RENAL AND SPLENIC SYMPATHETIC EFFERENT RESPONSES TO VISCERAL AND CAROTID SINUS AFFERENT STIMULI

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ABSTRACT

RENAL AND SPLENIC SYMPATHETIC EFFERENT RESPONSES
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Renal and splenic efferent nerve activity was simultaneously recorded while urinary bladder afferents, duodenal stretch receptors and carotid sinus baro- and chemoreceptors were stimulated. Differential responses shown earlier, to various stimuli in several different efferent nerves indicate, that the sympathetic nervous system is specifically organized. Renal nerves are significantly more excited by stimulation of urinary bladder afferents than are splenic nerves, in contrast to earlier data which showed greater excitation of splenic than renal nerves to other stimuli (Weaver et al., 1983a, 1983b). The responses of these two nerves were the same when carotid sinus chemoreceptors and baroreceptors were stimulated. Stimulation of additional receptors by norepinephrine inconsistently caused differential responses in renal and splenic nerves. This study indicates that the nerve response is dependent on both the afferent nerve stimulated and the efferent nerve, which suggests specific organization of the sympathetic nervous system.

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INTRODUCTION

Cannon first (1929) and others later (Schaefer, 1960) described the sympathetic nervous system as one which could function through uniform or mass activation. It has been shown since, however, that it is capable of specific discharge patterns to several different afferent stimuli. Using blood flow measurements to reflect the effects of sympathetic discharge to the renal and skeletal muscle vascular beds, Folkow (1961) found increased resistance in skeletal muscle arteries, but no change in renal artery resistance in response to carotid artery occlusion. The neurogenic basis for this differential response pattern was reveled by subsequent denervation. This type of study was important because it demonstrated the involvement of the sympathetic nervous system in control of cardiovascular function. It was also important because it demonstrated, specificity of sympathetic vasomotor activity to different organs.

Direct measurements of multifiber nerve activity became more accurate as advancements were made in instrumentation, and the specificity of the sympathetic nervous system could then be examined more directly and with greater precision. Karim, and coworkers (1972), were the first to demonstrate opposite responses of different sympathetic efferent nerves to the same stimulus. They found increased cardiac but decreased renal nerve activity in response to stimulation of atrial stretch receptors. Both differential responses in different

vascular beds and in different nerves were reported by Kullman, et al. (1970), and Walther, et al. (1970). Kollai and Koizumi (1977), completed one of the few studies in which nerve recordings and blood flow measurements were made simultaneously. Ninomiya, and coworkers (1971, 1973, 1974, 1975; Irisawa, 1973; Nisimaru,1971), reported differences in tonic discharge of multiple efferent nerves innervating different organs, as well as differences in responses to various afferent stimuli. This further demonstrates specific patterns of the sympathetic nervous system. The results of all of these studies indicate that the effects of sympathetic discharge are not uniform, as originally proposed, but are unique for each organ.

The primary goal of my research was to determine if and how reflex activation of sympathetic postganglionic efferents is differentially distributed in renal and splenic nerves in response to stimulation of bladder, duodenal, and colon afferents and carotid sinus chemoreceptor and baroreceptor afferents.

LITERATURE REVIEW

Differential Responses

Cannon (1929) was the first to describe the sympathetic nervous system as one which responded to stimuli primarily with a generalized or uniform activation. Many supported this concept of mass activation of the sympathetic nervous system (Schaefer, 1960) until other investigators (Celander and Folkow, 1953; Korner and Uther, 1975; Kullman et al., 1970) found directionally different blood flow changes in various vascular beds in response to autonomic stimulation. Among the first of these investigators were Celander and Folkow (1953), who electrically stimulated the lumbar sympathetic chain and measured blood flow changes in skin and muscle hindlimb vessels. They found a wider range of blood flow to the skin than to skeletal muscle in response to baroreceptor stimulation, as well as to lumbar chain activation. Blood flow in the denervated vessels indicated a higher tonic discharge in vasomotor fibers to skeletal muscle than in those to skin. A difference in resting tone, or number of fibers innervating the two regions, might explain the differences in blood flow changes to these different regions. Although these data were inconclusive and the experiments fragmentary, they nonetheless provided qualitative evidence to challenge Cannon's mass activation theory.

Additional data were provided by Löfving (1961), who found graded

vasodilation in vessels to skin, skeletal muscle, intestine and kidney during electrical stimulation of the cingulate area depressor regions in the limbic lobe. Skeletal muscle blood flow increased the most, whereas renal blood flow changed only slightly. Skeletal muscle blood flow decreased more in response to bilateral carotid artery occlusion than did blood flow to other beds, while renal blood flow was unchanged. These results also indicated that differences in tonic nerve discharge could cause the differences in the blood flow changes since the initial flow is dependent on the tonic activity level. In a later study, Folkow, et al. (1961), measured blood flow changes in skeletal muscle and renal vessels in response to carotid artery occlusion during normal, hyper- and hypoventilation. As in the previous study, renal blood flow was unchanged during carotid artery occlusion with normal ventilation; however, when the carotid arteries were occluded during hypoventilation, renal vessels constricted. This demonstrated that the combination of these two excitatory stimuli were needed to cause renal vasoconstriction and suggested that there were different medullary neuron pools innervating the functionally different vascular beds. They concluded that these neuron pools had different threshold levels for excitation which would allow a strong stimulus to cause mass activation.

Data which challenged Cannon's mass activation theory came not only from experiments which indirectly showed regional differences in central neural activity, but also from research in which nerve activity was measured directly. Green and Heffron (1966), simultaneously recorded cardiac and vertebral efferent nerve changes induced by hypoxia, and observed greater increases in vertebral than cardiac nerve activity. This provided direct evidence for quantitative regional differentiation

in the sympathetic nervous system. Further, Karim, et al., in 1972, were the first to demonstrate directional differentiation of the sympathetic nervous system, by showing cardiac nerve activity increases, but renal nerve activity decreases in response to stimulation of left atrial receptors. Lumbar and splenic efferent nerve activity were unchanged by this stimulus. These data indicate that activating left atrial stretch receptors is one stimulus which results in a directional differential response of two nerves innervating different regions.

Iriki, Riedel and Simon examined the effects of thermal stress, first on different vascular beds (Kullman et al., 1970), and then on Onerve activity to these beds (Iriki et al., 1971, 1972; Reidel and Peter, 1976; Simon and Reidel, 1975; Walther et al., 1970). They were particularly interested in comparing the relative blood flow changes in cutaneous and other vascular beds. The reflex effects of moderate and strong spinal cord heating and cooling were evaluated by simultaneously measuring changes in blood flow to skin, intestine and skeletal muscle. Spinal cord warming increased blood flow to the skin, but decreased flow to the intestine. Spinal cord cooling reversed the flow changes in both beds. Skeletal muscle flow was not affected by thermal stress. In a subsequent study, they recorded changes in cutaneous and intestinal efferent nerve activity during thermal stress, and found that the nerve responses paralleled changes in blood flow to the different organs. These studies indicate a complex organization of sympathetic nerve discharge, and suggested a "reciprocal innervation of functionally antagonistic autonomic effector systems" (Walther et al., 1970).

General observations by Korner and Uther (1975), who measured blood flow changes, suggested that asphyxia could also produce reciprocal

innervation effects. In 1971, Iriki, et al. (1971), simultaneously recorded cutaneous and splanchnic nerve responses to moderate and severe asphyxia. They found that intestinal nerve activity increased as expected, but cutaneous nerve activity decreased. This decrease in cutaneous nerve activity was consistent with the simultaneously measured increase in ear temperature. In response to severe hypoxia, however, both cutaneous and splanchnic nerve activity increased. This demonstrated that asphyxia was another stimulus which caused opposing responses of two efferent nerves; this opposition was dependent upon the stimulus intensity.

Iriki, et al. (1972), further investigated autonomic responses to hypoxia, hypercapnia and asphyxia, by comparing cardiac and intestinal efferent nerve changes. They found that cardiac nerve activity decreased in response to these respiratory stresses, as had cutaneous nerve activity, thereby showing this differential response was not unique to cutaneous nerves. Cardiac nerve activity, however, also increased in response to severe hypoxia.

They (Iriki and Simon, 1973) also investigated the responses of cardiac and intestinal nerves to spinal cord warming, and found that cardiac and intestinal nerve activity changed similarly: both increased. These investigators used two stressful stimuli to elicit responses in cutaneous, splanchnic and cardiac nerves and showed differences not only to the source of afferent stimulation, but also to the region the nerve innervated. These changes in nerve activity were correlated with blood flow changes, which suggests a functional relationship between nerve discharge and blood flow. The combined results from the blood flow and nerve recording experiments suggested a

functional regional differentiation of the sympathetic nervous system.

Ninomiya, et al. (1971), also investigated the specificity of the sympathetic nervous system. Their primary interest was to determine first if specificity of the sympathetic nervous system occurred at the organ level, then to study the responses to stimulating arterial baroreceptors in splenic, cardiac and renal nerves. Arterial baroreceptors were stimulated by several different methods: 1.) controlled occlusion of the ascending and descending aortas, 2.) isolated carotid sinus preparations, 3.) bilateral carotid artery occlusions, 4.) hemorrhage, 5.) reinfusion of blood and 6.) electrical stimulation of the aortic depressor nerves. They concluded that the threshold blood pressure for complete inhibition of splenic nerve activity was significantly lower than that for the renal nerve. They also found that baroreceptor reflex gain was significantly higher for splenic than for renal nerves. These results demonstrate quantitative, non-uniform responses of nerves innervating different organs to activation of arterial baroreceptors. Comparisons were made in other studies between either renal and gastric (Nisimaru, 1971), or renal and intestinal nerve activity. Gastric and intestinal tonic nerve discharge was shown to be correlated with contractions of the respective organs, and this was not completely inhibited by baroreceptor activation. This suggested a baroreceptor-independent component of these two nerves. In some experiments intestinal nerve activity increased in response to a systemic pressure increase (Irisawa et al., 1973). Ninomiya, et al. (1975), have demonstrated that qualitative and quantitative non-uniform responses can occur in two nerves innervating different organs.

Differences found in efferent nerve activity to different organs

indicated that the sympathetic nervous system could selectively change the discharge of certain fibers in a nerve bundle. Not all fibers responded similarly to hypercapnia, norepinephrine or thermal stimulation, some were excited and other inhibited by the same stimulus (Riedel and Peter, 1976; Simon and Riedel, 1975). That is, efferent nerve bundles contain a heterogenous fiber population. Jänig, et al. (1979), have completed several studies in which they recorded from cutaneous and muscle hindlimb single or few fiber preparations and also demonstrated heterogenous populations in cutaneous and skeletal muscle nerve bundles. Blumberg, et al. (1980), showed non-uniform responses of cutaneous nerve fibers in response to baroreceptor and chemoreceptor stimulation. In some cutaneous nerve fibers activity was only weakly inhibited, but in others it was completely inhibited by stimulation of arterial baroreceptors. Stimulation of arterial chemoreceptors or general hypoxia inhibited discharge of most cutaneous fibers; however, some were excited by these stimuli. Blumberg, et al. (1980), Kollai and Koizumi (1980), Riedel and Peter (1975), and Rogenes (1982) have demonstrated that even within a nerve there are non-uniform responses. They showed also that the response of the majority of fibers is similar to that of the whole nerve.

It has been shown that differential or non-uniform responses occur in nerves innervating different regions of the body, nerves innervating different organs within the same region and even in different fibers to the same organ. This indicates specificity as well as complex organization of the sympathetic nervous system.

Our laboratory has measured simultaneous renal and splenic nerve responses to various afferent stimuli during the past few years. Results

of these studies indicate that splenic efferent nerves are more easily excited than are renal nerves (Weaver et al., 1983a, 1983b). In the present study an attempt was made to find a stimulus that caused greater excitation of renal than splenic nerves to show that the splenic nerve was just not inherently more excitable. The second goal of the present study was to determine if activation of a chemoreceptor reflex could produce differential responses in the two nerves and if baroreceptor reflexes produced responses similar to those reported by Ninomiya. The subsequent sections review the literature involving the two organs and the reflexes tested in this research.

Kidney

Many of the functions which make the kidney an essential organ in the regulation of body water and electrolytes are under neural control. Several different techniques have revealed the diversity of renal innervation. Kuo, et al. (1982), using horseradish peroxidase, identified renal efferent neurons in the superior mesenteric ganglion and in the ipsilateral sympathetic chain ganglia from T_{12} to L_3. Spinal cord stimulation showed that there are renal nerve fibers derived from spinal cord segments T_5 to L_3 (Taksuchi et al., 1964). In addition, anatomical studies have shown small dense-cored vesicles in contact with tubular epithelial membrane of both the proximal and distal tubules (DiBona 1982), which implies sympathetic innervation of the tubules. Monoaminergic innervation of the juxtaglomerular apparatus has been shown by Barajas (1981) using fluorescent histochemistry, electron microscopy and autoradiography. Further, granular cells in the

juxtaglomerular apparatus are believed to be the source of renin. These results, combined with studies in which renal function was measured, suggest that renal nerve activity regulates renin release. Barajas (1981), found nerve bundles associated with arterioles in the renal cortex, suggesting neural control of renal blood flow. In fact, DiBona has stated that it is possible that one axon could innervate granular cells, smooth muscle cells in the glomerular arterioles and cells of the distal tubule (DiBona, 1982). He suggested that activity in one axon could affect renin release, sodium reabsorption and renal blood flow.

Slick, et al. (1975), studied the role of renal sympathetic efferent nerve activity in the regulation of sodium excretion by electrically stimulating renal nerves and measuring sodium excretion. At low level stimulation they found that urinary sodium excretion decreased without changes in renal blood flow or glomerular filtration rate. This decrease in sodium excretion was blocked by guanethidine, indicating that increased sympathetic nerve activity decreased sodium excretion. Osborn, et al. (1981), determined that low frequency stimulation of renal nerves also increased renin release without changing renal blood flow, glomerular filtration rate or uninary sodium excretion. These studies indicate that progressive increases in renal efferent nerve discharge increase first renin release, then sodium excretion and finally renal blood flow, (DiBona,1982; Slick et al.,1975; Osborn et al., 1981).

Gross, et al. (1980), measured renal blood flow changes and renal efferent nerve activity in separate groups of dogs. Carotid occlusion increased renal nerve activity but produced no change in renal blood flow. They found later (Gross, 1981) that neither renal blood flow nor

sodium excretion changed during carotid artery occlusion. They concluded that carotid artery occlusion was insufficient to affect renal blood flow or sodium excretion. Kopp and DiBona (1983), however, found decreased urinary sodium excretion and increased renin secretion when carotid sinus pressure was lowered by 41 $\pm$ 5 mmHg for 10 minutes, which was inadequate to produce renal hemodynamic changes. These changes in urinary sodium excretion and renin release are similar to those that occur in response to low-level electrical stimulation (< 1 Hz) of the renal nerves. Kopp and DiBona also showed that physiological activation of renal efferent nerves controls renal function (1983).

A current concept, based on physiological as well as on electrical activation of renal efferent nerves, indicates that they innervate renal arterioles, tubules and juxtaglomerular apparatus, and that their activity affects renin release, sodium excretion and renal blood flow (Kopp and DiBona, 1983).

Spleen

The spleen consists of arterioles, venules, areas of white pulp, areas of red pulp, trabeculae and capsule. It functions both in the breakdown (lysis) and storage of erythrocytes, and as a blood reservoir. Contraction of the cat splenic capsule, for example, increases cardiac output by increasing the circulating blood volume, which is advantageous during exercise, hemorrhage, asphyxia or other stresses. Information about the control of blood storage and flow in the spleen has come from anatomical as well as from denervation studies. Using degeneration studies, Utterback (1944) showed that splenic nerves pass through the

celiac plexus, but that there is no apparent vagal innervation of the spleen. He found also that there are nerve endings on arteries in the red pulp, venous tributaries and trabeculi. These data imply that innervation of the spleen is important in both its capacitive and its resistive functions.

Fillenz (1970) used histochemical techniques and electron-microscopy to correlate the structure of splenic innervation with characteristics of neurotransmission. The capsule and vascular trabeculae are loosely packed smooth muscle cells in connective tissue. Flourescence methods showed elastic tissue in the outer half of the capsule, the internal elastic lamina of arteries and around individual trabecular smooth muscle cells which would create a compliant vascular bed. Nerve endings were found on the splenic pedicle and along large branches of the arteries. There was also innervation of capsular and trabecular smooth muscle cells. Innervation of the splenic arteries both outside and inside the spleen was shown, as was innervation of central pulp arteries, however only noradrenergic nerve terminals were found in the spleen. It was concluded that both arteries and veins of the spleen are innervated and that the organ contains elastic tissue in its vascular walls and capsule. This suggests that the spleen sunctions as a blood reservior and its blood volume is regulated by the sympathetic nervous system.

Barcroft and Stephens (1927) showed that the spleen contracts in response to exercise and severe hemorrhage and determined that this contraction is dependent upon splenic nerve activity. They demonstrated also that splenic weight increased to 11 times that of the contracted spleen after splenic denervation, when the splenic veins were ligated.

These data clearly indicate that the spleen can change its blood volume.

Lewis, et al. (1942), found rapid and sustained contraction of the dog spleen during hypotension which was severe enough to produce dynamic and pathological signs of shock. They concluded that the spleen functions to increase venous return and thereby to increase cardiac output. Guntheroth, et al. (1963), investigated the blood reservoir functions of the spleen and liver. Changes in the diameter of these organs were measured in response to hemorrhage, hypoxia, and intravenous injection of epinephrine. They also measured changes in splenic vein flow, splenic venous hematocrit and arterial hematocrit, during hemorrhage and injections of epinephrine (Guntheroth, 1967). They found that there were prompt splenic contractions, that systemic hematocrit initially increased during hemorrhage and that hypoxia caused vigorous splenic contraction. They found also that splenic diameter decreased and systemic hematocrit increased after injection of epinephrine; these responses were maintained for at least 10 min. These results indicate that the spleen is important in increasing systemic hematocrit and suggest that this is caused by activation of sympathetic nerves.

Greenway, et al. (1968), electrically stimulated splenic nerves and measured splenic arterial blood flow and splenic weight. They found that maximal flow and weight changes occurred at a stimulus frequency of 3 impulses per second. Splenic weight remained decreased throughout a 2 hour stimulation period, but splenic arterial flow returned to control levels during this time. In a later study, Greenway and Lister (1974) examined splanchnic vascular resistance and the blood reservoir function of the spleen. They showed that there was splenic pooling of blood during a 10-34%, but not during a 6% increase in blood volume. They

found no change in splenic weight with a 4% blood volume loss, but with a more severe hemorrhage (15% blood volume) splenic weight decreased. The more rapid the hemorrhage, the larger the decrease in splenic weight. These data indicate that there are changes in splenic volume which are rate sensitive and that tonic splenic nerve activity is important in establishing splenic muscular tone.

Tkachenko, et al. (1976), simultaneously measured splenic efferent nerve activity, splenic arterial pressure and splenic venous pressure to determine the effect of sympathetic efferent nerve activity on the resistive and capacitive vessels during bilateral carotid occulusion. They found splenic nerve activity and splenic arterial pressure always increased in response to bilateral carotid occlusion, although both vasoconstriction and vasodilation occurred in splenic veins. They suggested that high (above 15 V) and low (15 V and lower) amplitude efferent impulses are associated with the control of resistance and capacitance vessels respectively, and that splenic nerves have an effect on both the resistive and capacitive functions of the spleen. Shoukas, et al. (1981), measured splenic and total body blood volume shifts during activation of the carotid sinus baroreceptor reflex. They compared the blood volume changes caused by variations in carotid sinus pressure before and after splenic isolation or splenectomy, and found no significant difference in total body vascular capacitance with or without the spleen. It was concluded that the spleen is not the major reservoir for blood volume shifts during the carotid sinus baroreceptor reflex.

Changes in splenic volume are under neural control and seem to compensate for changes in circlulating blood volume, but is not the

major reservior. The spleen is an elastic organ which functions to change circulating blood volume or to store blood when there are large blood volume increases. These changes in splenic blood volume are regulated by splenic efferent nerve activity.

Bladder

Distension of the urinary bladder has been long known to increase systemic arterial pressure reflexly. In 1928, Barrington (1928) identified seven reflexes involved in micturition. These are triggered by: 1.) activating the pelvic afferent nerves which are sensitive to bladder wall stretch to increase activity in pelvic efferent nerves and cause vesicle contraction; 2.) activating pudendal afferent fibers by running liquid through the urethra, thereby reflexly increasing activity in pelvic efferent nerves to result in vesicle contraction; 3.) distending the proximal urethra to activate hypogastric afferents and reflexly excite hypogastric efferent nerves to cause vesicle contraction; 4.) activating pudendal afferents by running liquid through the urethra also results in relaxation of the urethra by inhibiting pudendal nerve activity; 5.) distending the bladder to stimulate pelvic afferents which reflexly alter pudendal efferent nerve discharge and cause relaxation of the external sphincter; 6.) distending the bladder, which initiates a pelvic-pelvic reflex to relax the smooth muscle of the urethra; 7.) flowing liquid through the urethra, which also initiates a pelvic-pelvic reflex to stimulate vesicle contraction. These many reflexes are involved in micturition, and they assure emptying of the bladder.

The afferent fibers which initiate micturition are sensitive to many different modalities. Barrington (1928) found bladder afferents to be sensitive to the rate of distension, to bladder wall tension, to fluid in the urethra, to the temperature of fluid in the bladder and urethra as well as to pain. Floyd, et al. (1982), determined that the threshold pressure needed to activate reflexly hypogastric efferent fibers is greater than that to activate pelvic efferent fibers.

Kuru (1965), discusses the different modalities that excite bladder afferent nerves in his review on neural control of micturition. Bladder afferents are sensitive to bladder position, to pain, to temperature and to tension. There are both rapidly and slowly adapting tension receptors which are sensitive to vesical pressure. Afferents activated by bladder distension discharge in response to vesicle contraction in one of three ways: 1.) by steady discharge, 2.) by discharge during the initial stage of the contraction or 3.) by inhibition during the contraction. Bladder afferents are stimulated by several different modalities, have specific discharge patterns and initiate many different reflexes.

Mukherjee (1957), observed a small decrease in total kidney volume and a small rise in mean arterial blood pressure in response to bladder distension. After vagotomy and severing of both carotid sinus nerves, this effect increased. The renal vasoconstriction and blood pressure increase initiated by bladder distension were abolished after bilateral splanchnicotomy. This demonstrates the existance of a viscerovascular reflex, and implies that there is involvement of renal and possibly of splenic efferent nerves.

Reflexes initiated by bladder distension involve both spinal

(Wurster and Randall, 1975; deGroat, 1971) and supraspinal pathways (de Groat, 1976; Schondorf, 1983). de Groat (1971) and de Groat and Lalley (1972), showed both an inhibitory supraspinal reflex and an excitatory spinal reflex. In a later study (deGroat and Lalley, 1972), they found two distinct populations of lumbar preganglionic neurons; vesicle contraction excited one population and inhibited the other.

Laskey, et al. (1979), and Schondorf, et al. (1983), using both electrical and physiological stimulation, showed that pelvic afferents in a spinal animal reflexly activate fibers up to 10 spinal segments away. They recorded activity of single nerve fibers in the cervical sympathetic trunk while stimulating pelvic, radial and femoral afferent nerves and found the same precentage of fibers responding during pelvic and radial nerve stimulation. The pelvic nerve required a greater stimulus intensity, however, to elicit a reflex response. Schondorf (1983) studied these reflexes in intact and in decerebrate animals and showed both a supraspinal and spinal component with either electrical or physiological stimulation. Distension of the bladder is known to increase arterial pressure and renal efferent nerve activity. The excitatory spinal reflex is normally modulated by the inhibitory supraspinal reflex. This reflex has been shown to cause large blood pressure changes in paraplegics (Wurster and Randall, 1976).

Other Distensions

de Groat and Krier investigated intestinal motility (1978, 1979; de Groat et al., 1979) and found that mechanoreceptive afferent fibers in the pelvic and hypogastric nerves are important in initiating the

defecation reflex. Organization of this reflex is within the sacral spinal cord, and tonic activity in the lumbar colonic nerve has an inhibitory affect on motility.

Floyd, et al. (1982), recorded activity in hypogastric single fibers innervating the bladder, and measured the changes in discharge rate due to distension of the bladder and colon. They found that 50% of the fibers increased and 10% decreased their discharge rate during distension of the urinary bladder. In response to colon distension, 75% of the fibers increased their discharge rate. This is evidence for convergence of bladder and colon afferents onto bladder hypogastric efferent nerves.

Colonic afferents are not only important in regulating defecation but are also important in initiating micturition. Both the defecation and micturition reflexes involve spinal and supraspinal pathways.

Ninomiya, et al. (1974), simultaneously recorded renal and intestinal nerve activity, as well as intestinal pressure, and compared the responses of these two nerves to sinusoidal distension of the innervated intestinal segment. They found that intestinal nerve activity increased more than did renal during the distension and showed also that tonic intestinal nerve discharge was synchronous with intestinal pressure changes, but renal discharge was not. This is another example of differential responses and also shows differences in tonic nerve discharge to these organs.

Chemoreceptors

Pelletier and Shepherd (1972) stimulated arterial chemoreceptors by perfusing bilaterally isolated carotid sinus regions with hypoxic, hypercapnic or hypoxic-hypercapnic blood. In response to chemoreceptor stimulation, splenic venous and aortic pressures increased but cutaneous venous pressure decreased, indicating that chemoreceptor activation results in differential responses in two regionally different capacitance vascular beds.

Little and Öberg (1975) stimulated arterial chemoreceptors similarly by perfusing carotid sinus regions with venous blood and also electrically stimulated vasomotor nerve fibers to the kidney, intestine, skeletal muscle and skin while arterial blood flow was measured with a drop counter technique. They found that renal nerve activity was increased less than that in other beds. These data are similar to those of Pelletier and Shepherd, in that they demonstrate differential responses to arterial chemoreceptor stimulation.

Arterial chemoreceptor stimulation may produce either tachycardia or bradycardia. Kollai and Koizumi (1977) simultaneously recorded inferior cardiac and vertebral nerve activity, heart rate and forearm blood flow while arterial chemoreceptors were stimulated in a variety of ways. They found that cardiac nerve activity was inhibited while vertebral nerve activity was increased. These changes in nerve activity corresponded to observed decreases in forearm blood flow and heart rate, indicating that differential vasomotor responses are caused by changes in sympathetic nerve activity. Gregor and Jänig (1977) investigated the effects of hypoxia and hypercapnia on cutaneous and muscle

vasoconstrictor fibers. They found that most cutaneous nerve fibers decreased their firing rate, while most muscle fibers increased theirs in response to hypoxia or hypercapnia, providing even more evidence for a differential reflex caused by chemoreceptor stimulation. Hilton and Marshall (1982) examined cardiovascular responses to carotid chemoreceptor stimulation by measuring blood flow in renal, mesenteric, skeletal muscle and cutaneous arteries. They found there was vasodilation in skeletal muscle, but vasoconstriction in renal and the other arteries.

Baroreceptors

Both arterial pressure and its rate of change affect sympathetic discharge, which has been shown to be related to neural activity in baroreceptor afferent nerves. Ninomiya (1967) found a linear relationship between baroreceptor activity and mean blood pressure in a range from 80 to 240 mmHg. Further, Kezdi and Gellar (1968) demonstrated that postganglionic sympathetic nerve activity is inversely and linearly related to static pressure changes within a physiological range. These data provide information about baroreceptor responses without directly recording carotid sinus nerve activity. Ninomiya and Irisawa (1969) investigated the combined and separate effects that the 4 individual baroreceptor afferents have on renal efferent discharge. After randomly selecting and severing one of these 4 afferents, blood pressure was altered, while changes in renal efferent discharge were measured. This was repeated until all 4 nerves had been severed. They found that the sum of the individual inhibitory effects was larger than

the combined effect of all 4 nerves and that the left carotid sinus nerve had a stronger inhibitory effect on efferent nerve discharge. Guo, et al. (1982), also studied the relative roles of carotid sius, aortic depressor and cardiopulmonary baroreceptors on heart rate and hindlimb vascular resistance. They found that carotid sinus or aortic baroreceptors compensate in the absences of the other to decrease vascular resistance and therefore inhibit sympathetic sympathetic nerve activity. The baroreceptor afferents inhibit sympathetic efferent nerve activity, and there is apparently a compensating mechanism when a baroreceptor afferent nerve or receptor is damaged.

The effects of baroreceptor activity have been measured in a variety of vascular beds. Bond and Green (1969) measured renal, visceral, myocardial, cutaneous and skeletal muscle blood flow during bilateral common carotid artery occlusion. Carotid occlusion produced vasoconstriction in renal, visceral and skeletal muscle vascular beds, indicating an increase in sympathetic activity to them. Also Öberg and White (1970) measured changes in renal and skeletal muscle blood flow during moderate hemorrhage. There was no difference in blood flow changes in renal and skeletal muscle vascular beds when the baroreceptor reflex was activated, implying a uniform response to baroreceptor stimulation.

Similar studies have been made using an isolated carotid sinus, rather than the intact animal. Brender and Webb-Peploe (1969) used an isolated carotid sinus to study the effects of decreasing carotid sinus pressure on splenic capsule contraction, splenic venous resistance, limb vessel resistance and saphenous vein pressure. They found that splenic vein and limb resistance increased when sinus pressure decreased,

indicating an increase in splenic and hindlimb vasomotor nerve activity. Comparing the pressure changes in each vessel during decreased sinus pressure to those during electrical stimulation, they concluded that reflex excitation in the vasomotor nerve to the hindlimb was greater than that in splenic nerves. This would suggest a differential response to the baroreceptor reflex. Ninomiya, et al. (1971), simultaneously recorded activity in several nerves while baroreceptors were stimulated in different ways. They found that splenic nerves have a higher gain than do renal nerves and that splenic nerve activity is inhibited at a lower threshold pressure than is that for the renal nerve.

Kendrick, et al. (1972a), investigated differential blood flow responses to baroreceptor stimulation in renal and skeletal muscle vascular beds and found that reflex vasoconstriction in the kidney was less than that in skeletal muscle. After comparing this vasoconstriction to that induced by electrical stimulation, they determined that renal nerves were excited less by baroreceptor "unloading" than were skeletal muscle vasomotor nerves. In a later study (Kendrick et al., 1972b), they measured vascular resistance changes in a skeletal muscle vessel and in a renal artery when the isolated sinus was exposed to a range of pressures. They determined that threshold responses and maximal sensitivities were the same in both vascular beds. They found also that the response curves for renal and skeletal muscle vessels were identical when sinus pressures were changed using pulsatile flow stimuli. These results indicate that there are uniform responses to activation of the baroreceptor reflex.

There is still some disagreement, however, as to whether activation of the baroreceptor reflex produces a differential response in

sympathetic nerve activity. Irisawa and coworkers, found opposing responses to baroreceptor stimulation (1973), but there are others who suggest the baroreceptor reflex has a uniform inhibitory effect on sympathetic nerve activity (Iriki and Korner, 1979; Kendrick et al., 1972b).

Simultaneous Baro and Chemoreceptor Activation

Baroreceptor influence on the chemoreceptor reflex is important because stimulation of chemoreceptors increases blood pressure. When hypotension and hypoxia occur at the same time baroreceptors and chemoreceptors are often activated simultaneously. Heistad, et al. (1974), measured changes in perfusion pressure in the innervated gracilis muscle while activating both arterial baroreceptors and chemoreceptors to determine if the degree of baroreceptor activation affected the responses to chemoreceptor stimulation. Baroreceptor afferents were either attenuated by hemorrhage or stimulated by aortic occlusion. Carotid chemoreceptors were stimulated either by injecting nicotine into the common carotid artery, or by perfusing an isolated sinus with hypoxic-hypercapnic blood. To activate baroreceptor and chemoreceptors simultaneously, they stimulated baroreceptors in one sinus and chemoreceptors in the other. They found that the chemoreceptor responses were potentiated during hypotension and inibited during hypertension using either chemoreceptor stimulation method. These data suggest a central interaction of the two reflexes.

Mancia (1975) studied the effects of baroreceptor stimulation on responses to chemoreceptor activation in hind-limb and renal resistance

vessels and in cutaneous veins. He chose these vessels because the baroreceptors have been shown to have a non-uniform effect on them. He perfused isolated carotid sinus regions at three different constant pressures, and stimulated chemoreceptors by perfusing the sinus with hypoxic-hypercapnic blood. At an intermediate pressure, chemoreceptor stimulation decreased renal blood flow by 20% and hind-limb flow by 59%, suggesting differential responses in the two vessels. At both high and low sinus pressures, however, the response to chemoreceptor stimulation was diminished, thereby indicating a central interaction between baroreceptor and chemoreceptor inputs.

Wennergren, et al. (1976), perfused carotid sinus regions with venous blood, and recorded changes in renal and skeletal muscle blood flow while varying perfusion pressure from 50 to 220 mmHg. When sinus pressure was high, the vascular responses to chemoreceptor stimulation were eliminated, and at low sinus presures they were exaggerated. This further demonstrated increased gain and set point of the baroreceptor reflex during simultaneous chemoreceptor activation. These effects might be due to a "mutual summation" of chemoreceptor excitatory and bareceptor inhibitory inputs on a common neuronal pool.

These blood flow studies indicate there may be two neural pathways for the simultaneous activation of the two reflexes. One is that baroreceptors and chemoreceptors have independent inputs that synapse with a common central neuron pool. The other is that the inputs from either chemoreceptor or baroreceptor afferents is dependent upon the level of stimulation of the other receptor.

Information about the effect of simultaneous activation on efferent nerves can be obtained most directly only from neural recordings. Iriki,

et al. (1977, Iriki and Korner, 1979), simultantously recorded renal and cardiac nerve activity while changing arterial pressure during normal breathing and hypoxia. They found that arterial hypoxia affects the cardiac nerve baroreceptor response curve by decreasing the gain and range of the reflex, but opposite affects are indicated by the renal nerve response curve. The range of shmpathetic nerve activity , median blood pressure and gain of the baroreceptor curve were changed in both nerves during hypoxia. These results indicate a complex interaction of baroreceptor and chemoreceptor reflexes. The simultaneous activation of barorecptor and chemoreceptor reflexes produces differential responses in renal and splenic nerves, and involves a dependent relationship between the two reflexes.

METHODS

GENERAL METHODS

Thirty-five healthy adult cats of either sex were anesthetized with 60 to 80 mg/kg of alpha chloralose (Sigma Chemical Co., St.Louis, Mo) administered intravenously. The right brachial and/or femoral arteries and veins were cannulated, and the arterial cannula was connected to a pressure transducer (Model P23A; Statham, Inc., Hato Rey, PR) to monitor systemic arterial blood pressure which was displayed on a Grass polygraph Model 7D and recorded on magnetic tape. The venous cannula was used to administer drugs and to obtain blood samples. It also served as a site for drip infusion of lactated Ringers solution to maintain body fluid and electrolyte balance. A tracheal cannula was inserted and connected to a dual phase Harvard respirator (Model #665, Harvard Apparatus Co., South Natick, MA). A needle was inserted into the rubber tubing that connected the tracheal cannula and the respirator to monitor end tidal CO_2 fractions (Model 901 MK 2, P.K. Morgan, Ltd., Chatham, Kent, England). Respiratory frequency and tidal volume were adjusted to maintain end tidal CO_2 fractions between 3.5 and 4.0%. Once the tracheal cannula was connected to the respirator, a muscle relaxant was intravenously injected (4 mg/kg: gallamine triethiodide, Flaxedil;

Davis-Geck, Pearl River, New York, NY) and supplemented as required (1-2 mg/kg).

The animal's body temperature was monitored by an esophageal thermistor probe (Telethermometer Model 43TD; Yellow Springs Instrument Co., Yellow Springs, OH) and was maintained between 36.5 and 37.5° C by manual adjustment of a heating pad (Aquamatic K Module Model K-20; Hamilton Industries, Cincinnati, OH) and an infared heating lamp.

An incision was made in the animal's left flank to expose the kidney, greater splanchnic nerve and celiac plexus. Both renal and splenic nerves were dissected from the celiac (plexus) ganglion to their respective artery, and freed of connective tissue. If a renal ganglion was observed, a portion of the nerve distal to it was dissected. Each nerve was ligated with silk suture (Ethicon, Inc., Somerville, NJ) and cut distal to the tie (Figure 1). Noise artifacts in nerve recordings were minimized by pneumothorax and by stablizing abdominal viscera with sutures.

Neural Recordings

Dehydration of the nerves and other exposed tissue was prevented by filling the abdomen with mineral oil. The ligated nerves were placed on bipolar platinium electrodes for simultaneously recording multifiber nerve activity.

Each electrode was connected through a high impedance probe (model HI P511E; Grass Instruments, Quincy, MA) to a Grass P511 preamplifier (Grass Instruments, Quincy, MA) with a recording bandwidth setting of 30

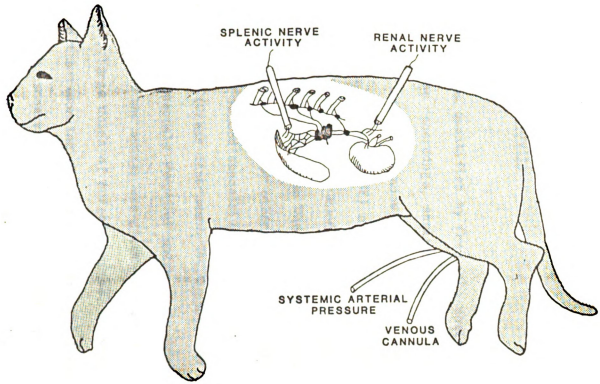


Figure 1. General methods. Nerve recording positions and techniques are indicated by this figure. Femoral or brachial arteries were cannulated to monitor mean arterial pressure and veins for drawing blood samples or infusing drugs.

Hz to 3 KHz. Amplified voltages were recorded on magnetic tape (Tandberg recorder, series 100; or Sangamo Sabre III; Sangamo Western, Sarasota, FL), as well as monitored visually (oscilloscopes: model 5100 series; Tektronix, Inc., Beaverton, OR) and audibly (Model AM 8 audio monitor; Grass Instruments, Quincy, Ma).

Quantification of nerve activity

Nerve activity was quantified by frequency analysis and by voltage integration. For some recordings, analysis of action potential spike dicharges were made using a window discriminator (Model 40-75-1, Frederick Haer & Co., Brunswick, ME) and a rate interval analyzer (Model 74-40-1, Frederick Haer & Co., Brunswick, ME) so that a voltage was generated which was proportional to the number of spikes recorded during sequential 2 second periods. For others, amplified voltages were summed during either 1 or 10 second successive periods with a calibrated Grass Model 7P10 integrator (Grass Instrument Co.,Quincy, MA). All data were corrected for simultaneously recorded noise artifacts by analyzing records obtained when the animal was given a ganglionic blocking agent (hexamethonium), large doses of norepinephrine, or when the nerve was cut central to the recording electrode at the end of each experiment. For quantification of nerve activity either by frequency analysis or by integration, the polygraph record was analyzed with the use of a PDP-11 computer (Digital Equipment Corporation), and a sonic digitizer. Records were evaluated separately for control, experimental and recovery periods.

Statistical Analysis

Differences between corresponding data were examinied using an analysis of variance test with a complete block design (Steele and Torrie, 1960). Mean values were compared with a least significant difference test. Differences were considered significant when $p < 0.05$. Variability was expressed by a standard error of the mean.

SPECIFIC METHODS

Bladder Distension

Bladder distension was used as a stimulus to compare renal and splenic nerve activities. In 14 cats, a mid-abdominal incision was made to expose the bladder. Then, a small incision was made in the bladder wall, a balloon-tipped catheter inserted through the incision and secured with a purse string suture. Carotid sinus and aortic depressor nerves were severed in 4 cats; of the remaining 10 cats, 4 were vagotomized and 6 had no denervations. In 8 cats, a T-tube connected to a Windkessel bottle was inserted into the aorta distal to the renal artery in order to stabilize blood pressure changes. A three-way stopcock was connected to the open end of balloon-tipped catheter to monitor continuously intravesicular pressure (pressure transducer Statham P23ID, Gould Inc., Medical Instruments Division, Oxford, CA). The bladder was distended for 30-120 sec by inflating the balloon with 10-20 ml of air.

Surgical trauma to the vesicle was avoided in 4 other cats by

inserting a double lumen catheter through the urethra and advancing it 5-7 cm into the bladder. One end of the catheter was connected to a pressure transducer, and the other was left open for urine drainage or infusion of 5-10 ml warmed saline which was just enough to initiate a bladder contraction. Bladder pressure was recorded on the Grass polygraph and nerve activity was quantified by the integration technique.

Duodenal Distension

Duodenal distension was used as a test to determine if there were differential responses in renal and splenic nerves. In 11 cats, 2 vagotomized and 9 intact, a mid-abdominal incision was made to expose the intestine. The duodenum was temporarily displaced from the abdomen, and a small incision was made in the duodenal wall so that a balloon tipped catheter could be inserted and secured with a purse string suture. A three-way stopcock was connected to the exposed open end of the catheter, which was in turn connected to a pressure transducer (Stathum P2ID, Gould, Inc., Medical Division, Oxford, CA) to allow duodenal pressure measurements and balloon inflation. The balloon was inflated for 90 sec by injecting 20 ml of air from a syringe.

Colon Distension

The colon was distended to test for colonic-renal reflexes or colonic-splenic reflexes. In 5 cats, a 2.5 mm balloon-tipped catheter was inserted through the anal canal and advanced approximately 4 cm into

the proximal colon. Colonic pressure was measured and displayed, as described previously for duodenal distension. The balloon was inflated (3-5 ml air) and the distension maintained for 20-60 sec.

Chemoreceptor stimulation

The left lingual artery was cannulated with PE 50 in 11 cats for injection of either lobeline (30 μg), sodium cyanide (50 μg) or .1-.4 ml of venous blood. A 1 ml anaerobic venous blood sample was drawn from the femoral or brachial vein, and the collecting syringe was connected to the left lingual cannula. First, 0.2 ml of venous blood was injected to fill the cannula, and then one to two minutes later, an equal volume of venous blood was injected, and the procedure repeated until the 1 ml sample had been depleted. This procedure was followed when the sinus was normally perfused, and when it was occluded or pressurized. In three cats, the right carotid sinus nerve was intact and the right lingual artery was also cannulated to allow comparisons of the right and left chemoreceptor afferent stimulation, as well as allowing simultaneous stimulation in one cat of both chemoreceptors and baroreceptors.

The confidence interval ($p < 0.05$) for integrated nerve activity during the 20 second control period immediately preceeding the injection was calculated (Steele and Torrie, 1960). The response was determined to be excitatory when integrated nerve activity exceeded the upper confidence limit and inhibitory when integrated nerve activity was less than the lower confidence limit.

Baroreceptor Stimulation

In 16 cats, baroreceptors were stimulated by systemic injections of norepinephrine (levophed bitartrate, Breon Laboratories, Inc., New York, NY). Only 2 of the 16 cats were vagotomized; the remainder were intact. Norepinephrine was infused to cause small (< 50mmHg) changes in systemic pressure, but was not enough to inhibit completely sympathetic efferent nerve activity. Nerve activity was quantified by frequency analysis in 10 cats and by integration in the remaining 6.

Carotid sinus preparations (Figure 2) were used in 12 cats to stimulate arterial baroreceptors. Right and left aortic depressor nerves were severed at their junction with the respective superior laryngeal nerves as they joined the vagal nerves. In 8 of the 12 cats, the right carotid sinus nerve was also severed to preclude reflex activation of the contralateral baroreceptors. The external carotid artery was cannulated (PE 160 tubing, Clay Adams division of Beckton Dickinson Company, Parssipany,NJ) and connected externally to a T-connector, one branch of which was connected to a pressure transducer, with the other branch connected to a heparinized saline filled reservoir. The reservoir was connected to a pressurized room air tank so that various pressures could be applied to the carotid sinus. A fluid-filled occluder (Model VO-3, Rhodes Medical Instruments, Inc., Woodland Hills, CA) was placed around the common carotid artery. The lingual artery was cannulated for chemoreceptor stimulation. The carotid sinus was stimulated by first occluding the carotid artery for 30 seconds, then opening the connection to the pressurized reservoir for

30–40 seconds, and finally by reversing this procedure so the sinus was again perfused with circulating blood.

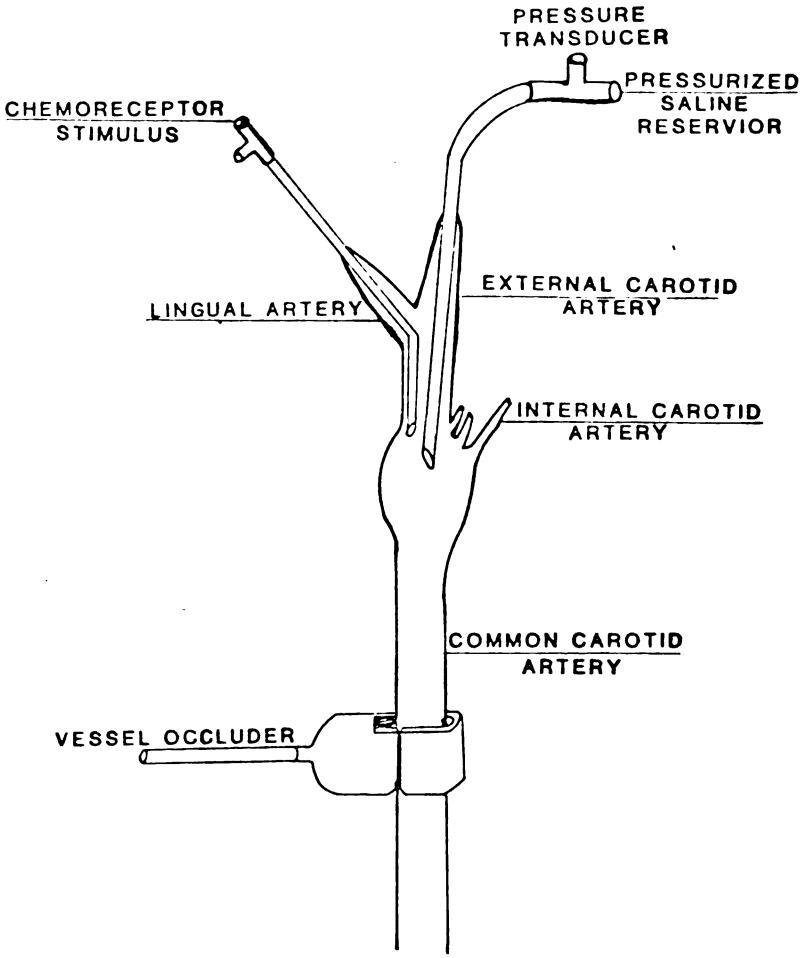


Figure 2. Carotid sinus preparation. A diagram of the preparation used to stimulate carotid sinus baroreceptors and chemoreceptors.

RESULTS

Bladder distension

Distension of the urinary bladder increased renal and splenic nerve activity, and systemic arterial pressure. The bladder in this group of cats was distended by inflating a balloon-tipped catheter that had been inserted through an incision in the bladder wall. Nerve activity was quantified by the frequency analysis technique during the response shown in Figure 3. In response to the distension, renal nerve discharge increased by 183%, but splenic nerve activity increased only by 93%. Systemic arterial presssure increased 35 mmHg. The initial rapid increase in both renal and splenic nerve activity indicates that there is a rate sensitive component of the reflex, as well as the sustained increase through out the 88 sec distension (bladder pressure 136 cm H_2O). Both renal and splenic nerve activity increased significantly above control discharge rate, as shown in Figure 4 (left panel). Mean control discharge rate of the two nerves was the same (renal 51, splenic 45 counts/sec), so that comparisons of the changes in nerve activity between the two nerves could be made. Figure 5 shows the mean maximum change in renal (16 counts) and splenic (8 counts) nerve activity during bladder distension. Renal nerve activity increased significantly more than did splenic nerve activity in response to the distension, as shown in the left panel (Figure 5).

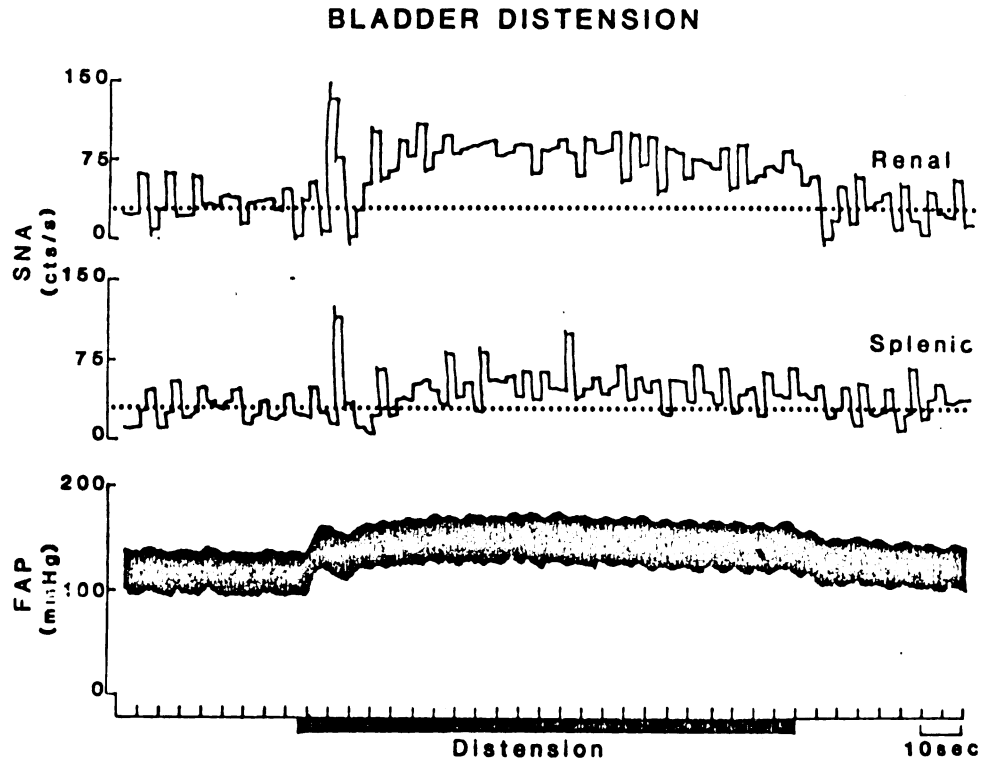


Figure 3. Bladder distension. Records of renal (top trace) and splenic (middle trace) nerve activity were quantified by frequency analysis (counts/sec) during distension of the urinary bladder. The percent change in renal nerve activity was 183%, and that for the splenic nerve, 93%. Femoral arterial pressure (bottom trace) increased 35 mmHg during the 88 sec distension (darkened area below the time line) at a pressure of 136 cm H_2O.

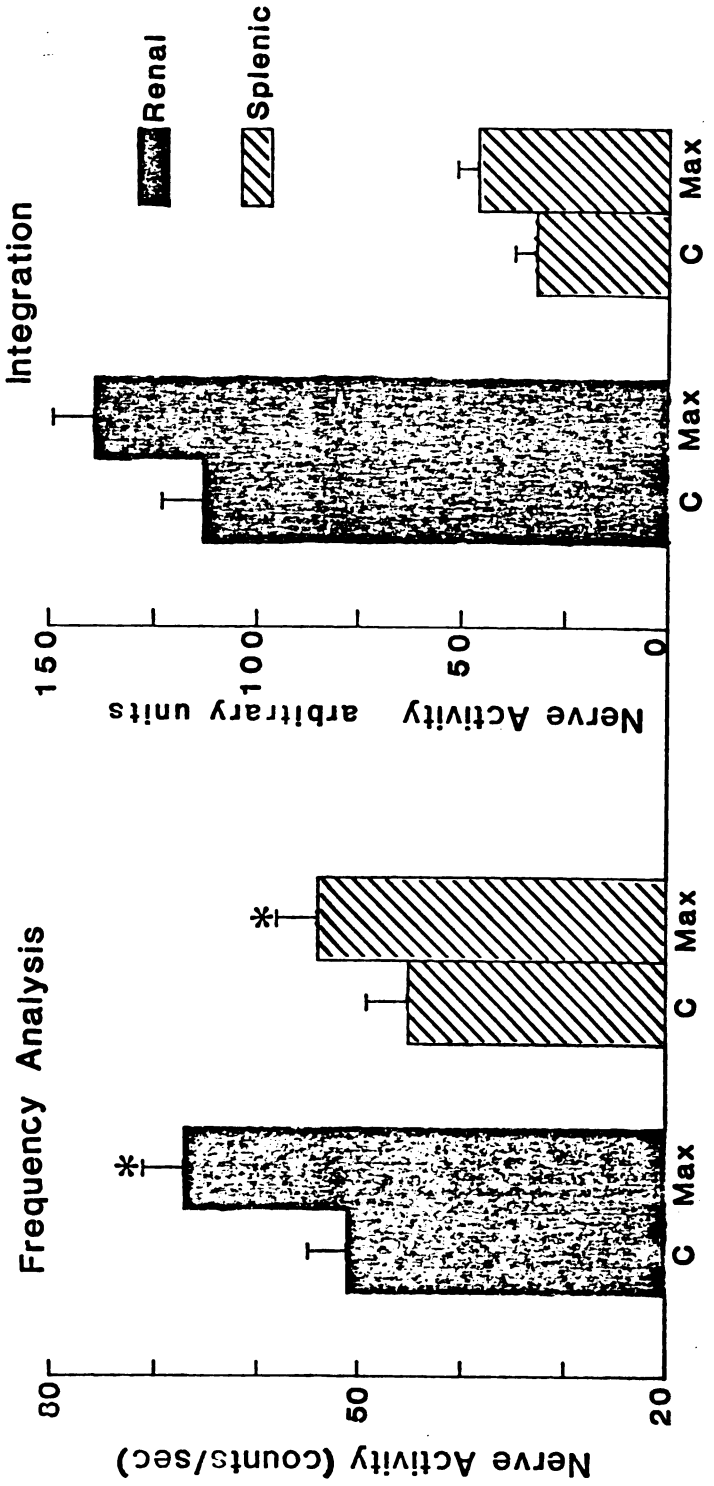


Figure 4. Bladder distension maximum responses. Mean maximum responses (Max) of renal (black area) and splenic (striped) nerves to urinary bladder distension are compared to control values (C). Responses significantly different from control are indicated by asterisks. Standard errors are indicated by the lines above the bar graphs. Left panel: Nerve activity was quantified by frequency analysis in 10 cats. Standard errors (renal SE=4; splenic SE=4) are indicated by the line above each bar. Right panel: nerve activity was quantified by integration in 4 cats. Standard errors for renal nerve responses =10 and that for splenic responses =5.

The changes in nerve activity of each cat are shown in the right panel of Figure 5. In 10 cats, the percent change in renal nerve activity was greater than that in splenic nerve activity. In three of these cats, splenic nerve activity decreased in response to the distension. Average bladder pressure during distension was 163 ± 58cm H_2O, and the average distension duration was 86 ± 38sec. Bladder distension caused a reflex mean increase in systemic arterial pressure of at least 23 ± 7mmHg (range 0-100mmHg).

Spontaneous bladder contractions were observed in 4 of 9 animals from this group. When spontaneous bladder contractions occurred, there was reflex excitation of both renal and splenic nerve activity, as shown by data reported in Figure 6. Even during spontaneous bladder contractions, the increase in renal nerve activity was greater than that in the splenic nerve. As shown by data in Figure 6 renal nerve activity changed by 50%, while splenic activity increased only by 19%; during the 20 sec contraction, and bladder pressure rose to 68 cm H_2O. In this group of cats, bladder pressure rose to 20-68 cm H_2O for 5-20 seconds during the spontaneous contraction. In four more cats, a cannula was inserted through the urethra and advanced into the bladder, saline was injected through the cannula to distend the bladder, and nerve activity was quantified by integration. Integrated nerve activity is reported in arbitrary units which have been corrected for differences in amplification of the two nerves. Data reported in the right panel of Figure 4 show that both renal and splenic nerve activity increased in response to bladder distension; mean bladder pressure was 95 ± 5cm H_2O and mean distension duration was 76 ± 19seconds. Neither renal or splenic nerve activity increased significantly above control

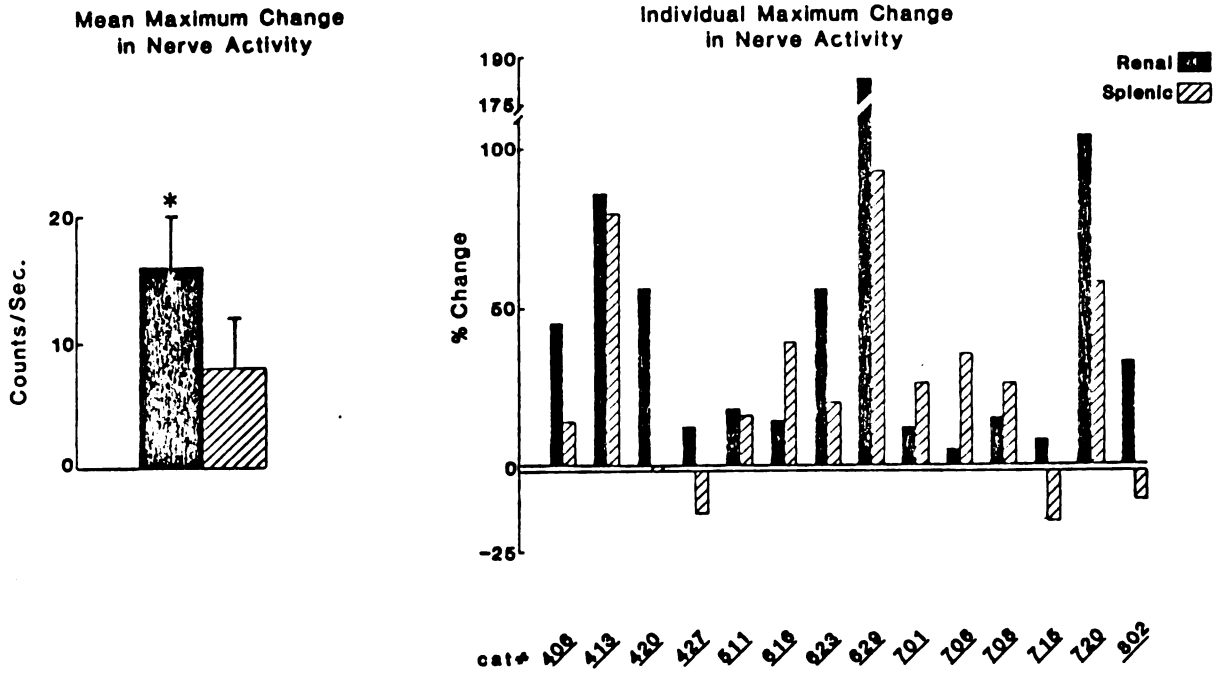


Figure 5. Comparisons of responses to bladder distension. Maximum change in renal (darkened area) and splenic (striped area) activity in response to bladder distension. Left panel: Mean change from control nerve discharge in response to bladder distension (SE=4). Asterisks indicate the change in renal nerve activity was significantly greater than that in splenic nerve activity. Right panel: Responses of individual cats expressed as a % change in nerve discharge. Each cat is identified by a 3 digit code.

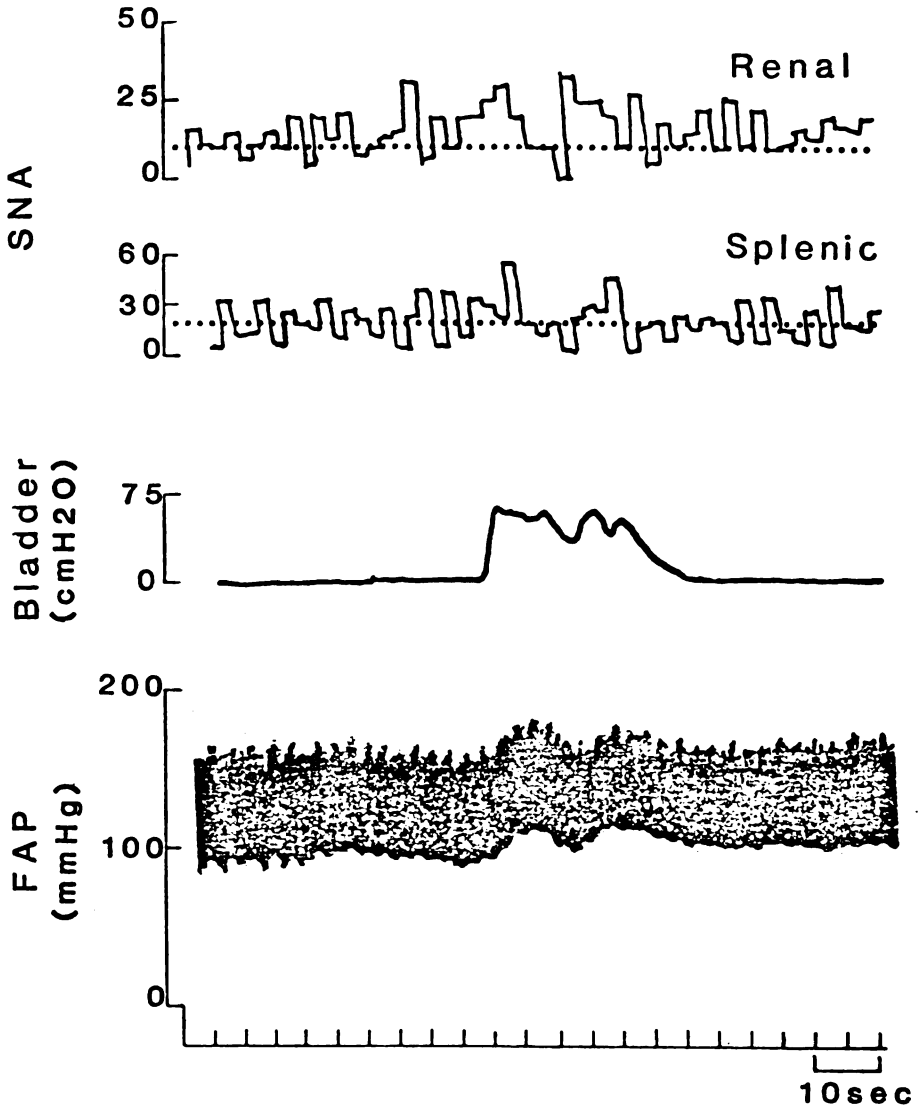


Figure 6. Spontaneous bladder contraction. Renal (50% change) and splenic (19% change) nerve activity (top 2 traces) is increased during spontaneous bladder contraction. Bladder pressure (center trace) rose to 68 cm H_2O during the 20 sec contraction.

values in this group. Spontaneous bladder contractions occurred in all 4 of these cats with bladder pressures rising as high as 102 cm H_2O and durations as long as 45 seconds.

Other distensions

In 11 cats, the duodenum was distended by inflating a balloon-tipped catheter that had been sutured in the duodenum. Nerve activity was quantified by frequency analysis in this group. The mean maximum responses of the two nerves to duodenal distension shown in Figure 7 indicate that splenic nerve discharge significantly increased by 23%, while renal nerve activity increased by 10%. The increase in renal nerve activity was not significantly different from control values. Mean maximum change in splenic nerve activity (13 counts) was greater than that of the renal nerve (6 counts), as shown in Figure 8. In 6 of the 11 cats, splenic nerve activity increased more than did that for the renal nerve when duodenal stretch receptors were activated. In 4 of these cats, renal nerve activity decreased, as shown by data reported in the right panel of Figure 8. Neither mean control discharge rate (renal 60, splenic 60 counts/sec) nor change in activity due to the response in the two nerves was significantly different from each other. The average duodenal pressure during distension was 140 $\pm$ 92cm H_2O, and average distension duration was 96 $\pm$ 26sec. The distension initiated a mean rise in systemic arterial pressure of 9 $\pm$ 4mmHg and as large as 40 mmHg.

In 5 cats the colon was distended by placing a balloon-tipped catheter in the proximal colon. Colon distension produced a bladder

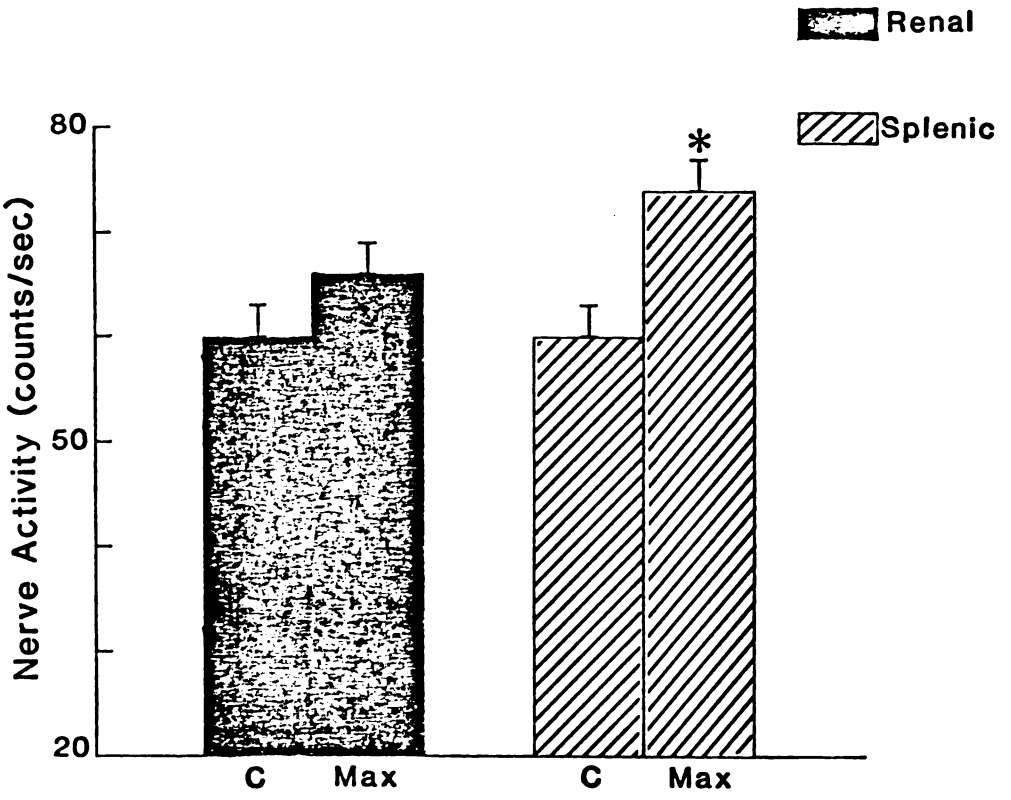
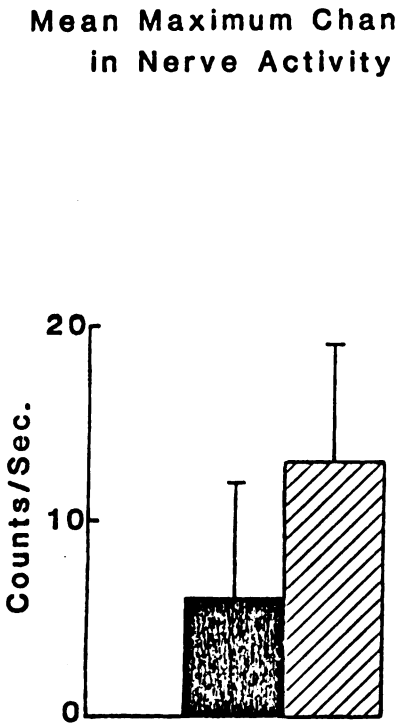


Figure 7. Duodenal distension maximum responses. Mean maximum (Max) responses of renal (darkened area) and splenic (striped area) to distension of the duodenum are compared to control (C) discharge values. Astericks indicated responses significantly different from control nerve discharge. Nerve activity was quantified by frequency analysis in these 11 cats. Standard errors for renal (SE=3) and splenic (SE=3) nerve responses are indicated by the lines above the bars.

Figure 8. Comparisons of responses to duodenal distension. Maximum change in renal (darkened areas) and splenic nerve activity in response to duodenal distension (mean distension pressure 140 +/- 92cm H_2O). Left panel: mean maximum change from control nerve discharge in response to duodenal distension (SE=6). Right panel: Responses of individual cats to duodenal distension expressed as a % change from control discharge values. Each cat is identified by a 3 digit code.


Mean Maximum Change
in Nerve Activity
Counts/Sec.
20
10
0

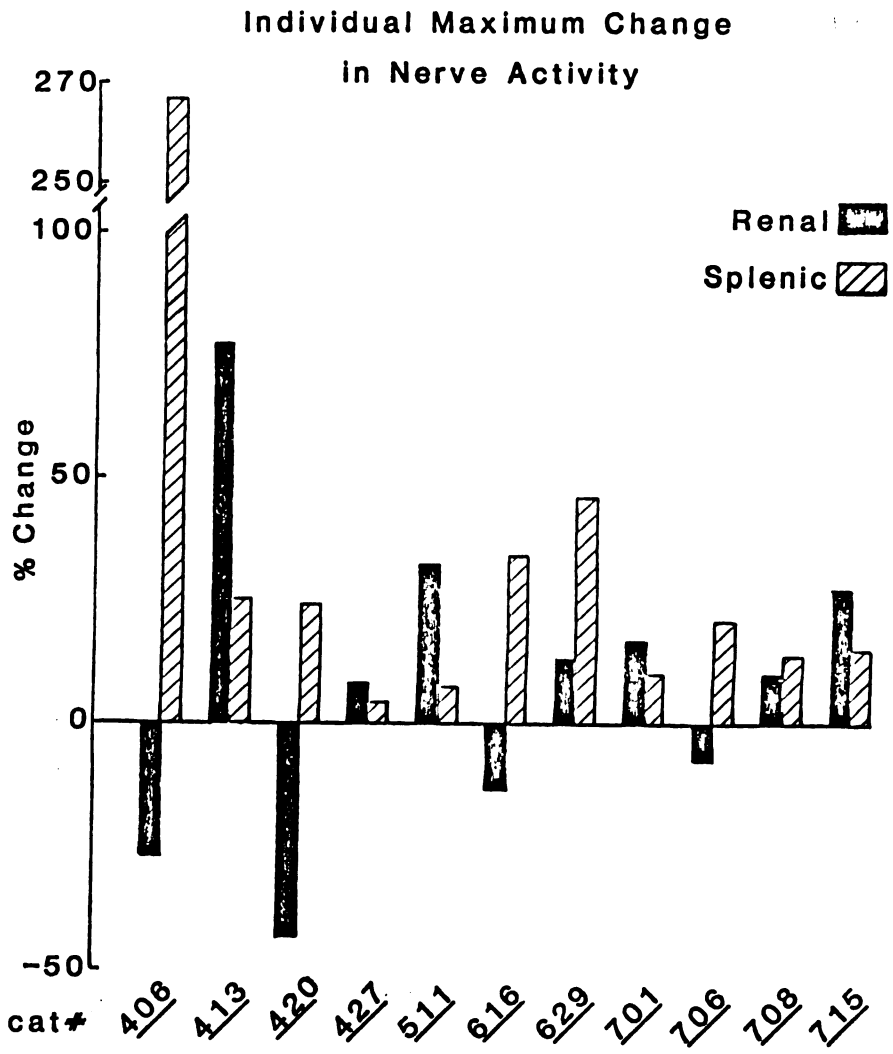

Individual Maximum Change
in Nerve Activity
% Change
270
250
100
50
0
-50
Renal
Splenic
cat#
406
413
420
427
511
616
629
701
706
708
715

Figure 8.

contraction and an increase in nerve activity in 3 cats. In the other 2 cats, distension did not initiate a bladder contraction, and there was no change in renal or splenic nerve activity. Therefore, the increase in nerve activity could not be distinguished from that caused by a bladder contraction. The average colon distension pressure was 56 ± 14cm H_2O, and the mean distension duration was 46 ± 15 sec.

Chemoreceptor stimulation

Carotid chemoreceptors were stimulated by injection of either venous blood, sodium cyanide or CO_2 saturated saline in 10 cats while nerve activity was quantified by integration. Venous blood injection was a repeatable and reliable carotid chemoreceptor stimulus, as shown by data reported in Figure 9 for which three 0.2 ml injections were given within a 5 minute period with the injections producing equivalent responses. Venous blood also is less toxic than sodium cyanide, and seemed to cause less deterioration of the animal. Seven of the 10 cats responded initially by increasing nerve activity and systemic pressure. Two cats responded by increasing systemic pressure only, and there was no response in only 1 cat. Chemoreceptor stimulation usually caused a biphasic response in both nerves; nerve activity increased for 3-8 sec, and then decreased for 2-5 sec. Mean responses of six cats are shown in Figure 10. In each cat, an average response was calculated from 3 or 4 repeated injections of a single blood sample. Renal nerve activity significantly increased above control levels and a few seconds later, significantly decreased below control values, in response to chemoreceptor stimulation. Significant responses were determined by

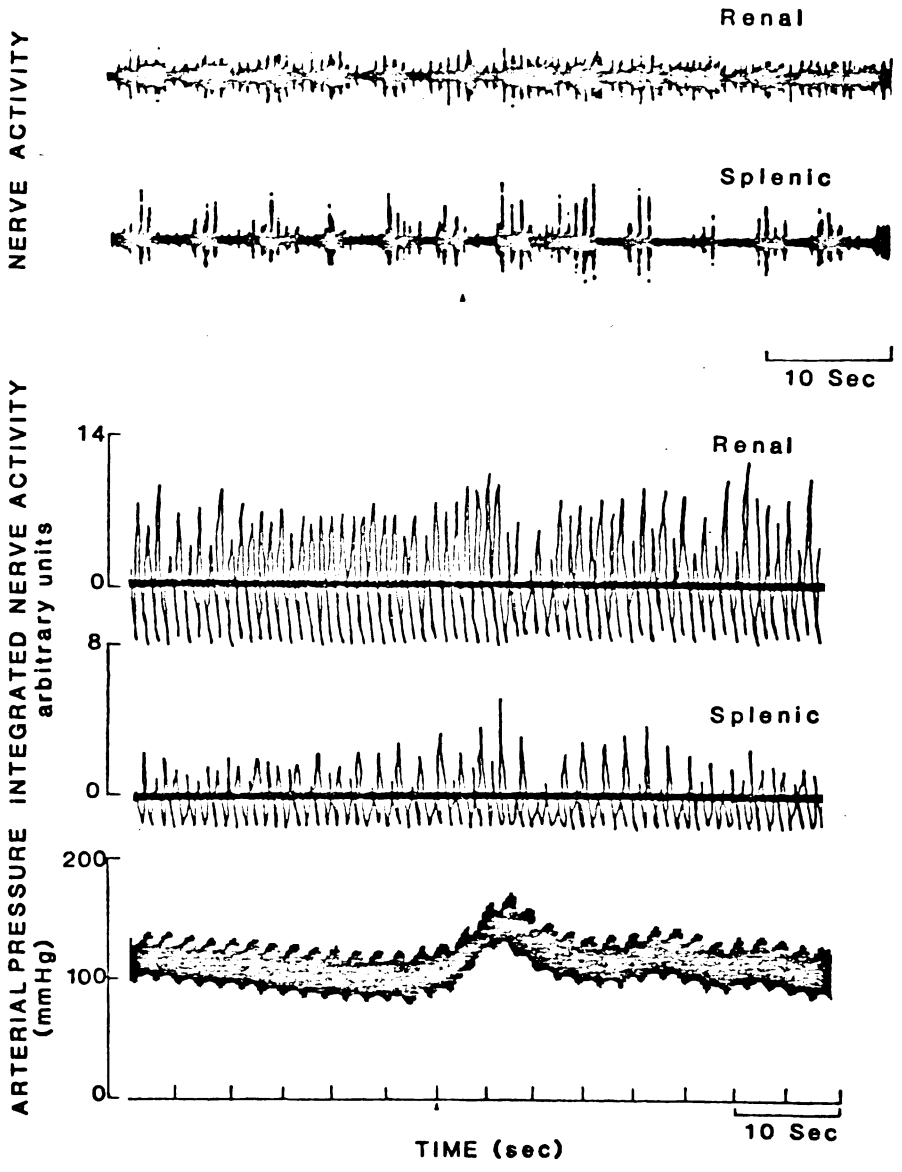


Figure 9. Chemoreceptor stimulation. Recordings of renal and splenic (amplified 2X that of renal) nerve activity, integrated nerve activity (middle) and systemic blood pressure (bottom), during an injection of 0.2ml of venous blood. Time of injection is indicated by the arrow on the time axis. The solid line indicates noise level. The arterial pressure recording indicates a 45 mmHg rise in pressure in response to venous blood injection.

calculating the confidence interval for the averaged control periods. Values that were greater than the upper confidence limit ($p < 0.05$) were determined to be significant increases and values that were less than the lower confidence limit were determined to be significant decreases. Splenic nerve activity increased above the upper confidence limit in individual cats, and later significantly decreased. The mean increase in splenic nerve activity for the 6 cats, however, was not significantly different from mean control values. The mean decrease in splenic nerve activity for the 6 cats was significantly different from mean control values. The mean control values of integrated renal nerve activity (4.59 units) was greater than that of splenic (1.67 units). There is no significant difference between the responses of renal and splenic nerves to activation of arterial chemoreceptors, as shown by data reported in the right panel of Figure 10. The ratio of renal to splenic nerve activity is not different from control (4.45) during either the excitatory (4.25) or inhibitory (4.47) periods. The mean percent of control for the renal nerve was 128% during excitation and 78% during inhibition, while the increase in splenic nerve activity was 138% of control, and the decrease 71%. This also indicates no difference in the responses of the two nerves. Chemoreceptor stimulation caused an average 22 $\pm$ 3mmHg increase in systemic pressure (range 5 to 50mmHg).

Baroreceptor stimulus

Arterial baroreceptors were stimulated by intravenous injection of norepinephrine and pressurization of the carotid sinus region. Data in

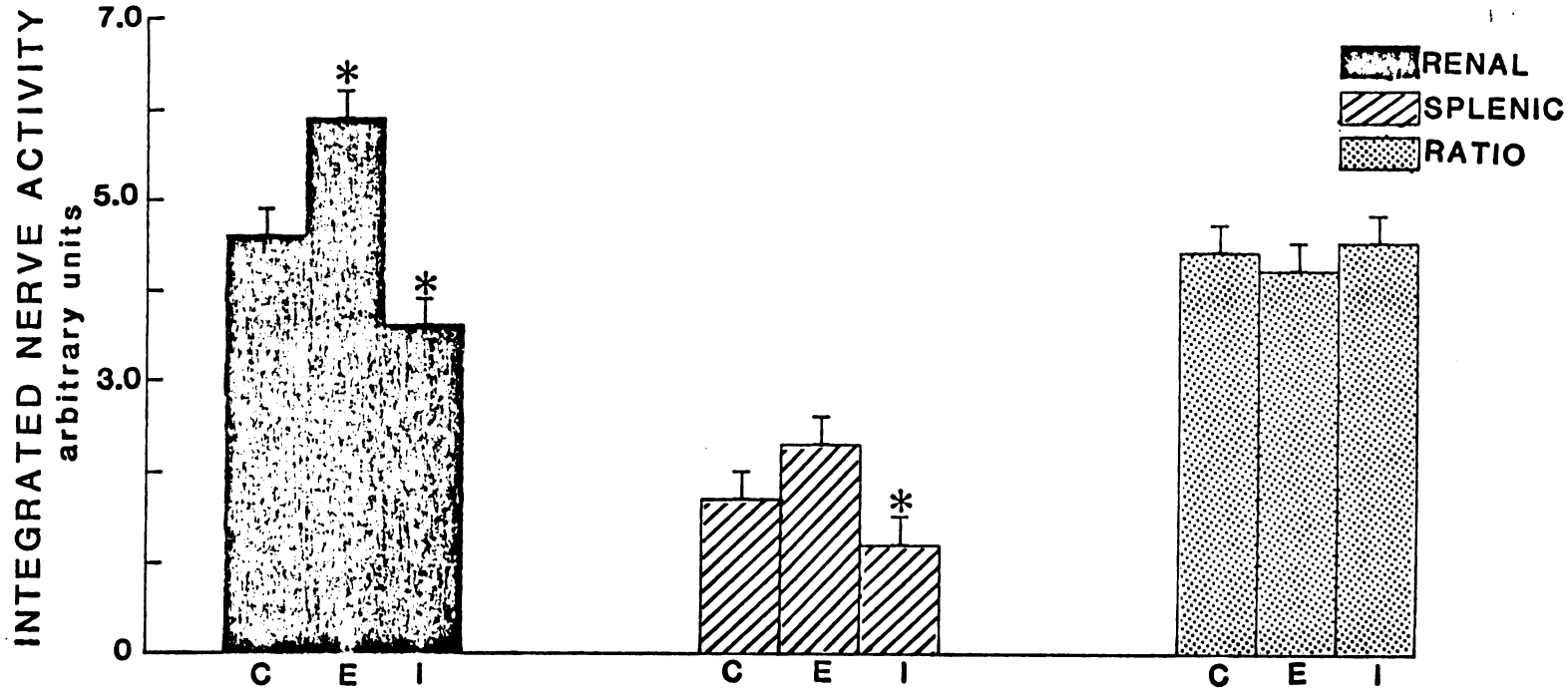


Figure 10. Mean maximum responses to chemoreceptor stimulation. Maximum excitatory (E) and inhibitory (I) responses of renal (darkened areas) and splenic (striped areas) nerves to injection of 0.2ml of venous blood in these 6 cats. The ratio (shaded areas) of renal to splenic nerve activity during the three periods is also shown. Asterisks indicate response values that are significantly different from control values. Standard errors for renal (SE=3) and the ratio (SE=3) are indicated by the lines above each bar.

Figure 11 show responses to injection of norepinephrine when nerve activity was quantified by the frequency analysis technique. Renal nerve activity was completely inhibited for 8 seconds, whereas splenic nerve activity was inhibited for only 2 seconds. Activity in both nerves could be competely inhibited with larger doses of norepinephrine. Renal nerve activity was inhibited to 26% of control, but that for the splenic nerve only 68% for the 20 second average period. Data in the left panel of Figure 12, show mean maximum responses to injection of norepinephrine when nerve activity is quantified by the frequency analysis technique. The mean systemic blood pressure increase due to the injection was 39 $\pm$ 18mmHg, from resting blood pressure of 118 $\pm$ 18mmHg. Activity of both nerves was significantly decreased in response to injection of norephrinephrine. Control discharge (renal 63, splenic 48 counts/sec) was not significantly different in the two nerves, and therefore comparisons were made between the percent change of the two caused by injection of norepinephrine. Results of this comparison are shown in Figure 13. The left panel shows that the mean decrease in renal nerve discharge is significantly more than that for the splenic nerve. Responses of individual cats in the right panel show that in 8 of the 10 cats, the % change in renal nerve activity was greater than that of the splenic nerve.

Data in the right panel of Figure 12 show the responses of 6 other cats to intravenous injection of norepinephrine when nerve activity was quantified by integration. Maximum responses for 10 sec integration periods after administration of norepinephrine show that activity in both nerves significantly decreased from a control discharge level. The

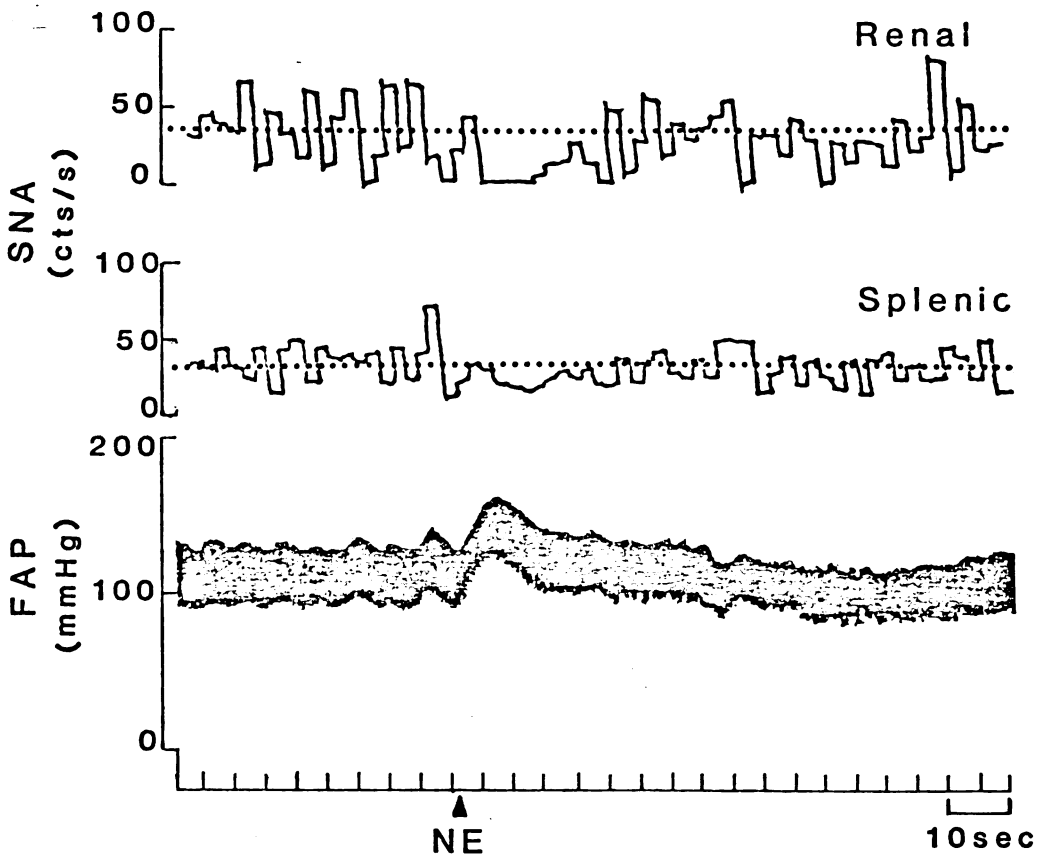


Figure 11. Norepinephrine (NE) pressor response. Records of renal (top trace) and splenic (middle trace) nerve activity quantified by frequency analysis during injection of NE. Femoral arterial pressure was recorded simultaneously (bottom trace) and the time of NE injection is indicated by the arrow.

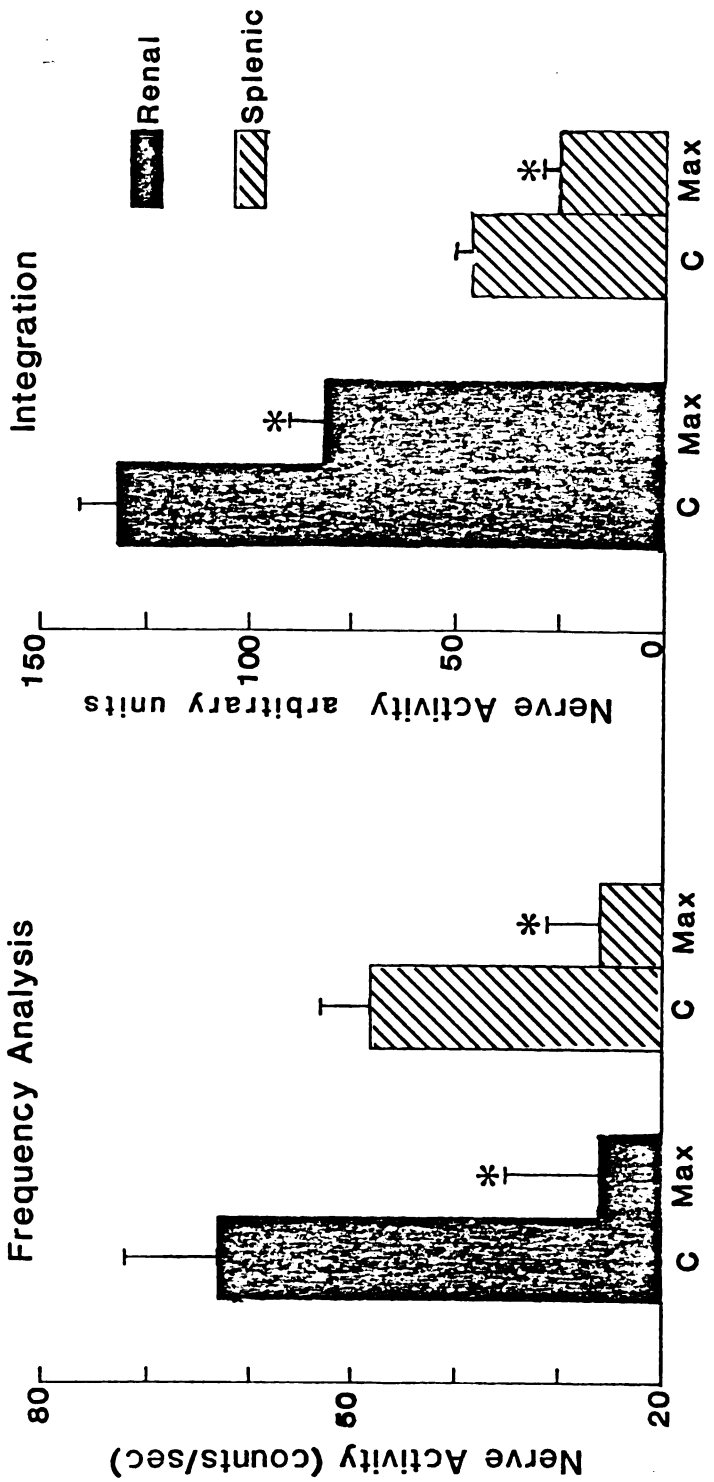


Figure 12. Norepinephrine maximum responses. Mean maximum (Max) responses of renal (darkened area) and splenic (striped area) to injection of norepinephrine (NE) are compared to control (C) values. Standard errors are indicated by the lines above each bar. Asterisks indicate responses significantly different from control values. Left panel: Renal (SE=9) and splenic (SE=5) nerve activity was quantified by frequency analysis in 10 cats. Right panel: Renal (SE=11) and splenic (SE=5) nerve activity quantified by integration in 7 cats.

Figure 13. Comparisons of responses to norepinephrine. Maximum change in renal (darkened area) and splenic (striped area) nerve activity to injection of NE. Left panel: Mean maximum responses of renal and splenic nerves. The asterick indicates that mean renal nerve changes were greater than mean splenic nerve changes. Right panel: Comparisons of renal and splenic nerve % changes of individual cats (identified by a 3 digit code).

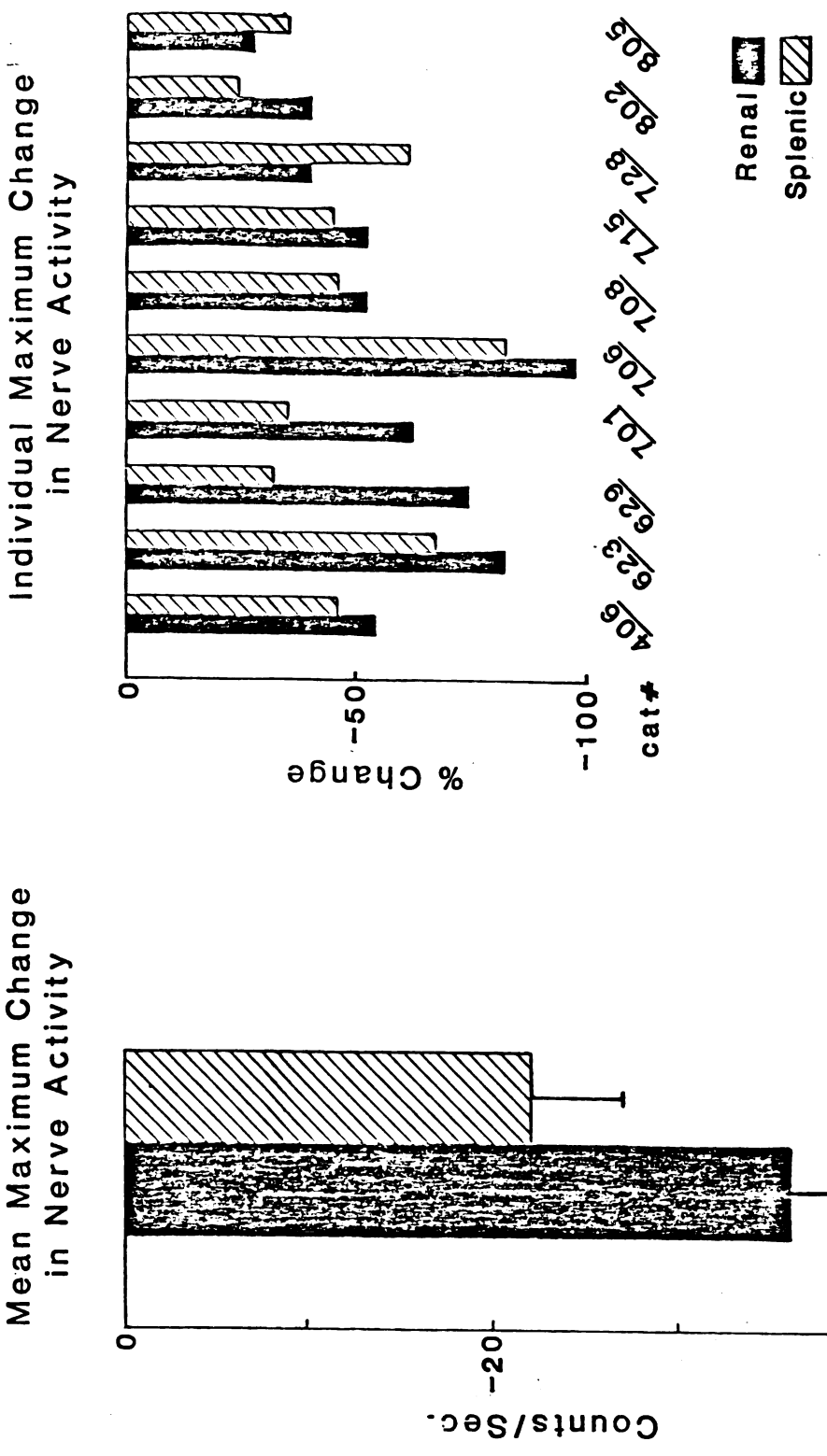

Individual Maximum Change in Nerve Activity
% Change
0
-50
-100
cat#
406
623
629
701
706
708
715
728
802
805
Renal
Splenic
Mean Maximum Change in Nerve Activity
Counts/Sec.
0
-20
-45
*

Figure 13.

mean systemic blood pressure increase due to injection of norepinephrine in this group was 23 ± 6mmHg, from a resting mean pressure of 138 ± 13mmHg. Control integrated activity of renal nerves (120 units) was significantly greater than that of splenic nerves (41 units). Therefore, a ratio of the integrated renal to splenic nerve activity was calculated and compared during the control and maximum response periods. The ratio during the maximum response period was 4.04 which is not significantly different from the control ratio (4.22).

Twelve animals were prepared for stimulation of carotid sinus baroreceptors. In six of these animals recordings were made when the sinus was pressurized at several different pressures. An example of this recording is shown in Figure 14 at a carotid sinus pressure of 150 mmHg. One of these cats had a left aortic depressor nerve intact. Integrated nerve activity was averaged for 5 sec during the initial change from the control or occlusion periods, and from 10 to 20 sec after the change in carotid sinus pressure. The general shape of the baroreceptor curve was similar in the 5 and 10 sec averages. The 5 sec averages (Figure 15 upper panel) showed a greater response to occlusion than did the 10 to 20 second averages (Figure 15 lower panel). The mean responses of five cats are shown in Figure 15. In this figure nerve activity (expressed) as a percent of control discharge is shown as a function of the percent of control carotid sinus pressure. This was done because control integrated nerve activity levels were different in the two nerves of the same cat as well as, in different cats. Values for control integrated activity and carotid sinus pressures are shown in Table 1. To show the response of the mean of 5 cats, groups with a

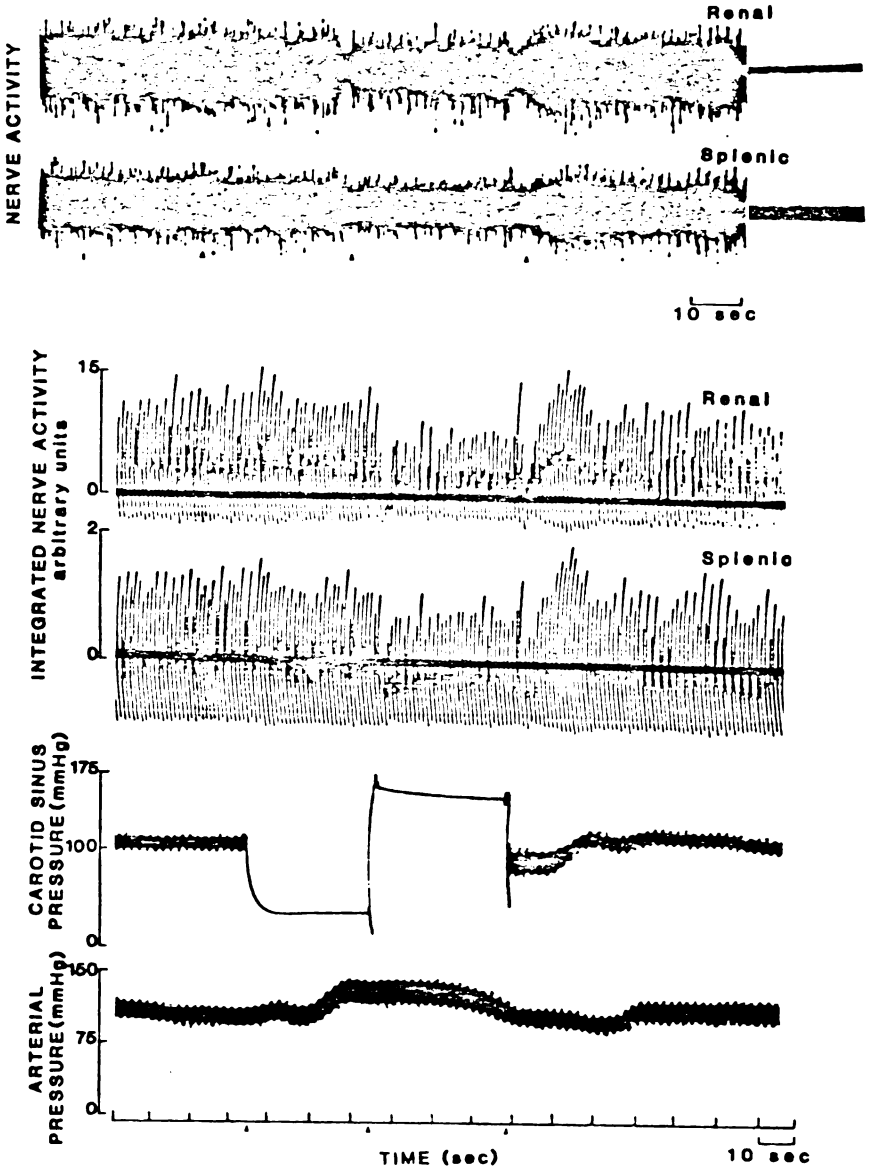


Figure 14. Baroreceptor stimulation. Renal and splenic (amplified 2X that of renal) nerve activity in top panel. Noise level for these recordings is at the far right. Renal and splenic integrated nerve activity shown next with a solid line indicating noise level. Pressure in the carotid sinus preparation (middle panel) and systemic arterial pressure (bottom panel). Arrows indicate times at which carotid sinus pressure was changed.

Figure 15. Mean carotid baroreceptor curves. Mean renal and splenic nerve responses in 5 cats expressed as a % of control discharge are shown as a function of carotid sinus pressure (expressed as a % of control). Renal values are indicated by diamonds and connected by solid lines. Splenic values are indicated by circles and connected by interrupted lines. Control is indicated by the point at (100,100). Top panel: Values for renal and splenic nerve activity are means of 5 sec averages of activity during the initial change in carotid sinus pressure. Bottom panel: Values for renal and splenic nerve activity are means of 10 sec averages beginning 10 sec after the initial change in carotid sinus pressure.

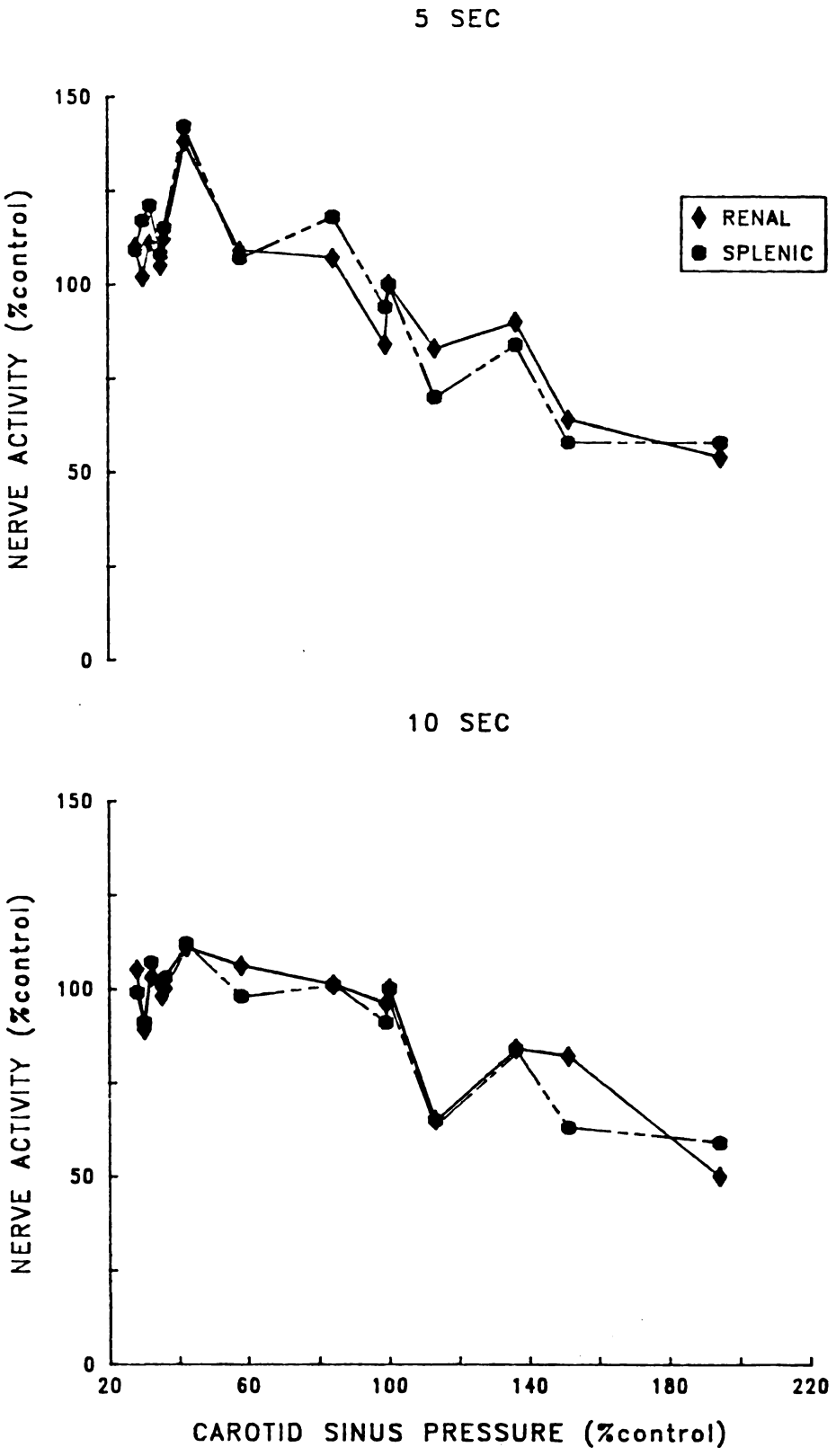

5 SEC
150
100
50
0
NERVE ACTIVITY (%control)
RENAL
SPLENIC
10 SEC
150
100
50
0
NERVE ACTIVITY (%control)
20
60
100
140
180
220
CAROTID SINUS PRESSURE (%control)

Figure 15.

Table 1. Absolute values. Carotid sinus pressure and renal and splenic nerve activity values during, control (C), occulsion (Occ) and carotid sinus pressurization (Csp), for 5 and 10 sec periods.

Carotid Sinus Pressure			Renal Nerve Activity					Splenic Nerve Activity				
C	Occ	Csp	C	Occ	Csp	Occ	Csp	C	Occ	Csp	Occ	Csp
			5 sec			10 sec			5 sec		10 sec	
Cat 316												
145	60	-	13.67	29.44	-	14.71	-	2.07	6.12	-	2.59	-
120	35	140	19.76	24.08	12.08	20.89	4.89	3.88	6.79	1.23	5.01	0.54
120	50	185	10.23	18.89	2.63	14.18	8.20	2.48	4.61	0.88	3.37	1.17
Cat 411												
70	25	95	10.31	11.59	9.85	10.88	8.98	2.75	3.02	2.82	2.84	2.57
70	25	105	9.69	10.70	10.30	10.23	8.64	3.22	3.41	3.17	3.23	2.68
63	20	135	8.15	8.99	7.97	8.86	7.08	3.00	3.35	2.84	3.25	2.76
Cat 420												
157	55	-	3.33	3.18	-	3.09	-	1.54	1.57	-	1.62	-
165	60	100	2.49	2.53	2.32	2.35	2.73	1.85	2.04	1.93	1.96	1.77
142	45	140	2.24	2.39	1.82	2.13	2.28	1.54	1.87	1.29	1.57	1.49
Cat 422												
135	70	-	3.46	4.29	-	3.60	-	0.74	0.86	-	0.74	-
148	55	145	1.80	1.80	1.58	1.55	1.62	1.06	1.20	1.21	1.03	0.91
138	88	150	1.31	1.57	1.44	1.40	1.07	1.73	1.89	1.60	1.74	1.57
123	70	175	4.28	4.58	4.71	4.30	3.22	1.57	1.58	1.42	1.53	1.35
148	60	250	4.54	4.87	4.36	4.35	2.74	2.08	2.18	2.05	2.18	1.63
Cat 601												
108	85	-	6.51	8.80	-	6.56	-	0.72	1.15	-	0.77	-
105	45	85	9.42	13.11	9.69	10.70	9.86	1.65	2.12	1.69	1.88	1.77
108	40	100	8.51	11.52	7.14	9.76	8.14	1.54	1.99	1.40	1.65	1.38
105	35	120	8.44	9.67	6.58	8.86	7.57	1.28	1.61	1.10	1.41	1.13
107	30	140	8.43	9.85	5.50	8.90	7.48	1.17	1.42	0.71	1.16	0.87
100	30	150	8.95	10.30	5.32	8.97	6.88	1.20	1.38	0.49	1.04	0.72
100	35	190	9.78	10.86	1.01	10.04	2.82	1.46	1.64	0.27	1.49	0.49

range of % of control carotid sinus pressure were formed and data for each group was averaged, so that in each, an individual cat contributes equally. That is, if there were more than one % of control carotid sinus pressure for one cat in the group, the pressure and nerve responses were averaged prior to averaging data for the group. Because of the difficulty in producing the exact sinus pressure and the differences in mean blood pressures between cats, each datum point does not necessarily represent the mean of all 5 cats. The % of control carotid sinus pressure groups were; 25-29%, 30-31%, 32-33%, 34-35%, 36-39%, 40-45%, 50-65%, 79-94% (1 cat), 95-100%, 109-120%, 130-145%, 150-155%, 160-215%. Ten sec averages were chosen to determine the mean values during a steady state period. Mean control systemic blood pressure and carotid sinus blood pressures ranged from 70mmHg to 135mmHg, and changes in sinus pressure were attempted relative to mean pressure. The response curves for the two nerves are the same. In three of these 5 cats, the sinus was stimulated 3 or four times at the same pressure and responses were averaged. Results from these experiments are shown in Figure 16. There is no difference between the response curve for splenic and renal nerves. Data from cat #420, #422 and #601 (Table 1) were used to create Figure 16, which was calculated similar to data in Figure 15. In this figure, the % of control carotid sinus pressure groups were; 30-33%, 34-35%, 36-39%, 40-45%, 50-65%, 79-94% (1 cat), 95-100%, 109-120%, 130-145%, 150-170% and 190-203%. In the one cat in which the left aortic depressor nerve was intact, a higher %-above-control pressure was needed to inhibit nerve activity, and there was inhibition in response to occlusion of the left common carotid artery.

Figure 16. Averages of repeated carotid sinus pressures. Baroreceptor curves for averages of 2 or 3 trials at each pressure for an individual cat are represented by the light curves. Points used to generate these curves are 10 sec averages discribed in Figure 15 bottom panel. The mean curve for the 3 cats is represented by the dark curves. Control is indicated by the point at (100,100). Top panel: Renal nerve response curve with mean values indicated by solid diamonds. Bottom panel: Splenic nerve response curve with mean values indicated by solid circles.

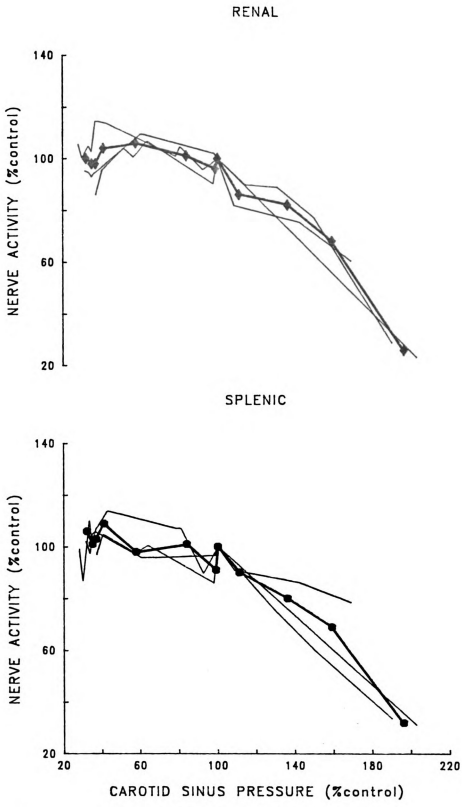

RENAL
140
100
60
20
NERVE ACTIVITY (%control)
SPLENIC
140
100
60
20
NERVE ACTIVITY (%control)
20
60
100
140
180
220
CAROTID SINUS PRESSURE (%control)

Figure 16.

Simultaneous baro and chemoreceptor stimulation

In one cat, right carotid chemoreceptors were stimulated by injecting 0.2 or 0.3ml venous blood through a cannula in the right lingual artery, while arterial baroreceptors were stimulated in the left sinus. Data in Figure 17 show the responses of the two nerves and compare the responses before and after pressurizing the sinus (dark curve) and during exposure to a static pressure of 75 mmHg in the left sinus (interrupted curve). Control values for renal (5.39 units) and splenic (0.55 units) integrated nerve activity are expressed as 100%. The response is only attenuated by increased sinus pressure, not abolished. Figure 18 shows the mean responses to carotid chemoreceptor stimulation for this cat the averaged of before and after pressurizing the left sinus (dark line), and during carotid baroreceptor stimulation (interrupted line). Mean control values (average of 30, 1 sec integrations) for renal (5.76 $\pm$ 0.92) and splenic (0.49 $\pm$ 0.11) integrated nerve activity are significantly different and therefore are expressed as 100%. The 4 sinus pressures were 75, 80, 100 and 105 mmHg.

Figure 17. Simultaneous activation of carotid baro and chemoreceptors. All curves were generated by connecting the percent of control values at 1 sec interval of integrated nerve activity for 30 sec. The dark solid curve indicates an average of the responses to chemoreceptor stimulation immediately preceding and following the response during pressurization of the left sinus. The time of injection was at 3 sec. The interrupted curve indicates the response to venous blood during sinus pressurization. Top panel: Renal responses. Bottom panel: Splenic responses.

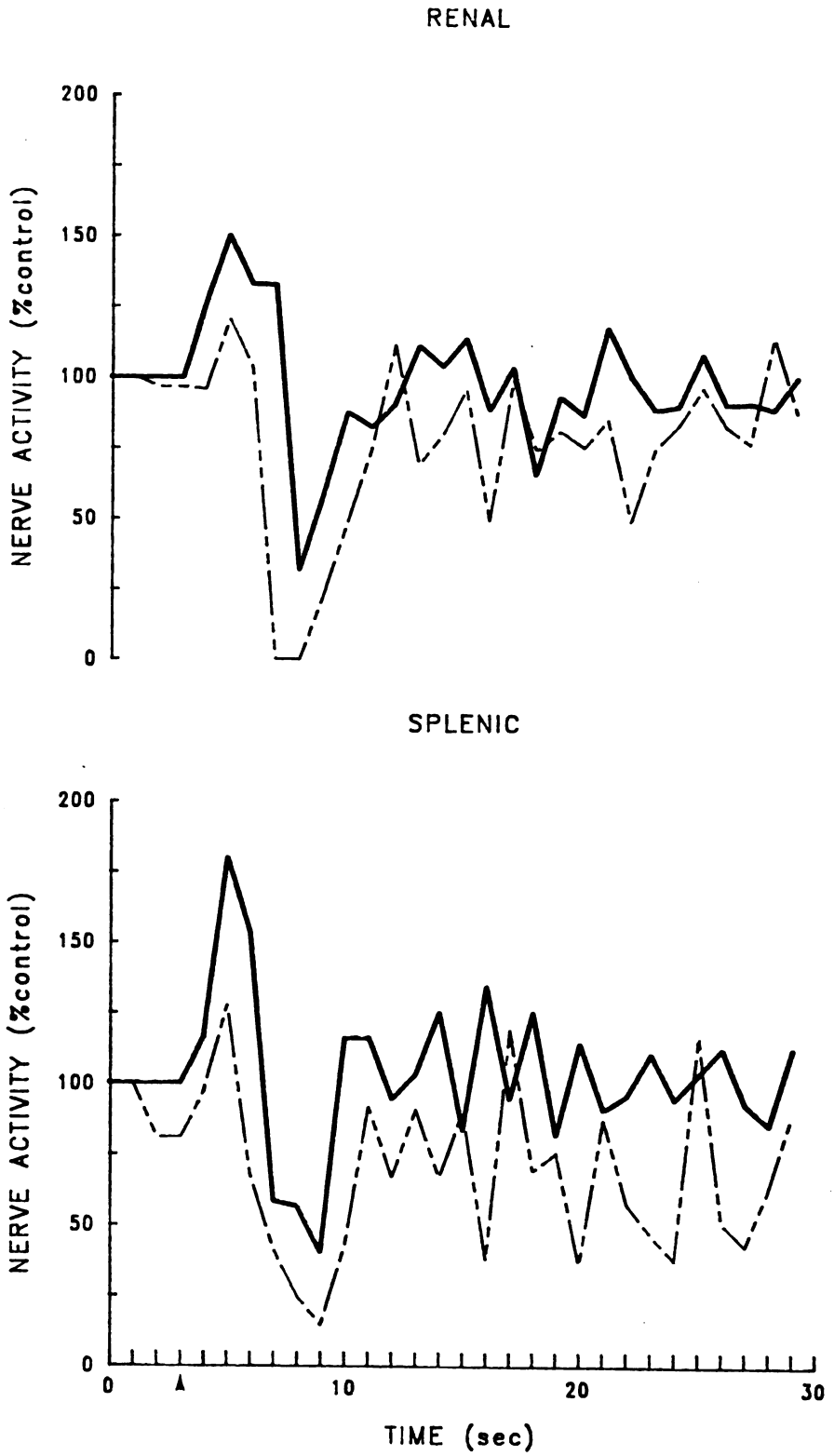

RENAL
200
150
100
50
0
NERVE ACTIVITY (%control)
SPLENIC
200
150
100
50
0
NERVE ACTIVITY (%control)
0
10
20
30
TIME (sec)

Figure 17.

Figure 18. Mean responses to simultaneous activation of carotid baro and chemoreceptors. Mean of 1 sec integrated activity of renal and splenic nerves to 4 different carotid sinus pressures for 20 sec. As in Figure 17, dark solid curves indicate the mean responses to venous blood immediately prior to and following the test during pressurization of the sinus. The interrupted curve is an average of the responses to venous blood at 4 different carotid sinus pressures.

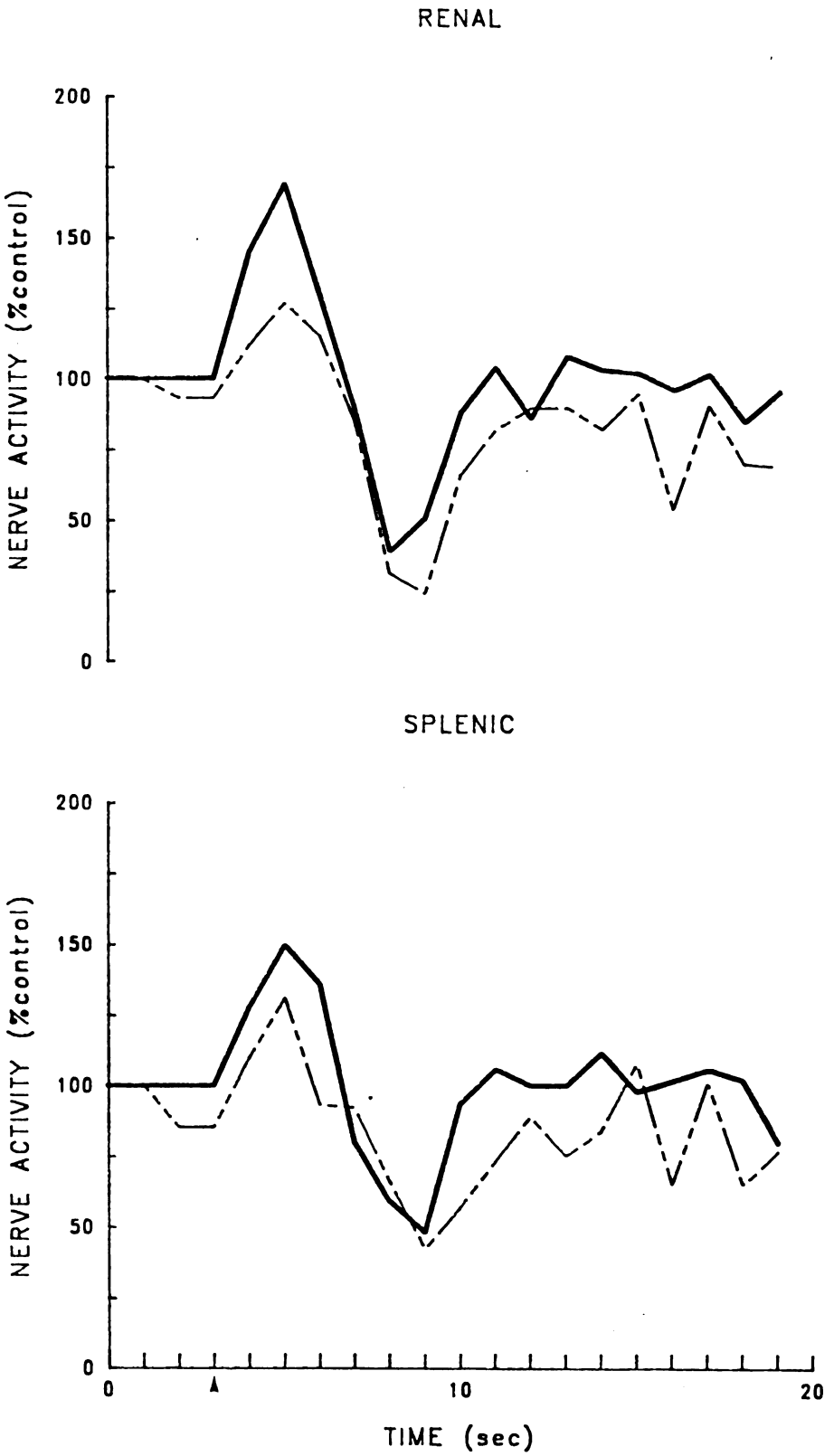

RENAL
200
150
100
50
0
NERVE ACTIVITY (%control)
SPLENIC
200
150
100
50
0
NERVE ACTIVITY (%control)
0
10
20
TIME (sec)

Figure 18.

DISCUSSION

Bladder distension

Data reported in Figures 3-6 show responses to stimulation of bladder afferents. Data in Figure 3 and Figure 5 indicate that renal nerve activity increased significantly more than did splenic nerve activity during distension of the urinary bladder. Data in Figure 4 (right panel) show that activity in both nerves increased in response to bladder distension with saline, but neither renal nor splenic nerve activity increased significantly above the control level discharge. This could be due either to the small number of animals in this experimental group, or because not as many afferents were stimulated with this distension method. Figure 5 (right panel) shows responses of individual cats, in some of which there was only slight nerve excitation. Data in Figure 5 (left panel) show that with the frequency analysis technique and balloon distension, excitation of renal nerve activity is greater than that of splenic nerve activity. This method of distension would activate more pain afferent fibers, which might explain why neither renal nor splenic nerve activity increased above the control discharge shown in Figure 4 (right panel).

The pressures used to distend the bladder in this study were sufficient to activate both pelvic and hypogastric afferents, as shown by Floyd, et al. (1982). It is likely that the balloon distension

method distended the bladder at a faster rate which would result in a shorter delay before a response occurred, and might also stimulate more afferents. Mukherjee (1957) suggests it is actually the tension in the bladder wall that is the stimulus for the increased blood pressure. The saline distension group was done more carefully to attempt a more physiological distension. Mukherjee (1957) saw evidence for renal vasoconstriction in only 5 of 15 animals when vagi and carotid sinus nerves were intact, however, vasoconstriction was observed in all 15 cats when the buffer nerves were cut. One would predict that increases in nerve activity could be more easily observed than vascular responses, however, it is possible that either a larger group of animals or animals in which vagi and carotid sinus nerves have been cut should be tested.

The experiments in which a balloon-tipped cateter was placed in the bladder probably activated more pain fibers, because of the incision, and possibly activiated more afferents sensitive to the rate of stretch, since the rate of distension was faster. Several investigators (Barrington, 1928; Kuru, 1965) have shown that different bladder afferents should have been activated with either method of distension. These data indicate there is a differential reflex response in renal and splenic nerve activity when various bladder afferents are activated, and show that renal nerves are sometimes more excitably than are splenic nerves. The excitation of renal nerve activity in response to bladder distension would help decrease urine production while also increasing urine osmolarity which would be benefical to prevent further bladder filling.

Floyd, et al. (1982), also showed that the distension pressures necessary to cause an increase in hypogastric efferent activity is

greater than that needed to increase pelvic or hypogastric afferent activity. Kuru (1965) accepted that free endings are pain receptors and that the existance of encapsulated nerve endings suggest that these receptors are sensitive to deformity of their capsules.

Nerve quantification

Selecting an appropriate method for quantifying nerve activity is a current problem in analysing and interpreting multifiber nerve activity (Hopp et al., 1983; Dick et al., 1974). Both integration and frequency analysis techniques are accepted methods for quantification of multifiber nerve activity (Iriki et al., 1972, 1973, 1977, 1979; Ninomiya et al., 1967, 1969, 1975; Weaver et al., 1983a, 1983b). A few new methods have been developed (Dick et al., 1974) and have been compared with computer simulated nerve activity. These new methods will most likely give a more accurate quantification of sympathetic nerve activity, so that a better correlation of nerve activity and organ functional responses may be made. The advantage of the frequency analysis technique is that it counts each action potential equally, and does not bias recording from nerve fibers which have larger amplitudes, since this might only be because a fiber is closer to the electrode. The frequency analysis method requires the amplified nerve signal to be passed through a window discriminator; an action potential that exceeds a set threshold level is counted as it drops below the level. Therefore, in multifiber nerve preparations 2 impulses could summate so that they would be counted as only one impulse. This would result in underestimating nerve activity when the impulses were synchronous, as

well as when the frequency of impulses increased.

Quantification of nerve activity by voltage integration continually sums the amplified signal voltage until a set voltage or time period occurs. A 1 sec time period was used in this study. This method can quantify synchronous activity and high frequency activity, but it also is determined mainly by the larger voltage spikes (Ninomiya et al., 1967). For example, a 3 fold increase in the larger amplitude spikes could increase the integrated signal to twice that of the control value, whereas a 3 fold increase in the same number of smaller amplitude spikes might only increase the integrated signal to 1 1/2 times the control value. The difference between the smaller and larger amplitude spikes might only be their relative distance from the electrode, instead of a functional difference in nerve activity. More accurate methods of quantifying nerve activity which allow for synchronization, high frequency signals and differences in spike amplitude will probably advance with the increased use of computer simulated and analyzed nerve activity.

Other Distensions

Data in Figure 7 and Figure 8 suggest that there is only slight excitation of renal and splenic nerve activity in response to duodenal distension. Splenic nerve activity increased significantly above control levels, whereas renal activity did not increase, however, there is no significant difference in the responses of the two nerves. These findings are similar to those of Ninomiya, et al. (1974, 1975), who also found that duodenal distension increased renal nerve activity only

slightly (7%), even though intestinal nerve activity increased 108%. Ninomiya, et al. (1975), found no change in splenic nerve activity and only slight increases in renal nerve activity. The results of this study showed increased splenic nerve activity which is probably because a larger distension pressure was used.

Colon distension showed no response in either renal or splenic nerve activity, but must have activated pelvic and/or hypogasteric afferents sufficiently to initiate bladder contractions. The bladder contractions indicate that the balloon-tipped catheter had been placed in the proximal colon which has been shown to be sensitive to stretch (de Groat and Krier, 1978, 1979; de Groat et al., 1979). There apparently is not a colonic-renal or colonic-splenic reflex.

Data from these different distension experiments support the hypothesis that the response of the two nerves is dependent on the afferent stimulus. There is not always the same response on the two nerves to distension of visceral organs, which suggests that the sympathetic nervous system is organized so that a specific response of an efferent nerve occurs depending on what afferent fibers are stimulated.

Multifiber or single fiber preparations

There is also a question about what information can be obtained from multifiber nerve preparations, and if this is more critical than the information obtained from single fiber recordings. Multifiber preparations give a better idea of changes in nerve activity as seen by the whole organ, whereas single fiber preparation experiments give

information about the specific responses of individual fibers within a nerve bundle (Blumberg et al., 1980; Iriki and Korner, 1979). Both multifiber and single fiber nerve recordings have been used to evaluate the effects of nerve impulses on organ function. More complete information about reflex responses and organization of the sympathetic nervous system can be obtained if both multifiber and single fiber activity are recorded in similar preparations. Multifiber recordings reflect the responses of the majority of nerve fibers (Blumberg et al., 1980; Riedel and Peter, 1976; Rogenes, 1982).

Responses of a few fibers might not be observed in this type of recording but these changes might be functionally important and might only be discovered by single or few fiber preparations. Several attempts were made to record single fibers from renal postganglionic nerves but none were successful. At best, few fiber preparations were recorded which would require analysis by a computer program for spike separation (Capowski, 1976). This analysis is not available. The information that can be gained from single fiber nerve preparations is subject to criticism and limitations. The most successful reported technique for single fiber recordings involves disecting fibers from the nerve bundle which could damage a fiber and prevent it from responding normally. It would be difficult to correlate the responses of renal and splenic fibers to the functional response because single fiber preparations are technically difficult and the measurements of renal and splenic function are complex.

Chemoreceptor stimulation

Data in Figure 9 and Figure 10 indicate that stimulation of arterial chemoreceptors initiates a biphasic response in both renal and splenic nerves. The responses of these two nerves is the same, even though responses of vertebral and cardiac nerves to chemoreceptor stimulation reported by Kollai and Koizumi (1977) were differential. Hilton and Marshall (1982), however, found in blood flow experiments that skeletal muscle vascular beds constricted whereas renal, mesenteric and cutaneous vascular beds vasodialated. Little and Öberg (1975) concluded that vasomotor fiber activity in skeletal muscle increased more than that in renal vessels. These results suggest that not only is the afferent stimulus important in determining the response of the efferent nerves but also that the efferent nerve is important in determining a differential response. This data along with data from others (Iriki et al., 1971,1979; Kollai and Koizumi, 1977), further emphasizes that the sympathetic nervous system is very organized and specialized to produce a specific response at one organ while a different response occurs at another organ. These studies suggest that the sympathetic nervous system integrates information from various afferent inputs to produce the most beneficial response at each individual organ.

Baroreceptor stimulation

Data in Figures 11-13 indicate that renal nerve activity is inhibited more than splenic nerve activity in response to intravenous injection of norepinephrine. The data in Figure 13 (left panel) show that inhibition of renal nerve activity is significantly greater than that of splenic nerve activity. The individual responses however, show that in only 3 cats inhibition of renal nerve activity was almost twice that of the splenic nerve.

Norepinephrine is a direct vasoconstrictor, and therefore will activate baroreceptors throughout the circulatory system. Norepinephrine is also a potent excitatory stimulus which can produce many effects at various organs (Goodman et al., 1980). This stimulus activates several different types of receptors including arterial and cardiac vagal pressure receptors. This could be why the responses to norepinephrine were not differential when nerve activity was quantified by integration. This response was just slightly differential in most of the animals in the frequency analyzed group, and it is possible that the group in which nerve activity was quantified by frequency analysis had more input from other afferents, and therefore the greater inhibition of renal than splenic nerve activity. Stimulation of left atrial receptors has been shown to inhibit renal nerve activity and cause no change in splenic nerve activity (Karim et al., 1972). This would explain the differential reflex found by the frequency analysis technique along with reports by Little and Öberg who suggested that baroreceptors usually predominate over cardiac vagal afferents. Future experiments might be

to test stimulation of cardiopulmonary receptors to determine if they produce differential reponses in simultaneously recorded renal and splenic nerve activity.

In contrast, the data in Figure 15 and Figure 16 indicate that the responses of renal and splenic nerves to stimulation of only carotid baroreceptors were the same. This is shown best by Figure 15, in which several points in both nerves have the same coordinates. This agrees with results reported by other investigators who also found that stimulation of arterial baroreceptors caused a generalized inhibition of nerve activity (Iriki et al., 1979; Oberg and White, 1970). Ninomiya, et al. (1971), reported that when pressure was increased steeply, complete inibition of splenic nerve activity lagged that of renal by 100 msec. They also reported that resting discharge of splenic nerve activity was less than that of renal discharge. This may be due to differences in the number of active fibers, or the inter-electrode resistance between the two nerves. Ninomiya, et al. (1971), however, found differential responses in splenic and renal nerve activity to stimulation of arterial baroreceptors. In their experiments, they changed static pressure in the carotid sinus at 3 different rates and produced changes in pulse pressure and moderate pressure increases by aortic occlusion. This might enable them to detect more discrete differences in renal and splenic nerve responses. A more physiological stimulation of carotid sinus baroreceptors would be to use a system in which both mean pressure and pulsatile pressure could be precisely controlled. This would better simulate changes in blood pressure that occur during daily disturbances.

It would be interesting to determine if these discrete changes in

nerve activity have an effect on the functions of the kidney and spleen. Measurements of renal and splenic functional responses to baroreceptor stimulation would present information helpful in determining the importance of the discrete differential responses. One approach to studying the relationship between multifiber or single fiber nerve activity and organ function has been to electrically stimulate nerve fibers to an organ and measure changes in organ function (Greenway et al., 1968; Osborn et al., 1981; Slick et al., 1975). Electrical stimulation activates fibers simultaneously which does not occur during physiological activiation. Electrical stimulation of renal nerves has been shown to cause increased renin secretion, increased sodium reabsorption and renal vasoconstriction (Osborn et al., 1981; Slick et al., 1975). Electrical stimulation of splenic nerves has been shown to cause contraction of the splenic capsule.

The slight differences in gain of the baroreceptors reflex and critical threshold (pressure) for complete inhibition were determined by aortic occlusions and isolated sinus preparations, in which all four baroreceptor afferents were intact. Maybe the combined effects of at least two baroreceptor afferents are needed to produce a differential response, or possibly aortic baroreceptors are necessary. Ninomiya, et al. (1975), indicated that with injections of norepinephrine, splenic inhibition was greater than that in the renal nerves which confirmed their previous reports. Results from these experiments are not described in as much detail, and therefore it is difficult to determine why they found greater inhibition of splenic than renal nerve activity which disagrees with our findings in these experiments.

Oberg and White (1970) have reported that cardiac vagal afferent

nerves have a particularly strong effect on vasomotor neurons controlling the heart and renal vessels. If cardiac vagal afferents have a stronger effect on renal efferent nerves than splenic nerves, this could explain why pressure increases caused by injections of norepinephrine caused greater inhibition of renal than splenic nerve activity.

Simultaneous Activation of Baroreceptors and Chemoreceptors

Data in Figure 17 and Figure 18 show that even in the presence of baroreceptor activation, stimulation of arterial chemoreceptors causes an increase in both splenic and renal nerve activity. The chemoreceptor response was attenuated, but not abolished, when carotid sinus pressure was high. These results indicate that there are central interactions between these two reflexes. These data are consistant with data reported by others (Heistad et al., 1974; Iriki et al., 1977, Iriki and Korner, 1979; Mancia, 1973; Wennegren et al., 1976) which all agree that there are central interaction of chemoreceptor and baroreceptor reflexes. Iriki and Korner (1979) further discribed this interaction as one in which there are both neurons that receive input from specific afferent groups (independent), and neurons that receive input from several afferent groups (dependent) involved in the baro and chemoreceptor reflexes. This could be evaluated better if more than one animal had been tested at several different sinus pressures. The combination of these two reflexes did not cause a differential response, as reported by Iriki, et al. (1979), who found differential responses in renal and cardiac nerves, however, the response of these two nerves to

chemoreceptor stimulation alone was differential.

The results of this study show that both specific responses and similar responses occur in renal and splenic nerves to various visceral afferent stimuli. Whether a differential response occurs or not, is dependent on both the afferent and efferent neurons involved in the reflex. This study also shows that there is no differential response in renal and splenic efferent activity when carotid sinus baroreceptors are activated. Even combinations of reflex inputs which have been shown to cause differential responses in other nerves might not cause differential responses in renal and splenic nerves. This study also indicates the need for a more accurate quanitification of multifiber nerve activity.

CONCLUSION

1. Renal nerves can be more excitable than splenic nerves.
2. The afferent stimulus is important in determing the occurance of a differential response.
3. A differential response is also dependent upon the efferent nerves.
4. This suggests that the sympathetic nervous system is organized to be capable of producing specific responses at each organ.

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