent changes in splenic nerve activity in animals with intact neuraxes and in spinal animals. Similar results were found with bradykinin and congestion (Table 7a). Stimulation of splenic receptors by capsaicin also caused equivalent changes in renal nerve activity in the intact and spinal states (Table 7a). Similar results were observed with bradykinin and congestion. Thus, activation of splenic receptors produced reflex changes in sympathetic nerve activity which were similar in the neuraxis intact and spinal states but the basal renal nerve activity exhibited a larger decrease than basal splenic after spinal cord transection.

Widespread visceral and somatic receptor stimulation by injections of capsaicin into the descending aorta produced greater splenic than renal nerve responses when the neuraxis was intact. After spinal cord transection this activation of visceral and somatic receptors caused a 161 $\pm$ 42% increase in splenic nerve activity and a 236 $\pm$ 62% increase in renal nerve activity.

When nerve activity is composed of large superimposed spikes, voltage integration method of quantifying nerve activity often provides

Table 6. Spike counted responses before and after spinal transection.

	NEURAXIS INTACT			SPINALIZED		
	Splenic NA (sp/s)		Renal NA (sp/s)	Splenic NA (sp/s)		Renal NA (sp/s)
Capsaicin						
Control	50		107	27		15
Maximum	89*	†	130*	67*	NS	47*
Recovery	49		98	25		17
CV	0.24		0.16	0.38		0.59
n = 12						
Bradykinin						
Control	47		94	19		15
Maximum	77*	†	116*	42*	NS	32*
Recovery	50		97	18		17
CV	0.20		0.11	0.49		0.36
n = 12						
Congestion						
Control	45		97	17		20
Maximum	59*	NS	107*	26*	NS	33*
Recovery	42		96	17		19
CV	0.26		0.08	0.24		0.32
n = 11						

These data are spike counted values statistically analyzed for the illustrations in Figure 11. Format is similar to that of Table 5. sp/s, spikes per second.

Table 7. Sympathetic responses before and after spinal transection expressed as absolute changes.

	CAPSAICIN n=12 (Δ voltage)	BRADYKININ n=12 (Δ voltage)	CONGESTION n=11 (Δ voltage)
a. Integrated voltages			
Splenic nerve responses:			
Intact neuraxis	13	11	7
Spinal	10	6	4
CV	0.63	0.83	1.02
Renal nerve responses:			
Intact neuraxis	15	14	15
Spinal	12	7	4
CV	0.67	1.18	1.79
	n=9 (Δ sp/s)	n=9 (Δ sp/s)	n=9 (Δ sp/s)
b. Spike counted			
Splenic nerve responses:			
Intact neuraxis	39	30	15
Spinal	31	23	10
CV	0.41	0.66	1.0
Renal nerve responses:			
Intact neuraxis	23	22	10
Spinal	31	17	13
CV	0.37	1.06	0.46

Δ voltage, change in integrated voltage value determined by subtracting control value from maximum value; Δ sp/s, change in spike counted value determined by subtracting control value from maximum value.
There was no significant difference of reflex changes in nerve activity between neuraxis intact and spinalized states.

Table 8. Comparisons of basal nerve activity before and after spinal cord transection.

	Neuraxis intact	Spinalized	CV
Basal Splenic NA (μV•s/10s) n=35	27	11*	0.47
Basal Renal NA (μV•s/10s) n=35	115	12*	1.07
Basal Splenic NA (sp/s) n=27	47	21*	0.72
Basal Renal NA (sp/s) n=27	99	17*	1.05

*, significant change from neuraxis intact; n, number of control periods for experimental procedures; sp/s, spikes per second; NA, nerve activity. Comparison of decreases of basal nerve activity with Friedman test determined that decrease of renal NA was greater than decrease of splenic NA.

a better estimate of neural activity. Superimposition of spikes especially during responses may result in underestimation of nerve activity using a spike counting method. However, when individual spikes of varying amplitude are present, the spike counting method provides a better estimate of activity. With the voltage integration method, responses of large amplitude spikes would be quantified as contributing more to the total response than the smaller spikes. The voltage of the renal nerve activity after spinal cord transection tended to be lower because there were fewer spikes, and so the neural data also were analyzed using the spike counting method. Stimulation of splenic receptors by capsaicin produced greater splenic than renal nerve responses in the animals with intact neuraxes (Table 6), and produced similar splenic and renal nerve responses in the spinal animals (Table 6). Essentially, the spike counting method revealed the same pattern of neural responses as that demonstrated by voltage integration (compare Figures 8 and 11).

The absolute changes in number of spikes (sp/s) during reflex responses to splenic receptor stimulation were compared also. Reflex changes in splenic nerve activity before and after spinal cord transection were equivalent (Table 7b). Reflex changes in renal nerve activity before and after spinal cord transection were equivalent also. Statistical comparisons showed that control activity of splenic and renal nerves both decreased significantly after spinal transection but renal activity decreased more than splenic (Table 8).

Figure 11. Spike counted nerve activity of responses in neuraxis-intact and spinal animals.

Mean responses to stimulation of splenic receptors with capsaicin before and after spinal cord transection. Format is similar to that of Figure 8. The axis indicates sympathetic nerve activity expressed in spikes per second (sp/s).

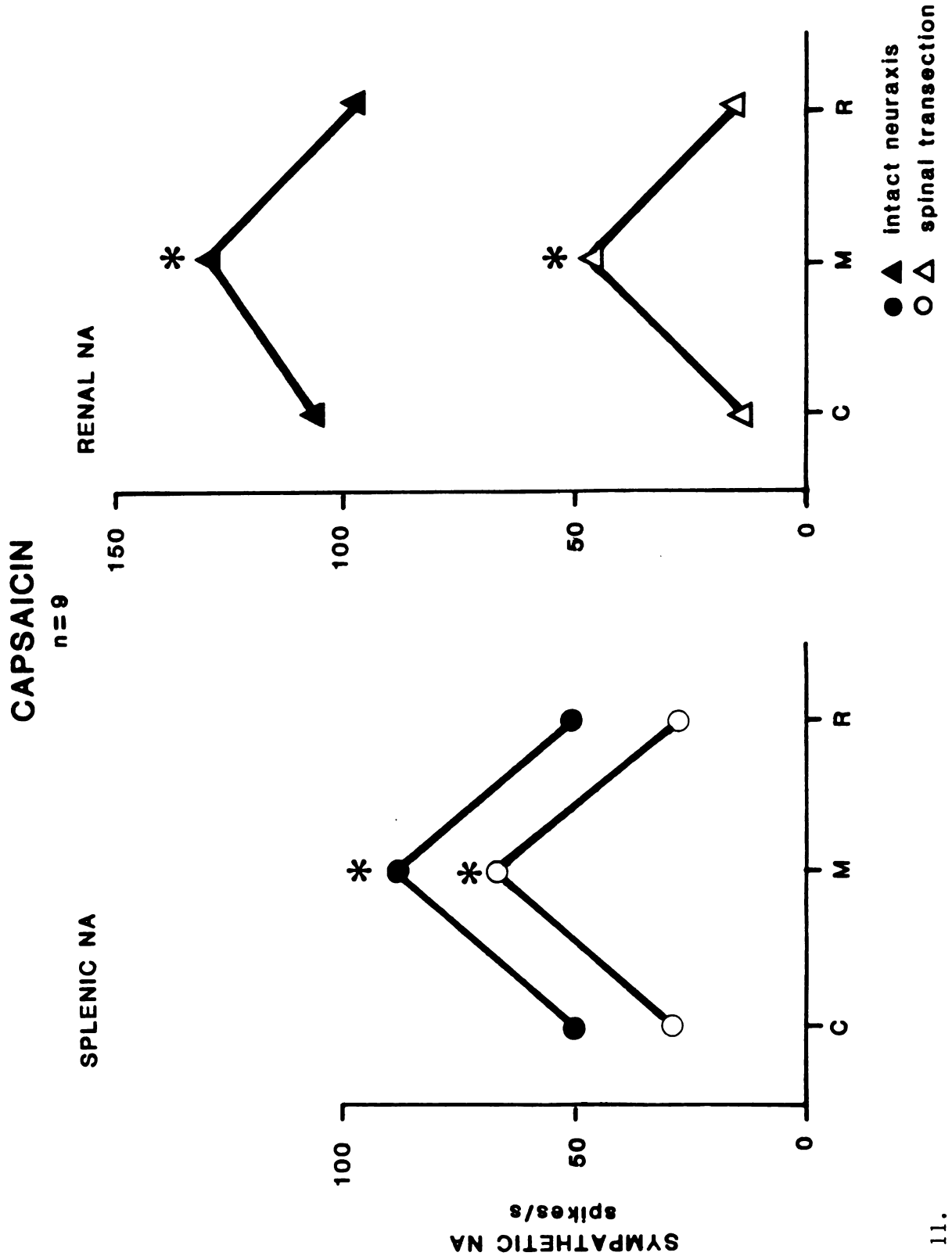
CAPSAICIN
n=9
SPLENIC NA
RENAL NA
SYMPATHETIC NA
spikes/s
100
50
0
150
100
50
0
C
M
R
C
M
R
intact neuraxis
spinal transection

Figure 11.

Figure 11. Spike counted nerve activity of responses in neuraxis-intact and spinal animals.

Mean responses to stimulation of splenic receptors with capsaicin before and after spinal cord transection. Format is similar to that of Figure 8. The axis indicates sympathetic nerve activity expressed in spikes per second (sp/s).

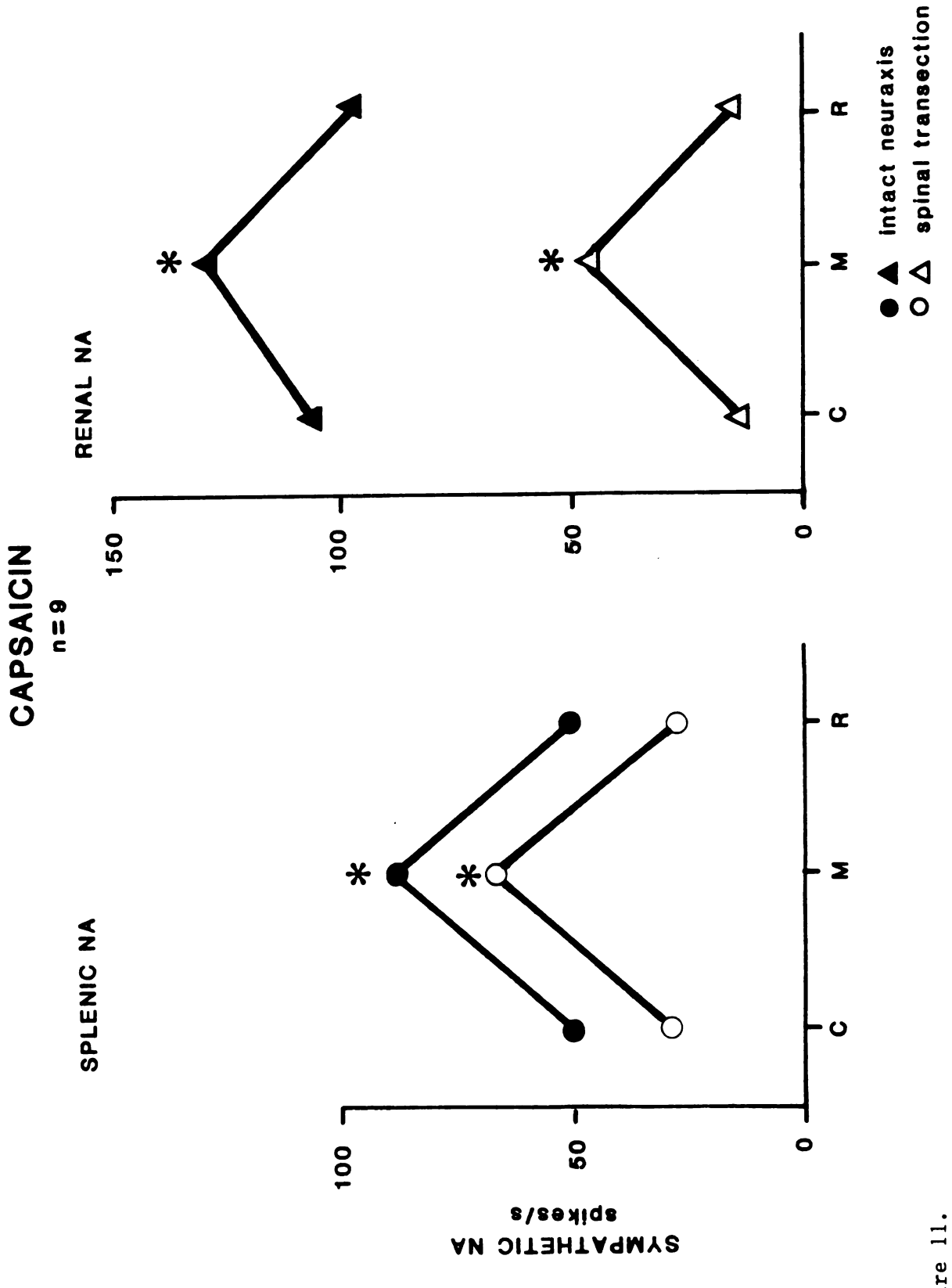
CAPSAICIN
n=9
SPLENIC NA
RENAL NA
SYMPATHETIC NA
spikes/s
100
50
0
150
100
50
0
C
M
R
intact neuraxis
spinal transection

Figure 11.

Neural responses are illustrated as percentage change in Figure 12, which shows that the neural responses to capsaicin and bradykinin tended to be unequal when the neuraxis was intact but were similar after spinal cord transection. A comparison of the reflex responses using ratios of neuraxis intact/spinalized for each nerve indicated that the splenic nerve responded similarly in both states while the renal nerve responded with a greater magnitude after spinal cord transection than in the intact state (Table 6).

Stimulation of the splenic receptors by capsaicin, bradykinin, or congestion produced significant pressor responses before spinal cord transection (Figure 12, Table 5). After spinal cord transection, pressor responses were still produced by capsaicin and bradykinin (Figure 12, Table 5). Splenic contractions and increases in splenic venous pressure accompanied splenic reflexes in the intact and spinal states (Figure 12, Table 5). Before spinal cord transection, there were heart rate increases in response to capsaicin (4 $\pm$ 1 bpm) and to bradykinin (3 $\pm$ 1 bpm) but not to congestion. After cord transection, there were no significant heart rate increases in response to stimulation of splenic receptors. The mean heart rates were 245 bpm before and 214 bpm after cord transection.

In summary, reflexes initiated from the spleen have a major spinal component, and the spinal reflex can be produced by mechanical or chemical stimulation of splenic receptors.

Figure 12. Mean responses to stimulation of splenic receptors before and after spinal cord transection.

Mean changes in sympathetic nerve activity, systemic arterial pressure and splenic venous pressure caused by stimulation of splenic receptors by capsaicin, bradykinin and congestion before and after high spinal cord transection. Percent changes are calculated from the data in Table 5. Splenic nerve responses are shown by bars with horizontal lines and renal nerve responses by bars with vertical lines. Format is similar to that of Figure 5. Numbers at bottom of NA bars are mean control values for the group.

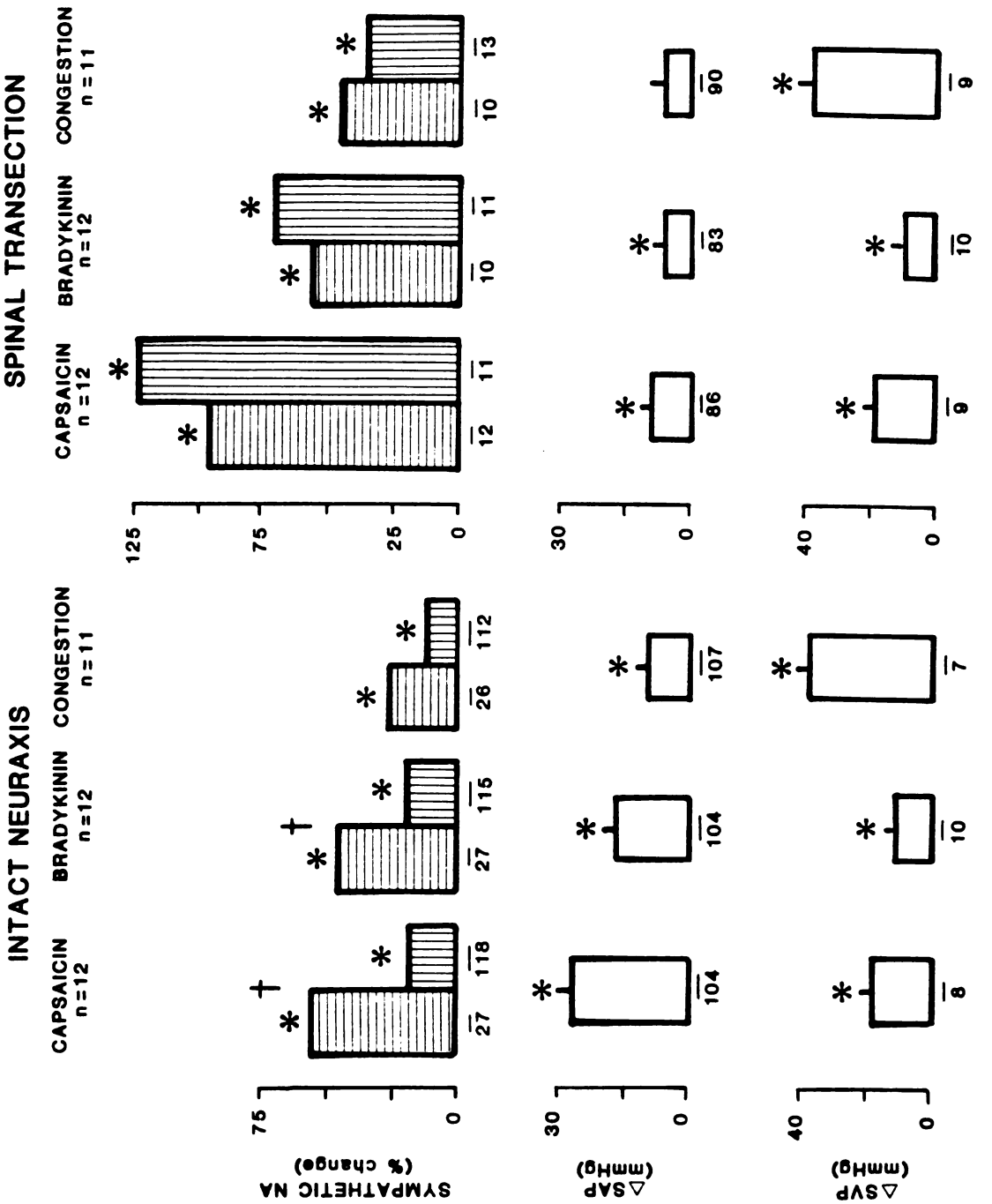
INTACT NEURAXIS
SPINAL TRANSECTION
CAPSAICIN n=12
BRADYKININ n=12
CONGESTION n=11
CAPSAICIN n=12
BRADYKININ n=12
CONGESTION n=11
SYMPATHETIC NA (% change)
ΔSAP (mmHg)
ΔSVP (mmHg)
75
0
30
0
40
0
125
75
25
0
30
0
40
0
27 118
27 115
26 112
12 11
10 11
10 13
104
104
107
86
83
80
8
10
7
9
10
9

Figure 12.

Afferent Nerve Responses to Stimulation of Splenic Receptors

The data presented previously indicate that chemical stimulation of splenic receptors more reliably produces reflex neural responses than does mechanical stimulation. It is possible that mechanical and chemical stimuli activate different populations of afferent neurons to produce the observed reflex responses. An alternative hypothesis is that chemical stimulation activates splenic afferent nerves more intensely than does mechanical stimulation. Forty-six splenic afferent fibers were tested. Of these, 8 were classified as multiunit and 37 were classified as single units. Afferent units were grouped into three broad categories according to their responses to mechanical and chemical stimuli: (a) mechanosensitive, (b) polymodal, and (c) chemosensitive/mechanosensitive which contributed to reflex responses to chemicals but did not contribute to reflex responses to congestion. Individual receptive fields were not located because the experimental set-up was such that manipulation of the spleen pulled the nerve fiber away from the electrode (thus interfering with the recording).

Multiunit Bundles

One of the multiunit bundles responded only to congestion. The discharge rate increased from 0.9 sp/s to 97.2 sp/s. The threshold for response to congestion was 10 mmHg, and the increased discharge continued for 70 s until the splenic venous pressure decreased below threshold. In this bundle, previously silent units were activated by

the increase in splenic venous pressure. The recruited units did not respond to capsaicin or bradykinin.

Six of the multiunit bundles responded to both mechanical and chemical stimulation of the splenic receptors. In response to congestion, the fibers increased their firing rates from 4.2 ± 2.0 to 12.4 ± 2.7 sp/s. The threshold pressure for activation ranged from 12 to 34 mmHg and the duration of increased discharge was 20-75 s (43 ± 11 s). Approximately half of the fibers had adapting discharge rates while the others maintained an increase in firing rate as long as the splenic venous pressure was above threshold. Two of the bundles also were tested with norepinephrine. Their firing rate increased from 1.3 ± 1.2 to 4.1 ± 2.3 sp/s and the increase lasted 5 s. The threshold was approximately 15 mmHg. Activation of the receptors by capsaicin increased the discharge rate from 4.8 ± 1.7 to 11.0 ± 2.6 sp/s. The increased discharge lasted for 5-50 s (19 ± 7 s). Two of the fibers also were tested with bradykinin. The discharge rate increased from 3.4 ± 2.0 to 11.3 ± 4.7 sp/s and lasted for 5-10 s.

One multiunit bundle responded to chemical and mechanical stimulation of receptors but likely did not contribute to the reflex responses to congestion because the afferent response to mechanical stimulation occurred well after efferent reflex responses to congestion. In response to capsaicin, the bundle increased its firing rate from 0.2 to 1.4 sp/s. Bradykinin caused the bundle to increase its discharge rate from 11.1 to 27.6 sp/s. Injection of norepinephrine

into the spleen to cause a splenic contraction increased the firing rate from 5.8 to 10.0 sp/s.

Single Units

Mechanosensitive

Thirteen units were tested which were categorized as mechanosensitive (Table 9). Of these, five were A-delta fibers and eight were C fibers. The range of conduction velocity for the A-delta fibers was 7-10 m/s and for the C fibers was 0.6-1.0 m/s. The mean response of the afferent units to congestion was an increase in discharge rate from 1.0 $\pm$ 0.3 to 4.0 $\pm$ 0.5 sp/s. The threshold pressure ranged from 6 to 35 mmHg and the maximum pressure achieved during congestion ranged from 28 to 49 mmHg. The stimulus duration was 25-125 s and the duration of response was 5-100 s (average 36 $\pm$ 9 s). Three types of afferent responses to congestion were found: quickly adapting units which increased their discharge rates for one 5-s interval and returned to control values; adapting units which returned to control discharge rates within 10-30 s in spite of maintained splenic venous pressure above threshold; nonadapting units which maintained increased discharge rates as long as the splenic venous pressure was above threshold. One unit was classified as quickly adapting and was an A-delta fiber. Seven units were adapting; three were A-delta and four were C fibers. Of the five units which were nonadapting, one was an A-delta fiber and four were C fibers.

Norepinephrine was injected into the splenic vasculature to examine the effects of splenic contraction on 5 of the 13 units. This

Table 9. Responses of splenic afferent units to congestion, norepinephrine, capsaicin and bradykinin.

	Control (sp/s)	Maximum (sp/s)	Threshold (mmHg)	Duration (sec)	Efferent response (% change)	n
Mechanosensitive						
Congestion	1.0 ± 0.3	4.0 ± 0.5	6-35	36 ± 9	54%	13
Norepinephrine	1.1 ± 0.6	2.5 ± 1.0	18-37	6 ± 1	18%	4
Polymodal						
Congestion	3.6 ± 1.1	16.1 ± 3.1	7-43	61 ± 7	27%	19
Norepinephrine	3.4 ± 1.0	7.0 ± 1.5	9-46	39 ± 4	15%	16
Capsaicin, 1 µg	2.6 ± 0.7	9.1 ± 1.9	----	22 ± 4	25%	20
2 µg	4.0 ± 1.2	13.6 ± 2.7	----	14 ± 2	45%	19
Bradykinin	5.0 ± 1.3	11.9 ± 2.5	----	33 ± 6	32%	16
Chemosensitive/ Mechanosensitive						
Capsaicin, 1 µg	4.4 ± 1.2	10.1 ± 1.7	----	15 ± 3	25%	3
2 µg	2.0 ± 0.8	6.7 ± 2.0	----	15 ± 3	38%	3
Bradykinin	1.4 ± 0.7	3.8 ± 1.1	----	7 ± 2	20%	3
Norepinephrine	1.8 ± 1.1	5.3 ± 1.7	7-40	8 ± 2	35%	3

Dosages used are listed in Methods. Control and Maximum are the mean discharge rates of the afferent units ± standard error of mean. Threshold is the splenic venous pressure at which the units increased their discharge rates. Duration is the length of time of increased afferent discharge. Efferent response is the increased activity of the splenic efferent nerve during the protocol. sp/s, spikes per second; sec, seconds; n, number of afferent units.

was done to determine if the response of the mechanosensitive units to congestion was due to stretch of the vasculature and spleen or due to increased intrasplenic pressure. Responses were obtained in four of the five units tested. The discharge rate increased from 1.1 ± 0.6 to 2.5 ± 1.0 sp/s. The threshold pressure was 18-37 mmHg and the maximum pressure obtained during contraction was 22-37 mmHg. The units responded for 5-10 s and the contraction duration was 20-60 s. The units responding to contraction reached maximum discharge rate during the increase in intrasplenic pressure or at peak pressure. The afferent response to norepinephrine was a 141% increase in discharge rate compared to a 479% increase of the same four units to congestion.

Efferent splenic nerve activity was monitored during the protocols. The efferent nerve activity increased 54% in response to congestion. In response to norepinephrine, the efferent activity increased 18%.

These mechanosensitive afferent units were not part of the population initiating reflex responses to chemicals because their responses to capsaicin or bradykinin consisted of no increases or very small increases in firing rate 30-60 s after prominent reflex responses were observed. These late afferent responses may have been produced by contraction of the spleen.

The responses of the mechanosensitive units were divided into A-delta and C fiber groups to determine if the two fiber types responded differently to the stimuli. In response to congestion, the discharge of A-delta fibers increased from 1.3 ± 0.8 to 4.8 ± 0.9 sp/s

and the discharge rate of the C fibers increased from 0.8 ± 0.3 to 3.5 ± 0.5 sp/s; therefore, responses of A-delta fibers to congestion were equivalent to those of C fibers (Table 10). All four of the units tested with norepinephrine were C fibers.

Polymodal

Twenty-one units were classified as polymodal, indicating that the units responded to mechanical and chemical stimuli and potentially contributed to reflex responses (Table 9). These units consisted of 7 A-delta and 14 C fibers. The range of conduction velocity for the A-delta fibers was 6-14 m/s and for the C fibers was 0.7-2.0 m/s. The afferent units responded to congestion with an increase in discharge rate from 3.6 ± 1.1 to 16.1 ± 3.1 sp/s. Threshold pressure ranged from 7 to 43 mmHg and maximum pressure obtained was 27-46 mmHg. The stimulus duration was 20-100 s and the responses lasted 10 to 100 s. The response of one unit to congestion is illustrated in Figure 13. The control discharge rate was 2.1 sp/s and increased to 12.4 sp/s during congestion.

Responses of six of the units were classified as adapting and all were C fibers. Thirteen of the units were nonadapting; six were A-delta and seven were C fibers. Two of the 21 units were not included in this description because recruitment of silent units during congestion obscured the responses of the two, previously discriminable, units. The efferent splenic nerve activity increased by an average of 27% in response to congestion.

Table 10. Comparison of responses of A-delta and C afferent splenic fibers.

	Aδ:	Control (sp/s)	Maximum (sp/s)	C:	Control (sp/s)	Maximum (sp/s)
Mechanosensitive						
Congestion	(n = 5)	1.3 ± 0.8	4.8 ± 0.9	(n = 8)	0.8 ± 0.3	3.5 ± 0.5
Polymodal						
Congestion	(n = 6)	3.3 ± 0.8	24.4 ± 6.0	(n = 13)	3.7 ± 1.6	12.2 ± 3.1
Capsaicin 1 μg	(n = 7)	2.7 ± 0.8	11.7 ± 2.2	(n = 13)	2.6 ± 1.1	7.7 ± 2.7
2 μg	(n = 7)	3.5 ± 1.3	16.7 ± 4.7	(n = 12)	4.2 ± 1.7	11.8 ± 3.3
Bradykinin	(n = 7)	4.6 ± 1.8	13.0 ± 3.7	(n = 9)	5.3 ± 2.0	10.2 ± 3.0

Format is similar to that of Table 8.

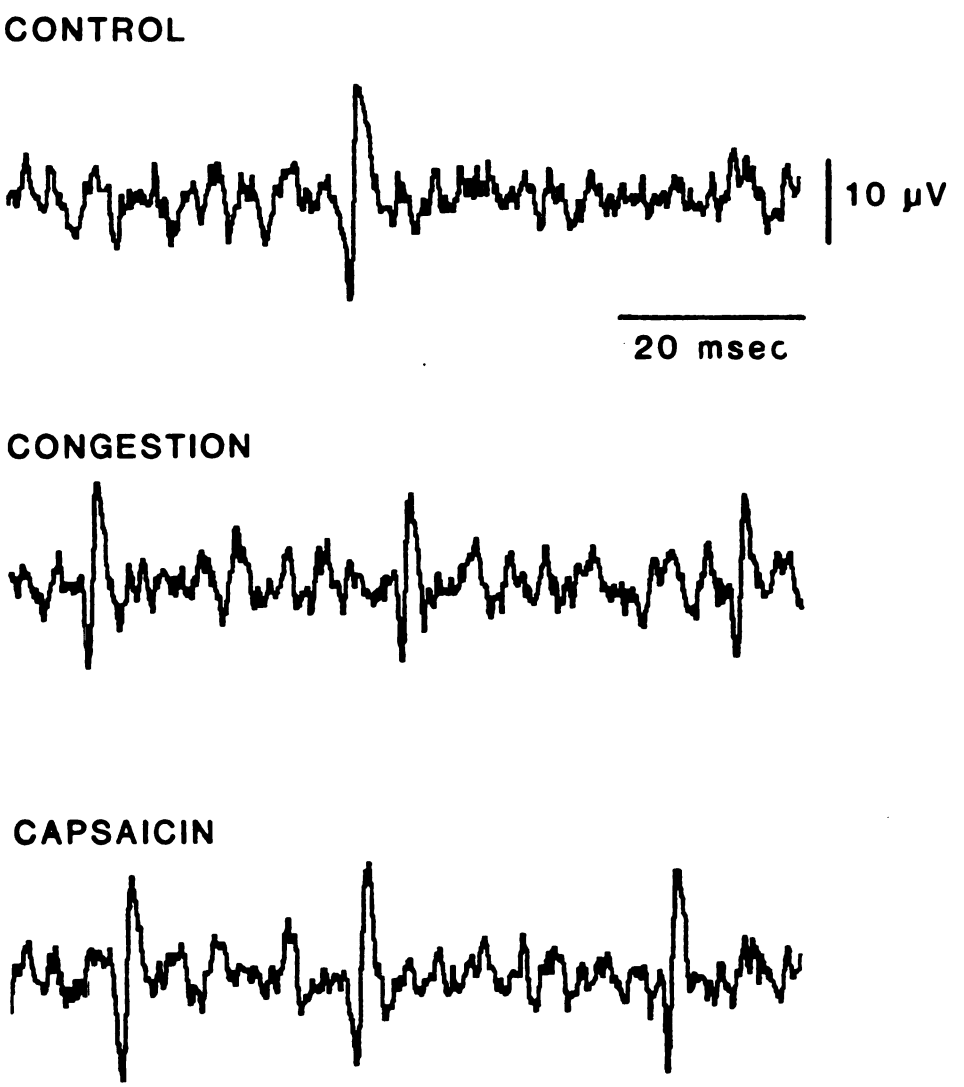

Figure 13. Responses of single splenic afferent unit to congestion and capsaicin.

The top panel illustrates a sample of control activity of the splenic afferent unit. The middle panel illustrates the activity of the unit during response to congestion. The bottom panel illustrates the response to capsaicin. The vertical bar represents 10 μV and the horizontal bar represents 20 msec.

The time course of the increase in splenic venous pressure and the afferent response is illustrated in Figure 14. After the splenic vein pressure increased above 15 mmHg the discharge rate of the unit gradually increased, reached its peak rate, then returned toward control values although the splenic venous pressure remained above threshold. In this example the efferent nerve response to congestion was barely discernible.

The effects of splenic contraction were examined in 18 of the 21 units. Fifteen of the 18 units responded to contraction which was produced by intrasplenic injection of norepinephrine. Their discharge rate increased from 3.4 $\pm$ 1.0 to 7.0 $\pm$ 1.5 sp/s. The threshold pressure was 9 to 46 mmHg and the maximum pressure reached ranged from 14 to 46 mmHg. The duration of the response lasted 5-55 s and the stimulus duration was 10-55 s. The maximum discharge rates occurred during the increase in intrasplenic pressure (n=8) or at peak pressure (n=7). In nine units, the peak responses to norepinephrine were an increase of 185% compared to an increase of 1089% in response to congestion. The peak responses were similar in six units, 240% increase for norepinephrine and 292% increase for congestion. Stimulation by norepinephrine caused 15% (average) increases in efferent splenic nerve activity.

The response of one unit to capsaicin is illustrated in Figure 13. The control discharge rate increased from 1.8 to 7.6 sp/s when 2.0 µg of capsaicin was injected into the spleen. In most of the protocols responses to 1.0 µg and 2.0 µg of capsaicin were tested. The mean

response to 1.0 μg was an increase from 2.6 ± 0.7 to 9.1 ± 1.9 sp/s. The mean response to 2.0 μg was an increase from 4.0 ± 1.2 to 13.6 ± 2.6 sp/s. The duration of increased discharge was 5-65 s. Dose-response relationships were observed in 13 of the 21 units, similar afferent responses to both doses of capsaicin were found in five units, and only one dose was tested in three units. The time course of an afferent response, efferent nerve response, splenic vein pressure, and systemic arterial pressure is illustrated in Figure 15. This is the same afferent unit that was shown in Figure 14. After 2.0 μg of capsaicin was injected, afferent discharge increased rapidly, a prominent efferent nerve response occurred, splenic venous pressure increased slightly, and a pressor response occurred. The afferent responses of the same unit to congestion and capsaicin are compared in Figure 16 by expanding the time scale. As the splenic venous pressure was increased by congestion, the afferent unit gradually increased its firing rate until the maximum was reached. In contrast, capsaicin caused an immediate burst of afferent activity. The mean efferent response to 1.0 μg of capsaicin was an increase of 25% in splenic efferent nerve activity and the response to 2.0 μg of capsaicin was an increase of 45%.

Afferent responses to bradykinin were tested in 18 of the 21 units, and 16 of the 18 responded to bradykinin. The control discharge rate of 5.0 ± 1.3 increased to 11.9 ± 2.5 sp/s. The duration of increased afferent discharge was 5-80 s. The two units which did not

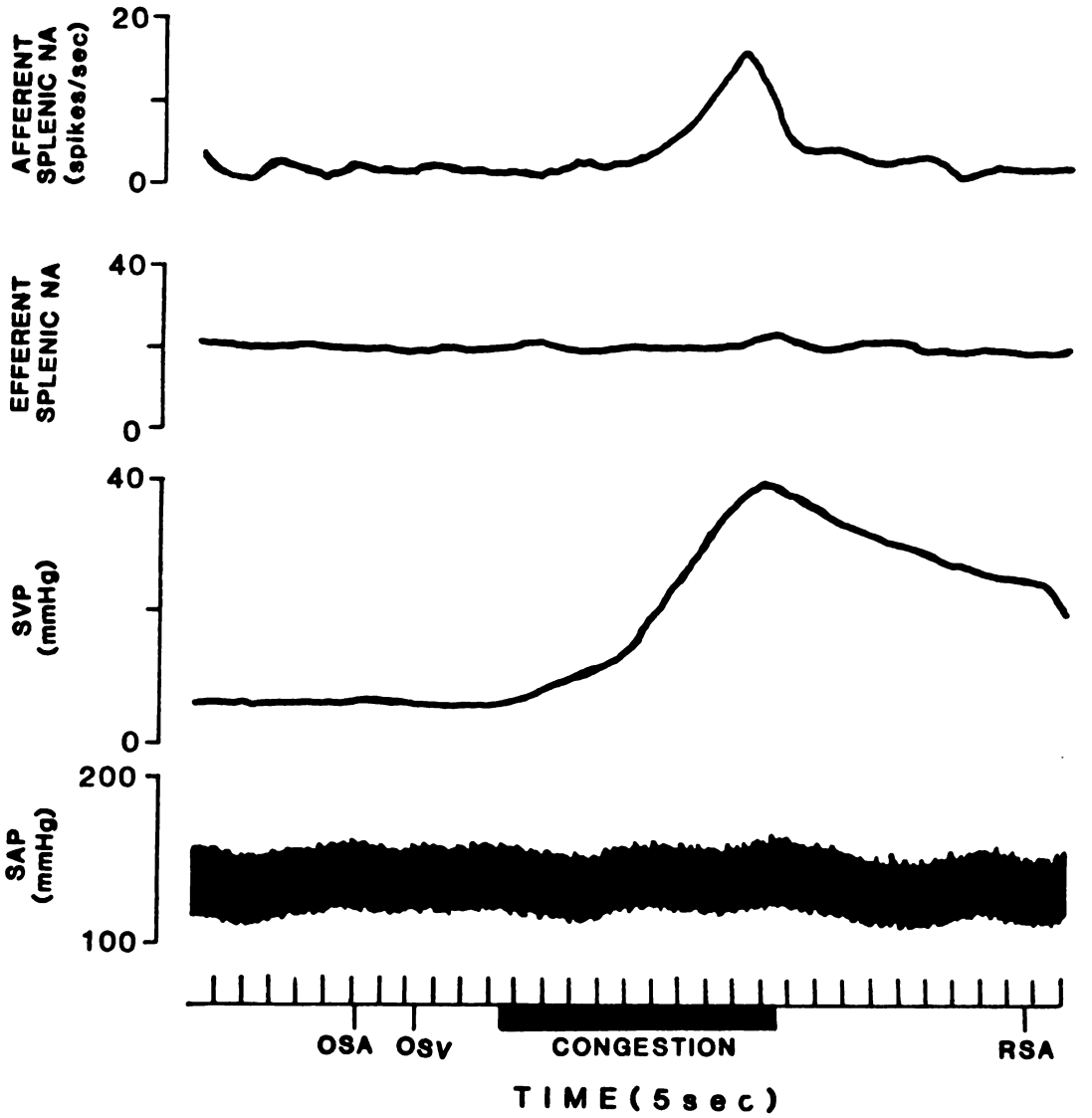

Figure 14. Response of splenic afferent unit to congestion.

Time course of responses of single splenic afferent unit, efferent splenic multifiber nerve activity, and systemic arterial pressure to increases in splenic venous pressure. Afferent splenic nerve activity is expressed as spikes per second and is activity averaged during 5 second intervals. Efferent splenic nerve activity is expressed as arbitrary units of voltage integration. Format is similar to that of Figure 3. The darkened bar under the time-base indicates infusion of saline into the spleen.

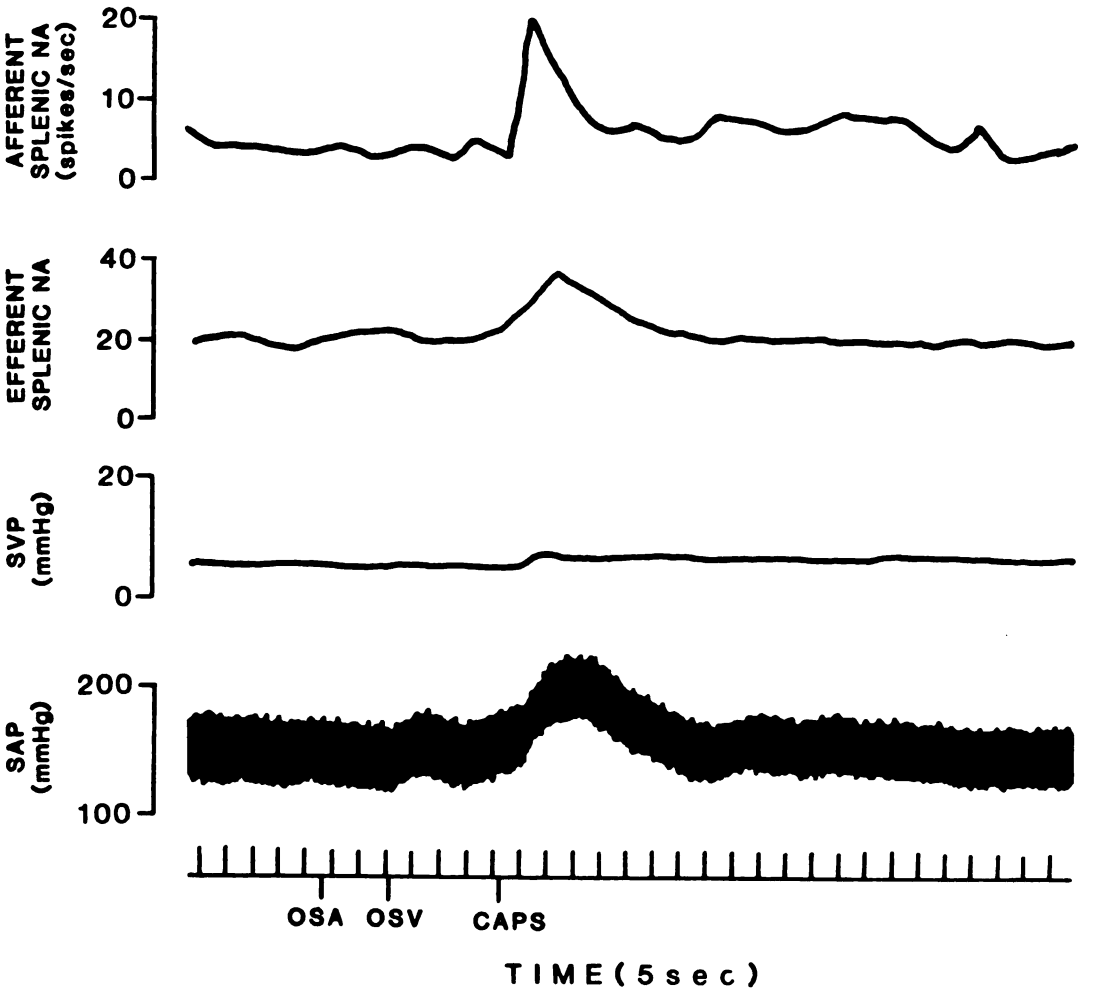

Figure 15. Response to splenic afferent unit to capsaicin.

Time courses of responses of single splenic afferent unit, efferent splenic multifiber nerve activity, and systemic arterial pressure to injection of 2 μg of capsaicin into the splenic artery. Format is similar to that of Figure 14. CAPS, injection of capsaicin.

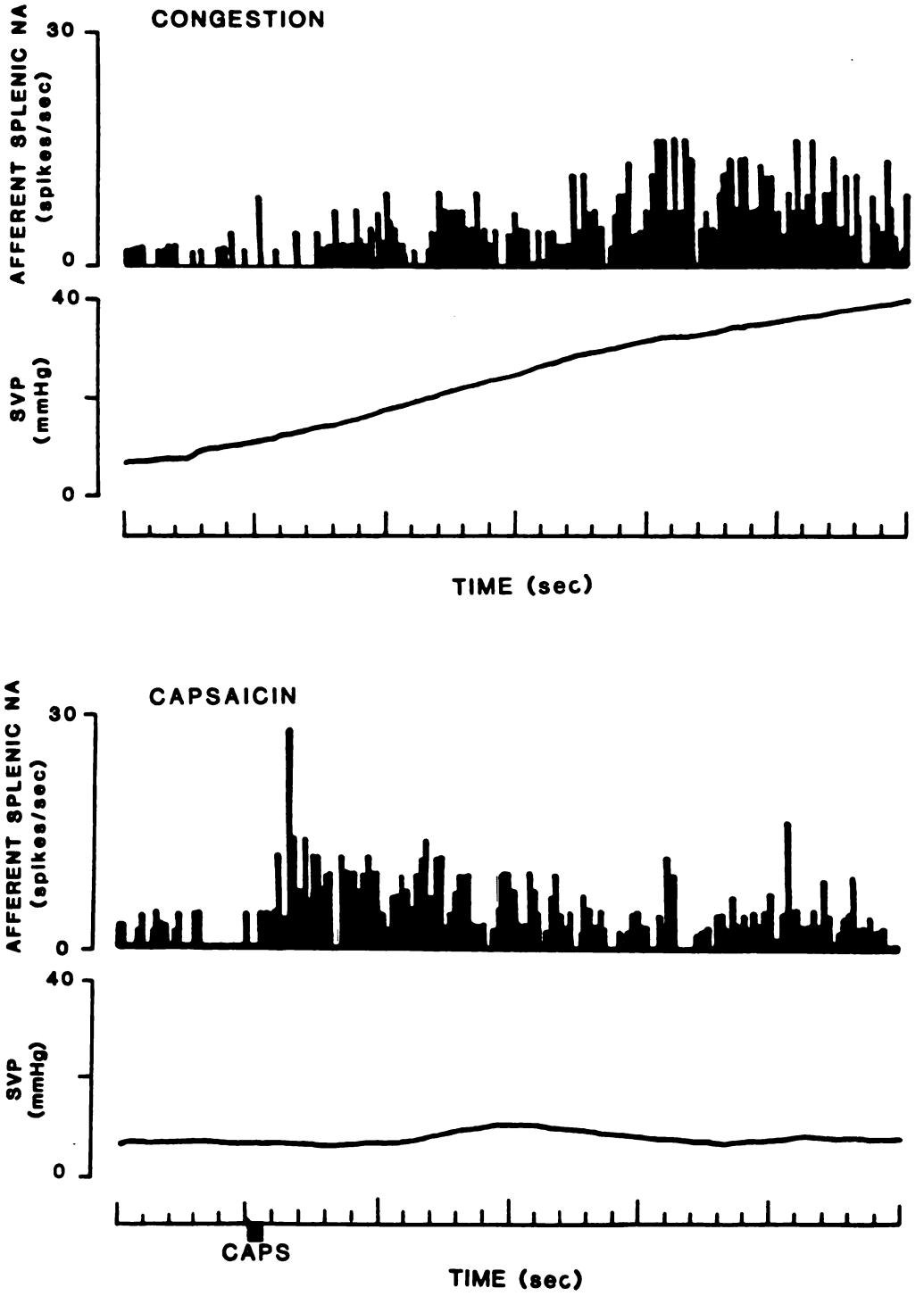

Figure 16. Responses of splenic afferent unit to congestion and capsaicin.

The time-base is expanded to illustrate responses of splenic afferent unit (shown in Figures 14 and 15) in spikes per second averaged during 0.5 second intervals. Format is similar to that of Figures 14 and 15.

respond to bradykinin were both C fibers. The efferent splenic nerve activity increased by 32% in response to bradykinin.

The units were grouped into A-delta and C fiber groups to determine if the responses of the two fiber types differed. In response to congestion, the A-delta group increased their discharge from 3.3 ± 0.8 to 24.4 ± 6.0 sp/s and the C group increased their discharge rate from 3.7 ± 1.6 to 12.2 ± 3.1 sp/s. The response of the A-delta group to 1.0 μg of capsaicin was an increase from 2.6 ± 0.8 to 11.7 ± 2.2 sp/s and the response of the C group was an increase from 2.6 ± 1.1 to 7.7 ± 2.7 sp/s. Capsaicin (2.0 μg) caused the discharge rate of the A-delta group to increase from 3.5 ± 1.3 to 16.7 ± 4.7 sp/s and the C group to increase from 4.2 ± 1.7 to 11.8 ± 3.3 sp/s. Bradykinin increased the discharge rate of the A-delta fibers from 4.6 ± 1.8 to 13.0 ± 3.7 sp/s and the C fibers from 5.3 ± 2.0 to 10.2 ± 3.0 sp/s. Therefore, the responses of A-delta fibers to congestion were greater than those of C fibers, and the responses of A-delta fibers to chemicals were equivalent to those of C fibers (Table 10).

Chemosensitive/Mechanosensitive

Three units were found which responded to chemical and mechanical stimulation of splenic receptors (Table 9). These units contributed to reflex responses to chemicals but did not contribute to reflex responses to congestion because the afferent response to mechanical stimulation occurred well after reflex responses to congestion. All three were C fibers. The conduction velocity of the C fibers was

0.8-1.0 m/s. Capsaicin (1.0 μg) caused the units to increase their firing rate from 4.4 ± 1.2 to 10.1 ± 1.7 sp/s for 10-20 s. The mean response to 2.0 μg of capsaicin was an increase from 2.0 ± 0.8 to 6.7 ± 2.0 sp/s. A dose-response relationship was found for two of the units, and the third exhibited similar responses to both doses of capsaicin. The efferent nerve responses were 25% and 38% increases in splenic nerve activity for 1.0 and 2.0 μg of capsaicin, respectively. All three units responded to bradykinin. The discharge rate increased from 1.4 ± 0.7 to 3.8 ± 1.1 sp/s for 5-10 s. Efferent splenic activity in response to bradykinin increased 20%.

The units responded to norepinephrine-induced contraction by increasing their firing rate from 1.8 ± 1.1 to 5.3 ± 1.7 sp/s. The threshold pressure for activation was 7-40 mmHg and maximum pressure achieved by contraction was 40-41 mmHg. The responses lasted for 5-10 s. The afferent responses to contraction could account for any increased discharge observed during congestion. The average efferent splenic nerve response was 35% increase in nerve activity.

In summary, reflex responses to congestion appeared to be mediated by two groups of afferent nerves, the mechanosensitive and polymodal groups, and reflex responses to capsaicin and bradykinin appeared to be mediated by two groups, the polymodal and primarily chemosensitive groups. Both A-delta and C fibers contribute to reflex responses to capsaicin, bradykinin, and congestion.

Influence of Cardiovascular Pressoreceptors on Reflex Sympathetic Responses

Initiation of reflexes from the spleen produces pressor responses, and these pressor responses presumably activate cardiovascular pressoreceptors. The resultant interaction of visceral (splenic) and pressoreceptor reflexes could affect splenic and renal sympathetic activity differently. The purpose of these experiments was to examine the influence of vascular pressoreceptors on reflexes initiated from the spleen and to examine the specific contributions of sino-aortic and vagally innervated pressoreceptors to reflex splenic and renal responses.

In eight animals with intact pressoreceptors, stimulation of splenic receptors by capsaicin consistently caused excitation of splenic nerve activity. However, responses of renal nerve activity were variable; inhibition of renal nerve activity occurred in two cats, excitation occurred in three cats, and a multiphasic pattern occurred in three cats. The neural responses lasted 10-60 s. Responses of a cat are illustrated in Figure 17. Splenic nerve activity increased, whereas renal nerve activity decreased. The peak inhibition of renal nerve activity occurred during the second component of the pressor response. Responses of another animal are illustrated in Figure 18. In this animal, excitation of splenic nerve activity occurred while a multiphasic pattern of renal nerve activity occurred; renal nerve activity was increased, then inhibited during the concomitant pressor response, and then increased again. Mean responses to this stimulation are shown in Figure 19 and Table 11.

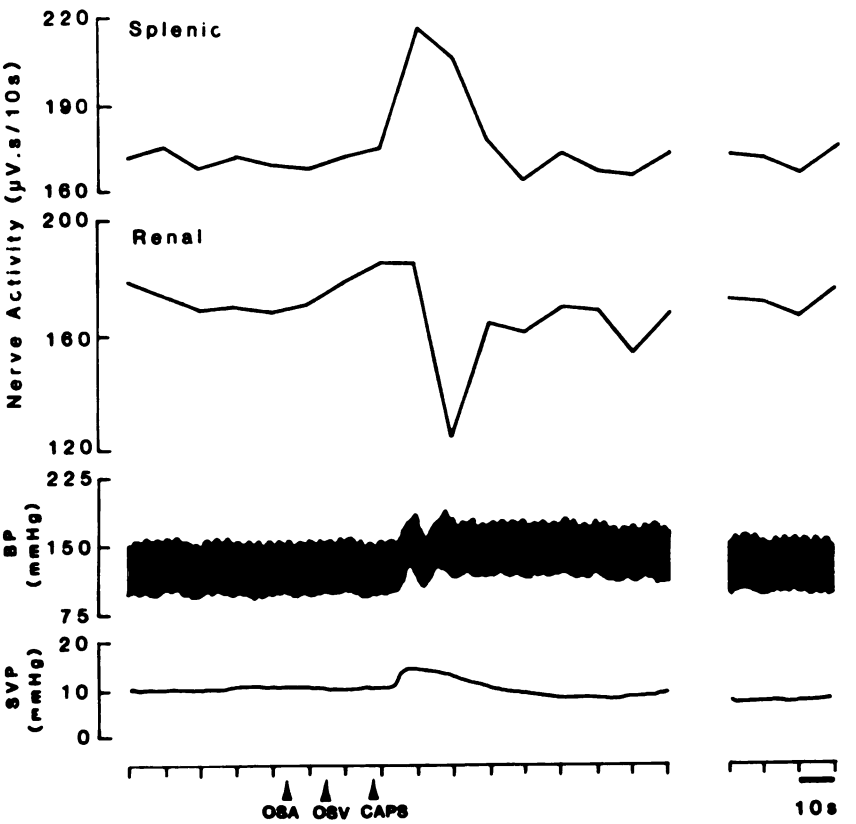

Figure 17. Responses of animal with intact pressoreceptors to intrasplenic capsaicin.

Effects of injection of 5 μg of capsaicin into splenic artery of a cat with intact pressoreceptors on integrated splenic and renal nerve activity, blood pressure, and splenic venous pressure. The format is similar to that of Figure 3. Recovery is shown at far right.

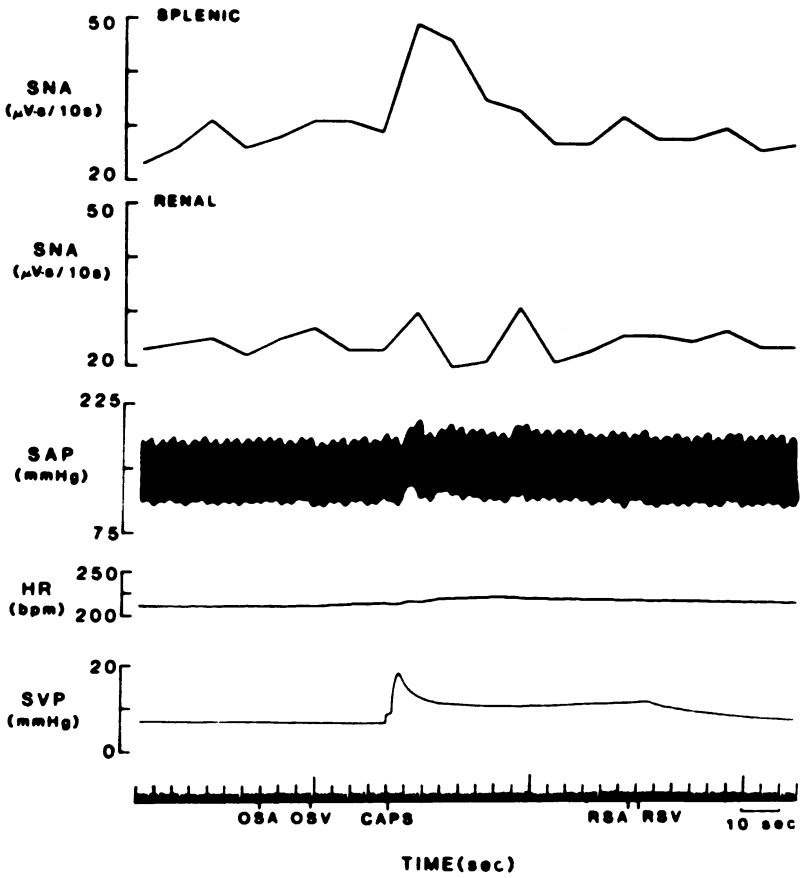

Figure 18. Responses of another animal with intact pressoreceptors to intrasplenic capsaicin.

Effects of injection of 5 μg of capsaicin into splenic artery of a cat with intact pressoreceptors on integrated splenic and renal sympathetic nerve activity (SNA), systemic arterial pressure (SAP), heart rate (HR), and splenic venous pressure (SVP). The time-base marks indicate occlusion of splenic artery (OSA) and vein (OSV), injection of capsaicin (CAPS) and release of splenic artery (RSA) and vein (RSV).

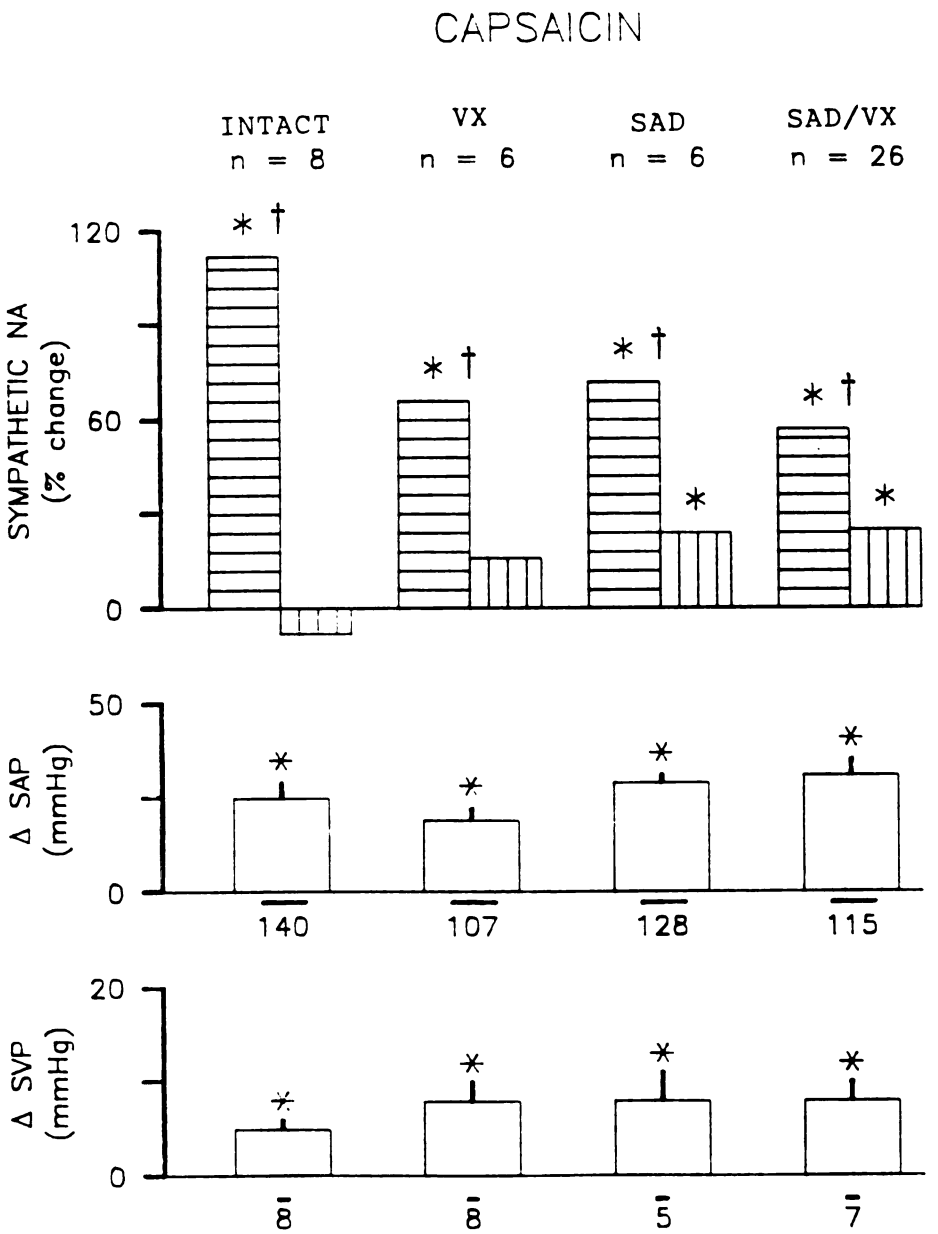

Figure 19. Mean responses to capsaicin in four states of pressoreceptor innervation.

Mean changes in sympathetic nerve activity, systemic arterial pressure, and splenic venous pressure caused by stimulation of splenic receptors by capsaicin in four states of afferent pressoreceptor innervation (see Methods). Percent changes are calculated from data in Table 11. Splenic nerve responses are shown by bars with horizontal lines and renal nerve responses by bars with vertical lines. Format is similar to that of Figure 5.

Table 11. Mean responses to capsaicin in four states of pressoreceptor innervation.

	CAPSAICIN			
	Splenic NA	Renal NA	SAP	HR
Intact				
Control	37	118	140	223
Maximum	60* †	131	165*	225
Recovery	38	110	139	225
CV	0.15	0.16	0.05	0.02
n = 8				
Vagotomized				
Control	28	122	107	225
Maximum	45* †	139	126*	228*
Recovery	27	119	108	224
CV	0.32	0.14	0.04	0.01
n = 6				
SAD				
Control	19	70	128	209
Maximum	31* †	85*	156*	212*
Recovery	19	72	132	209
CV	0.22	0.12	0.05	0.01
n = 6				
SAD/VX				
Control	28	115	115	237
Maximum	41* †	136*	146*	241*
Recovery	28	117	113	237
CV	0.17	0.10	0.10	0.01
n = 26				

These data are integrated voltage values statistically analyzed for the illustrations in Figure 19. The statistical methods used to compare these values are described in Methods.
CV, coefficient of variability; NA, nerve activity in μV•s/10s; *, significantly different from control; †, significant change in splenic/renal nerve activity ratio determined by Friedman test; NS, nonsignificant change in ratio, SAP, mean systemic arterial pressure in mmHg; HR, heart rate in beats/min; n, number of animals.

Stimulation of splenic receptors by capsaicin caused excitation of splenic nerve activity but caused no significant renal nerve response.

The specific contributions of sino-aortic and vagally innervated pressoreceptors to the reflex neural responses were examined. In the Vagotomized group, stimulation of splenic receptors by capsaicin caused significant excitation of splenic nerve activity and no significant renal nerve responses. These sympathetic responses were unequal when compared using the Friedman test (Figure 19, Table 11). Activation of splenic afferent nerves in the SAD group caused significant splenic and renal sympathetic responses (Figure 19, Table 11); splenic nerve excitation was greater than that of renal nerves. Stimulation of splenic receptors in the SAD/VX group produced significant excitation of splenic and renal nerves. These results are from Section A and are included here for purposes of comparison. These sympathetic responses also were unequal when compared using the Friedman test (Figure 19, Table 11). Simulation of splenic receptors by capsaicin produced comparable magnitudes of splenic nerve excitation among Intact, Vagotomized, SAD, and SAD/VX groups (Intact=VX=SAD=SAD/VX). However, this stimulation produced significantly less excitation of renal nerve activity in the Intact group than in the other three groups (Intact<VX=SAD=SAD/VX). In summary, the pattern of reflex responses was similar among the states of innervation, but the difference between the responses of the two nerves was exaggerated when sino-aortic baroreceptors or all pressoreceptors were intact. Pressor responses and contractions of the spleen accompanied the

splenic reflexes in all groups (Figure 19). Significant increases in heart rate accompanied the reflex responses except in the Intact state (Table 11).

Stimulation of splenic receptors by bradykinin (Figure 20, Table 12) produced neural responses similar to those caused by capsaicin. Stimulation of receptors by bradykinin in the Intact state consistently increased activity of splenic nerves, but renal nerve responses were variable. The renal nerve responses were inhibitory in one animal, excitatory in one, biphasic in one, and absent in five of eight animals. The neural responses lasted 10-120 s. The mean responses of the splenic nerves were greater than those of the renal nerves (Figure 20) when compared using the Friedman test (Table 12).

The reflex responses produced in the Vagotomized, SAD, and SAD/VX groups resembled those produced in the Intact group (Figure 20). Splenic nerves were excited in all three groups, and renal nerve responses were suppressed only when vagi were intact (SAD group). Thus, the differential pattern of reflex neural responses to bradykinin was exaggerated when vagi or all pressoreceptors were intact. Pressor responses and contractions of the spleen accompanied the splenic reflexes (Figure 20, Table 12). Significant heart rate increases accompanied the reflex responses in all the different states of innervation (Table 12).

In some states of pressoreceptor innervation, stimulation of the splenic receptors by congestion produced a pattern of neural responses which differed from that produced by chemical stimulation (Figure 21).

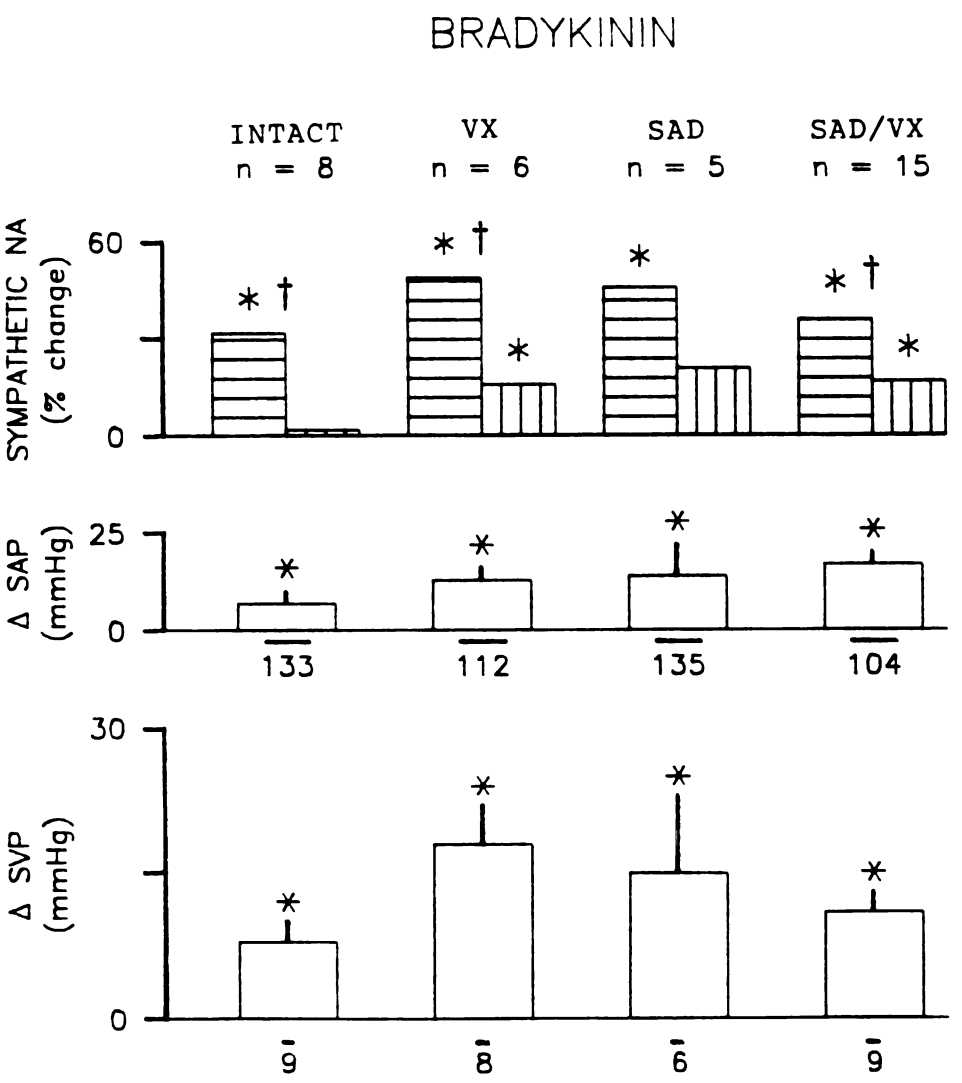

Figure 20. Mean responses to bradykinin in four states of pressoreceptor innervation.

Mean changes in sympathetic nerve activity, systemic arterial pressure, and splenic venous pressure caused by stimulation of splenic receptors by bradykinin in four states of afferent pressoreceptor innervation (see Methods). Percent changes are calculated from data in Table 12. Format is similar to that of Figure 18.

Table 12. Mean responses to **bradykinin** in four states of pressoreceptor innervation.

	BRADYKININ			
	Splenic NA		Renal NA	SAP	HR
Intact					
Control	39		134	133	221
Maximum	46*	†	131	139*	226*
Recovery	39		131	135	220
CV	0.08		0.10	0.04	0.01
n = 8					
Vagotomized					
Control	26		125	112	224
Maximum	39*	†	143*	123*	226*
Recovery	26		126	109	225
CV	0.23		0.09	0.04	0.01
n = 6					
SAD					
Control	19		61	135	211
Maximum	28*	NS	74	149*	217*
Recovery	19		63	134	212
CV	0.20		0.14	0.08	0.02
n = 5					
SAD/VX					
Control	28		112	104	247
Maximum	38*	†	126*	121*	250*
Recovery	28		114	105	249
CV	0.17		0.07	0.08	0.01
n = 15					

These data are integrated voltage values statistically analyzed for the illustrations in Figures 20. The statistical methods used to compare these values are described in Methods. Format is identical to that of Table 11.

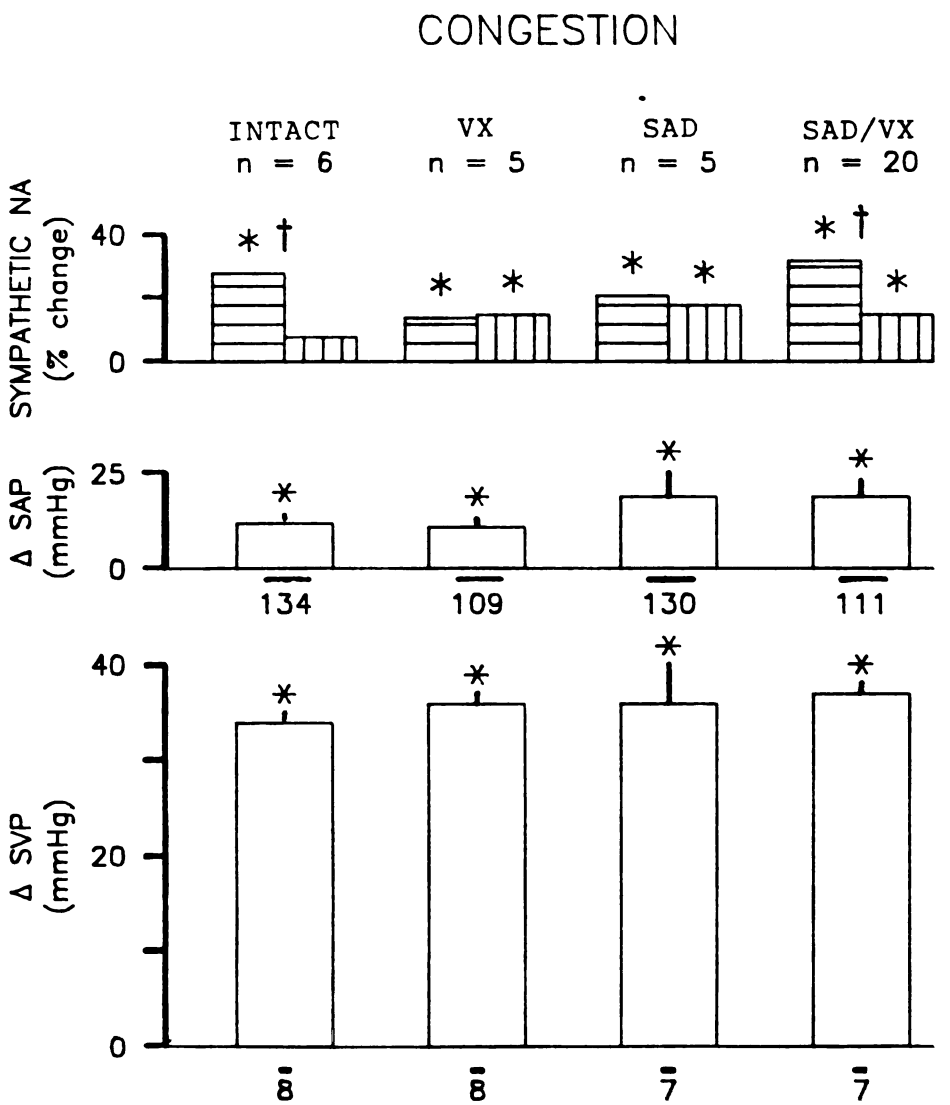

Figure 21. Mean responses to congestion in four states of pressoreceptor innervation.

Mean changes in sympathetic nerve activity, systemic arterial pressure, and splenic venous pressure caused by stimulation of splenic receptors by splenic congestion in four states of afferent pressoreceptor innervation (see Methods). Percent changes are calculated from data in Table 13. Format is similar to that of Figure 19.

In Intact animals congestion of the spleen produced consistent excitation of splenic nerves, but excitation of renal nerves in only one of six animals. Renal nerve activity was unaffected by this stimulus in the remaining five animals. The neural responses lasted 10-70 s. Mean responses are illustrated in Figure 21 and Table 13.

In the Vagotomized and SAD groups of cats, splenic and renal sympathetic responses to splenic congestion were variable in magnitude and not different from each other. After sino-aortic denervation and vagotomy (SAD/VX group), congestion produced unequal sympathetic responses (Figure 21). Thus, a differential response pattern was produced when sino-aortic and vagal receptors were denervated, and the differences between responses of renal and splenic nerves were exaggerated when all pressoreceptors were intact. Congestion also produced significant pressor responses and increases in splenic venous pressure (Table 13). Significant heart rate changes did not occur.

Influence of Cardiovascular Pressoreceptors on Tonic Sympathetic Activity

In the presence of all or part of the possible buffering influences from sino-aortic and vagally innervated receptors, stimulation of splenic receptors usually caused unequal sympathetic responses. These pressoreceptors appeared to attenuate the reflex responses of renal nerves preferentially; however, such influences on the reflexes could not be distinguished from influences on tonic activity of splenic and renal nerves. To determine if the vascular pressoreceptors had unequal influences on ongoing activity of splenic and renal

Table 13. Mean responses to congestion in four states of pressoreceptor innervation.

	CONGESTION				
	Splenic NA		Renal NA	SAP	HR
Intact					
Control	21		85	134	224
Maximum	25*	†	88	146*	227
Recovery	20		85	134	221
CV	0.09		0.07	0.03	0.02
n = 6					
Vagotomized					
Control	21		93	109	221
Maximum	24*	NS	104*	120*	221
Recovery	19		89	115	219
CV	0.09		0.08	0.06	0.01
n = 5					
SAD					
Control	16		67	130	216
Maximum	20*	NS	78*	149*	218
Recovery	14		64	139	213
CV	0.13		0.12	0.10	0.02
n = 5					
SAD/VX					
Control	25		127	111	237
Maximum	31*	†	144*	130*	239
Recovery	24		133	110	234
CV	0.16		0.17	0.10	0.02
n = 20					

These data are integrated voltage values statistically analyzed for the illustrations in Figures 21. The statistical methods used to compare these values are described in Methods. Format is identical to that of Table 11.

nerves, the inhibitory effects of sino-aortic baroreceptors and vagally innervated receptors on tonic activity of these nerves were compared. Small increases in blood pressure (15-21 mmHg) produced by intravenous injections of 3 ml of 6% dextran in saline caused significant inhibition of activity of both splenic and renal nerves in each of three states of innervation; i.e., in Intact, Vagotomized, and SAD groups (Figure 22). In the SAD/VX group only the slight inhibition of splenic nerve activity (9% decrease) was statistically significant. The change in splenic nerve activity was equivalent to that in renal nerve activity in all four states of afferent innervation, as determined by the Friedman test (Table 14). The neural inhibition elicited in the Intact state was greater than that produced in the other three groups. The relative degrees of sympathoinhibition among the groups were: Intact > Vagotomized = SAD = SAD/VX. Significant bradycardia occurred during the small increases in arterial pressure in the Intact and SAD groups (Table 14).

In 11 additional animals neural responses to dextran-induced pressor responses were compared to neural responses to equipressor doses of norepinephrine to evaluate possible preferential stimulation of cardiopulmonary receptors by the volume of dextran injected. The pressor responses were compared in six animals in the SAD group and in five animals in the Vagotomized group. In the SAD group increases in arterial pressure of 22 ± 3 mmHg by dextran resulted in 18% and 8% decreases in splenic and renal nerve activity, respectively. Increases in arterial pressure of 21 ± 4 mmHg by norepinephrine

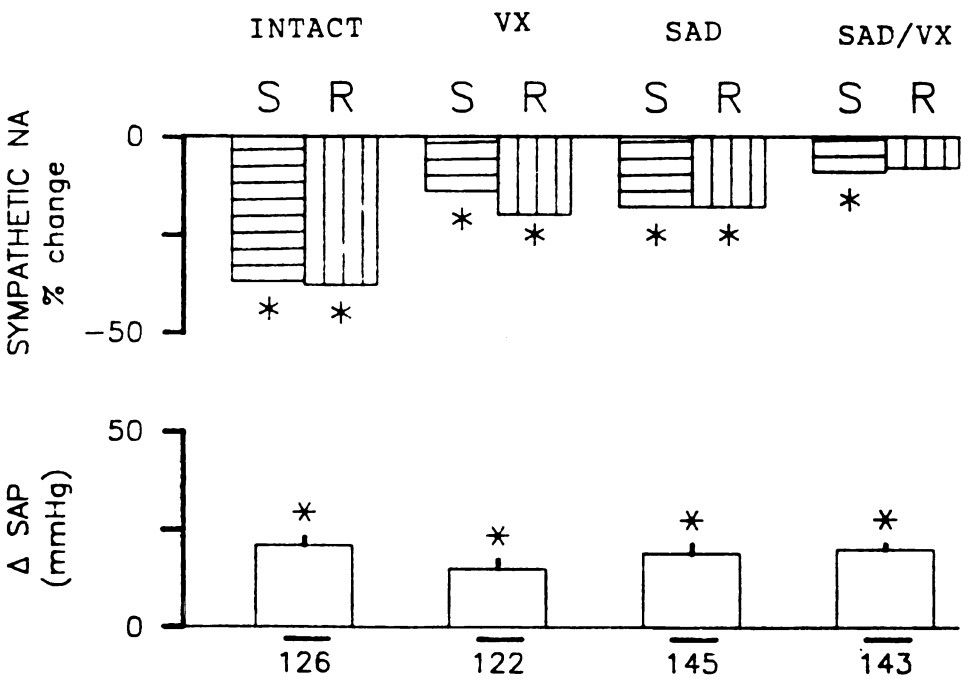

Figure 22. Mean nerve responses to small increases in blood pressure.

Mean changes in splenic (horizontal lines) and renal (vertical lines) nerve activity caused by small increases in systemic arterial pressure produced by injection of dextran in four states of afferent pressoreceptor innervation. Percent changes are calculated from data in Table 14. Format is similar to that of Figure 19.

Table 14. Sympathetic responses to small increases in systemic arterial pressure.

	SMALL INCREASES				
	Splenic NA		Renal NA	SAP	HR
Intact					
Control	19		101	126	225
Maximum	13*	NS	70*	147*	213*
Recovery	17		88	134	220
CV	0.14		0.26	0.05	0.01
n = 10					
Vagotomized					
Control	23		68	122	221
Maximum	21*	NS	54*	137*	216
Recovery	24		64	122	223
CV	0.06		0.09	0.04	0.01
n = 4					
SAD					
Control	26		127	145	224
Maximum	22*	NS	102*	164*	218*
Recovery	26		125	155	221
CV	0.04		0.14	0.04	0.01
n = 5					
SAD/VX					
Control	26		99	143	223
Maximum	24*	NS	89	163*	222
Recovery	26		103	149	223
CV	0.06		0.11	0.03	0.02
n = 8					

These data are integrated voltage values statistically analyzed for the illustrations in Figure 22. Format is similar to that of Table 11.

resulted in 14% decreases in splenic and renal nerve activity. In the Vagotomized group, increases in arterial pressure of 19 $\pm$ 5 mmHg induced by dextran injections resulted in 23% and 17% decreases in splenic and renal nerve activity, respectively. Increases in arterial pressure of 18 $\pm$ 3 mmHg by norepinephrine resulted in 27% and 22% decreases in splenic and renal nerve activity, respectively. All of the decreases in nerve activity were significant, and responses to dextran injection were not significantly different from those to norepinephrine administration.

Large increases in blood pressure (50-66 mmHg) produced by intravenously administered norepinephrine caused significant inhibition of nerve activity in all four states of pressoreceptor innervation (Figure 23). The inhibition of renal nerve activity was significantly greater than that of splenic nerve activity in the Intact, Vagotomized, and SAD groups (Table 15). In contrast, the large pressor response caused equivalent inhibition of splenic and renal nerve activity in the SAD/VX group. The large pressor responses produced comparable magnitudes of inhibition among Intact, Vagotomized, and SAD groups, and a lesser degree of inhibition in the SAD/VX group (Intact = VX = SAD > SAD/VX). Bradycardia accompanied the large increases in arterial pressure only in the Intact and the SAD groups (Table 15).

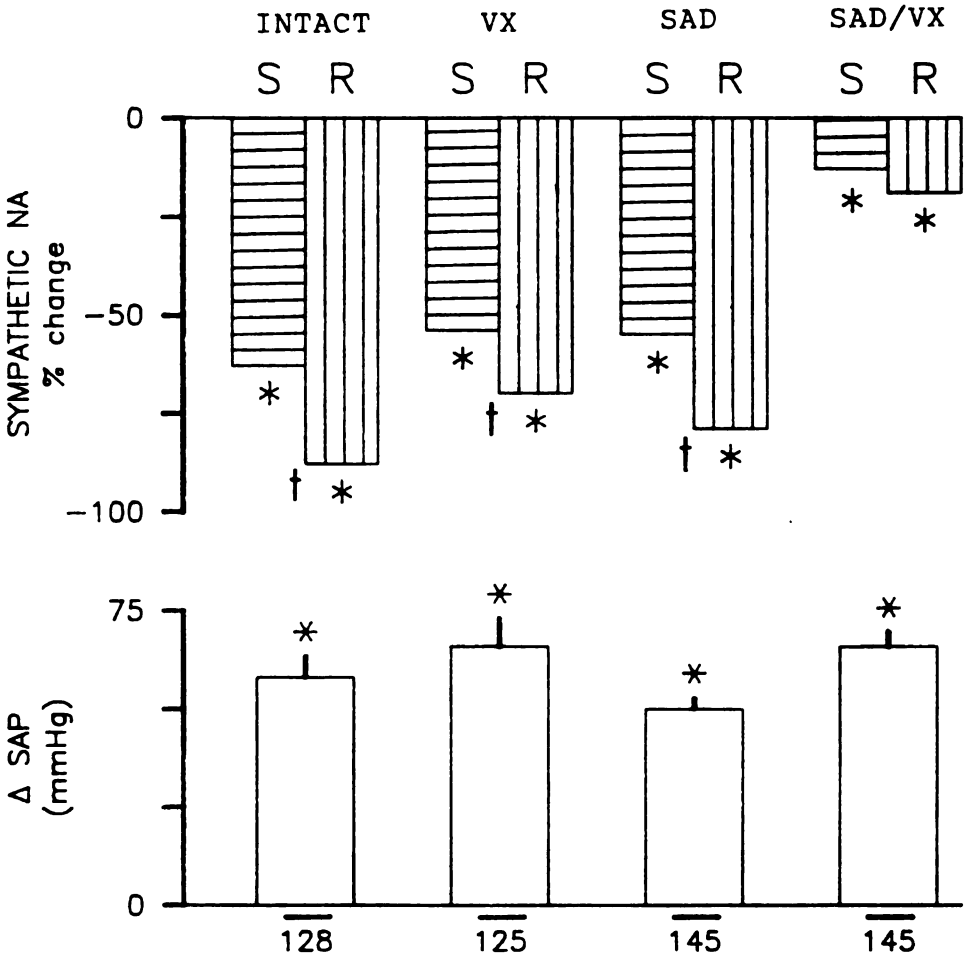

Figure 23. Mean nerve responses to large increases in blood pressure.

Mean changes in splenic and renal nerve activity caused by large increases in systemic arterial pressure produced by injection of norepinephrine in four states of afferent pressoreceptor innervation. Percent changes are calculated from data in Table 15. Format is similar to that of Figure 19.

Table 15. Sympathetic responses to large increases in systemic arterial pressure.

	LARGE INCREASES				
	Splenic NA		Renal NA	SAP	HR
Intact					
Control	16		155	128	229
Maximum	6*	†	7*	186*	210*
Recovery	17		140	128	230
CV	0.41		1.22	0.10	0.02
n = 7					
Vagotomized					
Control	20		49	125	222
Maximum	10*	†	10*	191*	217
Recovery	19		41	121	218
CV	0.31		0.51	0.11	0.05
n = 5					
SAD					
Control	36		80	145	217
Maximum	22*	†	11*	195*	187*
Recovery	35		65	132	212
CV	0.12		0.74	0.05	0.03
n = 7					
SAD/VX					
Control	24		90	145	225
Maximum	20*	NS	73*	211*	233*
Recovery	24		86	142	227
CV	0.09		0.13	0.07	0.02
n = 9					

These data are integrated voltage values statistically analyzed for the illustrations in Figure 23. Format is similar to that of Table 11.

Comparison of Splenic and Mesenteric Reflex Responses to Splenic Receptor Activation

Does a sympathetic nerve innervating another component of the abdominal capacitive circulation have a response to splenic receptor simulation similar to the splenic nerve response? Simulation of splenic receptors by capsaicin caused reflex excitation of both splenic and mesenteric nerves, pressor responses, tachycardia, and contraction of the spleen (Figure 24, Table 16). The magnitude of the mesenteric nerve responses tended to be greater than that of the splenic nerve responses (larger in six of eight animals). Bradykinin stimulation of splenic receptors similarly produced excitatory reflex responses of splenic and mesenteric nerves (Figure 24, Table 16). Mesenteric nerve responses were significantly greater than those of splenic nerves (Table 16). Significant increases in systemic arterial pressure, heart rate, and splenic venous pressure occurred in response to bradykinin stimulation of splenic receptors. In contrast, congestion of the spleen produced equivalent increases in splenic and mesenteric nerve activity as well as increases in heart rate and splenic venous pressure (Figure 24, Table 16).

The influence of cardiovascular pressoreceptors on splenic and mesenteric tonic activity was studied (Figure 25, Table 17). Hemorrhage to decrease systemic arterial pressure (-70 $\pm$ 10 mmHg) resulted in 56% increase in splenic nerve activity and 31% increase in mesenteric nerve activity (Figure 25), and heart rate increased by 19 $\pm$ 5 bpm. Increases in systemic blood pressure of 25 $\pm$ 11 mmHg above

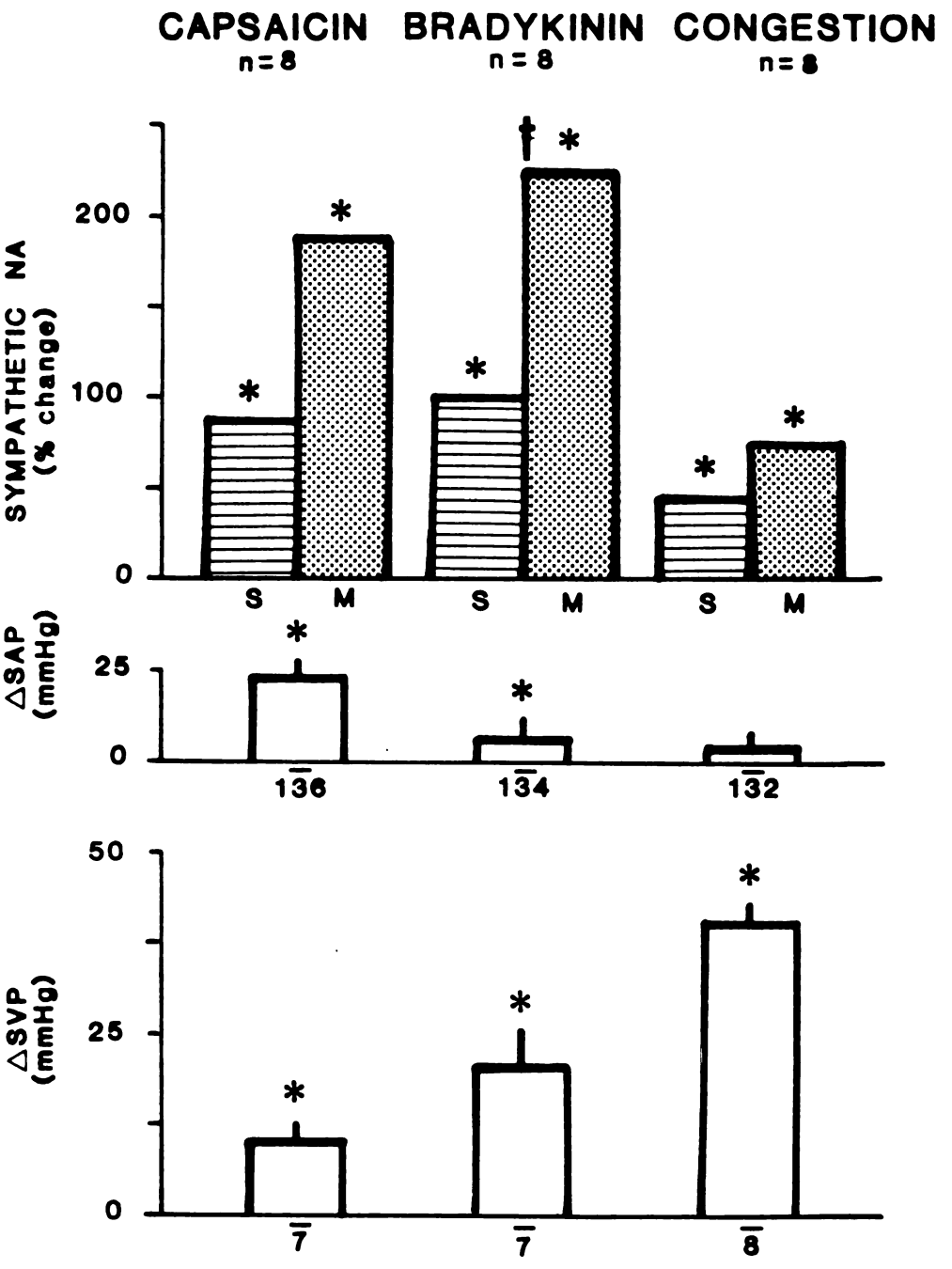

Figure 24. Mean responses of mesenteric and splenic nerves to stimulation of splenic receptors.

Mean changes in sympathetic nerve activity, systemic arterial pressure, and splenic venous pressure caused by stimulation of splenic receptors by capsaicin, bradykinin and congestion. Percent changes are calculated from the data in Table 15. S, splenic nerve activity; M, mesenteric nerve activity. Format is similar to that of Figure 5.

Table 16. Splenic and mesenteric responses to stimulation of splenic receptors.

n = 8	Splenic NA (μV•s/10s)		Mesenteric NA (μV•s/10s)	SAP (mmHg)	HR (bpm)	SVP (mmHg)
Capsaicin						
Control	12		37	136	232	7
Maximum	20*	NS	112*	159*	239*	16*
Recovery	12		41	133	235	8
CV	0.27		0.59	0.04	0.02	0.26
Bradykinin						
Control	14		34	134	237	7
Maximum	28*	†	113*	139*	241*	28*
Recovery	12		37	133	235	8
CV	0.79		0.55	0.05	0.02	0.54
Congestion						
Control	14		38	132	231	8
Maximum	20*	NS	67*	135	234*	48*
Recovery	12		37	133	227	7
CV	0.34		0.42	0.04	0.01	0.14

These data are integrated voltage values statistically analyzed for the illustrations in Figure 24. Format is similar to that of Table 1. †, significant change in splenic/ mesenteric nerve activity ratio determined by Friedman test; NS, nonsignificant change in ratio.

Figure 25. Mean nerve responses to hemorrhage and infusion of blood.

Mean changes in splenic and mesenteric nerve activity (NA) caused by hemorrhage (HEM) and reinfusion of blood (INF) to decrease and increase blood pressure (see Methods). Percent changes are calculated from data in Table 17. SAP, mean systemic arterial pressure; n, number of animals; *, significant change from control; †, significant change in splenic/mesenteric nerve activity ratio determined by the Friedman test.

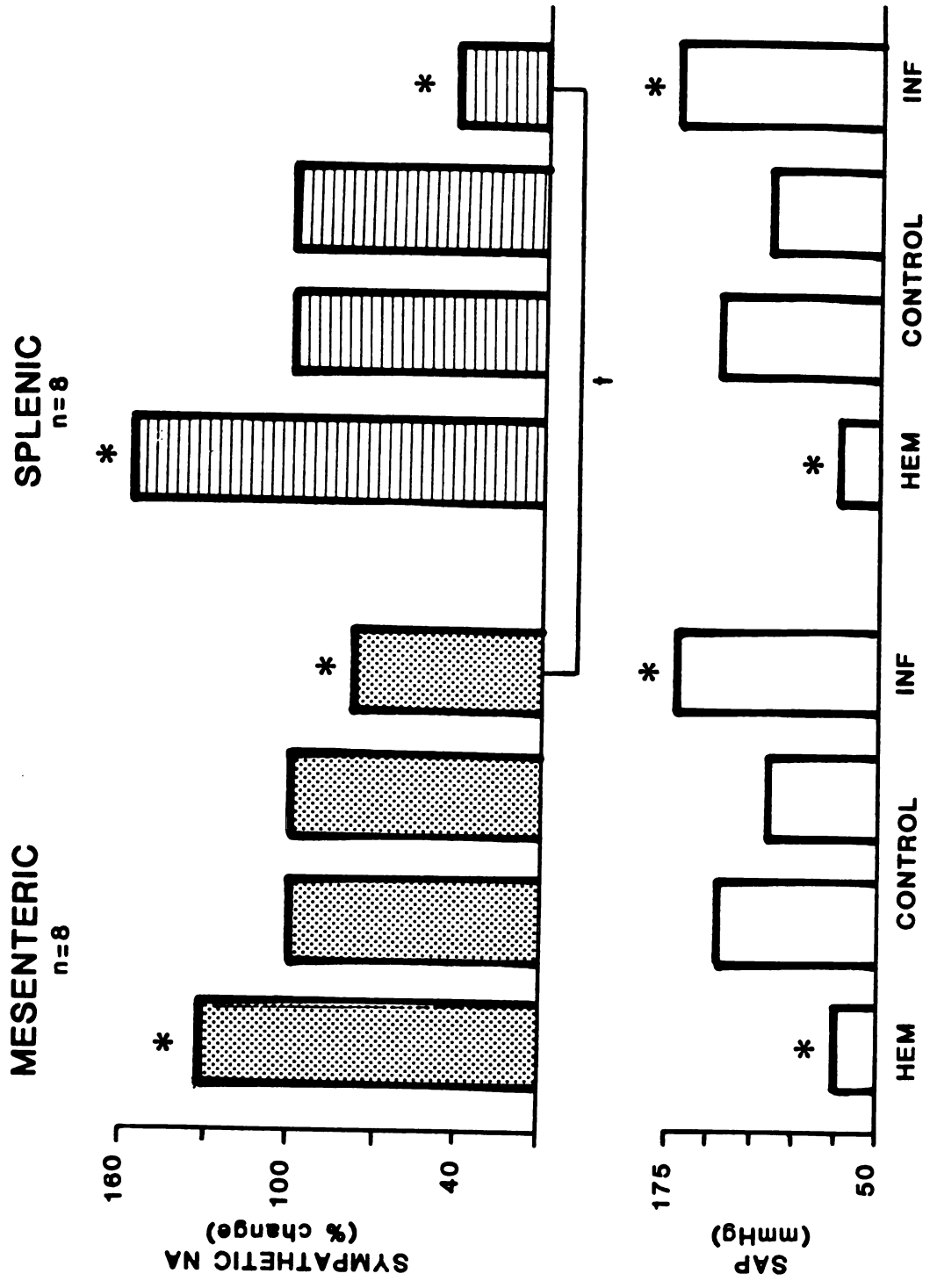
MESENTERIC
n=8
SPLENIC
n=8
SYMPATHETIC NA
(% change)
160
100
40
SAP
(mmHg)
175
50
HEM
CONTROL
INF
HEM
CONTROL
INF
*
*
*
*
*
*
†

Figure 25.

Table 17. Splenic and mesenteric responses to hemorrhage and infusions.

n = 8	Splenic NA (μV•s/10s)		Mesenteric NA (μV•s/10s)	SAP (mmHg)	HR (bpm)
Hemorrhage					
Control	16		40	144	229
Maximum	23*	NS	54*	74*	248*
Recovery	16		41	114	241
CV	0.14		0.13	0.21	0.03
Infusions					
Control	13		41	113	241
Maximum	6*	†	31*	169*	215*
Recovery	11		38	141	221
CV	0.23		0.11	0.15	0.05

These data are integrated voltage values statistically analyzed for the illustrations in Figure 25. Format is similar to that of Table 1.

initial control MAP were produced by reinfusion of blood or by intravenous injection of phenylephrine. These increases in systemic arterial pressure resulted in 58% and 23% decreases in splenic and mesenteric nerve activity, respectively (Figure 25). The decrease in splenic nerve activity was greater than the decrease in mesenteric nerve activity (Table 17). Heart rate decreased by 26 $\pm$ 6 bpm.

In summary, activation of splenic receptors produced excitatory mesenteric and splenic nerve responses. The mesenteric responses to bradykinin stimulation of splenic receptors were greater than splenic responses. Activation of pressoreceptors by increases of arterial pressure inhibited splenic nerve activity more than mesenteric nerve activity, and decreasing the influence of pressoreceptors by hemorrhage increased splenic and mesenteric nerve activity similarly.

DISCUSSION

Review of rationale. Few reports in the literature have described the contribution of splenic afferent nerves to the control of the circulation. Preparation of the vascularly isolated, neurally intact spleen made it possible to evaluate reflex responses to stimulation of exclusively splenic receptors. This preparation contrasts with the method of electrical stimulation of splenic afferent fibers used by Herman et al. (1982) to produce reflex responses. Electrical stimulation activates fibers simultaneously without regard to fiber type or modality; most large fibers will be activated when smaller fibers are activated, and thus, many fibers are activated to produce much temporal and spatial summation. Therefore, electrical stimulation may result in inaccurate conclusions because it may not produce the degree or quality of activation which would occur in a natural or physiological situation. Guzman et al. (1962) injected chemicals into the spleen but examined generalized responses such as vocalization, and changes in blood pressure and respiration. Therefore, the present investigation is among the first to examine reflex responses directed back to the spleen (see Calaresu et al., 1984) as well as to other abdominal organs (kidney and intestine). Although the concept of stimulation of visceral abdominal receptors is not new or unique, few investigators have studied the responses of two nerves simultaneously

to detect patterns of responses. And of the abdominal viscera, the spleen is one of the least investigated organs. This may be related to the inaccessibility of receptors (compared to other abdominal viscera) and to the vast vascular connections from the spleen to the stomach, pancreas, and intestine. Because of the connective tissue capsule, surface application of chemicals is often ineffective. When chemicals are injected into the splenic circulation, receptors in the stomach, pancreas, and intestine will be activated also unless the vascular connections to these organs are all ligated.

Summary of hypotheses and findings. To better understand reflexes originating from abdominal viscera and patterns of sympathetic outflow, this study formulated questions based on initial findings by Calaresu et al. (1984). The rationale for the questions is presented in detail in the Literature Review. Does the intensity of receptor stimulation determine the magnitude of reflex responses and thus determine (or contribute to) unequal neural responses? Although splenic and renal responses were similar in magnitude at lower levels of splenic receptor stimulation, that finding may result from limitations of quantification of multifiber nerve responses. However, intensifying the receptor stimulation revealed and then exaggerated the differences in the splenic and renal sympathetic responses. Does activation of splenic receptors by mechanical and chemical stimuli produce sympathetic reflexes which are mediated by spinal pathways? The spleno-splenic and spleno-renal reflexes were shown to include a major spinal component while potential supraspinal

components could not be definitively determined. Do mechanical and chemical stimulation activate different populations of splenic afferent nerves? If not, do the afferent fibers respond more intensely or with a different pattern of response to the chemical stimulation? Mechanical and chemical stimuli both activated some splenic afferent fibers, whereas others were preferentially activated by mechanical stimulation. Chemical stimulation did not activate the fibers to a greater degree than did mechanical stimulation. Mechanical stimulation produced a pattern of afferent responses different from that produced by chemical stimulation. Does activation of vascular pressoreceptors suppress excitatory sympathetic responses equivalently? Excitatory splenic and renal sympathetic responses to splenic receptor stimulation were not suppressed equally by pressoreceptor activation; renal reflex responses were suppressed while splenic were not. Is the influence of pressoreceptors on tonic splenic and renal nerve activity the same as that on reflex nerve responses? Sino-aortic baroreceptors and vagally innervated pressoreceptors had greater inhibitory influences on tonic renal than splenic nerve activity. Does a sympathetic nerve innervating another component of the abdominal capacitive circulation have a response to splenic receptor stimulation similar to the splenic nerve response? Mesenteric and splenic nerve responses to stimulation of splenic receptors were similar.

Spleno-splenic and spleno-renal reflexes. The results of the present study extended the findings of Calaresu et al. (1984) regarding sympathetic reflexes initiated by splenic receptors and detected

unequal reflex responses of splenic and renal nerves to congestion of the spleen. Because responses to congestion tend to be small in magnitude, a large number of animals needed to be tested to statistically detect the unequal neural responses which were not revealed in the earlier study. Thus, the unequal neural responses produced by splenic receptor stimulation did not depend on the character of the stimulus; i.e., congestion was as capable of producing unequal responses as was bradykinin or capsaicin.

Because in these experiments the control values of renal nerve activity often were higher than those of splenic nerve activity, it was questioned whether differences in baseline values of the two nerves could have been responsible for the observations of unequal responses. It could be speculated that the renal nerve responded less to the stimuli because it was closer to its maximal firing rate. Moreover, a given change from control values would constitute a smaller proportional change from a higher initial value (renal) than from a lower initial value (splenic). It is our contention that the tendencies for splenic nerves to be more readily excited or renal nerves more easily inhibited by afferent influences were not related to the higher voltage of basal renal nerve activity usually recorded. In several individuals the basal voltages recorded from splenic and renal nerves were almost identical or the voltage of the splenic nerve was greater than that of the renal nerve. In these animals activity of splenic nerves was still more excited, illustrating that "differential" reflexes observed were not related to unequal initial baseline

values of nerve activity. The renal nerve responses to splenic receptor stimulation were not maximum responses for those nerves, as injections of capsaicin into the aorta to cause widespread receptor stimulation always produced larger renal responses than had splenic receptor stimulation. The same proportional differences were obtained when nerve responses were quantified by spike counted averages, implying that limitations of the voltage integration or spike counting methods of quantifying neural activity did not lead to a false conclusion that differential reflexes occurred. Electrophysiological studies of single splenic and renal postganglionic neurons have shown that these neurons have similar resting discharge rates (range, 1-7 Hz; Weaver et al., 1986b). Therefore, the greater control voltage recorded in renal multifiber nerve bundles probably relates to a larger number of axons or smaller amounts of connective tissue in these bundles than in the splenic nerve bundles. Conclusions based on multifiber recordings are valid as previous work by Kidd et al. (1981) and studies in progress in this laboratory (Meckler, unpublished observations; Weaver et al., 1986b) of characteristics of ongoing and reflex firing of single renal and splenic postganglionic neurons have confirmed that the responses observed in multifiber recordings of splenic and renal nerves do reflect those of single neurons. Studies in progress (Meckler, unpublished observations) also indicate that splenic neurons respond more than renal neurons to stimulation of splenic receptors, thus verifying our contention that unequal

responses are not an artifact of multifiber techniques used to record neural discharge.

Renal sympathetic responses may have been smaller than splenic because of descending sympathoinhibition originating from supraspinal neurons. While descending excitation can be presumed or implied by a decrease in activity after spinal cord transection, descending inhibition to sympathetic outflow is more difficult to detect. One well-known source of inhibition of sympathetic reflexes (Coote & Downman, 1966), vascular baroreceptors, had been eliminated by sino-aortic denervation and vagotomy. However, baroreceptor-independent sources of tonic sympathoinhibition descending from the medial medulla have been described (Barman & Gebber, 1978). And Dembowsky et al. (1980) have described inhibition of somatosympathetic reflexes by baroreceptor-independent, tonic descending inhibition from the medulla. These sources of sympathoinhibition possibly may affect reflex activity of sympathetic nerves as well as tonic activity and may affect renal sympathetic reflexes more than splenic, thus contributing to the unequal neural responses observed. Another possible source of sympathoinhibition is somatic afferent input to the spinal cord due to surgical procedures. Somatic spinal afferent nerves can inhibit sympathetic activity (Kirchner et al., 1975a, 1975b; Wyszogrodski & Polosa, 1973).

Intensity-response characteristics. The intensity of splenic receptor stimulation used in the initial experiments in sino-aortic denervated, vagotomized animals (5 μg of capsaicin, splenic congestion

of 40 mmHg) produced unequal neural responses. If the intensity of receptor stimulation determines the magnitude of neural responses, does the intensity of afferent stimulation also determine the pattern of sympathetic responses? Would a less intense stimulation of receptors cause equivalent responses? And would an even greater stimulation of splenic receptors exaggerate the differential pattern of reflex responses? The data show that lower doses of capsaicin (1.0-2.0 μg) caused equivalent excitation of splenic and renal nerves. Although the splenic responses tended to be greater than the renal responses, it is possible that significant differences were not demonstrated due to limitations of multifiber recording techniques and limitations of quantifying mass activity. At the other end of the stimulus-response curve, the renal nerve responses reached a plateau at 100 μg of capsaicin whereas the splenic nerve responses continued to increase at the higher doses of capsaicin. Thus, there was a tendency for the renal nerve responses to reach a ceiling before the splenic nerve responses during very intense stimulation of splenic receptors (100 μg of capsaicin). However, the data also confirm that the smaller renal than splenic sympathetic responses to the lower doses of capsaicin used throughout the remainder of these studies could not be attributed to limitations of the maximum discharge rate of renal nerves or to an inability of the renal nerve to respond to this stimulus.

Khayutin and colleagues (Baraz et al., 1968) generated a dose-response curve of pressor responses to stimulation of intestinal

receptors with capsaicin. The shape of that dose-response curve is similar to the one generated in this study. The two studies differed in the threshold dose needed to elicit responses and in the dose which produced the maximal pressor responses. The curve in the present study is located to the right of the curve presented by Baraz et al. (1968). Some of the differences between these studies may be attributed to the stimulation of intestinal receptors by Baraz et al. and the stimulation of splenic receptors in the present study.

One puzzling aspect of these data is that the curves of the pressor responses and the neural responses were not parallel at the higher dosages of capsaicin. Above 50 μg of capsaicin, the neural responses either increased or reached a plateau but the pressor responses declined. The decline cannot be attributed to tachyphylaxis because efferent nerves still were responding to the capsaicin at these doses. Apparently the effector site (vascular smooth muscle) was unable to respond to the increased efferent nerve activity. This may have been due either to a noradrenergic feedback mechanism affecting alpha-2 receptors of the presynaptic terminal to decrease the release of norepinephrine or to a toxic action of capsaicin on the vascular smooth muscle caused by prolonged direct effect of previous doses of the irritant.

The other intensity-response relationship to be examined in the present study was the effect of different degrees of splenic congestion on sympathetic responses. Lower degrees of splenic congestion to splenic venous pressures of 15 and 25 mmHg did not produce reflex

neural responses suggesting that the threshold of splenic venous pressure for neural responses was between 25 and 40 mmHg. Unequal neural responses can often be detected when splenic venous pressure is increased to 40 mmHg (Table 1). The more intense stimulation of splenic receptors (splenic venous pressure of 50 mmHg) exaggerated the unequal neural responses.

Significant pressor responses were detected at stimulus intensities much lower than those which caused significant responses of splenic and renal nerves. This occurred with stimulation of splenic receptors by either capsaicin or congestion (Figures 6 and 7, Table 4). Several mechanisms may account for this observation. Sympathetic outflow to other vascular beds, e.g., intestinal, may have been increased at the lower stimulus intensity, or release of adrenal catecholamines may have caused the pressor responses. Another possibility is that limitations of multifiber recording techniques and limitations of quantifying mass activity may have precluded detection of neural responses to very-low-intensity afferent stimulation. However, the pressor responses produced by low-intensity splenic receptor stimulation supports the conclusion of Khayutin and colleagues that a change in systemic arterial pressure is a sensitive indicator of "tissue receptor excitation" (Khayutin et al., 1976).

Spinal reflexes. Sympathetic reflexes originating from the spleen have a spinal component. This is not surprising if one examines the information available about other visceral sympathetic reflexes. Spinal sympathetic reflexes have been initiated by stimulation of

mechanoreceptors and chemically sensitive receptors in the kidney (Beacham & Kunze, 1969; Recordati et al., 1982), intestine and mesentery (Andrews et al., 1972; Stein et al., 1986), urinary bladder (Laskey et al., 1979; Schondorf et al., 1983), and heart (Brown & Malliani, 1971; Malliani et al., 1972; Pagani et al., 1974; Weaver, 1981; Weaver et al., 1983a, 1983b).

Although it is generally accepted that tonic sympathetic activity decreases after spinal cord transection, this study demonstrated a greater decrease in basal renal than basal splenic nerve activity. The difference between the two sympathetic nerves parallels that found by Meckler and Weaver (1985). The splenic nerve activity appears to be less dependent on supraspinal sources of excitation than is renal nerve activity. This contrasts with the conclusions of Ninomiya and Irisawa (1975), who suggested that tonic activities of both splenic and renal nerves are highly dependent on medullary or supramedullary levels in the pentobarbital anesthetized cat. The spleno-splenic reflexes were similar before and after spinal cord transection (Figures 8, 9, and 10). In contrast, the spleno-renal reflexes differed in the two states; the reflex changes in activity were similar but the baseline discharge rates differed considerably. One could speculate that renal multifiber activity may contain at least two populations of renal neurons. One population would be tonically active and would respond to splenic receptor stimulation in neuraxis-intact and spinal animals. The other population would be driven by supraspinal sources, would contribute to the tonic activity, but would not

contribute to reflex responses. This population would not be active in the spinal state. Single unit studies (Weaver et al., 1986b) have demonstrated two populations of renal neurons; one is dependent upon supraspinal excitation for ongoing discharge, and the second maintains ongoing activity after spinal transection as well as in the intact state. However, both populations of renal neurons responded to visceral afferent activation and so contributed to tonic and reflex activity in the neuraxis-intact state. Thus, data from single unit studies refute the hypothesis that renal neurons driven by supraspinal sources would not contribute to reflex responses.

Reflex responses of splenic and renal nerves after cord transection appear to be of similar magnitudes (Figures 8, 9, 10, and 12). This conclusion conflicts with data from single unit studies of abdominal visceral reflexes. Stimulation of splenic receptors in spinal animals produced greater increases in the firing rates of splenic neurons than renal neurons (Meckler, unpublished results), a pattern which was similar to that observed in animals with intact neuraxes. Stimulation of intestinal receptors in spinal animals also produced neural reflexes similar to those produced in animals with intact neuraxes; i.e., the mesenteric neurons responded more than the renal neurons (Weaver et al., 1986b). Thus, the recording of electrical activity of multifiber bundles is not a sensitive enough technique to allow detection of differences in neural responses which can be observed when responses of single neurons are studied.

Splenic afferent nerves. Not only is the spleen an effector organ of the sympathetic nervous system, it is a source of afferent signals which can lead to reflex changes in the cardiovascular system. Stimulation of splenic afferent nerves by capsaicin, bradykinin, or congestion causes reflex excitation of portions of sympathetic outflow, specifically to the spleen, kidney, and intestine, as well as pressor responses and tachycardia. Previous studies of splenic afferent nerves (Herman et al., 1982; Lim et al., 1964) did not reveal the type of fibers or modality of receptors which could transmit information to the central nervous system to produce the sympathetic reflex responses. Studies of abdominal visceral afferent nerves (Lew & Longhurst, 1986; Longhurst et al., 1984a) have suggested that capsaicin stimulates more C fibers than A fibers, and that capsaicin stimulates a greater portion of C fibers than does bradykinin. Bradykinin stimulated similar percentages of A and C fibers in the studies. The A fibers in those studies were sensitive to light touch whereas the C fibers were classified as mechanically insensitive. Longhurst and coinvestigators concluded that C fibers were chemosensitive and A fibers were polymodal. This leads to the assumption that splenic reflexes initiated by capsaicin are mediated mainly by C fibers; reflexes produced by bradykinin are mediated by both C and A fibers; and those reflexes produced by congestion are mediated by mechanosensitive A fibers. In contrast, Janig and co-workers (Blumberg et al., 1983; Haupt et al., 1983) found colonic afferent nerves, both

small myelinated and unmyelinated, which responded to distension of the colon as well as chemical stimulation (bradykinin).

The present study was designed to test afferent responses to several different stimuli and to observe concurrent efferent responses to these stimuli. The latter allowed the designation of splenic afferent units potentially involved in initiating reflex responses by virtue of the latency of their response to stimulation of splenic receptors compared to the onset of the efferent response. Splenic afferent nerves carry information from at least three different types of receptors: mechanoreceptors, polymodal receptors, and preferential chemically sensitive receptors. Both A-delta and C fibers innervated mechanoreceptors and polymodal receptors. Due to the small number of chemosensitive units found, definitive conclusions cannot be reached about the fibers innervating the chemosensitive receptors. Their small number is unlikely to reflect their actual incidence (see Baker et al., 1980).

In the mechanosensitive and polymodal groups, resting discharge rates of A-delta and C fibers were not different. However, the resting discharge rate of the mechanosensitive units (1.0 $\pm$ 0.3 sp/s) differed from that of the polymodal units (3.6 $\pm$ 1.1 sp/s). In addition, the polymodal units achieved higher peak discharge rates during mechanical stimulation than the mechanosensitive units, although the percentage increases for mechanosensitive units (476 $\pm$ 130%) were not different from those for polymodal units (703 $\pm$ 202%). Both groups had similar thresholds but the duration of response for the polymodal

units was longer. In the polymodal group, the A-delta fibers exhibited a greater response to congestion than did the C fibers.

Few studies have utilized congestion of the vascular system of an organ to activate afferent fibers. Andrews et al. (1972) described spontaneously active intestinal afferent nerves which responded to increased mesenteric venous pressure, but they did not quantify the nerve activity. Herman et al. (1982) increased splenic venous pressure while recording from multifiber splenic afferent nerves. They described a linear relationship between splenic venous pressure and afferent activity. We agree with their conclusion that splenic receptors were slowly adapting or nonadapting because, of the single splenic units responding to congestion (32 total), 13 were slowly adapting and 18 were nonadapting. In contrast to the data presented here, Herman et al. (1982) found no tonically active afferent fibers in the naturally perfused, uncongested dog spleen. Also, according to their histological studies, they recorded responses from bundles containing only C fibers.

Contraction of the spleen induced by norepinephrine appeared to activate mechanosensitive and polymodal units similarly (141 $\pm$ 60% increase versus 224 $\pm$ 48% increase), although a difference in control discharge rates of these two types of afferent neurons existed. These data address a controversy in the literature. While Herman et al. (1982) reported that contraction of the spleen increased multifiber splenic afferent activity, Calaresu et al. (1984) did not detect reflex responses to splenic contraction, and thus concluded that the adequate

or effective stimulus to splenic receptors was stretch of splenic vessels and capsule. The data presented here demonstrate that contraction of the spleen activated receptors to produce small, very brief reflex responses. These responses were detected when the data were analyzed in shorter (5 s) time intervals than those used by Calaresu et al. (10 s). However, the afferent responses to contraction were almost always less than those to congestion, and therefore, congestion appears to be a more effective stimulus.

It is likely that a number of afferent units from the spleen are the mechanically sensitive units described by Longhurst and coinvestigators (Lew & Longhurst, 1986; Longhurst et al., 1984a) because punctate pressure on the spleen can cause increased afferent splenic discharge (Herman et al., 1982; Tobey, unpublished observations). However, the experimental arrangement precluded testing all units for responses to mechanical punctate pressure, and the connective tissue of the spleen would have interfered with transmission of the pressure to intrasplenic receptors.

The splenic afferent units responded to capsaicin with a latency of 0-5 s which corresponds to the onset of efferent responses. A dose-response relationship was noted for most of the afferent units. In contrast to results of Longhurst et al. (1984a), capsaicin stimulated only 70% of the C fibers, not all of them, and stimulated 58% of the A-delta fibers compared to their 38%. Afferent responses to bradykinin had a latency of 5-30 s to peak responses. This corresponds to the onset of efferent responses to bradykinin. The

proportion of A-delta and C fibers which responded to bradykinin was similar to that reported by Longhurst and colleagues (Lew & Longhurst, 1986; Longhurst et al., 1984a). The A-delta and C fibers responded to capsaicin with similar increases in discharge rates. Their responses to bradykinin also were similar in magnitude. Thus, both fiber types contributed to the reflex responses to these chemicals.

Longhurst et al. (1984a) concluded that abdominal visceral C fibers were chemosensitive and mechanically insensitive. In contrast, the present data showed that C fibers from the spleen included polymodal and mechanosensitive afferent units. The differences between the two studies may relate to the criteria for afferent units being tested and the method of administering the capsaicin. In their search for responsive units in abdominal organs, they found only one afferent unit from the spleen which responded to punctate mechanical stimulation and to intra-aortic injection of capsaicin (Lew & Longhurst, 1986), but found many more responsive afferent units from the mesentery, pancreas, stomach, duodenum, jejunum, ileum, liver, and gall bladder. The present study used localized injections of capsaicin and recorded from only splenic afferent nerves. Longhurst and colleagues tested a broad cross-section of abdominal afferent nerves, but their data may lack accuracy for the conclusions they have drawn pertaining to the effects of capsaicin. First, they only tested afferent units which could be activated by punctate pressure. Although this criterion may identify a large percentage of the total abdominal afferent units, it obviously does not include receptors within thickly encapsulated

organs such as the spleen and liver. Second, the data from the present investigation documented C fibers which responded to mechanical stimulation but not to capsaicin. The conclusions from the present study imply that the investigations of Longhurst and colleagues overlooked a subpopulation of abdominal afferent nerves which are C fibers, can respond to mechanical stimulation of receptors within an organ, and do not necessarily respond to capsaicin.

Data presented in this project indicate that chemical stimulation of splenic receptors can more reliably produce reflex responses than can mechanical stimulation in a given animal. Although both chemical and mechanical stimuli are capable of producing unequal neural responses, the responses obtained with capsaicin and bradykinin are more easily detected. Characteristics of afferent responses might help explain these differences. Chemical and mechanical stimuli activated a common group of afferent fibers; in fact, maximum responses to stimulation were similar. Is it possible that within the total population of splenic afferent nerves the chemicals stimulate more receptors? This study could not accurately answer the question because not every single unit within the population was tested and analyzed. The pattern of afferent responses to chemical and mechanical stimuli may contribute to the differences. Capsaicin produced an abrupt increase in discharge of the afferent unit whereas congestion caused a more gradual increase in afferent firing rate. Possibly the pattern of afferent activation evoked by capsaicin results in more temporal summation on the sympathetic preganglionic or postganglionic

neurons to produce reflex responses. These data do not eliminate the possibility that splenic afferent nerves respond to both types of stimuli at different thresholds and that mechanosensitive fibers are not truly different from polymodal fibers. The threshold of mechanosensitive afferent units for excitation by chemicals may not have been achieved by the doses used in this study.

This study examined responses of mechanosensitive, chemosensitive, and polymodal afferent splenic units. Another possible modality of afferent fibers would be specifically nociceptive. It has been postulated that a group of afferent fibers which specifically respond only to nociceptive stimuli exist. Such nociceptors are typically quiescent (Perl, 1983). Because this study investigated primarily tonically active units, the possible contribution of specific nociceptors to splenic afferent input to the central nervous system was not determined. The only obvious example of recruitment of silent units occurred with congestion. It is not likely that tonic activity of splenic afferent units is due to continuous noxious stimulation throughout the duration of the experiment.

Visceral afferent units could have a wide range of activity including nociception. It is probable that the stimulation of splenic receptors used here is a noxious or painful stimulus as injection of bradykinin (2 μg) into the spleens of conscious dogs evokes hyperpnea, hypertension, and vocalization (Guzman et al., 1962). However, it is doubtful that the splenic afferent units subserve only nociceptive functions. Lim et al. (1964) demonstrated that although bradykinin

causes splenic contraction at the same time as it evokes pain, a dose of epinephrine which produces a greater contraction of the spleen is not accompanied by pain. The problem is to understand how the central nervous system interprets incoming information. The splenic afferent nerves possess some resting activity, react to mechanical stimuli which could occur in physiological situations and not be noxious, and they also exhibit chemosensitivity. The afferent nerves may participate in homeostatic regulation of the spleen (Calaresu et al., 1984) or positive feedback mechanisms to maintain a high level of autonomic activity during conditions requiring a dynamic cardiovascular state (Malliani, 1982). In pathological situations which increase splenic venous pressure, such as cirrhosis of the liver or portal hypertension (Wanless & Bernier, 1983), or which result in production of bradykinin, e.g., inflammation of the spleen, or carcinoid syndrome (Oates et al., 1966; Zeitland & Smith, 1966), visceral pain may be elicited by excessive stimulation of afferent fibers. In these circumstances, pain could be postulated to result from excessive stimulation of receptive structures and not from stimulation of specific nociceptive afferent nerves (Malliani, 1982; Malliani & Lombardi, 1982). However, it has been shown that non-noxious cutaneous stimulation can cause a higher discharge rate of dorsal horn neurons than noxious stimulation (Le Bars and Chitour, 1983). Therefore, the mechanisms by which noxious visceral stimulation leads to the perception of pain remain to be determined.

Pressoreceptor influences on reflex activity. Neural control of the circulation can involve the integration of well-known reflexes initiated by cardiopulmonary and arterial baroreceptors, as well as reflexes originating from a variety of receptors within the viscera. Reflexes induced by excitation of receptors in the stomach (Longhurst & Ibarra, 1984; Longhurst et al., 1981; Longhurst et al., 1984b), liver (Ashton et al., 1982; Kostreva et al., 1980), gall bladder (Newman, 1974; Ordway & Longhurst, 1983), intestine (Baraz et al., 1968; Khayutin et al., 1969; Ninomiya & Irisawa, 1975; Ninomiya et al., 1974), mesenteric circulation (Andrews et al., 1972), and kidney (Kostreva et al., 1981; Kopp et al., 1984, 1985; Recordati et al., 1982) result in changes in blood pressure, heart rate, myocardial contractility, and vascular resistance. As these afferent influences can cause opposite sympathetic responses, the direction of changes occurring when several reflexes are activated simultaneously cannot be predicted easily. Although it is known that baroreceptor activation can attenuate sympathetic reflexes (Abboud et al., 1981; Barman & Wurster, 1978; Coote & Macleod, 1974b; Khayutin et al., 1969), such effects have not been studied extensively. The data presented in this section are examples of the response patterns in abdominal visceral sympathetic outflow that can occur when visceral afferent nerves from the spleen and from vagally innervated and sino-aortic pressoreceptors are activated simultaneously. The vagally innervated vascular receptors most likely to have produced significant reflexes in this study are those within the cardiopulmonary circulation (Abboud &

Thames, 1983). Reflexes initiated from sino-aortic and vagally innervated receptors had no influence on the reflex excitation of splenic nerves, whereas activation of these receptors, separately or together, caused significant suppression of renal nerve responses. Thus, the unequal pattern of renal and splenic nerve responses to splenic receptor stimulation was exaggerated by activation of cardiovascular pressoreceptors.

Pressoreceptor influence on tonic activity. The observation that pressoreceptors had preferential influences on reflex renal nerve responses led to the investigation of pressoreceptor influences on tonic activity of splenic and renal nerves. Intense afferent stimulation by large pressor responses demonstrated that stimulation of both vagally innervated and sino-aortic receptors can cause preferential inhibition of renal nerve activity (Figure 23). These results were consistent with the selective pressoreceptor suppression of renal excitatory responses to splenic receptor stimulation observed in the first part of this section. These data support previous suggestions that cardiopulmonary afferent nerves have selective influences on renal nerve discharge (Karim et al., 1972; Little et al., 1975; Weaver et al., 1984). In support of these present findings, Brown and Thames (1982) demonstrated in rabbits that activation of sino-aortic and cardiopulmonary receptors cause greater inhibition of renal than lumbar sympathetic activity and that each group of receptors contributes to the nonuniform responses of renal and lumbar nerves. In contrast, these findings were not consistent with those of Ninomiya

and co-workers (Ninomiya & Irisawa, 1975; Ninomiya et al., 1971), which suggested greater arterial baroreceptor influences on splenic than on renal nerve activity. Part of the discrepancy may be explained by differences in experimental design, because Ninomiya and coinvestigators employed several approaches to activate baroreceptors (aortic occlusion, pressurizing isolated carotid sinuses, and systemic administration of norepinephrine). However, such differences in design do not easily account for the disparate results. The difference in anesthetic agents, the pentobarbital anesthesia used by Ninomiya and coinvestigators, and the chloralose anesthesia used in our experiments may provide an explanation for the different results. First, chloralose has a minimal depressant action on the autonomic nervous system and augments baroreceptor-mediated reflexes predominantly at the level of medullary and spinal centers (Balis & Monroe, 1964; Brown & Hilton, 1956; Cox & Bagshaw, 1980; Greisheimer, 1965). In contrast, pentobarbital produces a depression of baroreceptor reflex mechanism compared to chloralose (Cox & Bagshaw, 1980; Greisheimer, 1965; Ngai & Bolme, 1966; Peiss & Manning, 1964). Second, Ninomiya and Irisawa (1975) suggested that discharge rates of both splenic and renal nerves are highly dependent on medullary or supramedullary drive in pentobarbital-anesthetized cats. Recent experiments in our laboratory have shown that ongoing renal nerve activity in chloralose-anesthetized cats is more dependent on supraspinal sources of excitation than is ongoing discharge of splenic nerves (Meckler & Weaver, 1985; Tobey, this study). Thus, medullary

integration of inhibitory baroreceptor inputs (Granata et al., 1983, 1985; Reis et al., 1984) appears more likely to affect renal than splenic sympathetic outflow under chloralose anesthesia.

The relative degrees of sympathoinhibition produced by each group of pressoreceptors were compared statistically. When receptors were stimulated by small increases in arterial pressure, the degree of inhibition produced by the sino-aortic receptors was equal to that produced by vagally innervated receptors (Table 14). The magnitude of sympathoinhibition caused by activation of either group of receptors was smaller than that produced when all receptors were innervated and did not differ significantly from that remaining after all these receptors had been denervated. The inhibition of sympathetic nerve activity remaining after sino-aortic denervation and vagotomy may be attributed to activation of visceral low-pressure vascular receptors (Herman et al., 1982; Kostreva et al., 1980, 1981; Tuttle & McCleary, 1975; Weaver, 1977). The degree of sympathoinhibition caused by stimulation of sino-aortic receptors by large increases in arterial pressure was equal to that caused by stimulation of vagally innervated receptors. However, this inhibition, produced in states of partial pressoreceptor denervation, was as great as that occurring when all pressoreceptors were intact (Table 15). This suggests that vagally innervated and sino-aortic baroreceptors have equal, overlapping inhibitory influences on sympathetic outflow, and that concomitant intense activation of all these inputs sums to produce neural occlusion. "Occlusive summation" of inputs from sino-aortic and

cardiopulmonary receptors as well as inputs from carotid sinus and aortic arch baroreceptors in rabbits has been described by Thames and coinvestigators (Brown & Thames, 1982; Guo et al., 1982; Thames & Ballon, 1984).

Ninomiya and Irisawa (1969) concluded that selective denervation of vascular pressoreceptor afferent nerves caused changes in baselines of renal nerve activity and mean arterial pressure. Their results showed that as inhibitory pressoreceptor influence was removed there was a progressive increase in renal nerve activity and mean arterial pressure. If the conclusions of Ninomiya and Irisawa regarding pressoreceptor influences on renal nerve activity could be extrapolated to reflex changes in nerve activity or to other sympathetic nerves, one could speculate that different states of pressoreceptor innervation could result in differing baselines which might influence the magnitude of reflex responses to visceral receptor stimulation. However, our data showed that control nerve activity did not differ significantly among the four states of pressoreceptor innervation. Consistent with our observations, Guo et al. (1982) found that acute denervation of carotid sinus or aortic baroreceptors, or vagotomy caused abrupt increases in arterial pressure and hindlimb perfusion pressure which tended to decline toward control values within 20 minutes. They concluded that there was readjustment of tonic sympathetic activity to a lower level independent of baroreceptors or a change in the responsiveness of vascular smooth muscle to sympathetic outflow. In a few individuals in the present study, sequential

selective pressoreceptor denervations were done which allowed comparison of basal nerve activity in two different states of pressoreceptor innervation in the same animal. In two animals, vagotomy was followed by sino-aortic denervation. In six animals, sino-aortic denervation was followed by vagotomy. The small number of observations warrants restraint in the conclusions; however, there was a tendency for basal renal nerve activity to be increased after selective pressoreceptor denervation. In contrast, basal splenic nerve activity did not change. These data permit the possibility that preferential influence of pressoreceptors on renal nerve activity exists, but these data are not conclusive.

Although denervation of sino-aortic and vagally innervated receptors did not conclusively increase basal renal and splenic nerve activity, activation of intact sino-aortic and vagally innervated receptors definitely inhibited tonic renal and splenic nerve activity and the renal nerve activity was preferentially inhibited. The vascular pressoreceptors also had preferential inhibitory influences on reflex renal nerve responses. This effect was most pronounced when both sets of receptors responded to increases in arterial pressure, or if pressor responses were particularly large. However, the pressor responses which were produced by splenic receptor stimulation were not large. Stimulation with capsaicin produced the largest pressor responses, and these were only slightly larger than the increases in arterial pressure produced by dextran or norepinephrine injections which had caused equal inhibition of tonic renal and splenic nerve

activity. Why, then, were renal reflex responses to splenic receptor stimulation always selectively suppressed, despite small pressor responses? A possible explanation is that splenic receptor stimulation excited efferent sympathetic nerves innervating the carotid sinus, thereby enhancing the sensitivity of baroreceptors (Sampson & Mills, 1970), and increasing the afferent activation by small pressor responses. Splenic receptor stimulation probably has widespread excitatory effects on sympathetic outflow, as it causes increased activity of mesenteric (see Results) and cardiac sympathetic nerves (Tobey, unpublished observations), as well as that of splenic and renal nerves. Visceral receptor stimulation often has such widespread effects (Weaver, 1985; Weaver et al., 1983b).

The preferential pressoreceptor-induced inhibition of tonic renal nerve activity was suggested in this discussion to be related to the greater dependence of renal nerves on medullary sources of tonic excitation and, consequently, to greater susceptibility to brainstem sources of sympathoinhibition (Granata et al., 1983, 1985; Reis et al., 1984). An explanation for the greater influences of pressoreceptors on excitatory renal nerve reflexes is more speculative. Data presented in an earlier section demonstrated that a major portion of the splenic and renal reflex responses to splenic receptor stimulation is mediated through spinal pathways. Splenic nerve reflex responses may be unaffected by pressoreceptors because these spinal reflexes are not affected by spinal components of baroreceptor inhibition (Coote et al., 1981). In contrast, the renal nerve reflex responses may be

affected by spinal sites of baroreceptor inhibition and/or may contain a supraspinal component susceptible to brainstem sites of inhibition.

Splenic and mesenteric responses. The splenic and renal circulations have different vascular functions; the splenic circulation has primarily a capacitive function whereas the renal circulation has primarily a resistive function. The difference in vascular function warrants the difference between reflex splenic and renal nerve responses. Splenic and mesenteric nerves both innervate capacitive beds, so one might expect similar reflex responses based on similar functions. Comparison of splenic and mesenteric sympathetic responses revealed excitatory responses to stimulation of splenic receptors. Mesenteric responses to chemicals were often greater than splenic responses whereas congestion produced equivalent neural responses. The pattern of greater mesenteric than splenic responses to chemical stimulation of splenic receptors is similar to that produced by stimulation of mesenteric receptors by bradykinin in which greater mesenteric than renal multifiber responses occurred (Stein et al., 1986). And this pattern also was seen in responses of single mesenteric, splenic, and renal neurons to stimulation of mesenteric receptors by bradykinin. Mesenteric responses were greater than splenic or renal unit responses (Weaver et al., 1986b).

Splenic and mesenteric reflex responses were examined in the presence of functioning pressoreceptors to ensure that sympathetic responses were recorded from mesenteric nerves with vasomotor components. The observation that mesenteric sympathetic responses tended

to be greater than splenic responses could be due to: (a) greater excitatory responses of postganglionic mesenteric nerves than splenic nerves to any excitatory afferent input, or (b) less inhibition of mesenteric than splenic nerve activity by activation of pressoreceptors. These data indicate that both situations contribute to the observed responses. Activation of pressoreceptors by increases of arterial pressure inhibited tonic splenic nerve activity more than tonic mesenteric nerve activity in this study. These findings agree with those of Ninomiya and Irisawa (1975) which suggested that intestinal nerve activity is less affected by baroreceptor input than is splenic or renal nerve activity. Thus, it is plausible that splenic congestion resulted in equal neural responses because the small pressor responses failed to activate pressoreceptors which would inhibit splenic activity more than mesenteric. The tendency for mesenteric responses to be larger than splenic responses to chemical stimuli could be attributed to greater excitatory responses of mesenteric than splenic nerves.

Reduction of cardiovascular pressoreceptor influence by hemorrhage caused similar increases in splenic and mesenteric nerve activity, so it appears that mesenteric nerve activity responds more to decreases in blood pressure than to increases. This observation is consistent with single unit studies of mesenteric neurons (Weaver et al., 1986b).

Use of drugs. Bradykinin is an endogenous substance which is released during inflammatory processes (Lewis, 1970) and causes

sensations of pain (Guzman et al., 1962). Prostaglandins are likely to be involved in the mechanism of action of bradykinin. Ferreira et al. (1973) demonstrated that in an in vitro spleen preparation, a basal level of prostaglandins is released and the release is increased by bradykinin in a dose-dependent manner. However, prostaglandins do not directly mediate the pain-producing activity of bradykinin because epinephrine-induced contraction of the spleen which causes release of prostaglandins (Ferreira et al., 1973) does not cause pain (Guzman et al., 1962). It is thought that prostaglandins accentuate the pain-producing activity of bradykinin by sensitizing the receptors that bradykinin activates. Prostaglandins also may be involved in the mediation of autonomic reflex responses because splenic contraction (which causes release of prostaglandins) can produce reflex sympathetic responses.

Of the two chemicals used to activate splenic receptors in this study, one occurs naturally in the body (bradykinin) and the other does not (capsaicin). That raises the possibility that they act by different mechanisms. Ohno et al. (1982) suggested that the mechanism of activation of thalamic nociceptive neurons produced by capsaicin is different from that of bradykinin. Their conclusions were based on a difference in latency to responses following administration, lack of suppression of capsaicin action by aspirin, and lack of cross-tachyphylaxis between capsaicin and bradykinin. However, the present investigation demonstrated that although capsaicin produced afferent and efferent responses with a shorter onset latency than bradykinin,

both chemicals activated the same afferent fibers to the same magnitude, and produced similar reflexes with spinal and supraspinal components. The present investigation also has demonstrated that congestion and chemicals can activate the same afferent fibers, and both types of stimuli produce similar magnitudes of afferent responses. This evidence implies that chemical (capsaicin and bradykinin) and mechanical stimulation of splenic receptors are two ways of producing the same reflex. The chemicals can exaggerate the reflex responses to allow them to be detected experimentally but the same neural relationships are evident. It could also be argued that capsaicin and bradykinin exaggerate a subliminal response which would never reach threshold under normal conditions. Even such subliminal inputs to central sympathetic neurons can provide important contributions to sympathetic excitability. However, capsaicin is an ideal pharmacological tool to study the pathways and effects of visceral afferent reflexes because it can be used to initiate reflexes repeatedly in the same animal.

Differential nature of reflexes. How does this differential neural reflex relate to responses of target organs? The autonomic nervous system controls functions of the spleen which are different from those of the kidney. The spleen acts as a reservoir of hemoconcentrated blood and has a capacitive function in dogs and cats. Major increases in splenic nerve activity cause expulsion of the stored red blood cells. Thus the spleen appears suited to mobilizing a critical volume of blood, mainly for its oxygen-carrying capacity, in times of

need. On the other hand, sympathetic innervation of the kidney influences renin release, sodium reabsorption, and renal hemodynamics. The kidney acts as a complex filter with concurrent excretory and endocrine functions. Reflex patterns that permit only small increases in renal nerve activity result in increases in renin release and sodium reabsorption (extracellular fluid and volume control), while large increases in renal nerve activity cause renal vasoconstriction as well. Unequal reflex influences would be consistent with different roles of the two organs in circulatory control. Studies in conscious dogs (Grignolo et al., 1982) provide physiological information that suggests unequal splenic and renal nerve responses in behaving animals. During shock avoidance experiments, the dogs responded with an increased hematocrit, most likely caused by splenic contraction, and with decreased sodium and water excretion without alterations in glomerular filtration rate, probably caused by small increases in renal nerve activity. A recent study by Weaver et al. (1986a) also provides evidence of a close relationship between neural responses and hemodynamic function. Mesenteric hemodynamic responses to activation of mesenteric receptors by bradykinin were greater than renal hemodynamic responses. The hemodynamic responses paralleled the pattern of neural responses in which mesenteric nerve responses were greater than renal nerve responses. This is evidence indicating that differences in responses of sympathetic nerves can have significant functional consequences.

Physiological significance of reflexes initiated from the spleen. The reflex initiated from the spleen is an excitatory visceral reflex even in the presence of buffering influences of cardiovascular presso-receptors. Activation of splenic receptors causes excitation of splenic, mesenteric, and renal nerves, pressor responses, and tachycardia. The physiological significance of excitatory viscero-visceral sympathetic reflexes may be to function as a positive feedback mechanism to maintain a high level of autonomic activity during conditions requiring a dynamic cardiovascular state (Malliani, 1982). Another view of excitatory visceral reflexes is that visceral afferent input contributes to the ongoing synaptic activity of sympathetic preganglionic neurons by providing widespread, subthreshold excitation (Dembowsky et al., 1985b). The physiological role of the spleen would include transmissions of afferent information to the central nervous system at levels which are not adequate to produce measurable reflex responses, but the input would impinge on the sympathetic preganglionic neuron to produce EPSPs and assist in bringing the neuron to threshold.

A homeostatic physiological role of spleno-splenic reflexes could occur in congestion of the spleen leading to an increase in sympathetic nerve activity which would, in turn, decrease arterial inflow to the spleen, increase venous outflow, and decrease splenic weight (Green et al., 1960; Greenway et al., 1968), thereby leading to elimination of the splenic congestion. In pathological conditions, increased intrasplenic pressure may contribute to symptoms such as

tachycardia. Conditions involving portal hypertension, e.g., cirrhosis of the liver and congestive heart failure, can increase portal venous pressure to pressures well above 25 mmHg and these conditions result in congestive splenomegaly and fibrous thickening of the splenic capsule caused by splenic congestion (Wanless & Bernier, 1983). The increased splenic venous pressure associated with cirrhosis of the liver (Womack & Peters, 1961) would result in increased splenic afferent traffic. This could contribute to the tachycardia observed in patients with cirrhosis of the liver (Womack & Peters, 1961), and the increased renal nerve activity caused by splenic congestion could result in increased sodium reabsorption and contribute to hypervolemia associated with splenomegaly (Donaldson et al., 1970). In injury or inflammation of the spleen, production of bradykinin could stimulate splenic receptors to evoke pain and cardiovascular responses. In addition, the large quantities of kinins that can be produced in the carcinoid syndrome (Oates et al., 1966; Zeitlin & Smith, 1966) could activate splenic as well as other visceral afferent nerves to produce alterations in cardiovascular parameters.

CONCLUSIONS

1. Stimulation of splenic receptors by capsaicin, bradykinin, or congestion produced greater splenic than renal nerve responses. The unequal pattern of reflex neural responses did not depend on unequal initial baseline values of nerve activity nor on the character of the stimulus.

2. Stimulation of splenic receptors by capsaicin or congestion produced a dose-response relation for splenic nerves which was greater than that for renal nerves. Intensity of afferent stimulation appeared to contribute to the pattern of unequal neural responses.

3. Reflex responses initiated from the spleen have a major spinal component, and the spinal reflex can be produced by stimulation of splenic receptors by capsaicin, bradykinin, or congestion.

4. Reflex responses to congestion were mediated by mechanosensitive and polymodal afferent nerves. Reflex responses to capsaicin and bradykinin were mediated by polymodal and primarily chemosensitive afferent nerves. Both A-delta and C fibers contribute to reflex responses to capsaicin, bradykinin, and congestion. Mechanical stimulation produced a pattern of afferent responses different from that produced by chemical stimulation; this difference may contribute to the higher probability of initiating reflex responses with chemical than mechanical stimulation.

5. Interaction of vascular pressoreceptor and splenic reflexes resulted in suppression of renal excitatory responses, but excitatory splenic responses were not attenuated.

6. Activation of vascular pressoreceptor reflexes had greater inhibitory influences on tonic renal than splenic nerve activity.

7. Stimulation of splenic receptors produced excitatory mesenteric and splenic sympathetic responses. The mesenteric responses to chemical stimulation of splenic receptors tended to be greater than splenic responses. Activation of pressoreceptors inhibited splenic nerve activity more than mesenteric nerve activity. Decreasing the influence of pressoreceptors increased splenic and mesenteric nerve activity similarly.

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