CARDIOPULMONARY SYMPATHETIC AFFERENT INFLUENCES ON THE KIDNEY

By

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ABSTRACT

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Activation of cardiopulmonary sympathetic afferent nerves (CPSAN) electrically or by intravascular volume expansion can alter renal efferent nerve activity, although it is not known if this reflex requires the integrity of the brainstem. To determine if this reflex requires supraspinal pathways, experiments were conducted in vagotomized, sino-aortic denervated cats prior to and following C_1 spinal cord transection.

Although the cardiopulmonary-renal reflex could be demonstrated prior to spinal transection, following transection this reflex could not be elicited either electrically or with intravascular volume expansion, indicating that this reflex requires supraspinal pathways.

Since CPSAN stimulation can alter renal nerve activity in intact cats, the influences of CPSAN on renal function was determined. Afferent stimulation at 1-2 trains per sec inhibited renal nerve activity and caused a diuresis and natriuresis. Afferent stimulation at 5-7 Hz had no net effect on renal nerve activity or renal function. Thus, CPSAN can influence renal nerve activity and renal sodium and water excretion, and therefore, may contribute to intravascular volume control.

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HISTORICAL REVIEW

General Background: Autonomic control of the thoracic and abdominal viscera has been studied extensively. Yet, our knowledge is still incomplete. Until very recently, it was thought that afferent information concerning the cardiovascular system was carried almost solely by nerves emanating from the carotid sinus and aortic arch, with a modest contribution made by other vagally innervated receptors.

However, Malliani and coworkers have shown that vascular structures innervated by sympathetic afferent nerves can reflexly influence the cardiovascular system (61,65). Often these influences do not require an intact neuraxis (62,65) indicating that spinal control of the circulatory system may be significant.

Very recently, it has been shown that cardiopulmonary sympathetic afferent nerves can reflexly influence renal sympathetic efferent nerve activity (101). Since evidence has been accumulating that renal nerve discharge can alter renal function (27), afferent fibers travelling with sympathetic nerves may possibly alter renal function. This would in turn indicate an influence of the sympathetic afferent system in intravascular volume control.

The Volume Receptor Hypothesis: Reflex Control of Renal Electrolyte and Water Excretion: In 1935, Peters suggested that one factor contributing to renal control of intravascular volume might involve the distribution of blood in

the vascular tree (84). Since that time numerous studies have been conducted in an attempt to locate receptors in the vasculature which might influence the kidney.

In 1954, Gauer et al. showed that exaggerated negative pressure breathing resulted in a diuresis in dogs (31). This procedure is believed to engorge the thoracic vasculature with blood. It was hypothesized that stretch receptors in the thoracic vasculature excited by the redistribution of fluid might form the afferent loop in a reflex resulting in diuresis. In 1956, Henry et al. found that distension of a small balloon placed in the left atrium also caused a diuresis (40). This procedure caused increases in pressure in the pulmonary circulation as well as in the left atrium. However, constriction of the pulmonary veins, causing the same elevation in pulmonary pressure as left atrial distension, did not cause a diuresis. Since distension of the left atrium resulted in increased afferent activity in the cardiac branch of the vagus nerve, and vagal cooling blocked the diuretic response to distension, it was concluded that vagally innervated receptors in the left atrium and terminal pulmonary veins were likely the initiators of the diuretic reflex (40,41). Previously, anatomical evidence of such receptors had been provided by Nonidez (79).

The diuresis resulting from negative pressure breathing had been assumed to be due to decreases in plasma concentrations of antidiuretic hormone, as the diuresis had resulted partly from increases in free water clearance

(16,31,98). With the use of balloon distension of the left atrium, workers from several laboratories were able to demonstrate decreases in plasma concentrations of antidiuretic hormone which corresponded to increases in left atrial pressure (2,43). Also, it was shown that the diuretic response to left atrial distension could be attenuated in some instances by infusion of antidiuretic hormone (55,58).

Not only was there evidence that renal excretion of water might be partially mediated by vagally innervated receptors in the thoracic vasculature, but there were also indications that renal excretion of salt might be regulated by similar receptors. In 1956, Davis et al. (25) had shown that constriction of the vena cava above the diaphragm stimulated aldosterone secretion. Mills et al. (73) confirmed this finding and added that the decrease in aldosterone secretion seen on release of caval constriction could be blocked by vagotomy. Aldosterone has long been known to increase sodium reabsorption by the kidney. Brennan et al. (17) later confirmed that antidiuretic hormone release could be inhibited by increases in left atrial pressure, and added that renin release could be inhibited by increases in right atrial pressure. Renin acts as an enzyme in the conversion of angiotensin I to angiotensin II, angiotensin II then causing secretion of aldosterone (24). Thus, vagally innervated receptors in the thoracic vasculature appear to be at least partially responsible for alterations

in both antidiuretic hormone and aldosterone secretion, and therefore, could influence renal excretion of sodium and water through humoral mechanisms.

In 1972, Karim et al. demonstrated that activation of vagally innervated left atrial receptors could alter renal sympathetic efferent nerve discharge (48). This study was particularly important in that it showed fractionation of the sympathetic response. That is, excitation of vagally innervated left atrial receptors caused an excitation of cardiac sympathetic efferent nerve discharge and an inhibition of renal sympathetic efferent discharge. More evidence of reflex vagal control of renal nerve activity was provided by Clement et al. (21), who demonstrated that volume expansion could inhibit and hemorrhage could enhance renal sympathetic nerve activity. That these changes in renal nerve activity could be eliminated or attenuated by vagal section again indicates a reflex alteration due to vagally innervated receptor activity.

Sympathetic nerves also carry afferent fibers which innervate receptors in the thoracic vasculature. These receptors have been demonstrated anatomically (76,79,80,81) and physiologically (19,20,65,100). By occluding the coronary arteries to produce myocardial ischemia, Brown demonstrated that activation of certain receptors in the coronary vasculature may be responsible for the sensation of cardiac pain (18). Malliani et al. (65) and Brown and Malliani (19) then showed that some sympathetically innervated receptors in

the coronary vasculature could respond to alterations in coronary arterial pressure and therefore appeared to be mechanoreceptors. The reflex response to pressure alterations was altered cardiac sympathetic efferent discharge.

Ueda et al. were able to locate receptors sensitive to mechanical stimuli whose fibers travelled in sympathetic nerves (100). These workers found receptors in the main pulmonary artery, pulmonary veins, pleura, superior and inferior venae cavae, thoracic aorta, aortic root, left coronary artery, and all four chambers of the heart. However, most of the fibers examined by this group were not spontaneously active. This would limit the amount of information received by the central nervous system from sympathetic afferent fibers and might indicate that these fibers serve to inform the central nervous system only of gross abberations in cardiovascular function. Several years following the study by Ueda et al., workers in Malliani's laboratory demonstrated tonic sympathetic afferent activity stemming from receptors in the atria and ventricles (63,64). Activity from these fibers was consistently in phase with identifiable parts of the cardiac cycle, although a given fiber did not necessarily fire during each cycle. Fibers from the atria discharged with atrial systole, closure of the artio-ventricular valves, or atrial filling. Fibers from the ventricles discharged 40-120 msec following the onset of the Q wave. Because discharge of these fibers can be linked to normal cardiac events, it is possible that these

sympathetic afferent fibers are involved in supplying the central nervous system with information about the normal cardiac cycle. Subsequently, tonic activity also was demonstrated in sympathetic afferent fibers from the pulmonary artery, pulmonary veins, and aorta (78,57,59) increasing the amount of information potentially conveyed to the central nervous system by sympathetic afferent nerves. That electrical activation of these afferent nerves could reflexly alter arterial pressure (18,85), heart rate (61), and cardiac contractility (62) coupled with the knowledge that these nerves are tonically active, indicates that they may be important in regulation of normal cardiovascular function.

Recently, it has been shown that electrical activation of cardiac sympathetic afferent fibers in cats in which cranial nerves IX and X have been severed also can alter renal sympathetic efferent nerve activity (101). High frequency stimulation of afferent fibers in the inferior cardiac nerve (a sympathetic nerve) increased, and low frequency stimulation decreased renal nerve activity. Also, in the same study, intravascular volume expansion caused an inhibition of renal nerve activity. This inhibition was not seen following section of dorsal roots T_1 through T_5. Sectioning these dorsal roots eliminates sympathetic afferent influences from the heart and lungs and, therefore, indicates that activation of sympathetically innervated mechanoreceptors in the thoracic vasculature may

have been responsible for reflex inhibition of renal nerve activity.

Previously, Gilmore and coworkers had shown that cardiac denervation could attenuate the natriuretic response to volume expansion only if cardiac afferent fibers traveling in sympathetic nerves were eliminated (35,72). These last studies suggest that sympathetic afferent fibers originating from receptors in the thoracic vasculature may contribute to renal control of intravascular volume by mechanisms other than systemic arterial pressure alteration.

Spinal Sympathetic Reflexes: In 1969, Malliani, Schwartz and Zanchetti demonstrated that coronary occlusion could cause an increase in preganglionic sympathetic activity to the heart (65). This reflex was present prior to and following spinal transection at the first cervical segment. In 1971, Brown and Malliani (19) showed that, in vagotomized cats with C_1 spinal transection, increases in coronary arterial pressure could cause increases in activity of both preganglionic and postganglionic sympathetic nerves to the heart. In addition, Malliani *et al.* (60) showed that increases in systemic arterial pressure could alter preganglionic sympathetic activity in vagotomized, spinal cats. Previously, Coote *et al.* (22) had shown that the size of sympathetic reflexes was reduced after decerebration, but that reflexes were returned to initial or

greater size upon high spinal transection. In consideration of Coote's findings, which indicated that supraspinal centers could have inhibitory influences on sympathetic reflexes, Brown and Malliani (19) postulated that a spinal sympathetic reflex arc might "represent the most elementary organization for circulatory reflexes, upon which supraspinal influences exert their effects."

The concept of spinal sympathetic control of circulation was not a new one. Fernandez de Molina and Perl (30) had noticed periodic changes in sympathetic preganglionic discharge and systemic arterial pressure in spinal cats which led them to suggest sympathetic control of the circulation at the spinal level.

The first report of a spinal sympathetic reflex is credited to Sherrington. In his Mammalian Physiology, he stated that stimulation of the splanchnic nerve in the spinal cat caused increases in arterial pressure (94). In 1924, Langley (53) confirmed Sherrington's observation. He also showed that increases in pressure could be evoked by stimulation of somatic nerves, indicating the existence of a spinal somato-sympathetic reflex arc. However, Langley pointed out that the increases evoked by somatic stimulation were much smaller and concluded that the splanchnic nerve likely contained more afferent fibers capable of evoking vascular reflexes.

More evidence of spinal sympatho-sympathetic reflexes was presented by Downman and McSwiney (28). By pinching

the abdominal viscera in spinal cats, these workers evoked large increases in arterial pressure. Mukherjee (74) then demonstrated that bladder distension in spinal cats could reflexly increase arterial pressure. In this study, it was pointed out that bladder distension also could cause renal vasoconstriction.

Up to this point, the ability of the sympathetic efferent system to respond to peripheral stimulation in the spinal animal had been shown only indirectly. Arterial pressure changes could be induced, but sympathetic efferent nerve activity had not been recorded. In 1964, Beacham and Perl provided direct evidence that the sympathetic efferent system was capable of responding reflexly in spinal animals (5). These investigators showed that afferent stimulation of dorsal roots, spinal nerves or limb nerves was capable of inducing a reflex discharge in sympathetic preganglionic fibers exhibiting tonic activity. However, possibly the most interesting finding was that both excitatory and inhibitory responses could be evoked. The observations that some of these neurons are tonically active and are capable of responding with an increased or decreased discharge greatly increase the possibilities for spinal sympathetic control of circulation.

The recent information provided by investigators in Malliani's laboratory makes the hypothesis of spinal sympathetic control of circulation extremely attractive. The work that most immediately prompted the hypothesis was the

demonstration of sympathetic discharge upon coronary occlusion (65) or increases in coronary or systemic arterial pressure (19,60) in spinal animals. Subsequently, Peterson and Brown (85) showed that, in spinal cats, central stimulation of either the inferior cardiac or pericoronary nerves could increase arterial pressure without effect on heart rate. Following the observation by Peterson and Brown, Malliani et al. (62) were able to show that dP/dt max could be reflexly increased in spinal animals by stimulation of the afferent fibers in cardiac sympathetic nerves. dP/dt max refers to the maximum rate of change in ventricular pressure with time and is considered a fairly accurate in vivo measurement of myocardial contractility (14). As the authors pointed out, increases in dP/dt max can result from changes in heart rate, changes in preload (ventricular pressure at the end of diastole), or changes in afterload (aortic pressure) (62). In this set of experiments neither heart rate nor preload changed. The authors felt that only a negligible contribution to the increase in contractility was made by changing afterload, since afferent stimulation produced no change in arterial systolic pressure. Also, increases in arterial systolic pressure were detectable only when dP/dt max had already reached peak value. It was concluded that the increase in myocardial contractility was reflex in nature because there were no direct connections between stimulated afferent fibers and efferent

fibers involved in the pathway, and also because a direct effect such as alteration in heart rate, preload or afterload, could be excluded.

A further study by Malliani et al. (61) showed that either chemical activation of cardiac sympathetic receptors, or electrical activation of cardiac sympathetic afferent nerves could cause a tachycardia. The tachycardia was reflexly evoked in cats with neuraxis intact and in cats with neuraxis severed at C_1. Stimulation also caused a transitory apnea and an increase in arterial pressure. Since removal of the right stellate ganglion eliminated the tachycardia without affecting the increase in arterial pressure or change in respiratory pattern, it was proposed that the right stellate ganglion was required for the efferent pathway. Also, since interruption of the upper 4 thoracic rami communicantes (through which afferent sympathetic fibers gain entry into the spinal cord through the dorsal roots) eliminated all responses to cardiac sympathetic afferent stimulation, it was suggested that these nerves form the afferent limb of the reflex.

Finally, in 1974, workers in Malliani's laboratory showed that, in spinal, vagotomized cats, sympathetic preganglionic fibers from the third or fourth thoracic sympathetic ramus could respond reflexly with either increases or decreases in frequency of discharge depending on the location of stimulated receptors (83). Specifically, activation mainly of cardiac receptors always

reflexly increased discharge of responsive preganglionic fibers. In contrast, simultaneous activation of cardiac and vascular receptors or activation of aortic receptors could either increase or decrease discharge of preganglionic fibers, although each fiber was consistent in response. Thus, much evidence has accrued in support of the hypothesis that the sympathetic nervous system is at least capable of reflexly altering cardiovascular function without input from higher centers.

Possibly, the recently described cardiopulmonary-renal sympathetic reflex (101) also contains a component which is mediated at the spinal level. Changes in renal nerve activity could be effected rapidly by alterations in cardiopulmonary receptor activity and would not necessarily require input from the brainstem.

Effect of Renal Nerves on Renal Sodium and Water Excretion: Renal nerves may contribute significantly to the control of renal function. Since Bernard first reported a diuresis after sectioning the splanchnic nerve in an anesthetized animal (12), the effect of renal nerve activity on the kidney has been in question.

One of the first attempts at complete renal denervation was made by Marshall and Kolls (68,69) who sectioned the splanchnic and all visible renal nerves. They found that, in dogs, either unilateral adrenalectomy, unilateral splanchnicotomy or unilateral section of all visible renal nerves resulted in a dilute urine in the denervated kidney,

such that the total amount of water, chloride, and urea excreted by the denervated kidney was greater than that excreted by the intact kidney. However, there was little difference in total creatinine or phenolsulphonphthalein excreted. Many of these effects were eliminated by applying pressure to the artery of the denervated kidney with a pressure cuff. However constricting the renal artery in this manner, also resulted in decreased total creatinine excretion. The authors attributed the changes in excretion of urea and sodium chloride to alterations in blood flow. However, neither creatinine excretion, (a measure of GFR), nor phenolsulphonphthalein (an indicator of renal blood flow) were increased as a result of denervation.

Due to the failure of Marshall and Kolls to demonstrate an increased GFR or renal blood flow in response to renal denervation, Kriss et al. (51) repeated the study using mannitol as a measure of GFR and para-amino hippurate (PAH) as a measure of renal plasma flow. These workers showed that unilateral splanchnicotomy resulted in a 447% increase in chloride excretion and a 205% increase in water excretion from the denervated kidney compared to the control kidney. In contrast, mannitol and PAH excretion were increased only 18% and 19% respectively, in the denervated kidney. These authors stated that, although the increases in chloride and water excretion could not always be explained by increases in glomerular

filtration rate or renal plasma flow, there was not enough evidence to conclude that alterations in excretion were due to specific inhibition of tubular reabsorption.

In 1951, Kaplan and Rapoport (47) again examined the effects of splanchnicotomy on renal function under conditions of osmotic diuresis. These investigators used creatinine as an index of GFR and PAH as an index of renal plasma flow. Creatinine is generally believed to be a better indicator of GFR than is mannitol (11). They showed that sodium and chloride excretion were increased in the denervated kidney although neither GFR nor renal plasma flow were increased above values determined in the innervated kidney. This indicated that changes did not result from alterations in filtered load. These authors proposed that the splanchnic nerve might control proximal tubular reabsorption of sodium.

The major problem with acceptance of the hypothesis that renal nerves can alter renal tubular functions stems from the concept that even undetectable changes in GFR might alter sodium excretion. This argument was used by Selkurt (93) in 1954 concerning the work of Kaplan and Rapoport. Selkurt pointed out that the GFRs in Kaplan's and Rapoport's dogs were always higher in the denervated kidney. In fact, this was the case although in one group of experiments, GFR was increased only 3% in the denervated kidney, whereas sodium excretion in the denervated kidney was increased 102% over that in the innervated

kidney. Thus, in spite of accumulating evidence, most investigators refused to accept the hypothesis that renal nerves could influence renal tubular reabsorption.

Another criticism was that differences in renal function following renal denervation could only be demonstrated in anesthetized dogs. Studies in unanesthetized dogs had failed to show differences between innervated and denervated kidneys (42,66,89). In 1952, Berne (13) conducted a study comparing the effects of chronic unilateral denervation in anesthetized and unanesthetized dogs. He confirmed the findings of others that renal function does not differ between denervated and innervated kidneys in the unanesthetized animal and felt that the differences he saw in anesthetized animals were due to differences in GFR. Kamm and Levinski (46) approached the problem by clamping the renal artery of a denervated kidney. The purpose of the clamp was to return sodium excretion to predenervation levels. When this was done, they found that the amount of sodium being filtered by the kidney also returned to predenervation levels. They reasoned that the denervation natriuresis must have resulted from an increase in filtered sodium, since clamping the renal artery caused filtered sodium to return to predenervation levels. Had decreased sodium reabsorption been the cause of denervation natriuresis, clamping the renal artery should have caused filtered sodium to fall below the predenervation level. More

credence was added to the idea that renal nerves did not affect renal tubular reabsorption when using histofluorescence techniques, Nilsson failed to demonstrate adrenergic innervation of the renal tubules in rats and rabbits (77). This finding was subsequently confirmed in dogs (71).

The Kamm and Levinski study seemed to be rather conclusive. However, as pointed out later by Bonjour et al. (15), Kamm and Levinski failed to report urine volume. Possibly, renal denervation produced a diuresis in their experiments. Also, a fall in renal arterial blood pressure might in itself be antinatriuretic independent of changes in GFR. By microsphere injection, Bonjour et al. were able to decrease GFR in a denervated kidney without altering renal arterial pressure. Using this procedure they found that even when GFR was decreased 40% there was still a significant diuresis and natriuresis in the denervated as compared to the innervated kidney. Apparently, alterations in GFR could not necessarily account for changes in function following denervation.

Renal denervation results not only in alterations in GFR but also in alterations in renin concentrations. In 1964, Taquini et al. (99) showed large differences in renin concentrations in innervated versus chronically denervated rat kidneys. At the same time, Barajas (3) showed that the juxtaglomerular apparatus was innervated. Possibly the increased sodium excretion seen following

denervation was due to decreased renin secretion, since a decrease in circulating renin would result in a decrease in angiotensin II production, and this, in turn would lead to a decrease in release of aldosterone. This, of course, would indicate an indirect effect of renal nerves on renal sodium excretion through the renin-angiotensin system.

Another possible mechanism through which renal nerves might alter sodium and water excretion, concerns blood flow distribution. Aukland (1) investigated the effects of renal nerve stimulation on blood flow distribution and found that, during renal nerve stimulation, both cortical and medullary flow were reduced to the same extent as measured by local clearance of hydrogen gas. Using a technique involving ^{85}Kr autoradiographs, workers in another laboratory found that stimulation of renal nerves resulted in relative increases in outer medullary blood flow (86). Bencsáth and Takács (10) approached the problem by examining the effects of unilateral splanchnicotomy on intrarenal distribution of blood flow in hydropenic, normovolemic, isotonic volume expanded and hypotonic volume expanded dogs. They found, using isotope indicators, that denervation resulted in an increased medullary blood flow in the denervated kidney especially in the hydropenic and normovolemic animals. However, although the increase in medullary flow was less in animals whose blood

volume had been increased, these animals showed a much higher level of diuresis and natriuresis than the hydropenic and normovolemic animals. They concluded that, although the diuresis seen following denervation might be due to increases in medullary blood flow, the natriuresis was not due to alterations in blood flow distribution. Again, it was suggested that renal nerves might exert a direct influence on renal tubules, or an indirect influence other than that associated with changes in blood flow.

This hypothesis was supported by work previously done by Gill and Bartter who showed that adrenergic blockade failed to decrease sodium excretion in patients on low sodium intake, although GFR was decreased and plasma aldosterone concentration was increased (33). Similarly, Gill and Casper showed that stimulation of alpha adrenergic receptors in the kidney resulted in increased reabsorption of sodium (34). Alpha adrenergic stimulation was accomplished by infusing the kidney with norepinephrine and propranolol (a beta adrenergic blocker).

Gill and Casper suggested that the decrease in urine flow and free water clearance along with the lack of change in GFR and urine osmolarity provided indirect evidence that alpha adrenergic stimulation increased proximal tubular sodium reabsorption. Bello-Reuss et al. (7) then showed by micropuncture that the increase in sodium excretion in denervated rat kidneys was due to decreased reabsorption

in the proximal tubule which was only partially compensated for by more distal segments of the nephron. In this study, GFR was unchanged. Prior to the study by Bello-Reuss et al., Müller and Barajas (75) had shown with electron microscopy that both proximal and distal tubules were directly innervated. When one considers that renal alpha-adrenergic stimulation may increase proximal tubular sodium reabsorption, that renal denervation does decrease proximal tubular sodium reabsorption, that renal tubules are innervated, and also that norepinephrine can stimulate active transport of sodium in epithelia similar to that of renal tubules (4,39), the possibility of a direct action of renal nerves on renal tubules influencing sodium movement becomes quite plausible.

Recently, it has been shown that direct low frequency (1-2 Hz) electrical activation of renal nerves in dogs can cause sodium retention without causing changes in GFR, renal blood flow or intrarenal blood flow distribution (95). Low frequency electrical activation of renal nerves in rats also was shown to cause an antidiuresis and antinatriuresis without changes in GFR or renal plasma flow (9). The study in rats which used whole kidney and single nephron techniques showed that the response appeared to be mediated by slowly conducting (0.7-1 m/sec), unmyelinated efferent renal nerves and that effects seen were due to action of these nerves on the proximal tubule. These studies were

important since questions have arisen as to adverse effects of renal denervation. Katz and Shear (49) have shown assymetric intra-renal blood flow distribution and renal damage resulting from denervation. They also suggested that denervation methods such as splanchnicotomy might result in incomplete denervation.

Because renal nerve stimulation can cause the release of renin (52) and prostaglandins (29) from the kidney, possibly the decreases in sodium excretion are not due to direct catecholaminergic influences on the renal tubule. Since both angiotensin II (70) and prostaglandin E_1 (38,56) increase sodium transport by toad bladder and frog skin, as does norepinephrine, (4,39) an indirect mechanism involving renal nerves still cannot be ruled out.

Zambraski and DiBona (102) showed that the antinatriuresis seen on low level renal nerve stimulation was not affected by angiotensin II inhibition. They suggested that the antinatriuresis seen with renal nerve stimulation was due to an increase in proximal tubular sodium reabsorption and was the direct result of the action of renal nerves on the proximal tubule. Kaloyanides et al. (45) then showed that the antinatriuresis could not be due to increases in prostaglandin by pretreating with indomethacin, a prostaglandin synthetase inhibitor. Thus, attention was turned back to direct catecholaminergic influences on the renal tubule.

Previously, Gill and Casper had shown that stimulation of alpha adrenergic receptors in the kidney caused decreases in sodium and water excretion (34). Recently, workers in DiBona'a laboratory have reexamined the specificity of the adrenergic effect with micropuncture techniques, using low frequency renal nerve stimulation in dogs (103). Their results showed that the antinatriuretic response to renal nerve stimulation could be blocked by phenoxybenzamine, an alpha adrenergic receptor blocking agent. A subsequent study by the same laboratory demonstrated that antinatriuresis evoked by reflex stimulation of the renal nerves also could be blocked by phenoxybenzamine or by guanethadine (104). Since guanethadine does not inhibit the response to circulating norepinephrine but specifically affects responses to sympathetic adrenergic stimulation, and phenoxybenzamine specifically blocks alpha-adrenergic receptors (37), the indication is that sodium reabsorption may in part be controlled by alpha adrenergic activity of renal nerves with endings directly on the tubules. Recent denervation studies in rats (8) and dogs (82) support the hypothesis that renal nerves may directly alter sodium excretion by acting on the proximal tubule.

RATIONALE

In cats with an intact neuraxis, electrical stimulation of cardiopulmonary sympathetic afferent nerves causes an excitation of renal sympathetic efferent nerve activity followed by an inhibition (101). Activation of these afferent nerves also has been shown to alter cardiac sympathetic efferent nerve activity via spinal and supraspinal pathways. Therefore, experiments were designed to determine the existence of a purely spinal component of the cardiopulmonary-reflex.

Also, since activation of cardiopulmonary sympathetic nerves can alter renal nerve activity (101), and alterations in renal nerve activity may alter renal function (27), experiments were designed to determine if cardiopulmonary sympathetic afferent stimulation was capable of altering renal function.

METHODS

GENERAL METHODS

Experiments were conducted in 33 cats. One cat was anesthetized with sodium pentobarbital (60 mg/kg) to allow spinal cord transection but was unanesthetized during the experiment. Thirty-two cats were anesthetized either with alpha chloralose (60 mg/kg) or with a mixture of sodium diallyl-barbiturate (50 mg/kg), urethane (200 mg/kg), and monoethyl urea (200 mg/kg). Cats of either sex were used and body weight ranged between 2-3 kg.

A tracheostomy was performed in all cats and both femoral veins were cannulated for delivery of drugs and infusates. One femoral artery was cannulated for monitoring arterial pressure and a second femoral artery was cannulated for withdrawing arterial blood samples. In some experiments the left jugular vein was cannulated with cannula advanced close to the right atrium for monitoring central venous pressure. Pressures were monitored with a pressure transducer (Model P23A; Statham, Inc. Hato Rey, P.R.) connected to a Grass polygraph (Model 7; Grass Inst. Co, Quincy, MA). To insure adequate muscle relaxation , cats were immobilized with 4 mg/kg gallamine triethiodide (Flaxedil, Davis-Geck, Pearl River, NY) and artificially respired with a Harvard respirator (model 607; Harvard Apparatus Co., Millis, MA). Subsequent doses of Flaxedil were administered as needed, following assessment of level of anesthesia. Respiration frequency was 18-20 breaths per

min. Tidal volume was approximately 45 ml and was adjusted to insure normal arterial PO_2 and pH as measured with a blood gas analyzer (Radiometer, Model MK2; Copenhagen). Arterial P_{CO_2} often was lower than normal due to slight hyperventilation. Esophageal temperature was monitored with a Telethermometer (model 43TD; Yellow Springs Inst. Co., Yellow Springs, OH) and maintained between 36° and 38°C with lamps and heating pads.

Influences of carotid sinus, aortic arch, and vagally innervated baro- and chemoreceptors were eliminated by severing glossopharyngeal and vagus nerves bilaterally at the jugular foramen. In some of the spinally transected cats, only the vagus nerve was severed, since severing the neuraxis eliminated reflex influences of the glossopharyngeal nerve on renal nerve activity.

In all cats access to the kidney was gained through a retroperitoneal approach. In most cats, one or two multifiber branches of a left renal nerve were severed and the central end was tied with saline-soaked thread. Nerves were tied with thread to facilitate attachment to electrodes. Nerves were immersed in a pool of mineral oil to prevent drying. In one group of experiments the left ureter was cannulated for collection of urine samples.

The left stellate ganglion was isolated in all experiments which utilized electrical stimulation of afferent cardiac sympathetic nerves. The ganglion was approached retropleurally after removal of portions of the second

and third ribs. Leaving the pleura intact, cardiac nerves emanating from the caudal edge of the ganglion (inferior cardiac nerve and/or ansa subclavia) were severed and the central ends were tied with saline-soaked thread. This procedure facilitated attachment of nerves to electrodes. Nerves were subsequently immersed in a pool of mineral oil

SPECIFIC METHODS I: SPINAL REFLEX EXPERIMENTS (FIGURE 1)

A. Activation of Afferent Nerves

1. Activation by Electrical Stimulation

In 7 cats cardiopulmonary or renal sympathetic afferent nerves were activated by electrical stimulation. The severed, tied central end of a cardiac or renal nerve was laid across one pole of a bipolar platinum electrode, with the attached thread loop being placed over the second pole. This technique allowed good contact of the nerve with the electrode and prevented the nerve from slipping.

Nerves were stimulated with trains of pulses. A train was 10 msec in duration and consisted of 3 equally spaced, square wave pulses. Each pulse was 0.5-1.0 msec in duration and 8-30V in intensity. Train frequency was 0.5 per sec. Stimuli were delivered to the afferent nerve from a Grass stimulator (Model S48) through a capacitance-coupled Grass stimulus isolation unit (Model SIU5).

2. Activation by Intravascular Volume Expansion

In 7 cats, cardiopulmonary sympathetic afferent

nerves were activated by increasing the circulating blood volume. All cats received an infusion of 3% dextran in isotonic saline. The infusion was delivered into a femoral vein at 4.4 ml per min using a Harvard infusion pump (Model 975; Harvard Apparatus Co.). The blood volume of 4 cats was expanded with 25 ml dextran per kg; the blood volume of 3 cats was expanded with 15 ml dextran per kg.

B. Neural Recording

The severed, tied central end of a cardiac or renal nerve was laid across one pole of a bipolar platinum electrode, with the attached thread loop being placed over the second pole. Spontaneous or evoked activity of efferent cardaic or renal nerves detected at the recording electrode was amplified by a capacitance-coupled Grass preamplifier (Model P511) using a bandwidth of 30 or 100 Hz - 1K Hz, displayed on a Techtronix oscilloscope (model D13; Techtronix Inc., Beaverton, OR), and stored on magnetic tape with a Tandberg recorder (series 100; Sangamo Data Systems, Columbus, OH, supplier).

C. Protocol

1. Responses to Activation of Afferent Nerves with Electrical Stimulation

Efferent nerve responses to cardiopulmonary sympathetic afferent stimulation were compared prior to

and following C_1 spinal cord transection. Prior to spinal cord transection, the ansa subclavia or inferior cardiac nerve was electrically stimulated and renal nerve activity was recorded in 5 anesthetized cats to verify the presence of a cardiopulmonary-renal sympathetic reflex. In two of these cats the presence of a cardio-cardiac reflex was verified by stimulating the ansa subclavia while recording from the inferior cardiac nerve. Similarly, for determination of a renal-renal reflex, one branch of a renal nerve was stimulated while evoked activity was recorded from a second branch of a renal nerve. Frequency of stimulation in all cases was 0.3-0.5 trains per sec and intensity was 8-30V. To insure that stimulus voltage was supramaximal, voltage was increased until the amplitude of the evoked efferent nerve response could not be increased further by further increases in stimulus voltage.

Following initial characterization of reflexes, the spinal cord was exposed at the first cervical segment (C_1). Immediately prior to spinal transection the dura was opened, taking care not to interrupt any venous sinuses. The spinal cord was then severed at C_1 using blunt dissection. The spinal cord also was severed in two cats in which reflexes were not characterized prior to spinal transection. Warmed dextran (3% in isotonic saline) or phenylephrine (0.06% Neo-

Synephrine, Winthrop Laboratories, NY, NY) were infused into a femoral vein to maintain arterial pressure above 70 mmHg, if necessary. Infusion rates were 2 ml/min and 0.01 ml/min, respectively.

Transection of the spinal cord often causes a marked but transient depression of spontaneous nerve activity. Following the recovery of spontaneous nerve activity, attempts were made to evoke a cardiopulmonary-renal reflex. In two cats, the presence of a cardio-cardiac reflex, and in three cats the presence of a renal-renal reflex also were determined. At the end of the experiment hexamethonium (a ganglionic blocking agent) was given to block neural transmission and thus allow accurate assessment of noise.

2. Responses to Activation of Afferent Nerves with Intravascular Volume Expansion

In 7 cats, in which the spinal cord was transected, cardiopulmonary sympathetic afferent nerves were activated by intravascular volume expansion in a further attempt to elicit a spinal cardiopulmonary-renal reflex. The spinal cord was transected at C_1 using blunt dissection. Arterial pressure was monitored and in cats whose blood pressure was less than 70 mmHg, pressure was supported with a phenylephrine infusion (0.06% Neo-Synephrine) at 0.01 ml/min. As previously described, nerve activity is often markedly depressed following

spinal cord transection. Thus, time was allowed for activity to recover. Following recovery of spontaneous nerve activity intravascular volume expansion (as described in Specific Methods I.A.2) was begun.

During the infusion, renal nerve activity was sampled every 2 min. Following the end of infusion, activity was sampled every 2 min for 15 min and every 5 min thereafter, for at least one hour. Throughout the experiment central venous pressure was continuously monitored. At the end of the experiment hexamethonium was given to block neural transmission and thus allow accurate assessment of noise level.

D. Data Analysis

1. Responses to Activation of Afferent Nerves with Electrical Stimulation

Nerve activity stored on magnetic tape, was later characterized by constructing histograms with the use of a Nicolet computer (Model 1070; Nicolet Inst. Corp., Madison, WI). Prior to computer analysis, nerve activity was processed by a window discriminator. Absolute noise level (10-20 μV) which was recorded at the end of each experiment, was used to facilitate proper adjustment of the threshold of the window discriminator. The threshold was considered to be adjusted properly when the window discriminator generated a pulse each time the voltage due to nerve activity exceeded that due to noise. These pulses were counted by the computer.

During electrical stimulation, a trigger signal from the stimulator was recorded simultaneously with efferent nerve activity. This signal, which corresponded in time with each stimulation of the afferent nerve, was used to trigger the computer to count pulses generated by the window discriminator. An internal trigger was used to initiate computer counting of spontaneous nerve activity. Once triggered, the computer was set to count for 200 msec or 1 sec. Histograms constructed consisted of summations of 50 or 100 triggered responses (evoked or spontaneous nerve activity). Histograms were then plotted with an x-y recorder (Model 7015A; Hewlett-Packard, San Diego, CA).

2. Responses to Activation of Afferent Nerves with Intravascular Volume Expansion

Nerve activity stored on magnetic tape was later processed by computer to determine frequency of discharge. Basic computer techniques are described in Specific Methods I.D.1. In these experiments the computer was used to sample and count spontaneous nerve activity for 30 sec periods. The number of counts registered by the computer was then displayed as a digital readout and used to calculate discharge frequency in counts per sec.

Mean control discharge frequency was calculated from four to six 30 sec samples obtained over a 5 min period. Following the initiation of intravascular volume expansion, discharge frequency was calculated every 2 min. Discharge frequencies obtained during intravascular volume expansion were transformed into percent of control discharge frequency. Central venous pressure also was recorded during the experiment. Following initiation of intravascular volume expansion, change in central venous pressure was determined at two minute intervals simultaneously with estimates of nerve activity.

Data were analyzed by constructing a confidence interval ($\bar{x} \pm t \cdot s_{\bar{x}}$) around central venous pressure (changes from control) or renal nerve activity (percent of control). Responses not contained within the confidence limits were considered significantly different from control ($P < 0.05$).

SPECIFIC METHODS II: RENAL FUNCTION EXPERIMENTS (FIGURE 2)

A. Activation of Afferent Nerves

In three groups of cats, cardiopulmonary sympathetic afferent nerves were prepared for electrical stimulation as described in Specific Methods I.A.1. In one group of cats, the afferent nerve was prepared for stimulation but was not stimulated. This group constituted a control group.

In a second group of cats afferent nerves were stimulated at 1-2 trains per sec. (Trains have been previously described in Specific Methods I. A.1.) Stimulus intensity in these experiments was 8-12V. In a third group of cats, afferent nerves were stimulated at 5-7 Hz. Stimulus duration was 0.5-1 msec and intensity was 8-12V.

B. Neural Recording

The effect of afferent nerve stimulation on renal efferent nerve activity was assessed in 7 cats. Nerves were prepared as previously described (Specific Methods I.B) and activity was amplified by a capacitance-coupled Grass preamplifier (Model 7) using a bandwidth of 10Hz-500Hz. Activity was displayed on a Grass polygraph (Model 7).

C. Sample Collection for Estimation of Renal Function

Urine and blood samples were obtained to allow estimation of glomerular filtration rate (GFR), urine flow rate, sodium excretion, and potassium excretion. Inulin clearance was measured to assess GFR. A solution of inulin (3% in isotonic saline) was infused into a femoral vein at 0.299-0.419 ml/min. Infusion was begun one hour prior to the initiation of an experiment to insure a constant plasma inulin concentration. Urine samples were collected from a cannula inserted into the left ureter. Urine collection periods were

10 min. Blood (1.5 ml) was sampled at the midpoint of each collection period from a cannula inserted into a femoral artery. Plasma was then separated from the blood sample and stored for later analysis.

D. Protocol

Experiments were conducted in three groups of cats to assess the effect of cardiopulmonary sympathetic afferent stimulation on renal function.

In a control group of 6 cats, consecutive urine and blood samples were obtained for 40-70 min. In cats in which afferent nerves were stimulated (1-2 trains/sec. or 5-7 Hz), after collecting two or three consecutive urine samples of constant volume, afferent stimulation was begun. Urine and blood samples were collected during the 10 min stimulation period and during two or three post-stimulation periods. Arterial pressure was continuously monitored in all cats and stabilized, if necessary, by infusing or withdrawing of a small volume (2 ml) of blood. Renal efferent nerve activity was continuously recorded in 5 of 7 cats in which afferent nerves were stimulated at 1-2 trains/sec. Renal nerve activity also was recorded from 2 additional cats in which afferent nerves were stimulated at 5-7 Hz. Renal function parameters were not measured in these 2 cats.

E. Data Analysis

1. Neural Recordings

Renal nerve activity was displayed on a Grass polygraph as described in Specific Methods II.B. This activity was simultaneously quantified by cumulative integration with a Grass integrator (Model 7P10). The threshold of this integrator was adjusted until only that voltage which exceeded noise level was accumulated. Voltage was integrated until a given preset voltage was reached. At this time the integrator reset and began accumulating voltage again. Changes in nerve activity were reflected as changes in time required for integrator resetting. This time is referred to as epoch time. Thus, inhibition of nerve activity would lengthen epoch time. Conversely, excitation of nerve activity would shorten epoch time.

2. Renal Function

Urine and plasma samples were analyzed for Na^+ and K^+ concentration by flame photometry with a Beckman photometer (Model 105; Beckman Inst., Palo Alto, CA). Urine and plasma inulin concentrations were determined by the method of Schreiner (92) (see Appendix). Total excretion of Na^+ ($U_{Na}V$) and K^+ (U_KV) were calculated as the product of ion concentration in a given urine

sample and the urine flow rate. Fractional excretion of Na^+ (FE_{Na}), which is the amount of Na^+ excreted per amount filtered, was calculated as:

$$FE_{Na} = \frac{U_{Na} \cdot V}{P_{Na} \cdot GFR} \cdot 100$$

where U_{Na} = urine sodium concentration (μEq/ml)

P_{Na} = plasma sodium concentration (μEq/ml)

V = urine flow rate (ml/min)

GFR = glomerular filtration rate (ml/min)

GFR was calculated as $\frac{U_{in} \cdot V}{P_{in}}$

where U_{in} = urine inulin concentration (mg%)

P_{in} = plasma inulin concentration (mg%)

V = urine flow rate (ml/min)

Data from the cats undergoing afferent stimulation were statistically analyzed using a randomized complete block design (96). Grouped data were expressed as a mean and variation was estimated with a coefficient of variability. Means were compared using the least significant difference test. The control state for each cat was estimated by averaging data obtained during the 10 min collection periods prior to stimulation. Similarily the data obtained during collection

periods following stimulation were averaged. A value of P less than 0.05 was considered significant.

Figure 1. General methods used in determining the existence of a spinal cardiopulmonary-renal reflex.

In anesthetized cats, cranial nerves IX and X were severed at the jugular foramen. One cannula was inserted into a femoral artery in order to monitor arterial pressure, and a second cannula was inserted into a femoral vein for administration of drugs. In electrical stimulation experiments one or two sympathetic nerves (CP) emanating from the left stellate ganglion were severed. One nerve was used for stimulation, and in some cats a second nerve was used for recording efferent activity. One or two branches of the left renal nerve were severed. One nerve was used for recording efferent activity, and in some cats a second renal nerve was used for stimulating. In most cats a cardiopulmonary-renal reflex was demonstrated prior to C_1 cord transection. In some cats cardio-cardiac and renal-renal reflexes also were demonstrated. The spinal cord was then severed at the first cervical segment and reflexes were reexamined. In intravascular volume expansion experiments, one branch of a left renal nerve was used for recording efferent activity and the left jugular vein was cannulated for monitoring central venous pressure. The spinal cord was then transected at C_1 and intravascular volume expansion was accomplished by infusing dextran into a femoral vein at 4.4 ml/min.

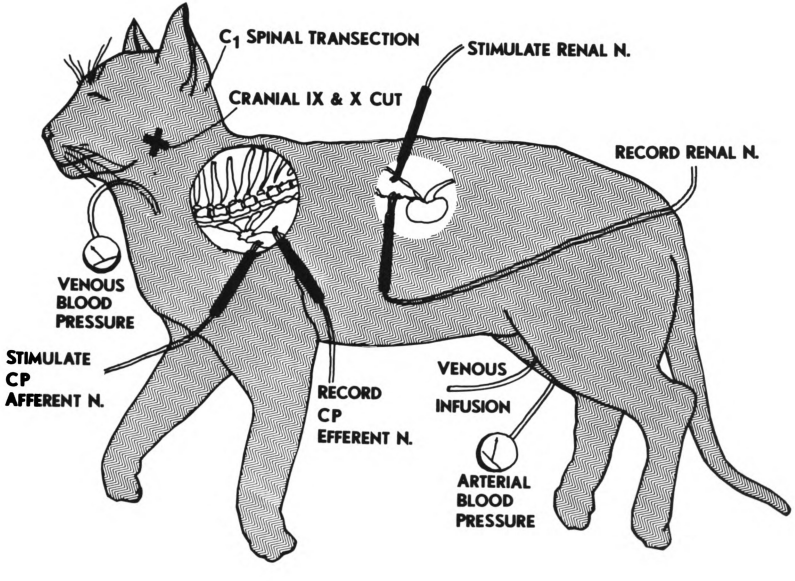
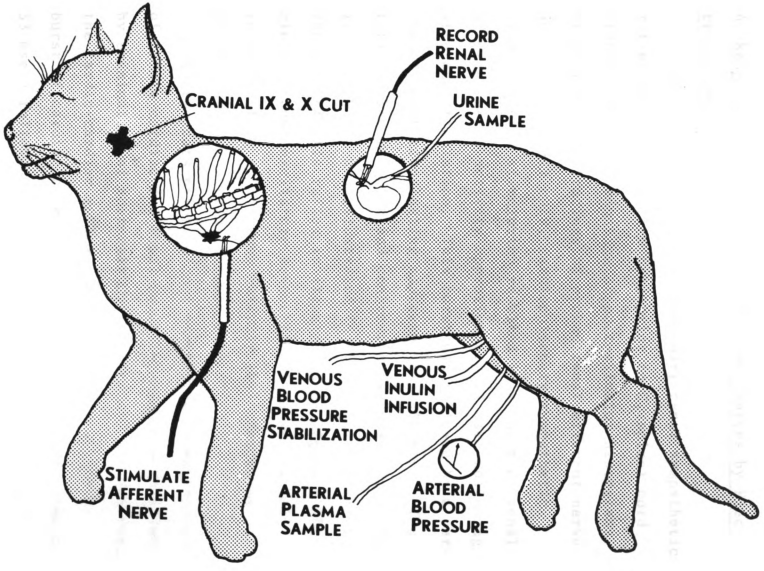

C1 SPINAL TRANSECTION
STIMULATE RENAL N.
CRANIAL IX & X CUT
RECORD RENAL N.
VENOUS BLOOD PRESSURE
STIMULATE CP AFFERENT N.
RECORD CP EFFERENT N.
VENOUS INFUSION
ARTERIAL BLOOD PRESSURE

Figure 2. General methods used in determining the influence of cardiopulmonary sympathetic afferent nerves on renal function.

In anesthetized cats, cranial nerves IX and X were severed at the jugular foramen. A left cardiac sympathetic nerve was severed and prepared for stimulation centrally and a small multifiber branch of the left renal nerve was prepared for recording efferent activity. The left ureter was cannulated for collection of urine samples. A femoral artery was cannulated to monitor arterial pressure, which was held constant by infusing or withdrawing small amounts of fluid from a femoral vein. Inulin was infused into the remaining femoral vein to establish constant plasma inulin concentration, for later assessment of glomerular filtration rate. Throughout the experiment, urine and arterial blood samples were collected every 10 min.


RECORD
RENAL
NERVE
CRANIAL IX & X CUT
URINE
SAMPLE
VENOUS
BLOOD
PRESSURE
STABILIZATION
VENOUS
INULIN
INFUSION
STIMULATE
AFFERENT
NERVE
ARTERIAL
PLASMA
SAMPLE
ARTERIAL
BLOOD
PRESSURE

RESULTS

I. SPINAL REFLEX EXPERIMENTS

A. Responses to Activation of Afferent Nerves by Electrical Stimulation

The presence of a cardiopulmonary-renal sympathetic reflex was verified in 5 cats prior to C_1 spinal cord transection by examination of post-stimulus histograms or oscilloscope tracings of evoked renal efferent nerve activity. In all cats, cardiopulmonary sympathetic afferent nerve stimulation resulted in a burst of renal efferent nerve activity approximately 82 msec following afferent stimulation. This burst was followed by a period of inhibition lasting approximately 400 msec. The total evoked response and contrasting spontaneous activity in one cat are illustrated in Figure 3. The first 200 msec of this reflex is illustrated in Figure 4 which clearly shows excitatory component of the reflex (A) in contrast to spontaneous activity (B). Responses of all cats are listed in Table 1.

In two cats prior to cord transection, the presence of cardio-cardiac and renal-renal reflexes was verified by examination of post-stimulus histograms. In one cat, the cardio-cardiac reflex consisted of an excitatory burst of cardiac efferent nerve activity (onset latency: 53 msec) followed by a 600 msec silent period (Figure 5, Table 1: cat 7). The excitatory component is

illustrated in Figure 6A in contrast to spontaneous nerve activity in Figure 6B. In a second cat, the cardio-cardiac reflex consisted of three components (Table 1: cat 6). The first response was an early excitatory burst of activity which followed stimulation with a latency of 28 msec. This early burst was followed at 64 msec by a second excitatory burst of longer duration. The second burst was followed immediately by a 400 msec period of inhibition.

In both cats (Table 1: cats 6,7) stimulation of the afferent fibers in one renal nerve evoked two excitatory bursts of efferent activity in a second renal nerve. The early response occurred approximately 30 msec following stimulation while the latency of the later response approximately 110 msec. The late burst was longer in duration than the early burst and was followed by a 300 msec period of inhibition. The total response in one cat is illustrated in Figure 7 although two separate excitatory bursts cannot be seen. The two separate excitatory responses are illustrated more clearly in Figure 8A and B.

Following verification of cardiopulmonary-renal, cardio-cardiac, and renal-renal reflexes, the spinal cord was transected at the first cervical segment. C_1 spinal transection also was performed in two cats in which reflexes had not been tested previously. Spinal

cord transection often caused a large, transient decrease in spontaneous nerve activity. Therefore, spontaneous activity was allowed to recover prior to testing reflexes.

After the recovery of spontaneous nerve activity, the cardiopulmonary renal reflex was tested in 7 cats. Attempts to evoke this reflex in one cat are illustrated in Figure 4. No difference was seen between evoked (Figure 4C) and spontaneous (Figure 4D) activity. This is in contrast to the demonstration of reflex excitation in the same cat prior to C_1 spinal cord transection (Figure 4A,B). The cardiopulmonary-renal reflex could not be demonstrated in any cat following C_1 cord transection (Table 1).

However, other sympathetic reflexes were demonstrated in several cats following spinal transection. A spinal cardio-cardiac reflex was demonstrated in two cats (Table 1: cats 6,7; Figure 6C,D), and a spinal renal-renal reflex was demonstrated in 3 cats (Table 1: cats 5,6,7; Figure 8C,D). Both of these reflexes consisted of one excitatory burst of efferent nerve activity. The onset latency of these responses was approximately 35 msec. The spinal renal-renal reflex was strikingly similar to the early excitatory component of the same reflex evoked in the intact animal. Although no late burst or period of inhibition was seen in any spinal

cat, the presence of early responses indicates that the failure to demonstrate a cardiopulmonary-renal reflex could not be attributed to poor viability of the afferent or efferent nerves.

B. Responses to Activation of Afferent Nerves by Intravascular Volume Expansion

Following C_1 spinal cord transection, the intravascular blood volume of 7 cats was increased by dextran infusion. Four cats received a total of 25 ml dextran per kg and three cats received a total of 15 ml dextran per kg. Although dextran infusion significantly increased central venous pressure, no consistent change occurred in renal nerve activity (Figure 9: During Infusion). In cats which received 15 ml dextran per kg. nerve activity and central venous pressure were monitored after volume expansion had been completed. Although central venous pressure began to return toward control, no change was seen in renal efferent nerve activity (Figure 9: After Infusion). The lack of a renal nerve response was not due to lack of excitability since, in two cats, renal nerve discharge increased with renal anoxia produced by occluding the renal artery.

II. Renal Function Experiments

A. Renal Nerve Responses to Afferent Stimulation

Electrical stimulation of the central end of severed cardiopulmonary sympathetic afferent nerves at 1-2 Hz

produced an inhibition of renal nerve activity (Figure 10). In three cats the mean change in renal nerve activity was a 16% inhibition from control as measured by cumulative integration. This inhibitory response was consistent throughout the 10 min stimulation period.

Stimulation of the central end of severed cardiopulmonary sympathetic afferent nerves at 5 Hz produced no net change in discharge frequency of renal nerves in 2 cats as analyzed by computer. This is in contrast to the 16% inhibition seen with stimulation at 1-2 trains/sec.

B. Renal Function Responses to Afferent Stimulation

Stimulation of the cardiopulmonary sympathetic afferent nerves at 1-2 Hz also produced a diuresis and a natriuresis. Responses of individual animals are shown in Figures 11 and 12, and grouped data are illustrated in Figure 13. During stimulation, total sodium excretion increased in 6 of 7 cats. This increase often was long-lasting, continuing for the duration of the experiment (20-30 min). Urine flow rate also increased during stimulation and remained higher than control. In contrast, total potassium excretion was unaffected by stimulation in all cats. In one cat, potassium excretion increased during most of the experiment and therefore, the increase could not be attributed to stimulation.

The natriuresis and diuresis could not be attributed to an increased GFR or systemic blood pressure since neither parameter changed. In contrast, fractional excretion of sodium increased as a result of stimulation and remained higher than control during the post-stimulation periods.

Stimulation at 5-7 Hz produced no change in either sodium or water excretion. Also, potassium excretion, GFR, and systemic arterial pressure were unaltered. Grouped data are illustrated in Figure 14.

In 6 cats cardiopulmonary sympathetic afferent nerves were not stimulated. In these cats no consistent changes occurred in any of the renal function parameters evaluated. Responses of individual animals are shown in Figures 15 and 16.

Figure 3. Effect of cardiopulmonary sympathetic afferent nerve stimulation on renal sympathetic efferent nerve activity prior to spinal cord transection.

Stimulation of a cardiopulmonary sympathetic afferent nerve evoked an excitatory burst in renal efferent nerve activity which was followed by a period of inhibition (A). The evoked response can be contrasted with spontaneous renal efferent discharge recorded from the same nerve (B).

Format: Traces on the left are oscilloscope recordings of one response of the efferent nerve to afferent stimulation (A) or one sweep of spontaneous efferent nerve activity (B). Summations of 50 such responses in the same cat were used to construct histograms illustrated on the right. Delivery of the stimulus to the afferent nerve is represented by a dot. Stimulus consisted of a 10 msec train of 3 pulses (30V; 0.5 msec per pulse). Vertical calibration for nerve activity is 25 μV and for histograms is counts of activity. Horizontal calibration is 80 msec in all traces.

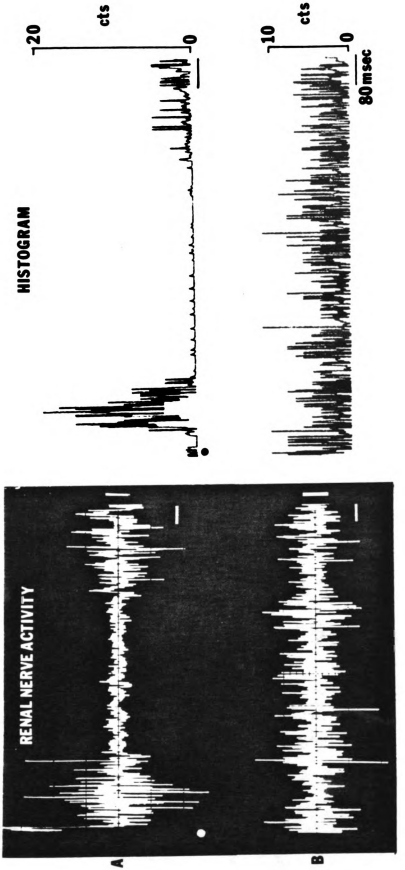

RENAL NERVE ACTIVITY
A
B
HISTOGRAM
20
cts
0
10
cts
0
80msec

Figure 4. Excitatory response of a renal sympathetic efferent nerve to cardiopulmonary sympathetic afferent nerve stimulation prior to and following spinal cord transection.

Intact: Prior to C_1 spinal cord transection, stimulation of a cardiopulmonary sympathetic afferent nerve evoked an excitatory burst in renal efferent nerve activity within the first 200 msec following stimulation (A). The evoked response can be contrasted with spontaneous renal efferent discharge recorded from the same nerve (B).

Spinal: Following C_1 spinal cord transection, stimulation of the cardiopulmonary sympathetic afferent nerve failed to evoke a reflex response in renal efferent nerve activity (C). The failure of afferent stimulation to produce a response is apparent when compared to spontaneous efferent activity (D).

Format: Traces on the left are oscilloscope recordings of one response of the efferent nerve to afferent stimulation (A,C) or one sweep of spontaneous efferent nerve activity (B,D). Summations of 100 such responses in the same cat were used to construct histograms illustrated on the right. Delivery of stimulus to the afferent nerve is represented by a dot. Stimulus consisted of a 10 msec train of 3 pulses (30V; 0.5 msec per pulse). Vertical calibration for nerve activity is 25 μV and for histogram is counts of activity. Horizontal calibration is 80 msec in all traces.

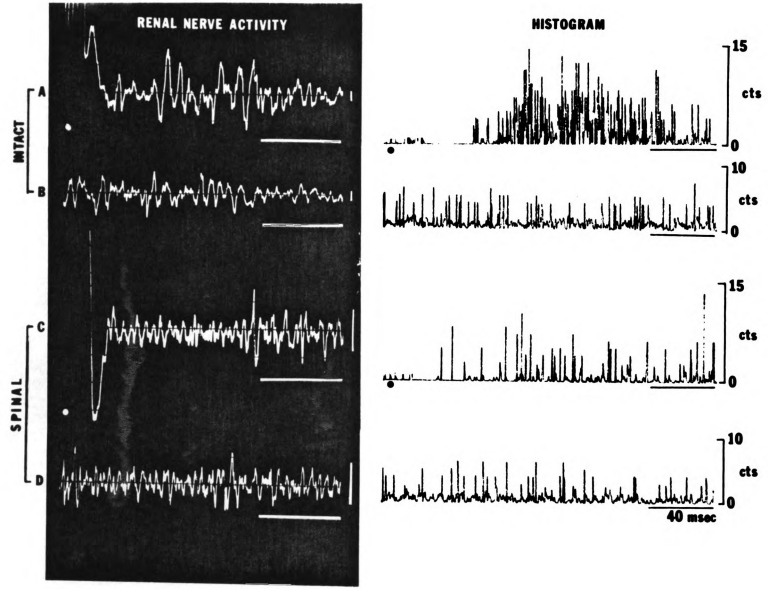
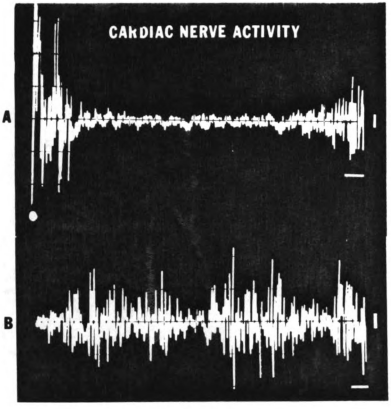

RENAL NERVE ACTIVITY
HISTOGRAM
INTACT
A
B
SPINAL
C
D
15
cts
0
10
cts
0
15
cts
0
10
cts
0
40 msec

Figure 5. Effect of cardiopulmonary sympathetic afferent nerve stimulation on cardiac sympathetic efferent nerve activity prior to spinal cord transection.

Stimulation of a cardiopulmonary sympathetic afferent nerve evoked an excitatory burst in cardiac efferent nerve activity which was followed by a period of inhibition (A). The evoked response can be contrasted with spontaneous cardiac efferent discharge recorded form the same nerve (B).

Format is the same as in Figure 3.


CARDIAC NERVE ACTIVITY
A
B

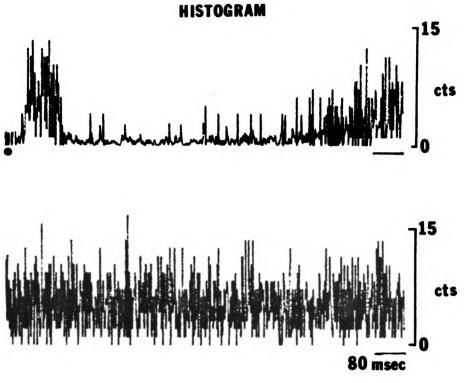

HISTOGRAM
15
cts
0
15
cts
0
80 msec

Figure 6. Excitatory response of a cardiac sympathetic efferent nerve to cardiopulmonary sympathetic afferent nerve stimulation prior to and following spinal cord transection.

Intact: Prior to C_1 spinal cord transection, stimulation of a cardiopulmonary sympathetic afferent nerve evoked an excitatory burst in cardiac efferent nerve activity within the first 200 msec following stimulation (A). The evoked response can be contrasted with spontaneous cardiac efferent discharge recorded from the same nerve (B).

Spinal: Following C_1 spinal cord transection, stimulation of a cardiopulmonary sympathetic nerve again evoked an excitatory burst in cardiac efferent nerve activity (C). This excitatory burst begins earlier than the excitatory burst seen prior to spinal cord transection (A), and can be contrasted with spontaneous efferent activity recorded from the same nerve (D).

Format is the same as in Figure 4.

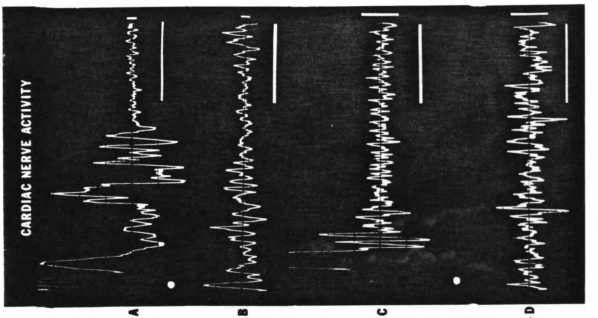
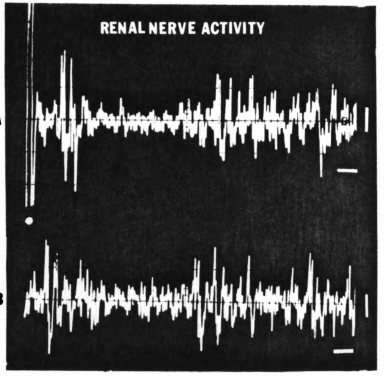

CARDIAC NERVE ACTIVITY
HISTOGRAM
INTACT
SPINAL
A
B
C
D
15
cts
0
10
cts
0
25
cts
0
5
cts
0
40 msec

Figure 7. Effect of renal sympathetic afferent nerve stimulation on renal sympathetic efferent nerve activity prior to spinal cord transection.

Stimulation of the afferent fibers in one renal nerve evoked an excitatory burst in efferent activity recorded from a second renal nerve. This excitatory burst was followed by a period of inhibition (A). The evoked response can be compared with spontaneous renal efferent discharge from the same nerve (B).

Format is the same as in Figure 3.


RENAL NERVE ACTIVITY
A
B

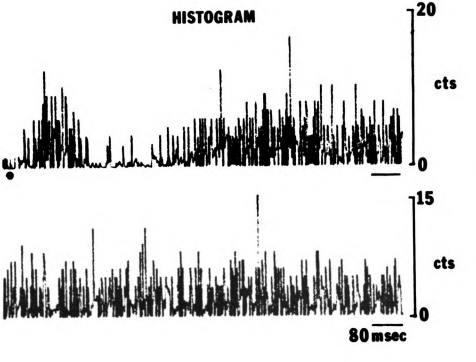

HISTOGRAM
20
cts
0
15
cts
0
80 msec

Figure 8. Excitatory responses of a renal sympathetic efferent nerve to renal sympathetic afferent nerve stimulation prior to and following spinal cord transection.

Intact: Prior to C_1 spinal cord transection, stimulation of a renal sympathetic afferent nerve evoked an early and late excitatory burst in renal efferent nerve activity within the first 200 msec following stimulation (A). These two responses appear as one response in Figure 7A due to the slower time base. The evoked response can be compared with spontaneous renal efferent discharge recorded from the same nerve (B).

Spinal: Following C_1 spinal transection, stimulation of a renal afferent nerve evoked only an early excitatory burst in renal efferent nerve activity (C). This burst was commensurate with the early burst seen prior to spinal transection (A) and can be compared with spontaneous renal efferent discharge recorded from the same nerve (D).

Format is the same as in Figure 4.

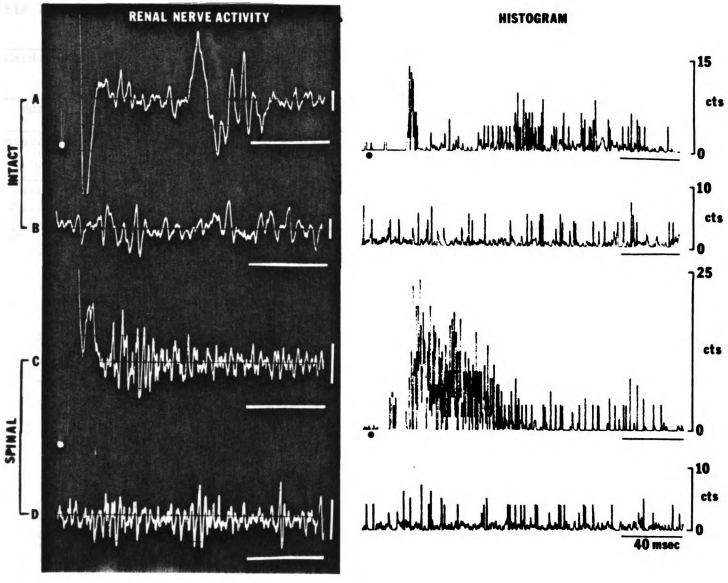

RENAL NERVE ACTIVITY
HISTOGRAM
INTACT
SPINAL
A
B
C
D
15
cts
0
10
cts
0
25
cts
0
10
cts
0
40 msec

Table 1. Sympathetic reflex characteristics prior to and following C_1 spinal cord transection.

	Supra Spinal									Spinal					
	Cardiopulmonary-renal (msec)			Cardio-cardiac (msec)			Renal-renal (msec)			Cardiopulmonary-renal (msec)		Cardio-cardiac (msec)		Renal-renal (msec)	
	Burst		Silent Period	Burst		Silent Period	Burst		Silent Period						
Cat No.	Onset	Duration	Duration	Onset	Duration	Duration	Onset	Duration	Duration	Onset	Duration	Onset	Duration	Onset	Duration
1	--[a]	--	--	--	--	--	--	--	--	X[b]	X	--	--	--	--
2	Present--Characteristics not determined			--	--	--	--	--	--	X	X	--	--	--	--
3	80	57	300	--	--	--	--	--	--	X	X	--	--	--	--
4	84	73	340	--	--	--	--	--	--	X	X	--	--	--	--
5	--	--	--	--	--	--	--	--	--	X	X	--	--	28	44
6	80	58	420	28 64	16 70	410	24 120	28 110	350	X	X	44	44	30	64
7	86	88	500	53	58	619	35 104	18 195	220	X	X	35	46	38	56

[a] Reflex not tested.

[b] Reflex tested but not present.

Figure 9. Effects of intravascular volume expansion on renal nerve activity and central venous pressure (CVP) following C_1 spinal cord transection.

Spontaneous nerve activity was quantified as spike frequencies by computer and is expressed as percent of control discharge. Time refers to the amount of time elapsed during or following infusion of 3% dextran in isotonic saline at 4.4 ml/min. Mean data are expressed $\pm$ standard error. Numbers in parentheses refer to the number of cats assessed at a given time period. Significance ($P < .05$) was determined by constructing a confidence interval and is indicated by an asterisk (*).

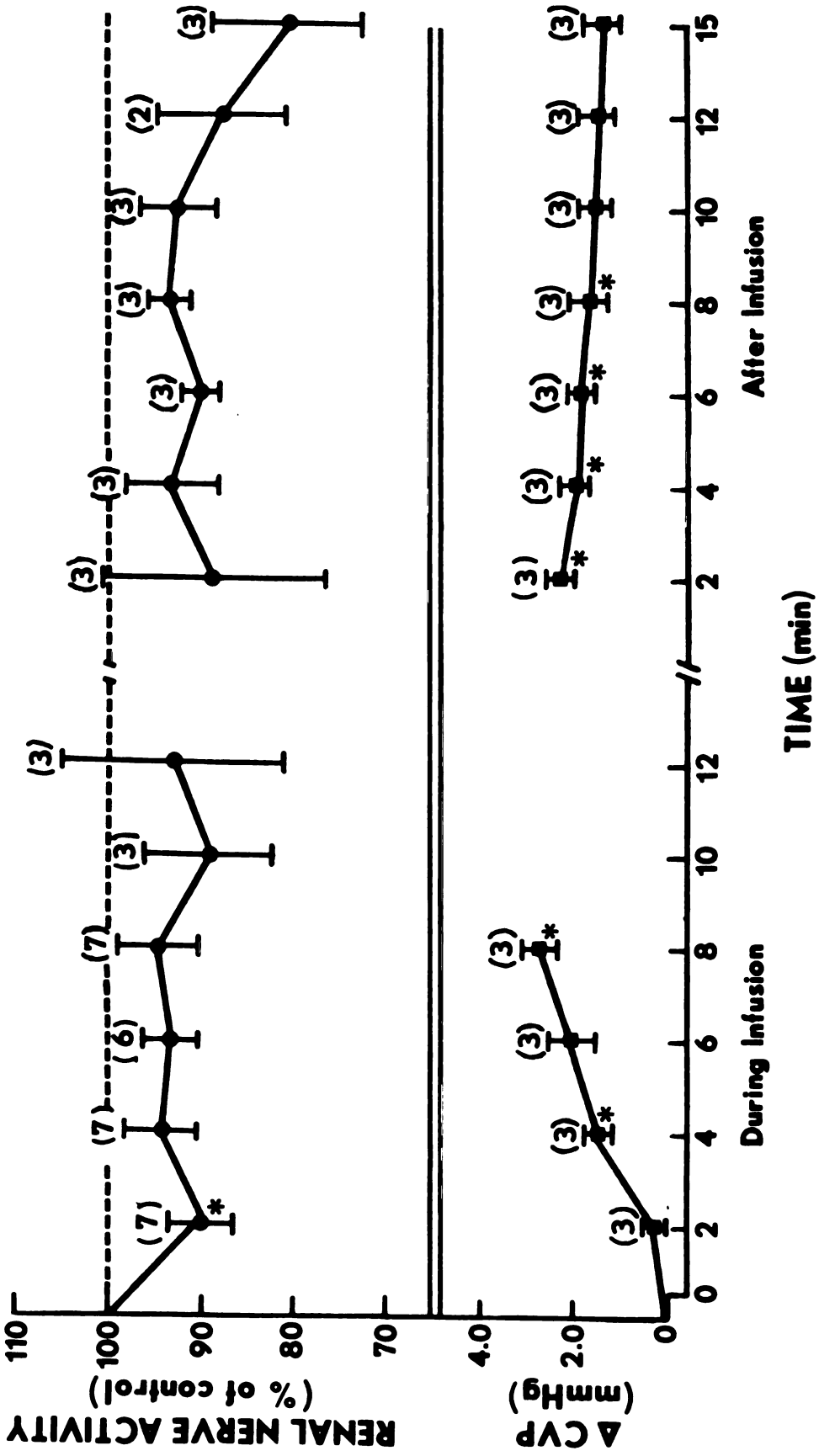

RENAL NERVE ACTIVITY
(% of control)
110
100
90
80
70
Δ CVP
(mmHg)
4.0
2.0
0
0
2
4
6
8
10
12
//
2
4
6
8
10
12
15
During Infusion
After Infusion
TIME (min)

Figure 9. Effects of intravascular volume expansion on renal nerve activity and central venous pressure (CVP) following C_1 spinal cord transection.

Spontaneous nerve activity was quantified as spike frequencies by computer and is expressed as percent of control discharge. Time refers to the amount of time elapsed during or following infusion of 3% dextran in isotonic saline at 4.4 ml/min. Mean data are expressed $\pm$ standard error. Numbers in parentheses refer to the number of cats assessed at a given time period. Significance ($P < .05$) was determined by constructing a confidence interval and is indicated by an asterisk (*).

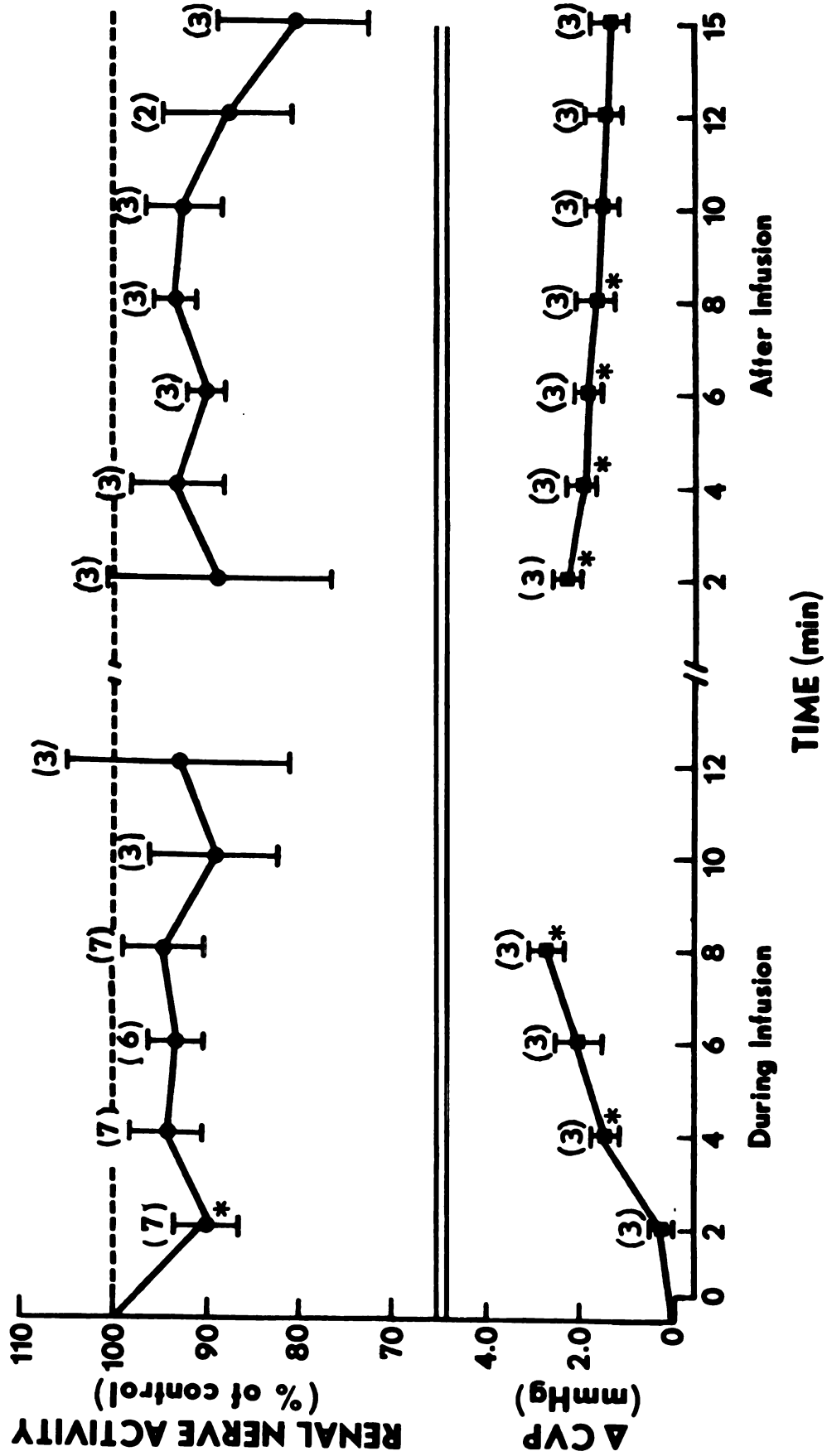

RENAL NERVE ACTIVITY
(% of control)
110
100
90
80
70
Δ CVP
(mmHg)
4.0
2.0
0
0
2
4
6
8
10
12
//
2
4
6
8
10
12
15
During Infusion
After Infusion
TIME (min)

Figure 10. Renal efferent nerve responses evoked by cardiopulmonary sympathetic afferent nerve stimulation at 1-2 trains/sec.

Spontaneous renal efferent nerve discharge is shown in Panel 1. Once each second, a 10 msec train of 3 pulses (8-15V, 0.5 msec) was delivered to the cardiopulmonary sympathetic afferent nerve. Inhibition of renal nerve discharge following afferent stimulation is shown in Panel B. Each dot represents the delivery of one train of stimuli to the cardiopulmonary sympathetic afferent nerve. Period of stimulation was 10 min.

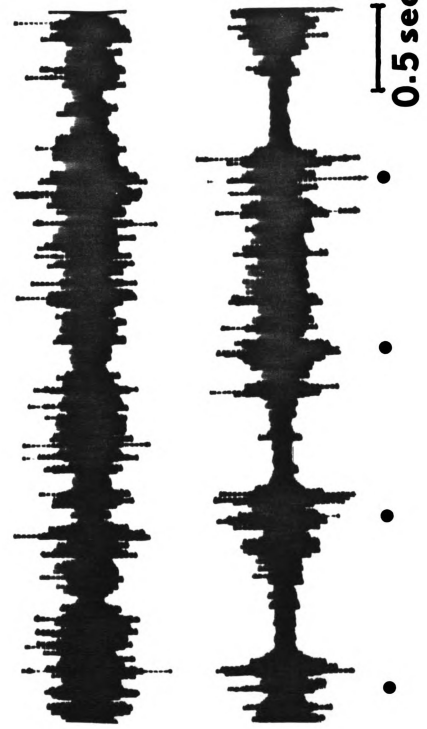

A
B
200 μV
0.5 sec

Figure 11. Effect of cardiopulmonary sympathetic afferent stimulation (1-2 trains/sec) on sodium excretion ($U_{Na}V$), potassium excretion ($U_K V$) and urine flow rate ($\dot{V}$) in 7 cats.

Each line connects individual data points from a single cat, and the stippled area delineates the 10 min stimulation period. Urine collection periods were 10 min and data points are plotted at the midpoint of the period. During stimulation, $U_{Na}V$ increased and remained increased for the duration of the experiment. In several cats $\dot{V}$ increased during stimulation, and in all cats V was greater than control during post stimulation periods. In contrast $U_K V$ did not change.

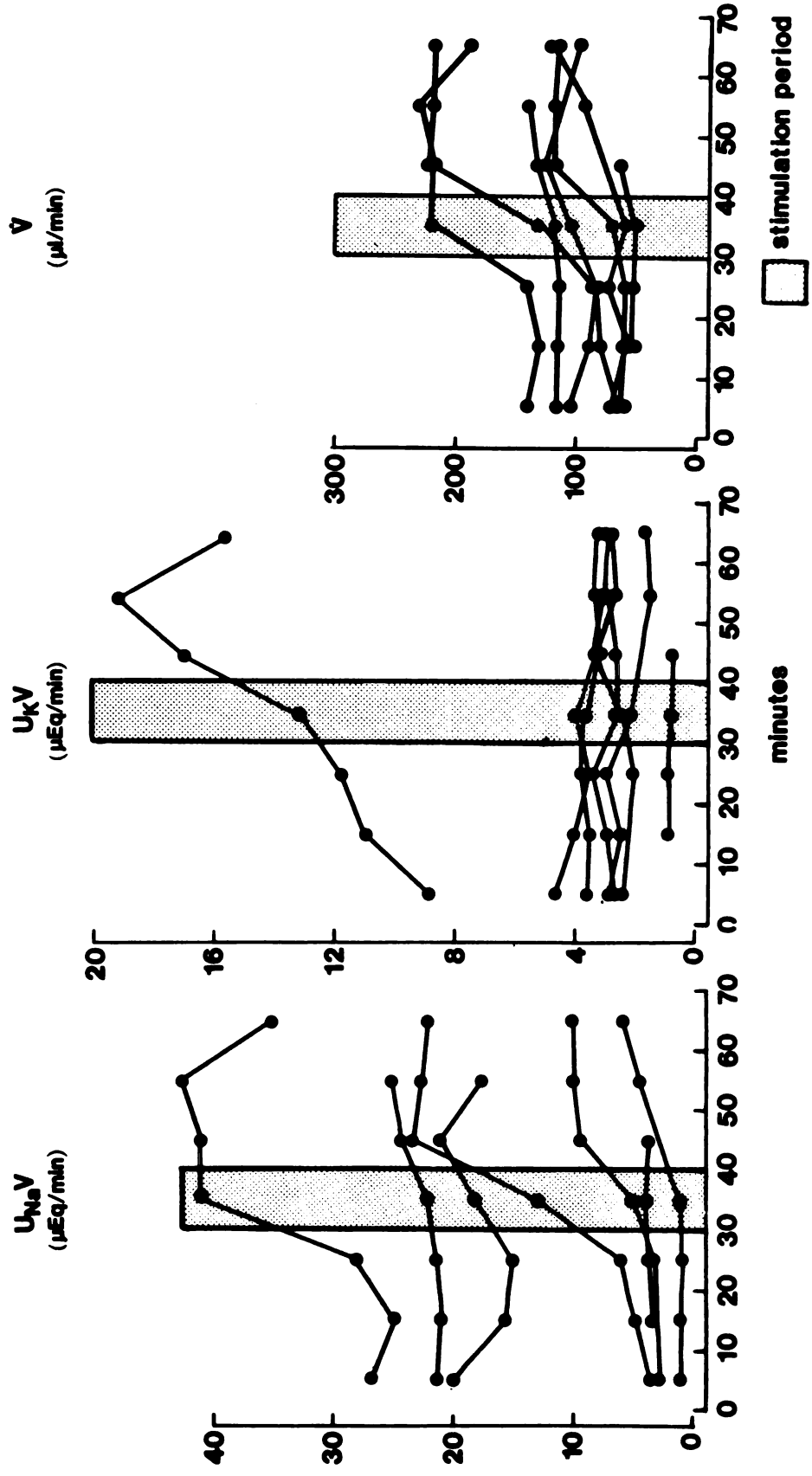

$\dot{V}$ (µl/min)
$U_K V$ (µEq/min)
$U_{Na} V$ (µEq/min)
minutes
stimulation period

Figure 12. Effect of cardiopulmonary sympathetic afferent stimulation (1-2 trains/sec) on glomerular filtration rate (C_{IN}), mean arterial pressure (MAP), and fractional excretion of sodium (FE_{Na}) in 7 cats.

The experimental format is the same as that of Figure 12. In most cats C_{IN} remained constant and MAP was stable. In contrast FE_{Na} increased markedly in most cats during the stimulation period, and did not return to control throughout the remainder of the experiment.

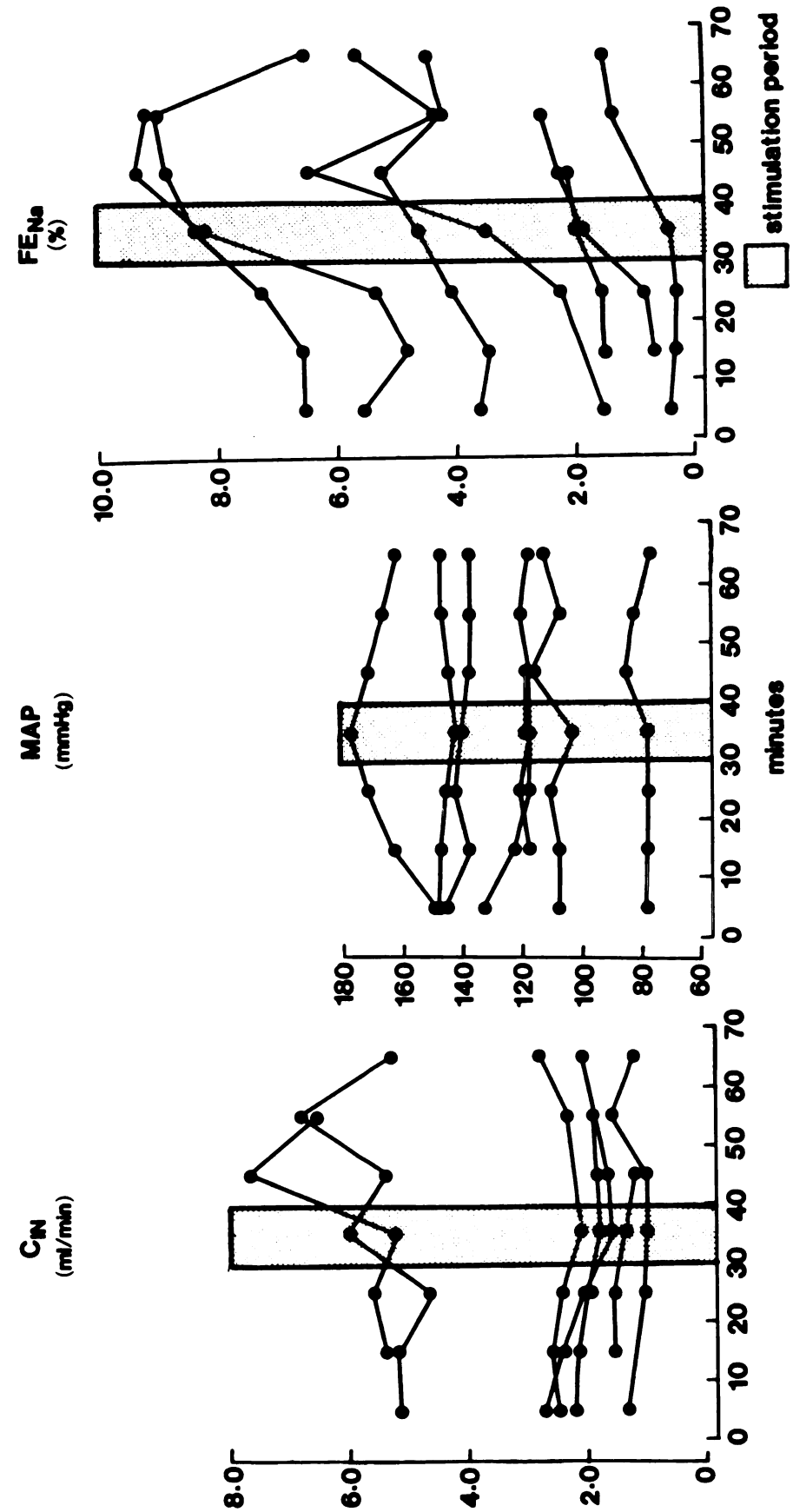
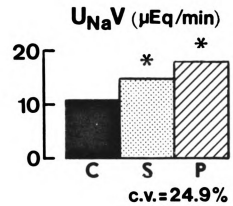
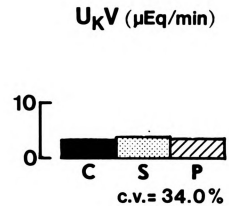
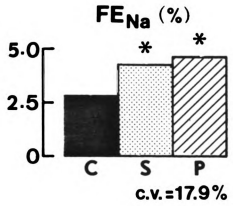

C_{IN} (ml/min)
MAP (mmHg)
FE_{Na} (%)
minutes
stimulation period

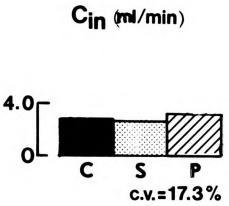
Figure 13. Summary of renal responses to cardiopulmonary sympathetic afferent stimulation at 1-2 trains/sec in seven cats.

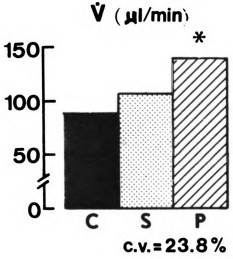
Control data are indicated by black bars, responses to stimulation are indicated by stippled bars and post-stimulation data are indicated by striped bars. Abbreviations are the same as those defined in Figures 11 and 12. Data are expressed as a mean. Coefficient of variation also is given. The number of replications is seven. Control for each cat consists of averaged data obtained from two or three samples collected prior to stimulation. The post-stimulation sample for each cat consists of averaged data obtained from two or three samples collected following stimulation. Stimulation data were obtained from the one sample collected during stimulation. Statistical significance (*) was attained at $P < 0.05$. Both $U_{Na}V$ and FE_{Na} were increased significantly during and after stimulation. Urine flow rate was significantly increased following stimulation. In contrast, U_KV, GFR and MAP did not change.


$U_{Na}V$ (μEq/min)
20
10
0
*
*
C
S
P
c.v.=24.9%


U_KV (μEq/min)
10
0
C
S
P
c.v.= 34.0%


FE_{Na} (%)
5.0
2.5
0
*
*
C
S
P
c.v.=17.9%


C_{in} (ml/min)
4.0
0
C
S
P
c.v.=17.3%


$\dot{V}$ (μl/min)
150
100
50
0
*
C
S
P
c.v.=23.8%

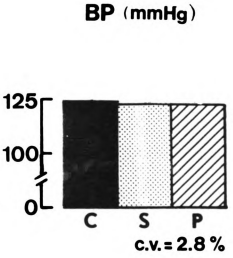

BP (mmHg)
125
100
0
C
S
P
c.v.= 2.8%

Figure 14. Summary of renal responses to cardiopulmonary sympathetic afferent stimulation at 5-7 Hz in 5 cats.

Control data are represented by black bars, responses during stimulation are represented by stippled bars, and responses following stimulation are represented by striped bars. Abbreviations are the same as those described in Figures 11 and 12. Data are expressed as a mean. Coefficient of variability also is given. Control for each cat is averaged data obtained from two or three samples collected prior to stimulation. Post-stimulation data for each cat is averaged data obtained from two or three samples collected following stimulation. Stimulation data were obtained from the one sample collected during stimulation. No significant change was seen in renal function parameters measured ($P > 0.05$).

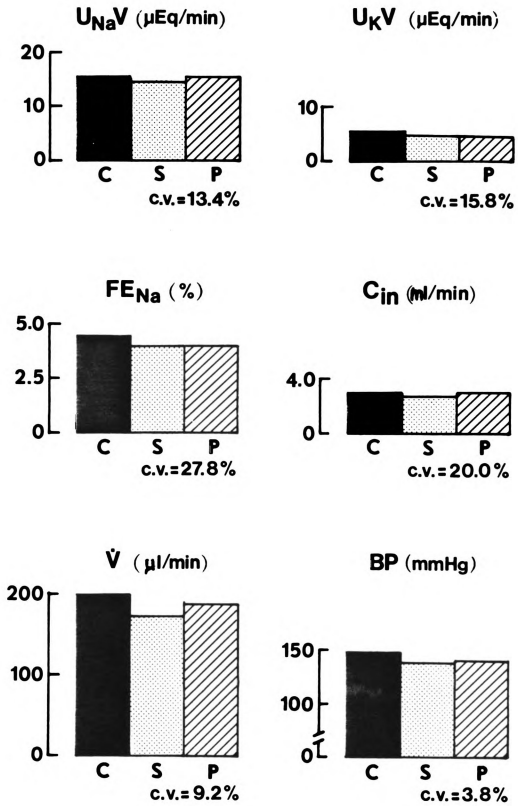

$U_{Na}V$ (μEq/min)
20
10
0
C
S
P
c.v.=13.4%
U_KV (μEq/min)
10
0
C
S
P
c.v.=15.8%
FE_{Na} (%)
5.0
2.5
0
C
S
P
c.v.=27.8%
C_{in} (ml/min)
4.0
0
C
S
P
c.v.=20.0%
$\dot{V}$ (μl/min)
200
100
0
C
S
P
c.v.=9.2%
BP (mmHg)
150
100
0
C
S
P
c.v.=3.8%

Figure 15. Effect of time on sodium excretion ($U_{Na}V$), potassium excretion (U_KV) and urine flow rate ($\dot{V}$) in 6 cats.

Each line connects individual data points from a single cat and the stippled area corresponds to the period during which stimulation would have occurred (Figure 11). Urine collection periods were 10 min and data points are plotted at the midpoint of the period. No consistent effect of time was seen in $U_{Na}V$, U_KV or V. Only one cat showed an increase in $U_{Na}V$ and $\dot{V}$. This is in contrast to the increase in $U_{Na}V$ and $\dot{V}$ seen with 1-2 trains per sec stimulation (Figure 11).

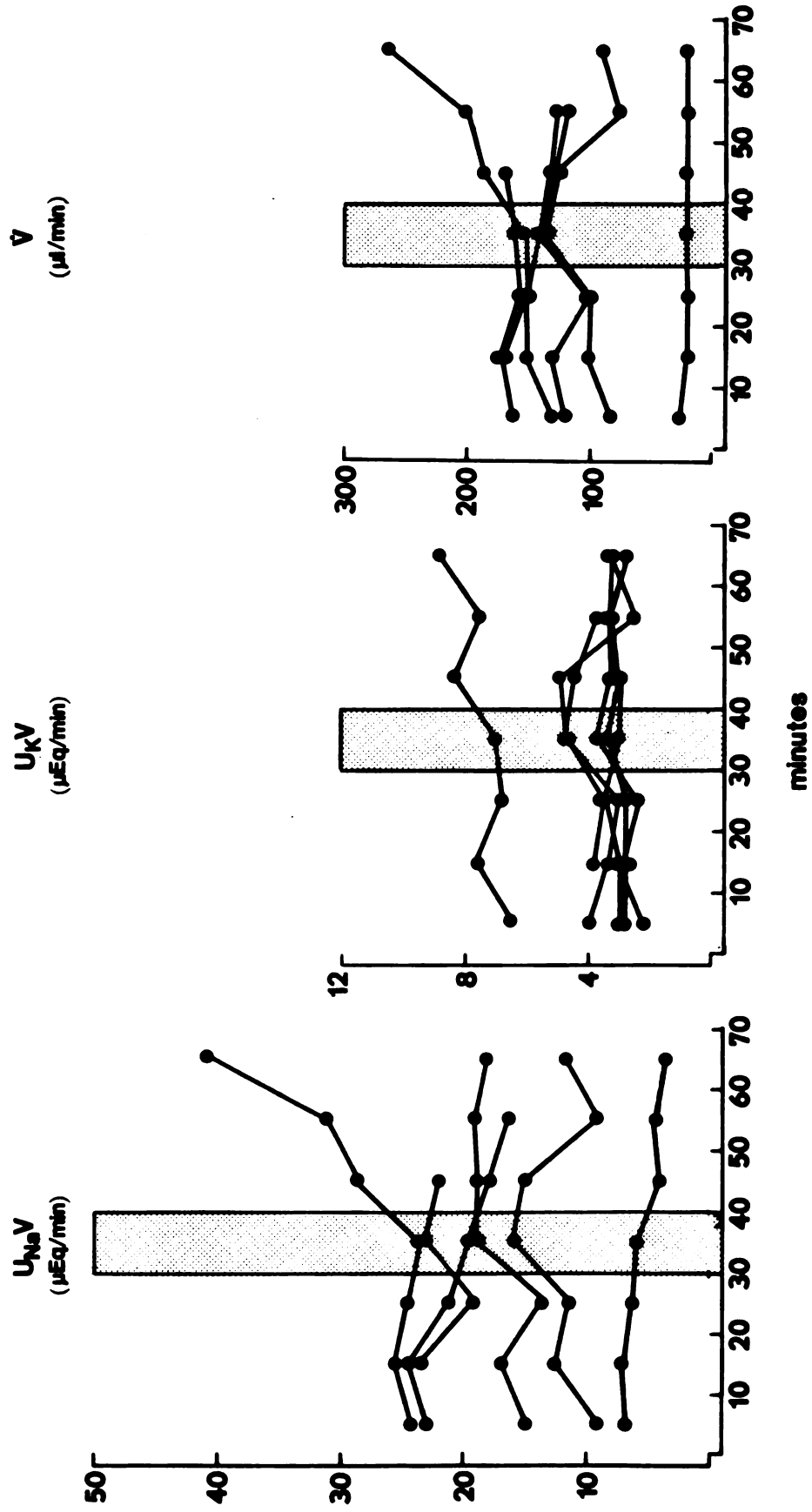
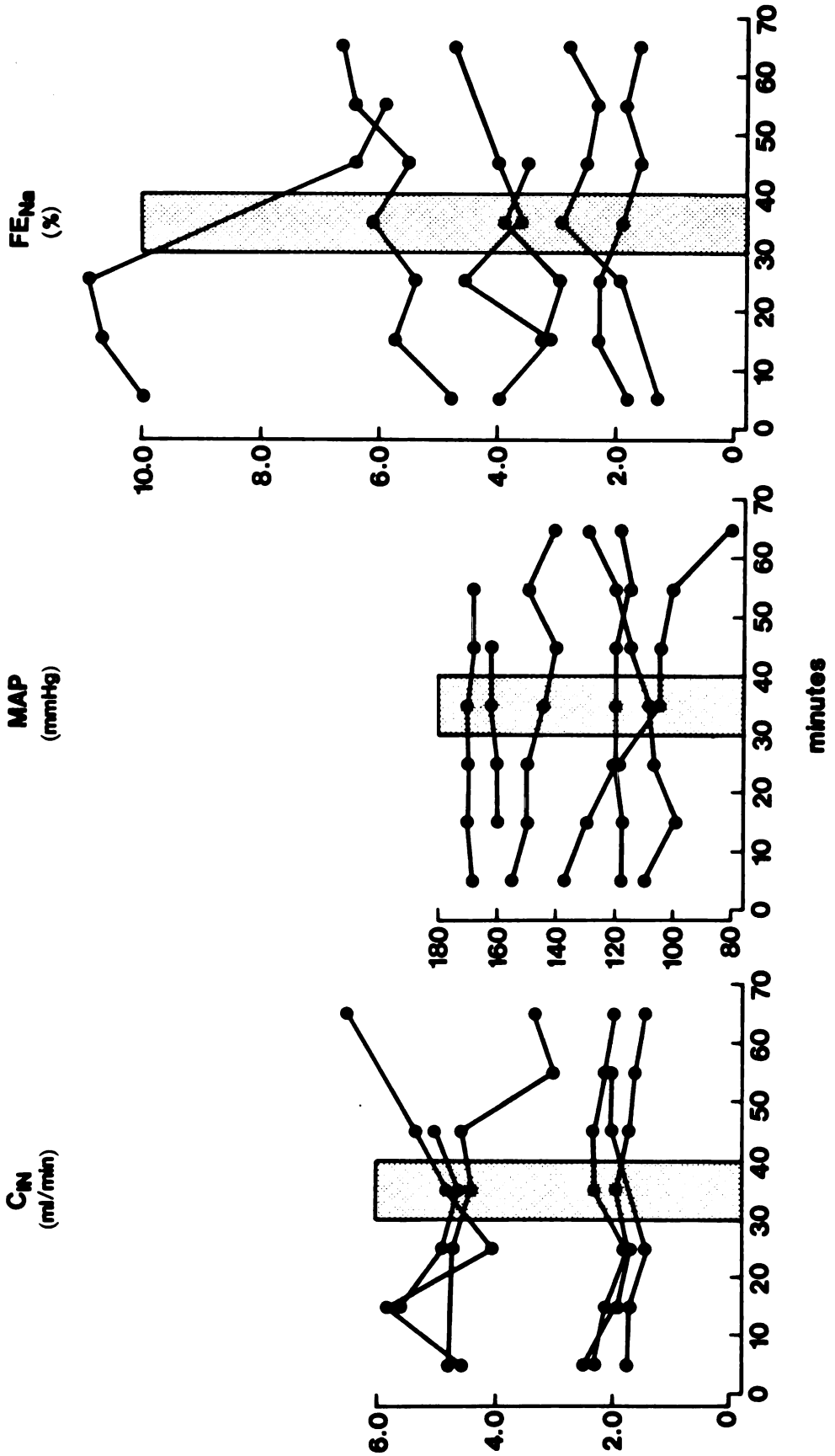

$\dot{V}$
(µl/min)
300
200
100
$U_K V$
(µEq/min)
12
8
4
$U_{Na} V$
(µEq/min)
50
40
30
20
10
10
20
30
40
50
60
70
minutes

Figure 16. Effect of time on glomerular filtration rate (C_{IN}), mean arterial pressure (MAP), and fractional excretion of sodium (FE_{Na}) in 6 cats.

The experimental format is the same as that of Figure 15. No consistent change was seen in C_{IN}, MAP or FE_{Na} as a result of passage of time. This is in contrast to the increase in FE_{Na} seen with 1-2 train per sec stimulation (Figure 12).


C_{IN} (ml/min)
6.0
4.0
2.0
0
MAP (mmHg)
180
160
140
120
100
80
FE_{Na} (%)
10.0
8.0
6.0
4.0
2.0
0
0 10 20 30 40 50 60 70
minutes

DISCUSSION

In a recent paper by Weaver (101), a cardiopulmonary-renal reflex was demonstrated in cats by activating sympathetic afferent nerves originating in the heart, lungs and thoracic vasculature. These afferent nerves were activated by electrical stimulation or by intravascular volume expansion. When elicited electrically, the cardiopulmonary-renal reflex began approximately 94 msec after afferent stimulation and consisted of an excitatory burst of renal efferent nerve activity followed by a long silent period. Previously, reflexes with these characteristics have been shown to require the brainstem for their integrity (50). Due to the various conduction velocities and lengths of the different nerves, a spinal reflex would be expected to occur before 94 msec (6,32,90). More specifically a spinal cardiopulmonary-renal sympathetic reflex would be expected to have an onset latency of approximately 40-50 msec, in contrast to 95 msec observed in Weaver's study and 80 msec observed in the present study. (The faster onset latency of the supraspinal reflex in these experiments may be due to the use of trains of stimuli as opposed to single pulses. Trains would promote greater temporal summation and thus possibly an earlier response.)

Coote et al. (22) have shown that the brainstem can exert inhibitory influences on spinal reflex arcs. Thus the possibility existed that the cardiopulmonary-renal reflex contained a purely spinal component which was being

masked. Therefore, it was necessary to attempt to evoke this reflex in cats with the neuraxis severed to eliminate possible descending inhibitory influences. With the spinal cord severed, electrical activation of cardiopulmonary sympathetic afferent nerves failed to elicit a reflex alteration in renal efferent nerve activity.

Activation of cardiopulmonary sympathetic afferent nerves by intravascular volume expansion also failed to inhibit renal nerve activity in spinal animals, whereas, in animals with the neuraxis intact, renal nerve activity was inhibited as much as 30% with this procedure (101).

The absence of the reflex in spinal cats did not result from poor condition of the animals. All cats showed vigorous spontaneous nerve activity and most cats were able to maintain their own blood pressure at greater than 70 mmHg. In addition, other sympathetic reflexes could be evoked. In three cats a renal-renal sympathetic reflex was demonstrated, and in two of these cats a cardio-cardiac reflex was demonstrated. Also, in two cats in which blood volume was expanded, anoxia produced reflex increases in renal nerve activity. Thus, the preparations were viable and reflexly excitable.

The absence of a cardiopulmonary-renal sympathetic reflex in spinal animals was expected. Beacham and Perl (6), and Sato and Schmidt, (91) showed that the largest reflex discharge in a preganglionic sympathetic nerve was

evoked from afferent fibers of the same or the adjacent segment. A possible explanation for this may be that the convergence of afferent neurons is greatest on efferent neurons of the same segment, and convergence decreases in more distant segments. This, in turn, would mean that spatial summation of excitatory influences on efferent neurons would decrease as the distance from the segment of entry of the afferent nerve increased. Thus, the presence of cardio-cardiac and renal-renal reflexes could be readily demonstrated in these experiments because of the relatively short intersegmental distances involved.

A decrease in renal efferent nerve activity occurred in most cats upon initiation of volume expansion. It is unlikely that this decrease was due to influences of cardiopulmonary sympathetic afferent nerves, since the cardiopulmonary-renal reflex, when evoked by intravascular volume expansion, is well correlated with increases in central venous pressure (101), and central venous pressure was not increased during the first 2 min of intravascular volume expansion. Also, electrical stimulation of cardiopulmonary sympathetic afferent nerves failed to alter renal nerve discharge. Possibly, the decrease in renal efferent nerve activity was due to reflex influences of afferent nerves from the liver, kidney, or other abdominal viscera. However, since central venous pressure was not immediately altered, it is difficult to ascertain what the possible stimulus would be. If the cannula for infusion rested in

the vena cava near the renal vein, renal venous pressure may have increased faster than central venous pressure. Possibly increases in renal venous pressure can influence renal nerve activity in the spinal cat through a spinal renal-renal reflex. Alternatively, afferent fibers from any of the abdominal viscera may have caused a reflex inhibition. That the inhibition during intravascular volume expansion lasted only 2 min may indicate accommodation of sensory fibers. Finally, the depression may have been merely a chance occurrence and not true inhibition.

The failure to demonstrate cardiopulmonary-renal sympathetic reflex in spinal cats may be related to a necessary spinal interneuron in the reflex arc, which is driven by descending excitatory pathways originating in the brain. Removal of the excitatory drive to the interneuron would eliminate the reflex. Thus, although the pathway might be complete at the spinal level, activation of a reflex which uses this pathway would be dependent upon descending excitation of one of its components. The existence of interneurons in sympathetic reflex arcs has been documented by Gebber and McCall (32), and such neurons may have excitatory or inhibitory influences on sympathetic outflow. Alternatively, the excitatory and inhibitory influences of cardiopulmonary sympathetic afferent nerves on renal nerve activity might be determined less on a spinal level and more by the brainstem. In support of this concept, Coote

and Macleod (23) have demonstrated sympatho-inhibitory pathways emanating from the brainstem. The possibility also exists that the characteristics of the cardiopulmonary-renal reflex are determined both spinally and supraspinally, spinal influences requiring tonic input from high centers.

Although cardiopulmonary sympathetic afferent nerves are unable to influence renal nerve activity in the spinal cat, these afferent nerves can influence both renal nerve activity and renal sodium and water excretion in cats with an intact neuraxis. Cardiopulmonary sympathetic afferent stimulation at 1-2 trains per sec caused an inhibition of renal nerve activity and an increase in both sodium and water excretion. The natriuresis and diuresis could not be attributed to an increased filtered load of sodium and water since afferent stimulation did not usually alter glomerular filtration rate. The lack of change in glomerular filtration rate suggests that the natriuresis was due to decreased sodium reabsorption. The reflexly increased fractional excretion of sodium in these cats supports this hypothesis. The natriuresis and diuresis also could not be explained on the basis of elapsed time, as, in cats in which cardiopulmonary sympathetic afferent nerves were not stimulated, the passage of time had no consistent effect on any renal function parameter measured. Since afferent stimulation at 5-7 Hz had no net effect on renal nerve activity or sodium and water excretion, the changes in renal function

seen with 1-2 train afferent stimulation may have been a direct effect of the evoked alterations in renal nerve activity. Since, in some situations cardiopulmonary sympathetic afferent nerves can alter renal nerve activity and renal function, these nerves may contribute to renal control of intravascular volume.

Sympathetic afferent stimulation at 1-2 trains per sec induced a natriuresis and diuresis regardless of the initial state of sodium and water balance of the animal. The continuous saline infusion used in all of these experiments caused a steady diuresis and natriuresis which continued throughout the experiments. This infusion was responsible for the high basal sodium and water excretion observed in many cats. The response of animals to stimulation at 1-2 trains shows that activation of sympathetic afferent fibers can induce a natriuresis and diuresis, even when basal rates of sodium and water excretion are high. This observation is similar to that of Bello-Reuss et al. (7,8) who were able to induce a denervation diuresis and natriuresis in normovolemic and volume expanded rats.

Stimulation of the sympathetic afferent nerves can alter systemic blood pressure as well as renal nerve activity (101). Blood pressure was stabilized when necessary in the present experiments to facilitate observation of those afferent influences on renal function not attributable to changes in systemic blood pressure. In normal physiological

situations, influences of the afferent nerves on renal function probably would not be mediated by changes in systemic blood pressure. Other baroreceptor reflexes, such as those from the carotid sinus, would attenuate such hemodynamic changes. In addition, afferent nerve stimulation often alters renal nerve activity without affecting systemic blood pressure (101).

The long-lasting renal response to sympathetic afferent stimulation at 1-2 trains per sec appears to require a complex explanation involving multiple mechanisms. Part of the increase in sodium and water excretion may be caused by the inhibition of renal nerve activity. The rapid onset of natriuresis and diuresis is consistent with such a mechanism as is the observation that no such changes were seen with 5-7 Hz stimulation (which did not alter renal nerve activity). DiBona (27) has recently reviewed the evidence indicating that renal sympathetic nerves can directly alter sodium reabsorption. Increased renal sympathetic nerve activity can produce an immediate antinatriuresis which appears to be caused by direct catecholaminergic influences on sodium reabsorption. Conversely, a decrease in sympathetic activity might elicit a natriuresis. It is well known that denervation of the kidney produces a diuresis and a natriuresis (13). At least a portion of the denervation diuresis probably is due to decreased sodium reabsorption in the proximal tubule and is independent of changes

in glomerular filtration rate, renal plasma flow and filtered load of sodium (7,8). This supports the hypothesis that diminished renal sympathetic activity might directly cause a rapid increase in sodium and water excretion. Such a mechanism could explain the rapid natriuresis and diuresis following cardiopulmonary sympathetic afferent stimulation.

Decreased traffic in renal nerves may induce a natriuresis and diuresis via several additional mechanisms. Sympatho-inhibition could lead to renal vasodilation and increased renal blood flow with resultant increments in glomerular filtration rate and sodium excretion (13). It has been shown that inhibition of renal nerve activity evoked by thoracic sympathetic afferent stimulation can produce increased renal blood flow (88). However, depression of renal nerve activity or even renal denervation does not necessarily increase renal blood flow or glomerular filtration rate (7,8,87). Although renal blood flow was not measured in the present study, the failure of afferent stimulation to alter glomerular filtration rate suggests that changes in total renal blood flow probably were not responsible for the natriuresis and diuresis.

Inhibition of renal nerve activity causing renal vasodilation also can lead to changes in blood flow distribution within the kidney (97). Such changes may lead to altered sodium excretion and may contribute to the natriuretic responses to afferent stimulation. However, Prosnitz and

DiBona (87) elicited reflex inhibition of renal nerve activity and observed a natriuresis which was not accompanied by changes in renal blood flow or blood flow distribution.

Reflexes which inhibit renal nerve activity may decrease the release of renin possibly causing diminished concentrations of angiotensin II (AII) in the circulation and in the kidney. Activation of cardiopulmonary vagal afferent fibers can inhibit renal nerve activity and renal secretion of renin (48,67,105). Such changes in AII concentration could produce a natriuresis and diuresis either by decreasing intrarenal vascular tone or possibly by reducing AII-mediated facilitation of renal sodium reabsorption (44). These changes might cause rapid increases in sodium and water excretion. A neurally induced decrease in AII concentration also can result in diminished plasma aldosterone concentration which could lead to increased sodium and water excretion. Because of the long half-life of aldosterone, changes in plasma concentrations of this hormone might explain the long duration of the natriuresis and diuresis. Presently, no definitive evidence is available to support or deny such hypotheses.

Alternatively, the effects of sympathetic afferent stimulation on renal function may be independent of changes in renal nerve activity. Stimulation may have altered the release of humoral agents from the central nervous sytem.

Activation of vagally innervated receptors has been suggested to inhibit antidiuretic hormone (ADH) release (28,36). Receptors innervated by sympathetic afferent fibers also may alter ADH release. Diuresis resulting from an inhibition of ADH release could be long-lasting. If the mechanism of the natriuretic and diuretic response to afferent stimulation at 1-2 trains per sec were humoral in nature, possibly 5-7 Hz stimulation had no apparent effect because afferent nerves in these cats were stimulated for a short period prior to the initiation of an experiment. (This was done in 5-7 Hz experiments to determine if afferent stimulation could alter arterial pressure, and was used as a measure of afferent nerve viability, since no nerve recordings were done during these experiments.) If afferent stimulation does cause the release of humoral agents, the effect of a second stimulation period might be masked by a long-term effect of the first stimulus. However, in all cats, the stimulus was tested for periods of less than 30 sec, and in several cats the 30 sec test was performed at least one hour prior to the 10 min experimental stimulation period. Thus effects of initial stimulation probably would have dissipated prior to the experimental stimulation.

Thus, the mechanisms by which cardiopulmonary sympathetic afferent nerves induce a natriuresis and diuresis remain in question. However, this reflex clearly resembles other known reflex influences on the kidney. Distension of the pulmonary vein-left atrial junction elicits a diuresis

which appears to be mediated by vagally innervated receptors (54). Inhibition of ADH release and of renal nerve activity may contribute to this diuresis (26,13). Activation of carotid sinus baroreceptors also causes natriuresis which has been attributed by Prosnitz and DiBona (87) to withdrawal of direct renal sympathetic influences on sodium reabsorption. The natriuresis observed by these investigators was similar in magnitude and duration to that elicited by cardiopulmonary sympathetic afferent stimulation (personal communication). Thus cardiopulmonary sympathetic afferent influences on the kidney may contribute to volume control in the same manner as other previously described reflexes.

In conclusion, activation of cardiopulmonary sympathetic afferent nerves can influence both renal nerve activity and renal function. The reflex influences of these nerves appear to require the integrity of the brainstem. Alterations in cardiopulmonary sympathetic afferent nerve activity are integrated by the brainstem which in turn influences renal nerve activity. It is likely that alterations in renal function are a direct effect of reflex alterations in renal nerve activity, with net decreases in renal nerve activity causing a diuresis and a natriuresis. However, the possibility also exists that cardiopulmonary sympathetic afferent nerves influence renal function by causing alterations in release of hormones. This study provides evidence that cardiopulmonary receptors innervated by sympathetic afferent nerves can influence the kidney, and thus, may contribute to the control of intravascular blood volume.

SUMMARY AND CONCLUSIONS

Cardiopulmonary sympathetic afferent nerves can reflexly alter renal efferent nerve discharge. This reflex requires the integrity of the brainstem.

Cardiopulmonary sympathetic afferent nerves also can alter renal function. Low frequency electrical stimulation (1-2 trains/sec) of these afferent nerves causes a natriuresis and diuresis. This stimulation frequency also causes a net inhibition of renal nerve activity. Since afferent stimulation at a frequency (5-7 Hz) which has no net effect on renal nerve activity also had no effect on renal function, it is likely that alterations in renal function are due to reflexly induced inhibition of renal nerve activity. In conclusion, cardiopulmonary sympathetic afferent nerves can alter renal nerve activity and renal function, and therefore may contribute to the control of intravascular volume.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Aukland, K. Effect of adrenalin, noradrenalin, angiotensin and renal nerve stimulation on intrarenal distribution of blood flow in dogs. Acta Physiol. Scand. 72:498-509, 1967.

2. Baisset, A., and P. Montastruc. Polyurie par distension auriculaire chez le chien; rôle de l'hormone antidiurétique. J. Physiol., Paris 49:33-36, 1957.

3. Barajas, L. The innervation of the juxtaglomerular apparatus. Lab. Invest. 13:916-924, 1964.

4. Bastide, F. and S. Jard. Actions de le noradrénaline et de l'ocytocine sur le transport actif de sodium et la permeabilité à l'eau de la peau de grenouille. Rôle du 3 , 5 -AMP cyclique. Biochim. Biophys. Acta. 150:113-123, 1968.

5. Beacham, W.S. and E.R. Perl. Background and reflex discharge of sympathetic preganglionic neurons in the spinal cat. J. Physiol., London 172:400-416, 1964.

6. Beacham, W.S., and E.R. Perl. Characteristics of a spinal sympathetic reflex. J. Physiol., London 173:431-448, 1964.

7. Bello-Reuss, E., R.E. Colindres, E. Pastoriza-Muñoz, R.A. Miller, and C.W. Gottschalk. Effects of acute unilateral renal denervation in the rat. J. Clin. Invest. 56:208-217, 1975.

8. Bello-Reuss, E., E. Pastoriza-Muñoz, and R.E. Colindres. Acute unilateral renal denervation in rats with extracellular volume expansion. Am. J. Physiol. 232:F26-F32, 1977.

9. Bello-Reuss, E., D.L. Trevino, and C.W. Gottschalk. Effect of renal sympathetic nerve stimulation on proximal water and sodium reabsorption. J. Clin. Invest. 57:1104-1107, 1976.

10. Bencsáth, P. and L. Takács. Intrarenal distribution of blood flow and cortico-medullary sodium gradient after unilateral splanchnicotomy in the dog. J. Physiol., London 212:629-640, 1971.

11. Berger, E.Y., S.J. Farber, and D.P. Earle, Jr. Renal excretion of mannitol. Proc. Soc. Exp. Biol. Med. 66:62-66, 1947.

12. Bernard, C. Lecons sur les Proprietes Physiologique et les Alterations Pathologiques des Liquides de l'Organisms. Paris, Bailliere, 1859.

13. Berne, R.M. Hemodynamics and sodium excretion of denervated kidney in anesthetized and unanesthetized dog. Am. J. Physiol. 171:148-158, 1952.

14. Berne, R.M. and M.N. Levy. Cardiovascular Physiology. St. Louis, C.V. Mosby, 1977.

15. Bonjour, J., P.C. Churchill, and R.L. Malvin. Change of tubular reabsorption of sodium and water after renal denervation in the dog. J. Physiol., London 204:571-582, 1969.

16. Boylan, J.W. and D.E. Antkowiak. Mechanism of diuresis during negative pressure breathing. J. Appl. Physiol. 14:116-120, 1959.

17. Brennan, L.A., Jr., R.L. Malvin, K.E. Jochim, and D.E. Roberts. Influence of right and left atrial receptors on plasma concentrations of ADH and renin. Am. J. Physiol. 221:272-278, 1971.

18. Brown, A.M. Excitation of afferent cardiac sympathetic nerve fibers during myocardial ischemia. J. Physiol., London 190:35-53, 1967.

19. Brown, A.M., and A. Malliani. Spinal sympathetic reflexes initiated by coronary receptors. J. Physiol., London 212:685-705, 1971.

20. Brown, A.M., and D.F. Peterson. Pressor reflexes produced by stimulation of afferent fibers in the cardiac sympathetic nerves of the cat. Circ. Res. 28:605-610, 1971.

21. Clement, D.L., C.L. Pelletier, and J.T. Shepherd. Role of vagal afferents in the control of renal sympathetic nerve activity in the rabbit. Circ. Res. 31:824-830, 1972.

22. Coote, J.H., C.B.B. Downman, and W.V. Weber. Reflex discharges into thoracic white rami elicited by somatic and visceral afferent excitation. J. Physiol., London 202:147-159, 1969.

23. Coote, J.H. and V.H. Macleod. The spinal route descending from the medulla oblongata. Pflugers Arch. 359:335-347, 1975.

24. Davis, J.O. Importance of the renin-angiotensin system in the control of aldosterone secretion. In: Hormones and the Kidney. P.C. Williams, ed. New York, Academic, 1963.

25. Davis, J.O., M.M. Pechet, W.C. Ball, Jr., and M.J. Goodkind. Increased aldosterone secretion in dogs with right-sided congestive heart failure and in dogs with thoracic inferior vena cava constriction. J. Clin. Invest. 36:689-694, 1957.

26. deTorrente, A., G.L. Robertson, K.M. McDonald, and R.W. Schrier. Mechanism of diuretic response to increased left atrial pressure in the anesthetized dog. Kidney Int. 8:355-361, 1975.

27. DiBona, G.F. Neurogenic regulation of renal tubular sodium reabsorption. Am. J. Physiol. 233:F73-F81, 1977.

28. Downman, C.B.B., and B.A. McSwiney. Reflexes elicited by visceral stimulation in the acute spinal animal. J. Physiol., London 105:80-94, 1946.

29. Dunham, E.W. and B.G. Zimmerman. Release of prostaglandin-like material from dog kidney during nerve stimulation. Am. J. Physiol. 219:1279-1285, 1970.

30. Fernandez de Molina, A., and E.R. Perl. Sympathetic activity and the systemic circulation in the spinal cat. J. Physiol., London 181:82-102, 1965.

31. Gauer, O.H., J.P. Henry, H.O. Seiker, and W.E. Wendt. The effect of negative pressure breathing on urine flow. J. Clin. Invest. 33:287-296, 1954.

32. Gebber, G.L., and R.B. McCall. Identification and discharge patterns of spinal sympathetic interneurons. Am. J. Physiol. 231:722-733, 1976.

33. Gill, J.R. and F.C. Bartter. Adrenergic nervous system in sodium metabolism. II. Effects of guanethidine on the renal response to sodium deprivation in normal man. New Engl. J. Med. 275:1466-1471, 1966.

34. Gill, J.R., Jr., and A.G.T. Casper. Effect of renal alpha-adrenergic stimulation on proximal tubular reabsorption. Am. J. Physiol. 223:1201-1205, 1972.

35. Gilmore, J.P., and W.M. Daggett. Response of the chronic cardiac denervated dog to acute volume expansion. Am. J. Physiol. 210:509-512, 1966.

36. Goetz, K.L., G.C. Bond, and D.D. Bloxham. Atrial receptors and renal function. Physiol. Rev. 55:157-205, 1975.

37. Goodman, L.S. and A.G. Gilman, eds. The Pharmacological Basis of Therapeutics. New York, Macmillan, 1975.

38. Hale, W.J. and J.D.G. Martin. PGE_1 and net water flow across isolated frog skin. J. Physiol., London 232:85-86P, 1973.

39. Handler, J.S., R. Besinger, and J. Orloff. Effect of adrenergic agents on toad bladder response to ADH, 3', 5'-AMP and theophylline. Am. J. Physiol. 215:1024-1031, 1968.

40. Henry, J.P., O.H. Gauer, and J.L. Reeves. Evidence of the atrial location of receptors influencing urine flow. Circ. Res., 4:85-90, 1956.

41. Henry, J.P. and J.W. Pearce. The possible role of cardiac atrial stretch receptors in the induction of changes in urine flow. J. Physiol. (London), 131:572-585, 1956.

42. Hiatt, E.P. The effect of denervation on the filtration rate and blood flow in dog kidneys rendered hyperemic by administration of pyrogen. Am. J. Physiol. 136:38-40, 1942.

43. Johnson, J.A., W.W. Moore, and W.E. Segar. Small changes in left atrial pressure and plasma antidiuretic hormone titers in dogs. Am. J. Physiol. 217:210-214, 1969.

44. Johnson, M.D. and R.L. Malvin. Stimulation of renal sodium reabsorption by angiotensin II. Am. J. Physiol. 232:F297-F306, 1977.

45. Kaloyanides, G.J., R.H. Ahrens, J.A. Shepherd and G.F. DiBona. Inhibition of prostaglandin E_2 secretion: failure to abolish autoregulation in the isolated kidney. Circ. Res. 38:67-73, 1976.

46. Kamm, D.E. and N.G. Levinsky. The mechanism of denervation natriuresis. J. Clin. Invest. 44:93-102, 1965.

47. Kaplan, S.A. and S. Rapoport. Urinary excretion of sodium and chloride after splanchnicotomy; effect on the proximal tubule. Am. J. Physiol. 164:175-181, 1951.

48. Karim, F., C. Kidd, C.M. Malpus, and P.E. Penna. The effects of the left atrial receptors on sympathetic efferent nerve activity. J. Physiol., London 227:243-260, 1972.

49. Katz, M.A., and L. Shear. Effects of renal nerves on renal hemodynamics II. Renal denervation models. Nephron 14:390-397, 1975.

50. Koizumi, K., A. Sato, A. Kaufman, and C.M. Brooks. Studies of sympathetic neuron discharges modified by central and peripheral excitation. Brain Res. 11:212-224, 1968

51. Kriss, J.P., P.H. Futcher, and M.L. Goldman. Unilateral adrenalectomy, unilateral splanchnic nerve resection and homolateral renal function. Am. J. Physiol. 154:229, 1948.

52. LaGrange, R.G., C.H. Sloop, and H.E. Schmid. Selective stimulation of renal nerves in the anesthetized dog. Circ. Res. 33:704-712, 1973.

53. Langley, J.N. Vasomotor centers. Part III. Spinal vascular (and other autonomic) reflexes and the effect of strychnine on them. J. Physiol., London 59: 231-258, 1924.

54. Ledsome, J.R., and R.J. Linden. The role of left atrial receptors in the diuretic response to left atrial distension. J. Physiol. (London) 198:487-503, 1968.

55. Ledsome, J.R., and J.M. Mason. The effects of vasopressin on the diuretic response to left atrial distension. J. Physiol., London 221:427-440, 1972.

56. Lipson, L.C. and G.W.G. Sharp. Effect of PGE_1 on sodium transport and osmotic water flow in the toad bladder. Am. J. Physiol. 220:1046-1052, 1971.

57. Lombardi, F., A. Malliani, M. Pagani. Nervous activity of afferent sympathetic fibers innervating the pulmonary veins. Brain Res. 113:197-200, 1976.

58. Lydtin, H., and W.F. Hamilton. Effect of acute changes in left atrial pressure on urine flow in unanesthetized dogs. Am. J. Physiol. 207:530-536, 1964.

59. Malliani, A., and M. Pagani. Afferent sympathetic nerve fibers with aortic endings. J. Physiol. London 263:157-169, 1976.

60. Malliani, A., M. Pagani, G. Recordati, and P.J. Schwartz. Evidence for a spinal sympathetic regulation of cardiovascular functions. Experientia 26:965-966, 1970.

61. Malliani, A., M. Parks, R.P. Tuckett, and A.M. Brown. Reflex increases in heart rate elicited by stimulation of afferent cardiac sympathetic nerve fibers in the cat . Circ. Res. 32:9-16, 1973.

62. Malliani, A., D.F. Peterson, V.S. Bishop, and A.M. Brown. Spinal sympathetic cardiocardiac reflexes. Circ. Res. 30:158-166, 1972.

63. Malliani, A., G. Recordati, and P.J. Schwartz. Nervous activity of afferent cardiac sympathetic fibers with atrial and ventricular endings. J. Physiol. 229:457-469, 1973.

64. Malliani, A., G. Recordati, P.J. Schwartz, and M. Pagani. Tonic sympathetic activity from the heart. Experientia 28:269-270, 1972.

65. Malliani, A., P.J. Schwartz, and A. Zanchetti. A sympathetic reflex elicited by experimental coronary occlusion. Am. J. Physiol. 217:703-709, 1969.

66. Maluf, N.S.R. Role of the renal innervation in renal tubular function. Am. J. Physiol. 139:103-108, 1943.

67. Mancia, G., J.C. Romero, and J.T. Shepherd. Continuous inhibition of renin release in dogs by vagally innervated receptors in the cardiopulmonary region. Circ. Res. 36:529-535, 1975.

68. Marshall, E.K., Jr., and A.C. Kolls. Studies on the nervous control of the kidney in relation to diuresis and urinary secretion. I. The effect of unilateral excision of the adrenal section of the splanchnic nerve and section of the renal nerves on the secretion of the kidney. Am. J. Physiol. 49:302-316, 1919.

69. Marshall, E.K., Jr. and A.C. Kolls. Studies on the nervous control of the kidney in relation to diuresis and urinary secretion. II. A comparison of the changes caused by unilateral splanchnotomy with those caused by unilateral compression of the renal artery. Am. J. Physiol. 49:317-325, 1919.

70. McAfee, R.D., and W. Locke. Effect of angiotensin amide on sodium isotope flux and short circuit current of isolated frog skin. Endocrinology 81:1301-1305, 1967.

71. McKenna, O.C., and E.T. Angelakos. Adrenergic innervation of the canine kidney. Circ. Res. 22:345-354, 1968.

72. Michaelis, L., and J.P. Gilmore. Cardiac sympathetics and control of sodium excretion. Am. J. Physiol. 218:999-1002, 1970.

73. Mills, I.H., A.G.T. Casper, and F.C. Bartter. On the role of the vagus in the control of aldosterone secretion. Science 128:1140-1141, 1958.

74. Mukherjee, S.R. Effect of bladder distension on arterial blood pressure and renal circulation in acute spinal cats. J. Physiol., London 138:300-306, 1957.

75. Müller, J. and L. Barajas. The innervation of the juxtaglomerular apparatus and surrounding tubules: a quantitative analysis by serial section electron microscopy. J. Ultrastruct. Res. 43:107-132, 1973.

76. Nettleship, W.A. Experimental studies on the afferent innervation of the cat's heart. J. Comp. Neurol. 64:115-131, 1936.

77. Nilsson, O. The adrenergic innervation of the kidney. Lab. Invest. 14:1392-1395, 1965.

78. Nishi, K., M. Sakanashi, and F. Takenaka. Afferent fibers from pulmonary arterial baroreceptors in the left cardiac sympathetic nerve of the cat. J. Physiol., London 240:53-66, 1974.

79. Nonidez, J.T. Identification of receptor areas in the venae cavae and pulmonary veins which initiate reflex cardiac acceleration. Am. J. Anat. 61: 203-232, 1937.

80. Nonidez, J. Studies on the innervation of the heart. I. Distribution of the cardiac nerves, with special reference to identification of the sympathetic and parasympathetic post ganglionics. Am. J. Anat. 65:361-413. 1939.

81. Nonidez, J. Studies on the innervation of the heart II. Afferent endings in the large arteries and veins. Am. J. Anat. 68:151-189, 1941.

82. Nomura, G., T. Takabatake, S. Arai, D. Uno, M. Shimao, and N Hattori. Effect of acute unilateral renal denervation on tubular sodium reabsorption in the dog. Am. J. Physiol. 232:F16-F19, 1977.

83. Pagani, M., P.J. Schwartz, R. Banks, F. Lombardi, and A. Malliani. Reflex responses of sympathetic preganglionic neurones initiated by different cardiovascular receptors in spinal animals. Brain Res. 68:215-225, 1974.

84. Peters, J.P. Body Water: The Exchange of Fluids in Man. Charles C. Thomas, Springfield, IL, 1935.

85. Peterson, D.F., and A.M. Brown. Pressor reflexes produced by stimulation of afferent fibers in the cardiac sympathetic nerves in the cat. Circ. Res. 28:605-610, 1971.

86. Pomeranz, B.H., A.G. Birtch, and A.C. Barger, Neural control of intrarenal blood flow. Am. J. Physiol. 215:1067-1081, 1968.

87. Prosnitz, E.H., and G.F. DiBona. Natriuresis due to reflex decreases in renal sympathetic nerve activity. Kidney Int. 12:570, 1977.

88. Purtock, R.V., J.H. vonColditz, J.L. Seagard, F.O. Igler, E.J. Zuperku, and J.P. Kampine. Reflex effects of thoracic sympathetic afferent nerve stimulation on the kidney. Am. J. Physiol. 233: H580-H586, 1977.

89. Rhoads, C.P., D.D. VanSlyke, A. Hiller, and A.S. Alving. The effects of novocainization and total section of the nerves of the renal pedicle on renal blood flow and function. Am. J. Physiol. 110:392-398, 1934.

90. Ruch, T.C., H.D. Patton, J.W. Woodbury, and A.L. Towe. Neurophysiology. Philadelphia, W.B. Saunders, 1965.

91. Sato, A. and R.F. Schmidt. Spinal and supraspinal components of the reflex discharges into lumbar and thoracic white rami. J. Physiol., London 212:839-850, 1971.

92. Schreiner, G.E. Determination of inulin by means of resorcinol. Proc. Exptl. Biol. Med. 74:117-120, 1950.

93. Selkurt, E.E. Sodium excretion by the mammalian kidney. Physiol. Rev. 34:287-333, 1954.

94. Sherrington, C.S. and E.G.T. Liddell. Mammalian Physiology; A Course of Practical Exercises. Oxford, Claredon Press, 1929.

95. Slick, G.L., A.L. Aguilera, E.J. Zambraski, G.F. DiBona, and G.J. Kaloyanides. Renal neuroadrenergic transmission. Am. J. Physiol. 229:60-65, 1975.

96. Steele, R.G.D. and J.H. Torrie. Principles and Procedures of Statistics. New York, McGraw-Hill, 1960.

97. Stein, J.H., T.F. Ferris, J.E. Huprich, T.C. Smith, and R.W. Osgood. Effect of renal vasodilation on the distribution of cortical blood flow in the kidney of the dog. J. Clin. Invest. 50:1429-1438, 1971.

98. Surtshin, A., J. Hoeltzenbein, and H.L. White. Some effects of negative pressure breathing on urine excretion. Am. J. Physiol. 180:612-616, 1955.

99. Taquini, A.C., P. Blaquier, and A.C. Taquini, Jr. On the production and role of renin. Can. Med. Assoc. J. 90:210-213, 1964.

100. Ueda, H., Y. Uchida, and K. Kamisaka. Distribution and response of the cardiac sympathetic receptors to mechanically induced circulatory changes. Jap. Heart. J. 10:70-81, 1969.

101. Weaver, L.C. Cardiopulmonary sympathetic afferent influences on renal nerve activity. Am. J. Physiol. 233: H592-H599, 1977.

102. Zambraski, E.J., and G.F. DiBona. Angiotensin II in antinatriuresis of low-level renal nerve stimulation. Am. J. Physiol. 231:1105-1110, 1976.

103. Zambraski, E.J., G.F. DiBona, and G.J. Kaloyanides. Specificity of neural effect on renal tubular sodium reabsorption. Proc. Soc. Exp. Biol. Med. 151:543-546, 1976.

104. Zambraski, E.J., G.F. DiBona and G.J. Kaloyanides. Effect of sympathetic blocking agents on the antinatriuresis of reflex renal nerve stimulation. J. Pharmacol. Exptl. Therap. 198:464-472, 1976.

105. Zehr, J.E., J.A. Hasbargen, and K.E. Kurz. Reflex suppression of renin secretion during distension of cardiopulmonary receptors in dogs. Circ. Res. 38: 232-239, 1976.

APPENDIX

Inulin Determination

INULIN DETERMINATION

Inulin is a large sugar (molecular weight approximately 5,000) which is filtered by the glomerulus of the kidney but is not reabsorbed. Because of this, inulin clearance by the kidney provides a fairly accurate estimate of glomerular filtration rate. To calculate inulin clearance, it is first necessary to determine inulin concentrations in plasma and urine. In this study, a method described by Schreiner (92) was used.

This method employs hydrochloric acid (HCL) which hydrolizes inulin into molecules of fructose. Resorcinol then reacts with the fructose producing a characteristic orange color. The intensity of color can then be used to determine the concentration of inulin in a given sample.

I Dilution of Samples

A. Dilute urine: 10 µl urine/10 ml H_2)

B. Precipitate protein and dilute plasma as follows

1. place in test tube:

   0.25 ml plasma sample
   2.5 ml distilled water
   1.0 ml trichloroacetic acid (11.25% in distilled water)

2. vortex

3. let test tube stand for 10 min

4. centrifuge for 10 min

5. draw off and save supernatant

II. Standards

A. Stock Solution: dissolve 100 mg inulin in 100 ml distilled water (100 mg% inulin)

B. Working Standards:

1 ml stock solution in 100 ml distilled water (1mg%)
2 ml stock solution in 100 ml distilled water (2mg%)
3 ml stock solution in 100 ml distilled water (3mg%)
4 ml stock solution in 100 ml distilled water (4mg%)

III. Procedure

A. Place 1.0 ml sample, working standard or distilled water (blank) in a large test tube

B. Add 1.0 ml resorcinol reagent[1] to each test tube

C. Add 2.5 ml hydrochloric acid[2] to each test tube and vortex

D. Stopper test tubes with loose fitting plastic stoppers

E. Place test tubes in a water bath ($80^{o}C$) for 25 min.

F. Remove test tubes from water bath and allow to cool

G. Absorbance of light (optical density) at 490 mμ should be determined for each test tube using a spectrophotometer.

H. Subtract optical density value of blank from optical density of samples and standards

I. Plot a standard curve with optical density of the standard on the x axis and mg% inulin on y axis

J. Calculate slope of the standard curve and use slope to calculate inulin concentration of plasma and urine samples:

$$m \cdot \begin{matrix}\text{optical density} \\ \text{of sample}\end{matrix} = \begin{matrix}\text{mg\% inulin} \\ \text{of sample}\end{matrix}$$

K. Inulin clearance in then calculated as $\frac{U_{IN} \cdot \dot{V}}{P_{IN}}$

where U_{IN} = urine concentration of inulin (mg%)
P_{IN} = plasma concentration of inulin (mg%)
V = urine flow rate (ml/min)

1. Resorcinol reagent: dilute 1 mg resorcinol in 1 ml ethyl alcohol (95%). Make up 1 ml for each sample, blank and standard. This reagent must be made fresh for each assay.

2. Hydrochloric Acid: 300 ml HCL (37%) in 67.2 ml distilled water.