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The role of the sympathetic nervous system in the control of renin secretion

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THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE CONTROL OF RENIN SECRETION

by

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THESIS

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PROLOGUE

Renin is a proteolytic enzyme with a molecular weight of approximately 35,000 which is secreted by the kidney. Its only established action involves the formation of angiotensin II. Its circulating half-life is about 80 minutes (Assaykeen et al., 1968). Attention was first drawn to the renin-angiotensin system through a relationship with hypertension. Subsequently effects on aldosterone secretion found in the early 1960's drew attention to its role in the control of body fluid volumes.

Renin has been found in the kidneys of all mammals studied (Schaffenburg et al., 1960). However, species variations have been noted, and relate to observations of structural variation (based on antirenin antibody formation) and pressor potencies of renin preparations in heterologous species (Ganong and Van Brunt, 1968). Renin from one mammalian species has been shown to be active in other mammals, but non-primate renin seems to be inactive in primates (Braun-Menendez et al., 1946).

Through its proteolytic action, renin acts on an α_2-globulin of hepatic origin, antiotensinogen, to form the decapeptide angiotensin I. By splitting off two C-terminal amino acids, converting enzyme changes approximately 90% of the angiotensin I into angiotensin II, an octapeptide. This latter conversion occurs in a single passage through the lungs. Angiotensin II is the most potent vasopressor agent known; it exerts this effect via direct action on peripheral arterioles. It is also one of the most powerful trophic agents known for the production and release of aldosterone from the zona glomerulosa of the adrenal

cortex, and also increases secretion of glucocorticoids. The mechanisms of these actions are uncertain. Angiotensin II seems to stimulate steroid synthesis as well as release. Kaplan and Bartter (1962) found that there was no increase in conversion of progesterone or corticosterone to aldosterone when angiotensin II was added to beef adrenal slices in vitro; however, an increase in conversion of cholesterol to aldosterone suggests that the action may be at some step in steroid biosynthesis between cholesterol and progesterone.

The effect of angiotensin II on sodium excretion is threefold: (1) by increasing aldosterone secretion and production, sodium excretion is decreased due to the greater reabsorption of Na^+ ions in the distal tubules, collecting ducts, and possibly proximal tubules; (2) by decreasing glomerular filtration rate and renal plasma flow (Del Greco, 1962) there is a decrease in sodium excretion due to the associated fractional reabsorption of sodium; and (3) under some circumstances it paradoxically causes a decrease in tubular reabsorption of sodium (see Ganong and Van Brunt, 1968). Angiotensin II is rapidly destroyed by various enzymes termed angiotensinases which are found in the blood and various tissues.

It is now well established that the juxtaglomerular (J-G) cells of the juxtaglomerular apparatus are the source of renin secretion in the kidney. These cells are located in the media of the afferent arteriole just before it enters the glomerulus. Similar cells surround the afferent arteriole and fill the angle between the afferent and efferent arterioles. Each distal renal tubule returns near the glomerulus from which it arises, and in the angle between the arterioles,

it comes into close contact with the J-G cells. This marks the beginning of the distal convoluted tubule, and the tubular epithelium contains tall columnar-type cells which form the macula densa (Fig. 1). Evidence demonstrating the presence of renin came from observations that the J-G cells contain granules which fluoresce intensively and selectively when kidneys are treated with fluorscein-labeled antirenin antibodies (Edelman and Hartroft, 1961; Hartroft, 1963). It was also shown that the degree of granulation parallels the renin content of the kidney (Tobian and Tomboulain, 1959). Investigators have also noted hyperplasia, hypertrophy, and hypergranularity of the J-G cells in animals which were actively or passively immunized with renin (Hartroft et al., 1964; Ganong et al., 1965; Schmid and Graham, 1962).

Investigations concerning the mechanisms involved in the control of renin secretion have led to two principal theories. One widely held theory, the "baroreceptor theory", was advanced by Tobian (1960a, b; 1962; 1964). It has been hypothesized that secretion is controlled by the pressure gradient across the J-G cells, and that these cells, functioning as receptors as well as secretory cells respond to this gradient. Due to their anatomic location, these specialized myoepithelial cells are believed to perceive pressure changes as distortions of the existing stretch on the arteriolar walls. For example, under conditions of decreased blood volume, with the resultant decrease in perfusion pressure, there would be a decrease in afferent arteriolar pressure. This could be perceived by the J-G cells as a decrease in stretch exerted on the afferent arteriolar walls. With the resultant increase in renin leaving the kidney via the renal veins and lymphatics, an increase in aldosterone

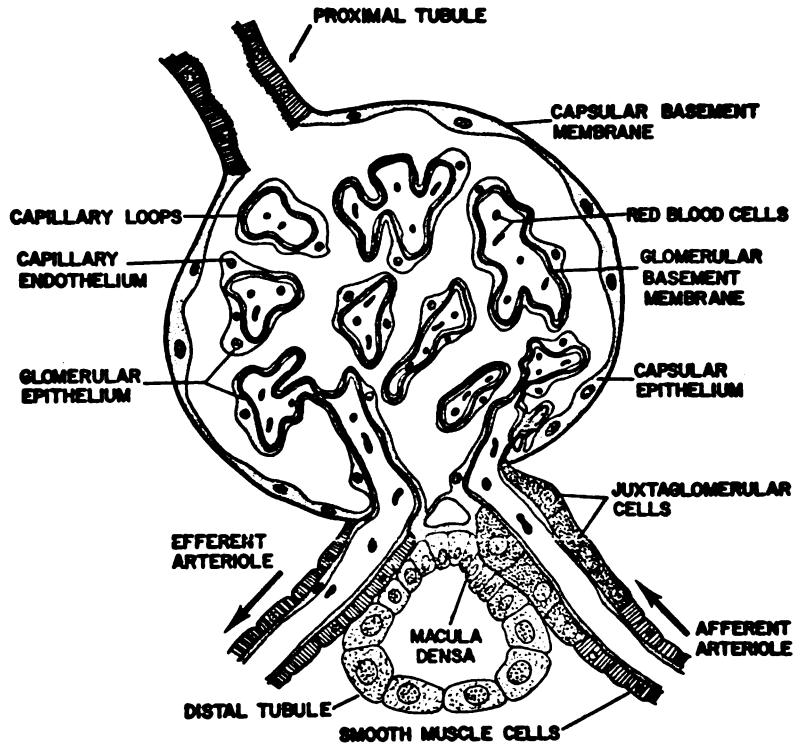


Figure 1. Diagram of the glomerulosa, showing juxtaglomerular cells and the macula densa. (From Ganong, 1971.)

secretion would follow with increased sodium retention and expansion of extracellular fluid volume--thus increasing the J-G stretch and decreasing the stimulus to renin. If blood volume were expanded, presumably the reverse would occur.

The other theory, supported by Vander and Miller (1964), suggests that the macula densa controls renin secretion. Recent variations on this theory maintain that there is an inverse relationship between sodium transport across the macula densa and renin secretion. Details of this "macula densa theory" will be discussed at a later point.

More recently, evidence has been mounting that the sympathetic nervous system is involved in the regulation of renin secretion. This evidence will be discussed in the following pages. It may be pointed out here (Assaykeen and Ganong, 1971) that evidence involving the role of the sympathetic nervous system and the control of renin secretion is not inconsistent with the above two theories. It is possible that changes in sympathetic nervous activity could be reflected in changes in the afferent arterioles or the transport of sodium at the macula densa.

INTRODUCTION

The role of the sympathetic nervous system in the control of renin secretion has been the subject of recent reviews and reports (Assaykeen and Ganong, 1971; Allison et al., 1970; Passo et al., 1971a,b; Assaykeen et al., 1971; Otsuka et al., 1970; Schmitt, 1968; Gordon et al., 1967; Bunag et al., 1966).

There are many lines of evidence implicating the sympathetic nervous system in the control of renin secretion. It has been shown that the kidney receives significant adrenergic innervation. McKenna and Angelakos (1968) demonstrated adrenergic nerve fibers traveling along interlobar, arcuate, and interlobular arteries and along the afferent arterioles. The presence of rich sympathetic innervation has also been observed in association with efferent arterioles (Barajas, 1964). There are also many adrenergic nerve fibers associated with the J-G cells, and synapse-like structures between the nerves and J-G cells have been described (Barajas, 1964). Furthermore, Wagermark et al. (1968), using a combination of a histochemical fluorescence method for biogenic monoamines and staining of J-G cell granules, demonstrated sympathetic nerve terminals in the walls of the parts of the juxtaglomerular arterioles that contain granulated cells (rat kidney). These observations could form a morphological basis for a direct influence of sympathetic nervous activity on the liberation of renin.

Further evidence suggests the presence of reflex control mechanisms for renin release involving the autonomic nervous system and the renal nerves. Vander (1965) and others (Lee, Loeffler, Stockigt, and Ganong,

unpublished observations) have shown that direct electrical stimulation of the renal nerves increases renin release. The renal nerves may also play a role in sodium conserving reflex mechanisms and the renin response to changes in intratubular sodium. Vander and Luciano (1967a,b) demonstrated that in the unilateral renal denervated dog mercurial diuresis caused no change in glomerular filtration rate or renal plasma flow in the denervated kidney, but a decrease in glomerular filtration rate and renal plasma flow occurred in the contralateral innervated kidney. The renin response was also less in the denervated kidney. Recently, Johnson et al. (1971) have demonstrated in papaverine treated dogs, whose renal baroreceptor mechanisms have supposedly been blocked by the vasodilatory effects of the drug, that hemorrhage and renal nerve stimulation cause increases in renin secretion. The data suggest that the renal nerves can increase renin independently of the macula densa or renal baroreceptor mechanism. Finally, previous experiments in the rat have shown that denervation of the kidney results in decreases in renin content of the kidney (Taquini et al., 1964) and in the degree of granularity of the J-G cells (Tobian et al., 1964).

Bunag, Page and McCubbin (1966a) reported that increased vasomotor activity induced by hemorrhage caused renin release in dogs whether or not there was measurable change in either arterial pressure or total renal blood flow. In this study release of renin was prevented by ganglion blockade or local anesthesia of the renal nerves. Further findings which suggest a "reflex system" controlling renin release and involving the autonomic nervous system have been presented by Hodge et al. (1969). They found that cooling of the vagus nerve or vagotomy

resulted in an increase in circulating angiotensin II, presumably via an increase in renin release.

Another line of evidence supporting the role of the sympathetic nervous system in the control of renin secretion involves findings that catecholamine infusions increase renin secretion. Vander (1965) produced increases in renal venous plasma renin concentration with intravenous infusions of either epinephrine (5-6 μg/min) or norepinephrine (12-16 μg/min) during maintenance of a constant renal arterial blood pressure by means of suprarenal aortic constriction. The effects of norepinephrine were confirmed by Bunag et al. (1966a) who showed an increase in renin release when 0.05-0.1 μg/kg/min were infused into a renal artery in dog. Gordon et al. (1967) demonstrated in normal human subjects that, despite concurrent increases in arterial blood pressure, plasma renin activity (PRA) increased in response to the infusion of catecholamines (norepinephrine : epinephrine, 10 : 1).

In studying the effect of hypglycemia on PRA in dogs, infusions of epinephrine at rates comparable to its secretion rate during hypoglycemia resulted in a significant increase in PRA. However, infusions of norepinephrine at this rate failed to increase PRA (Otsuka et al., 1970).

In addition, it has been shown that isoproterenol (0.3 μg/kg/min) causes a significant increase in PRA (Allison et al., 1970). However, in doses of 0.1-0.2 μg/kg/min (Bunag et al., 1966a) and 0.009 μg/kg/min (Reid, personal communication) no changes in renin secretion were observed. It has been suggested, however, that the increases in PRA observed with high doses are due to systemic effects from the drug passing from the kidney and circulated systemically. Serotonin and dopamine have not been shown to

directly affect renin secretion (Vander and Luciano, 1967a;Bunag et al., 1966a;Otsuka et al., 1970). Furthermore, Michelakis et al. (1969) found that epinephrine, norepinephrine and cyclic AMP caused striking increases in the "net production" of renin in dog renal cell suspensions in vitro. This finding in addition to the observed close association of adrenergic nerve endings and granulated J-G cells, support the view that the action of the sympathetic nervous system on the J-G cells is a direct one.

To further examine the role of the sympathetic nervous system in the release of renin,two stimuli which are known to increase sympathetic activity were studied: insulin-induced hypoglycemia and brain stem stimulation. Otsuka et al. (1970) found that insulin-induced hypoglycemia resulted in a significant increase in PRA in anesthetized dogs. The data indicate that this increase is associated with increased secretion of epinephrine from the adrenal medulla. Passo et al. (1971a) demonstrated that stimulation of the pressor region of the medulla oblongata also causes increased renin secretion. This increase was regularly associated with a marked increase in blood pressure, but no significant changes in circulating catecholamines was seen. In the latter study renin secretion was blocked by renal denervation, while the blood pressure response was not. The rise in PRA associated with insulin-induced hypoglycemia was still present with renal denervation, but was abolished with adrenal denervation, indicating that perhaps circulating catecholamines as well as those liberated at adrenergic nerve endings can both stimulate renin secretion.

Ahlquist (1948) demonstrated the existence of two types of receptor for sympathomimetic amines. This theory proposes that there are two

different adrenergic receptors (alpha and beta) with which molecules of adrenergic agonists act. The characteristic response that is observed, therefore, depends upon the specific receptor which is activated. The receptors which mediate vasoconstriction in the kidney have been shown to belong to the alpha-group (see Lees and Lockett, 1963; Ahlquist, 1948), and experimental results in rats indicated the presence of renal receptors which may properly be typified as beta-adrenergic, as shown by preferential activation by low concentrations of isoprenaline (Botting et al., 1961). The relative order of potency of three adrenergic agonists used to help classify adrenergic receptors is epinephrine > norepinephrine > isoproterenol for alpha receptors and isoproterenol > epinephrine > norepinephrine for beta receptors. It has been hypothesized that increases in renin secretion associated with sympathetic stimulation are mediated via a beta-adrenergic receptor (Assaykeen and Ganong, 1971).

Assaykeen et al. (1970) have shown that the rise in PRA associated with infusion of .6 μg/kg/min epinephrine in propranolol (beta-adrenergic blocking agent) treated dogs was blocked. In contrast, the use of the alpha-adrenergic blocking agent, phenoxybenzamine, may have potentiated the response to epinephrine. Dogs receiving propranolol infusion (0.3 mg/kg/kr) showed no renin response to .1-.3 μg/kg/min isoproterenol (Allison et al., 1970), but the rise in PRA was not affected by phenoxybenzamine (4 mg/kg). Furthermore, there was almost complete block of the increase in PRA associated with insulin-induced hypoglycemia when the animals were treated with propranolol; and a slight potentiation of the renin response was seen with the use of phenoxybenzamine (Assaykeen et al., 1970). A similar finding was seen in the renin response to brain stem

stimulation. The alpha-adrenergic blocking agent, phenoxybenzamine, was shown to have no effect on the increase renin secretion produced by stimulation of the "pressor area" of the medulla oblongata in dog. However, the beta-adrenergic blocking agent, propranolol, reduced the response and administration of both drugs completely abolished the response (Passo et al., 1971a).

Similar effects of alpha- and beta-blocking agents have been observed in the rat. Physostigmine, an anticholinesterase which in rats induces a rise in blood pressure probably due to activation of the sympathetic nervous system, also increases plasma renin activity. Phenoxybenzamine lowered blood pressure and increased PRA; propranolol and two other beta-adrenergic blocking agents induced a rise in blood pressure and a reduction in PRA (Alexandre et al., 1970). The response to stimulation of the renal nerves has also been shown to be blocked by the beta-adrenergic blocking agent, propranolol (Loeffler, Stockigt, and Ganong, unpublished observations).

Finally, some evidence has accumulated to suggest a similarity between adenyl cyclase, an enzyme which catalyzes the conversion of ATP to cyclic AMP which in turn leads to the activation of phosphorylase or lipolysis, and the beta-adrenergic receptor (Westfall, 1969). Theophylline, a xanthine which increases intracellular cyclic AMP by blocking the action of phosphodiesterase, has been shown to cause an increase in renin secretion. This rise in PRA was not blocked by propranolol, but may have been potentiated by phenoxybenzamine (Reid and Ganong, 1971). However, a conflicting report which has not been confirmed claims that both propranolol and phenoxybenzamine suppress an increase in PRA from a number of stimuli including

ethacrynic acid and theophylline in man (Winer et al., 1969a). Winer et al. (1969b) have suggested that because of the equipotent blocking effects of d- and l-propranolol, the ability of alpha- and beta-blocking agents to inhibit alpha-and beta-agonists respectively, and the failure of cyclic AMP to stimulate renin secretion in the presence of adrenergic blockade, adrenergic blocking agents suppress renin secretion by an action other than adenyl cyclase inhibition.

Does the sympathetic nervous system play a role in other stimuli to renin secretion? Some stimuli not as directly related to the sympathetic nervous system that have been examined include hemorrhage, upright posture and sodium depletion. Concerning the latter, there is indirect evidence in man and dog (Gordon et al., 1967; Bull et al., 1970; Mogil et al., 1969; Brubacher and Vander, 1968; Passo, Shackelford, Boryczka, and Ganong, unpublished observations) that the sympathetic nervous system is involved in the renin rises induced by low sodium diet. Sodium depletion and the corresponding renin response produced by diuretic drugs have also been studied. Attempts to study the role of the sympathetic nervous system under these conditions have generally included surgical (renal and adrenal denervation) and pharmacological (adrenergic end organ blockade by alpha- and beta-adrenergic blocking agents, and ganglion blockade) interruption of sympathetic output. Data presented here are the results of further studies examining the role of the sympathetic nervous system in changes in PRA in response to changes in volume and electrolyte balance associated with administration of diuretic drugs.

Earlier studies (Vander and Miller, 1964; Vander and Luciano, 1967a,b; Vander, 1967; Nash, 1968; Brown et al., 1966; Meyer et al., 1966) indicated

that changes in renin secretion associated with diuretic drugs appeared to be dependent upon the resultant salt and water (volume) depletion resulting from the diuresis. However, Meyer et al. (1968b) demonstrated that even with the prevention of these urinary losses through a ureteral-venous shunt, furosemide-induced renin secretion in the rabbit was not altered despite constancy of blood pressure, plasma sodium concentration and plasma volume. This increase in PRA was, however, prevented when extracellular or plasma volume expansion was combined with reinfusion of urine.

The renin stimulating action of furosemide and ethacrynic acid, a closely related and potent natruretic drug, has been demonstrated previously in man (Meyer et al., 1966; Fraser et al., 1965) and in dogs (Cooke et al., 1967, 1970; Vander and Carlson, 1969). Meyer et al. (1968b) and Vander and Carlson's (1969) results were not consistent with the conclusion that the stimulatory effects on renin secretion by these drugs were dependent on salt or volume depletion per se. Thus it appeared that furosemide could indeed stimulate renin secretion independent of salt or volume depletion. Further elaboration on this conclusion is found in the discussion of the experimental findings. The purpose of the present undertaking was to examine the role of the sympathetic nervous system in a renin response induced by furosemide in anesthetized dogs in which volume and sodium depletion were prevented by ureterovenous anastomosis. Data were obtained by examining the effects of alpha- and beta-adrenergic blocking agents on this response.

METHODS

Experiments were performed in sodium pentobarbital (30 mg/kg iv) anesthetized mongrel dogs weighing 12 to 18 kg. The animals were fasted overnight and allowed free access to water. Right and left ureters were catheterized (through a medial abdominal incision), the catheters being inserted to the level of the renal pelvis, and urine flow was shunted to the left femoral vein. Arterial blood pressure was recorded from a right femoral artery catheter using a Statham strain gauge transducer and Grass Model 5 polygraph. Arterial blood samples were also collected from this catheter. Drugs, fluids, blood, and all infusions were administered via a right femoral vein catheter. Central venous blood pressure was monitored from a catheter placed in the right jugular vein and manipulated until it was close to the right atrium. This pressure was also recorded via a Statham transducer and the Grass Model 5 polygraph. Blood was replaced volume for volume with whole blood taken from donor animals which had been nephrectomized four hours prior to collecting the blood.

Blood samples were collected in iced tubes containing Na_2EDTA (disodium ethylenediamine tetraacetic acid) in a saturated solution (.3 M neutralized to pH 7.4); one part EDTA per nine parts of blood collected. All samples were then centrifuged immediately under refrigeration and the plasma stored at -20 C until analyzed for PRA by radioimmunoassay of angiotensin I (Stockigt et al., 1971). Blood samples taken for the measurement of circulating catecholamines and plasma electrolytes were collected in siliconized tubes to which 2-3 drops of heparin had been added. These plasma samples were also frozen at -20 C and stored until analyzed.

Arterial plasma epinephrine and norepinephrine were measured by the

ethylenediamine method as modified by Goldfien (1961). Plasma and urine potassium and sodium levels were analyzed with an I-L Model 143 flame photometer. Urine samples were taken from the ureterovenous catheter and an accurate measurement of the volume taken was made. This loss was correlated with volume changes due to drug injections, blood sampling and replacement, and infusions so that no change in an animal's total fluid volume occurred. A urine flow was calculated from accurate time and volume measurements taken during periods of urine sampling. Standard analytical procedures were used to analyze all data, and statistical analysis of data within a protocol was accomplished by use of the "t" test for paired variates (Hodgman et al., 1956).

After preparation of an animal one of the following experimental protocols was followed after a 30-40 minute resting period following the surgical procedures.

Protocol 1 - Control

Following the initial resting samples, 14 animals were infused with 5% dextrose at a rate of 0.123 ml/min for the duration of the experiment. A stat dose of furosemide (Lassix, Hoechst), 5 mg/kg, was injected and the animals were followed over a four hour period. Blood samples for the determination of renin, catecholamines, and plasma electrolytes were taken. Urine samples were collected and urine flow was calculated. Hematocrit, arterial blood pressure, and central venous pressure were also followed. Input and output of fluids were measured and controlled so as to maintain zero net volume change. Time sequences for a typical experiment were as follows:

Sample #	Time	Samples taken*
1	-20	R,U
infuse Dextrose 5% @ 0.123 ml/min		
2	- 5	R,P,U
inject furosemide 5 mg/kg	0	
3	5	R,U
4	15	R,P,U
5	30	R
6	60	R
7	120	R
8	180	R,P
9	240	R,U

*Samples taken - Blood: R = Renin
P = Plasma catecholamine and electrolyte determination
Urine: U - Urine

Protocol 2 - Beta-adrenergic blockade

Preliminary preparation of these 8 animals was similar to that in Protocol 1. In this group, however, a loading dose of the beta-adrenergic blocking agent, propranolol (Inderal, Ayerst), 0.6 mg/kg iv, was given followed by infusion of 0.3 mg/kg/hr for the duration of the experiment. All of the parameters monitored in Protocol 1 were followed throughout these experiments. Upon completion of the final samples, a single dose of isoproterenol (10 μg iv) was injected in order to determine the completeness of the beta-blockade. With complete blockade the vasodilitation ordinarily produced by isoproterenol is blocked and the depressor response can no longer be elicited (Wang, 1967). It might be noted that the pressor response to epinephrine is potentiated in this situation because the depressor component is eliminated. In all cases abolition of the usual depressor response was noted. Time sequences were as follows:

8	1.0
7	120
6	60
5	30
4	15
3	8
2	4
1	2

Sample #	Time	Samples Taken
1	-40	R,P,U
infuse Dextrose 5% @ 0.123 ml/min		
2	-30	R
inject propranolol 0.6 mg/kg plus 0.3 mg/kg/hr		
3	- 5	R,U
inject furosemide 5 mg/kg	0	
4	5	R,U
5	15	R,P,U
6	30	R
7	60	R,P,U
8	90	R
9	120	R,U
10	180	R
11	240	R,P,U
inject isoproterenol 10 μg		

Protocol 3 - Alpha-adrenergic blockade

Preliminary preparations of these 7 animals were similar to that in Protocol 1. After the appropriate control samples were taken, these animals were infused with 5% Dextrose containing phenoxybenzamine, 5 mg/kg, for one hour at a rate of 0.247 ml/min followed by continuous infusion of 5% Dextrose at 0.123 ml/min for the duration of the experiment. Furosemide, 5 mg/kg, was injected in a single dose iv two and one-half hours following the start of the infusions. Samples were taken to follow the response for a period of four hours, and the parameters monitored in Protocol 1 were followed. Upon completion of the experiment the animals were injected with a single dose of epinephrine, 10 μg/kg, to check the completeness of the alpha blockade. Phenoxybenzamine reverses the pressor effect of epinephrine, but the vasodilating action of isoproterenol is not inhibited. In all cases a depressor response was observed, thus confirming the blockade of alpha-adrenergic receptors (Osal et al., 1967; Nickerson and Hollenberg, 1967; American Hospital Formulary, 1971). Time sequences for a typical protocol were as follows:

Sample #	Time	Samples taken
1	after anesthesia	R
2	before cannulation of ureters	R
3	-150	R,P,U
infuse phenoxybenzamine 5 mg/kg in 5% Dextrose @ 0.247 ml/min for one hour followed by 5% Dextrose @ 0.123 ml/min		
4	-120	R
5	- 90	R
6	- 30	R
7	- 5	R,P,U
inject furosemide 5 mg/kg	0	
8	5	R,U
9	15	R,P,U
10	30	R
11	60	R,P,U
12	90	R
13	120	R,U
14	180	R
15	240	R,P,U
inject epinephrine 10 μg/kg		

Protocol 4 - Repeat stimulation

Following either Protocol 1 or 2, four animals were subjected to a second injection of furosemide and followed in a fashion similar to the first response. Upon completion of the first four-hour period, these dogs were rested thirty minutes before pre-injection control samples were taken the second time. They were then injected with the second dose of furosemide, 5 mg/kg. Time sequences for the samples taken correspond to the respective protocol followed.

Protocol 5 - Volume expansion

Four animals which had been treated per Protocol 3 were infused with isotonic saline following the four-hour experimental period. Over a period of thirty minutes, extracellular fluid volume was expanded at least twenty percent. Furosemide, 5 mg/kg, was then administered and the response was followed for thirty minutes.

RESULTS

Protocol 1 - Control

Within five minutes following the injection of furosemide, plasma renin activity (PRA) had risen significantly ($P < .005$) from 22.9 $\pm$ 5.4 to 42.5 $\pm$ 8.1 (PRA is expressed in ng AI generated per ml of plasma per three hours incubation). PRA was greatest fifteen minutes following the injection (52.2 $\pm$ 10.9) and then declined to control levels (22.2 $\pm$ 3.5) after two hours (see Graph 1). At the end of four hours PRA had fallen to 13.5 $\pm$ 4.8; however, this was not a statistically significant decrease from control levels. At the same time PRA had reached its peak, sodium excretion had risen approximately twenty times control levels and potassium excretion increased four fold (see Table 1). Salt excretion remained elevated for the duration of the experiment in spite of the fact that PRA fell to or below control levels by the end of four hours. Increases in urine flow were of the same magnitude as the increases in sodium excretion. Plasma electrolytes were also examined. A significant rise in plasma potassium concentration occurred after two hours (3.81 $\pm$ .09 to 4.54 $\pm$ .17) and continued to the end of the experiment. However, no significant changes in plasma sodium concentration were observed.

Circulating catecholamines (epinephrine and norepinephrine) did not quite show statistically significant increases during the experiment. However, a significant transient fall in mean arterial blood pressure (132.8 $\pm$ 2.8 to 121.7 $\pm$ 4.4) was observed during the first fifteen minutes following the injection of furosemide. After this initial fall, mean arterial blood pressure returned to and continued at control levels. Changes in central venous pressure (CVP) were erratic and inconsistent,

Table 1 - Control

Time	-20	-5	5	15	30	60	120	180	240-270
Renin ng AI/ml/3hr	18.9 ±3.7	22.9 ±5.4	42.5* ±8.1	52.5* ± 10.9	51.6* ±7.8	38.5* ±7.2	22.2 ±3.5	14.4 ±4.1	13.5 ±4.8
Plasma Na^+ mEq/l		147.1 ±.93		146.6 ±1.0			147.1 ±.87		145.8 ±1.7
Plasma K^+ mEq/l		3.81 ±.09		3.89 ±.10			4.54* ±.17		5.24* ±.47
Excreted Na^+ mEq/min	.035 ±.011	.072 ±.023	.890* ±.183	1.58* ±.28					1.08* ±.26
Excreted K^+ mEq/min	.028 ±.014	.046 ±.012	.114* ±.045	.139* ±.043					.134* ±.048
MAPB mm Hg	135.4 ±3.3	132.8 ±2.8	121.7* ±4.4	129.1 ±3.2	133.9 ±2.7	135.6 ±3.7	136.4 ±3.4	135.6 ±6.2	133.9 ±6.3
Epinephrine µg/l		.553 ±.185	.967 ±.376	.688 ±.220	.400 ±200	.500 ±.265	.560 ±.144	.433 ±.340	.500 ±.265
Norepinephrine µg/l		2.07 ±.31		2.66 ±.34			3.40 ±.90		

All values ± one standard error

* $p < .05$

N = 14

as placement of the catheter varied from animal to animal. Changes in hematocrit throughout the experiments were not significant.

Protocol 2 - Beta-adrenergic blockade

Animals which had received the beta-adrenergic blocking agent showed responses similar to control dogs (see Graphs 1 and 2). Within fifteen minutes renin levels rose from 22.8 $\pm$ 7.8 to 42.7 $\pm$ 11.1 ($P < .005$), reached a maximum of 52.5 $\pm$ 12.0 by thirty minutes, and then fell to below control levels (12.4 $\pm$ 3.0) after four fours (See Table 2). However, this was not a statistically significant decrease from control levels.

During this time, sodium and potassium excretion had risen twenty and four fold respectively over basal levels. Maximum excretion was seen after one hour and remained elevated as seen in the control situation (see Tables 1 and 2). The same relationship between urine flow and sodium excretion was also seen. Again no changes were seen in plasma sodium concentration, but plasma potassium levels rose significantly by the end of the experiment (4.25 $\pm$ .45 to 5.49 $\pm$ .57; $P < .005$).

Mean arterial blood pressure fell significantly from 132.7 $\pm$ 2.7 to 119.4 $\pm$ 8.9 during the first five minutes following the furosemide injection, but quickly returned to resting values during the first hour. Hematocrit was stable throughout the four hours.

Protocol 3 - Alpha-adrenergic blockade

The animals treated with the alpha-adrenergic blocking agent, phenoxybenzamine, experienced a significant initial fall in mean arterial blood pressure from 140.7 $\pm$ 6.1 to a low of 84.3 $\pm$ 8.8 ($P < .005$) over a period of an hour following infusion of the drug. Prior to the injection

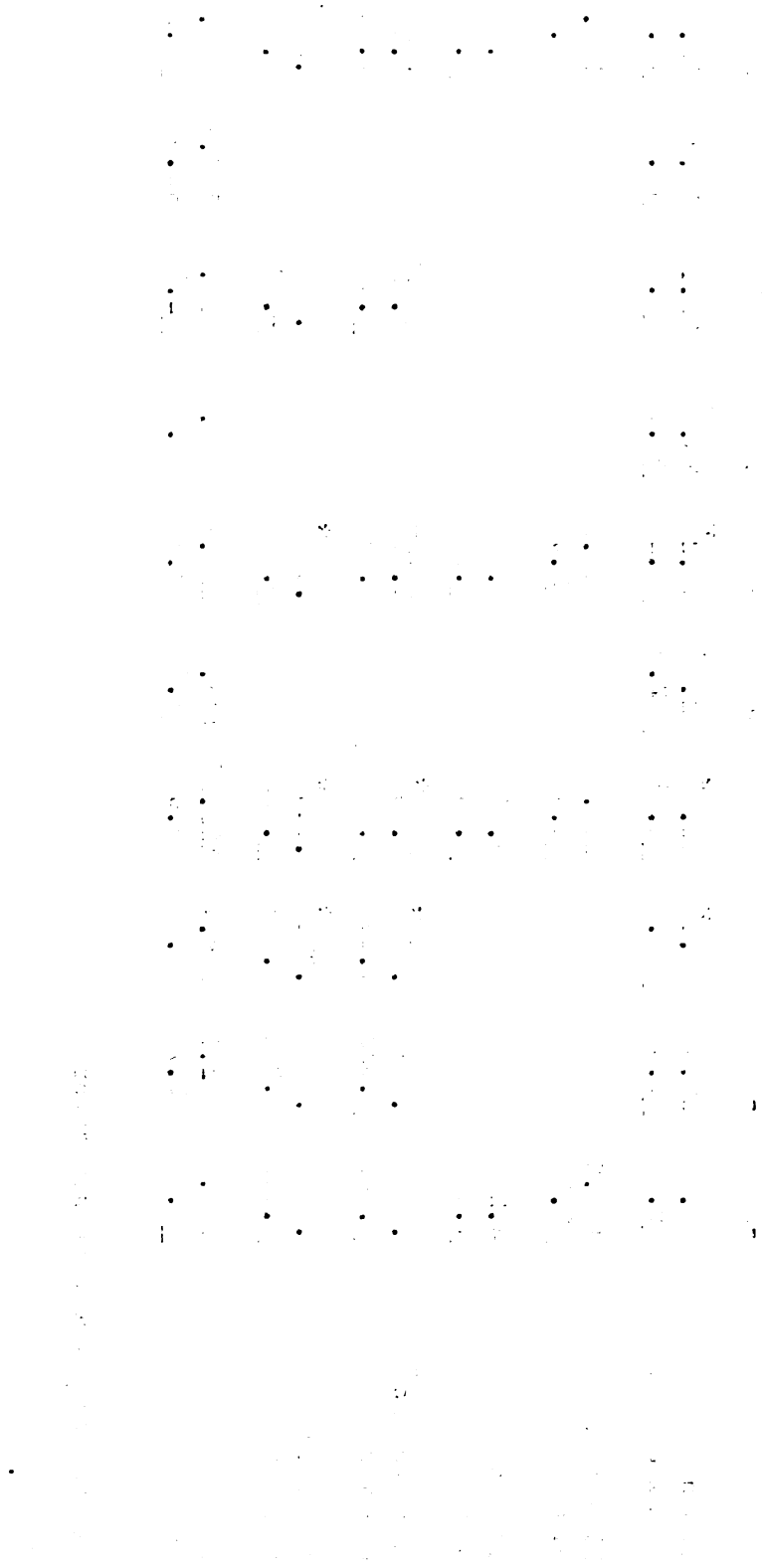
Table 2 - Beta-blockade

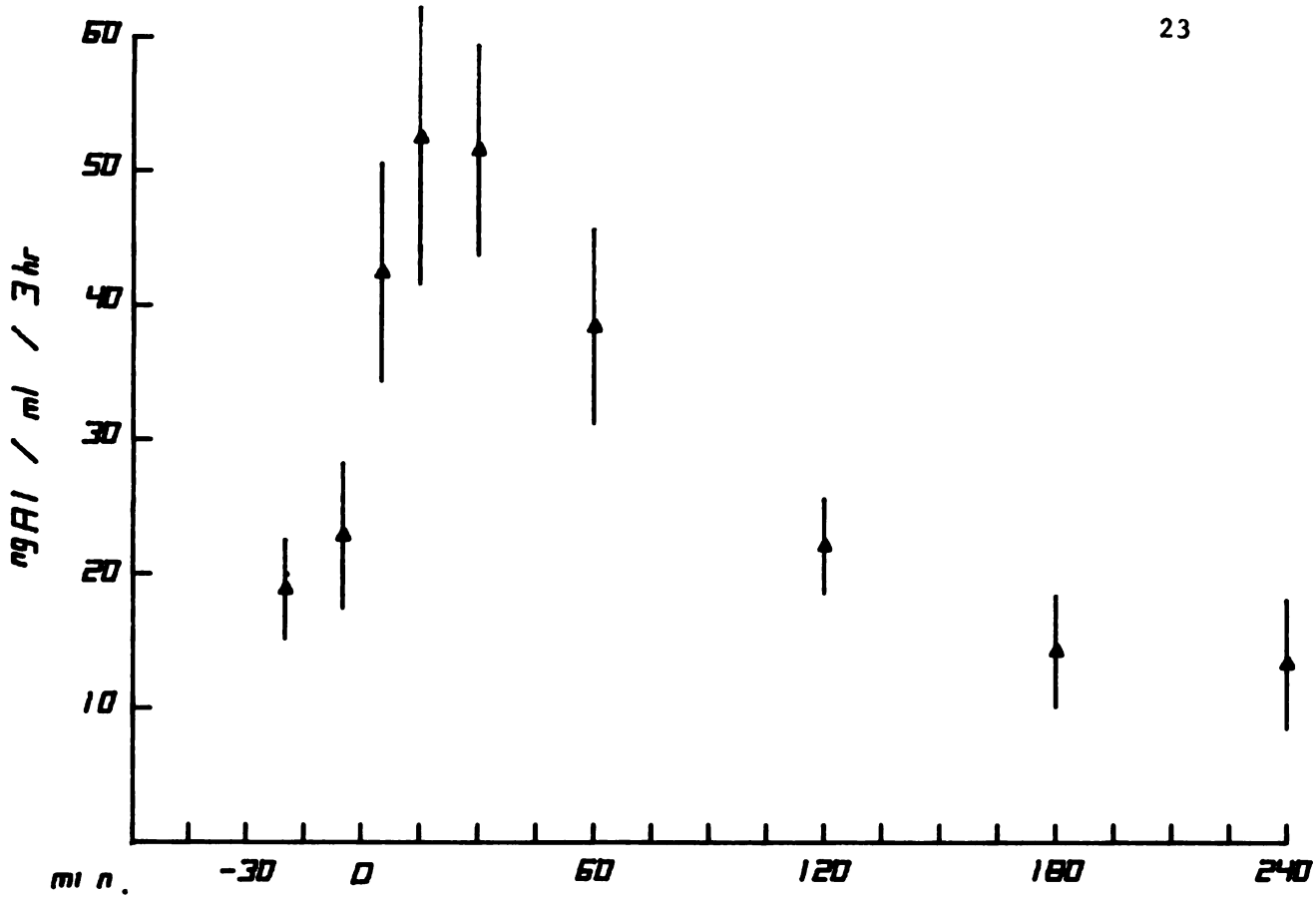
Time	-30	-5	5	15	30	60	90	120	180	240
Renin ng AI/ml/3hr	28.1 ±9.5	22.8 ±7.8	38.3* ±10.5	42.7* ±11.1	52.5* ±12.0	41.2* ±8.2	28.4 ±6.6	23.1 ±5.6	14.9 ±3.7	12.4 ±3.0
Plasma Na^+ mEq/l	146.0 ±2.1			142.4 ±2.6		145.3 ±2.4				144.1 ±2.4
Plasma K^+ mEq/l	4.25 ±.45			5.02 ±.63		4.70 ±.51				5.49 ±.57
Excreted Na^+ mEq/min	.031 ±.007	.062 ±.023	.897* ±.23	1.25* ±.27		2.12* ±.33		1.92* ±.29		1.22* ±.32
Excreted K^+ mEq/min	.004 ±.001	.006 ±.001	.022* ±.004	.023* ±.002		.031* ±.003		.033* ±.003		.033* ±.004
MABP mm Hg	136.0 ±3.4	132.7 ±2.7	119.4 ±8.9	123.5* ±3.6	128.6 ±3.0	133.7 ±2.3	135.4 ±2.5	136.3 ±2.2	135.4 ±2.0	133.1 ±1.9

All values ± one standard error

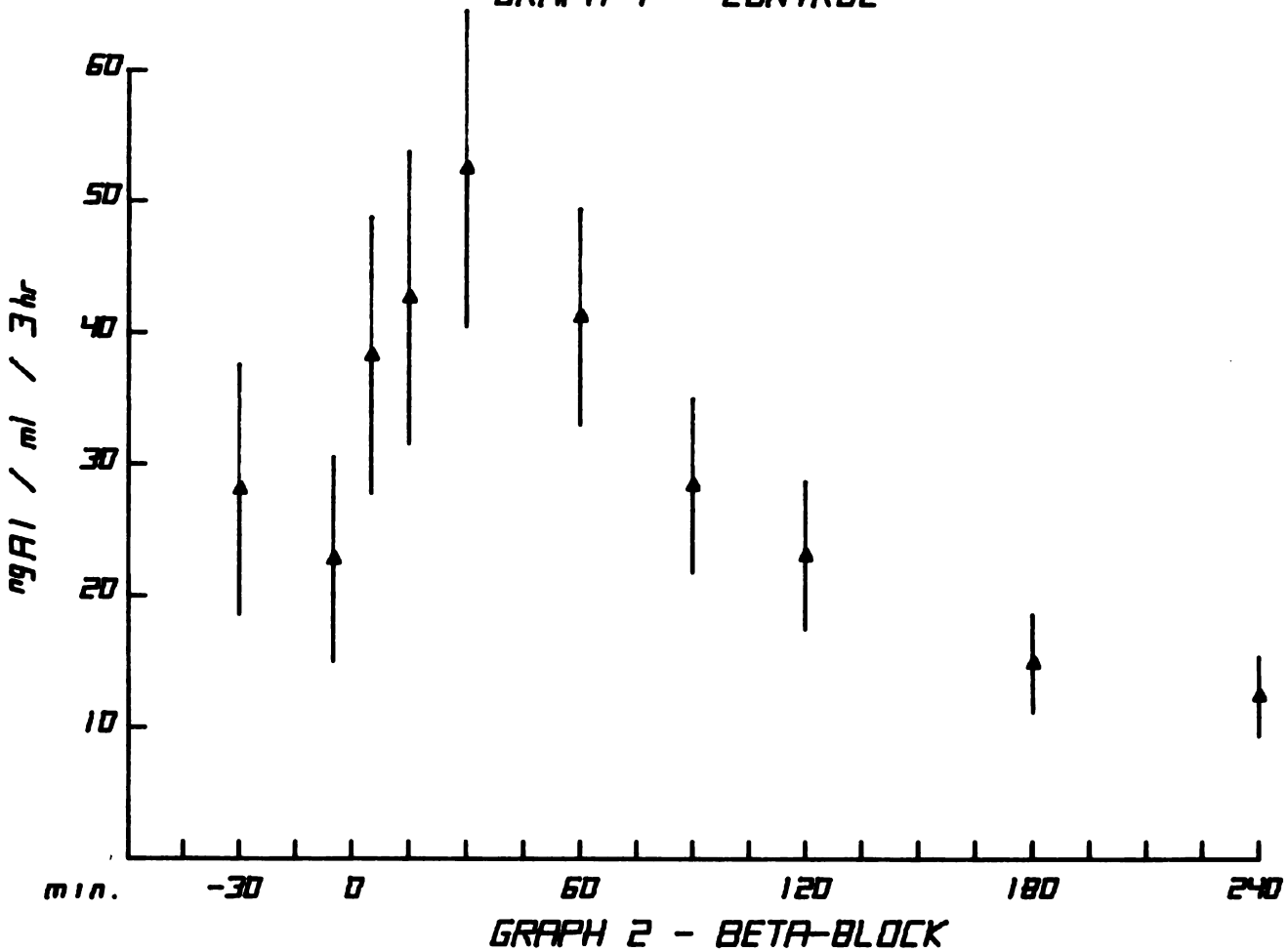
* $p < .05$

N = 8





GRAPH 1 - CONTROL



GRAPH 2 - BETA-BLOCK

of furosemide, the mean arterial blood pressure had risen to 103.1 ± 7.5, but this was significantly less than the pre-phenoxybenzamine values. As described in Protocols 1 and 2, a significant fall in mean arterial blood pressure (103.1 ± 7.5 to 78.6 ± 7.5; $P < .02$) occurred within five minutes following the injection of furosemide, and recovered within thirty minutes (see Table 3). However, after returning to pre-furosemide levels, mean arterial blood pressure eventually fell during the course of the experiment to 95.2 ± 10.1.

A significant increase in PRA was seen after administration of phenoxybenzamine and prior to the injection of furosemide (see Graph 3). Values rose from 27.0 ± 4.5 to 63.1 ± 15.2 ($P < .025$). PRA increased again following the injection of furosemide. However, this increase was not statistically significant when compared to the pre-furosemide value (see Table 3 and Graph 3). Sodium excretion increased thirty times and potassium excretion four times control values and remained elevated. Potassium excretion increased throughout the experimental period. However, no significant increases were seen in plasma potassium concentration, and plasma sodium concentration remained constant. There were also no significant changes in hematocrit.

Protocol 4 - Repeat stimulation

In animals receiving a second injection of furosemide, it was noted that by using the initial control levels of PRA as a basis for comparison that there was no significant rise in PRA from the second injection of the drug. However, in examining the second response, comparison of pre-injection values to the post-injection response showed a significant rise in PRA five and fifteen minutes after the injection. This response

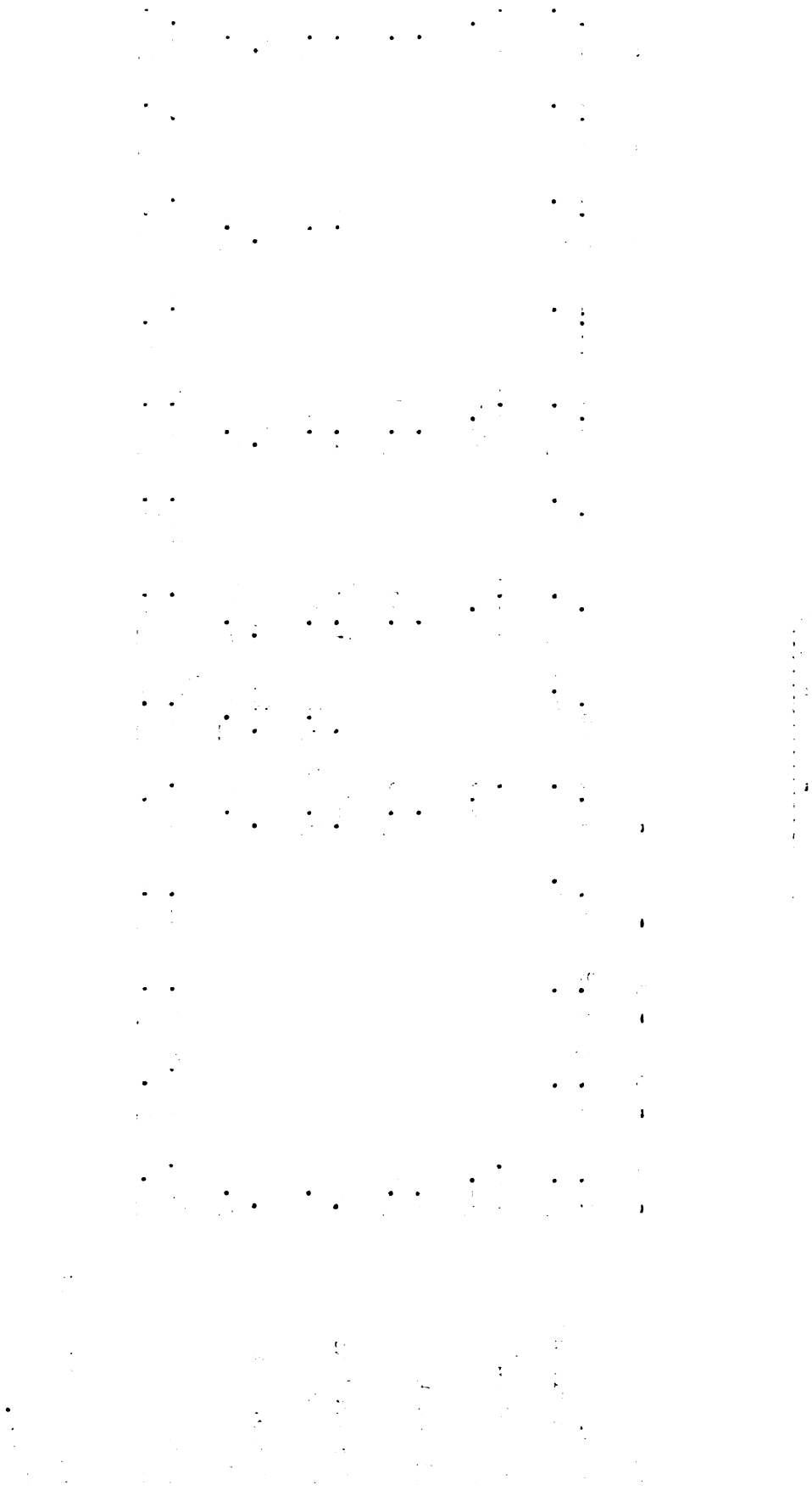
Table 3 - Alpha-blockade

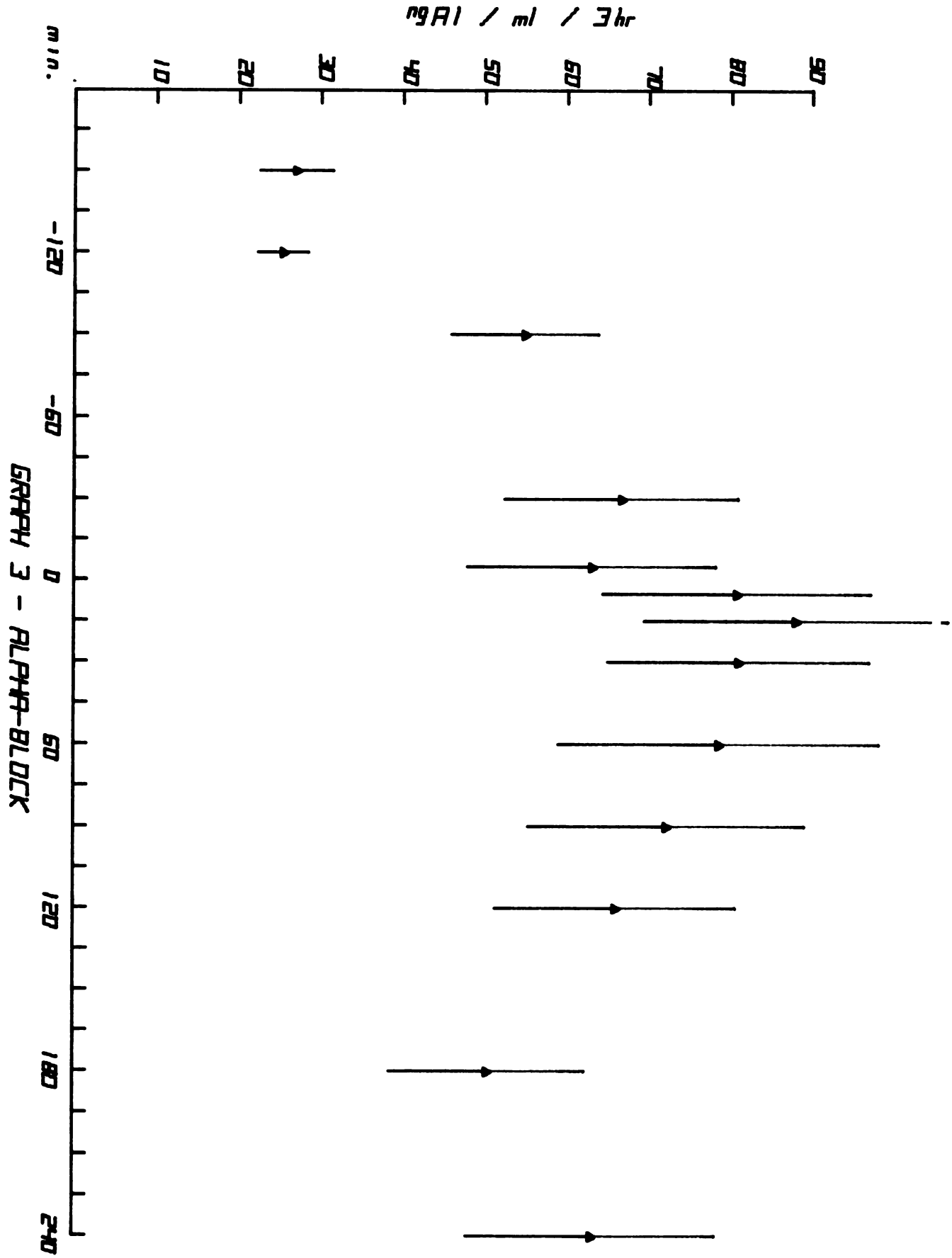
Time	-150	-120	-90	-30	-5	5	15	30	60	90	120	180	240
Renin ng AI/ml/3hr	27.0 ±4.5	25.4 ±3.1	54.9 ±9.0	66.7 ±14.3	63.1 ±15.2	80.8 ±16.4	88.0 ±18.5	81.0 ±16.0	78.6 ±19.6	72.2 ±16.9	66.0 ±14.7	50.4 ±11.9	63.1 ±15.2
Plasma Na^+ mEq/l	142.0 ±2.4				144.4 ±2.6		141.5 ±1.7		141.4 ±2.5				142.9 ±1.4
Plasma K^+ mEq/l	4.11 ±.35				3.89 ±.45		3.80 ±.43		4.14 ±.50				4.46 ±.31
Excreted Na^+ mEq/min	.018 ±.006				.024 ±.009	.717* ±.25	1.14* ±.35		1.22* ±.26		1.50* ±.37		1.30* ±.33
Excreted K^+ mEq/min	.003 ±.0004				.005 ±.001	.026* ±.008	.022* ±.004		.025* ±.006		.032* ±.008		.049* ±.014
MABP mm Hg	140.7 ±6.1	101.9 ±7.0	84.3 ±8.8	95.7 ±7.5	103.1 ±7.5	78.6* ±7.5	103.8 ±10.6	106.0 ±11.2	104.8 ±11.1	104.0 ±9.4	103.6 ±9.5	98.6 ±10.9	95.2 ±10.1

All values ± one standrd error

* $p < .05$

N = 7




GRAPH 3 - ALPHA-BLOCK
ng AI / ml / 3hr
min.
10
20
30
40
50
60
70
80
90
-120
-60
0
60
120
180
240

was similar in duration, but of a lesser magnitude than the initial response (see Graphs 1 and 4, and Table 4). It should also be noted that the decrease in PRA seen at the end of both experimental periods was not statistically significant.

It was also noted that in most cases sodium excretion was more elevated during the second response than the first (see Tables 1 and 4). In addition, the transient depressor effect of furosemide that was seen in the first response (see Table 1) was not significant in the second. The hematocrit was essentially unchanged during both periods.

Protocol 5 - Volume expansion

Following the second dose of furosemide, animals which had been markedly expanded (20% extracellular fluid volume) showed no increase in PRA during the thirty minute period in which they were followed (see Table 5 and Graph 5). In fact there was a significant decrease in PRA ($P < 0.25$) five minutes following the injection of furosemide (28.9 ± 5.2 to $15.5 \pm 15.5 \pm 2.6$). These findings confirm those of Meyer et al. (1968b) wherein significant expansion of extracellular volume or plasma volume was capable of depressing the rise in PRA caused by furosemide.

It should be pointed out here that the animals used in this protocol had been previously treated with phenoxybenzamine. It is the author's feeling that, while the rise in PRA following the injection of furosemide in Protocol 3 was not quite statistically significant, it was similar to the response seen in Protocols 1 and 2. However, without adequate control data care must be exercised in interpreting the decrease in PRA mentioned above.

Table 4 - Repeat stimulation

Time	(270) -5	5	15	30	60	90	120	180	240
Renin ng AI/ml/3hr	6.0 ±1.7	10.6* ±1.5	15.3* ±3.8	14.0 ±3.7	9.6 ±1.6	9.1 ±1.2	5.6 ±.8	5.9 ±.9	5.2 ±1.5
Plasma Na^+ mEq/l	146.2 ±1.7		146.0 ±1.2				145.8 ±1.1		
Plasma K^+ mEq/l	5.20 ±.49		5.18 ±.40				5.62 ±.74		
Excreted Na^+ mEq/min	1.09 ±.32	3.71* ±.80	4.14* ±1.1		3.91* ±.96				1.35* ±.21
Excreted K^+ mEq/min	.181 ±.091	.216 ±.155	.338 ±.170		.308 ±.153				.177 ±.139
MABP mm Hg	138.8 ±4.8	133.4 ±5.3	136.3 ±4.3	138.8 ±4.9	137.1 ±5.7	138.8 ±5.3	135.4 ±7.1	135.5 ±6.3	130.9 ±9.4

All values ± one standard error

* $p < .05$

N = 4

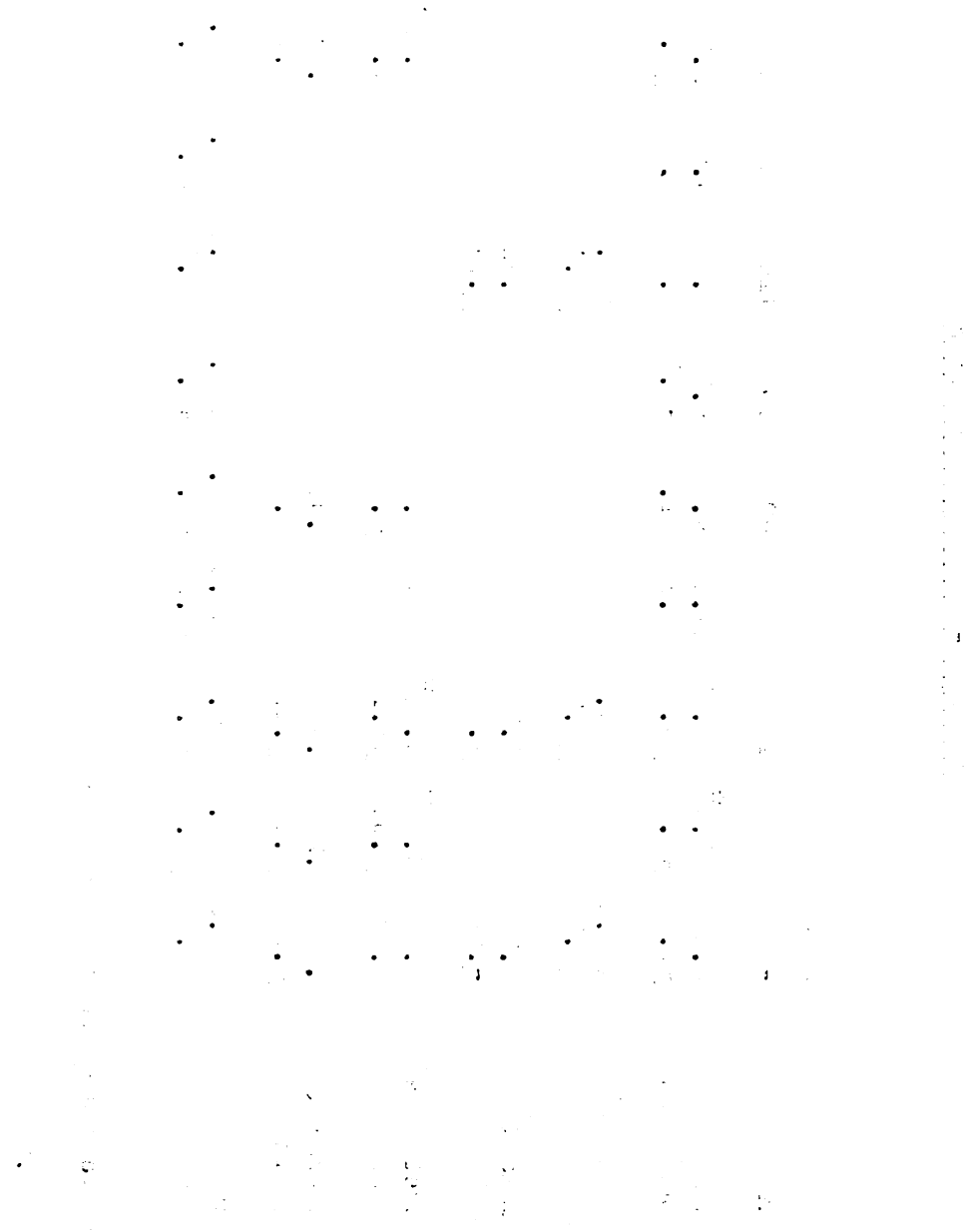


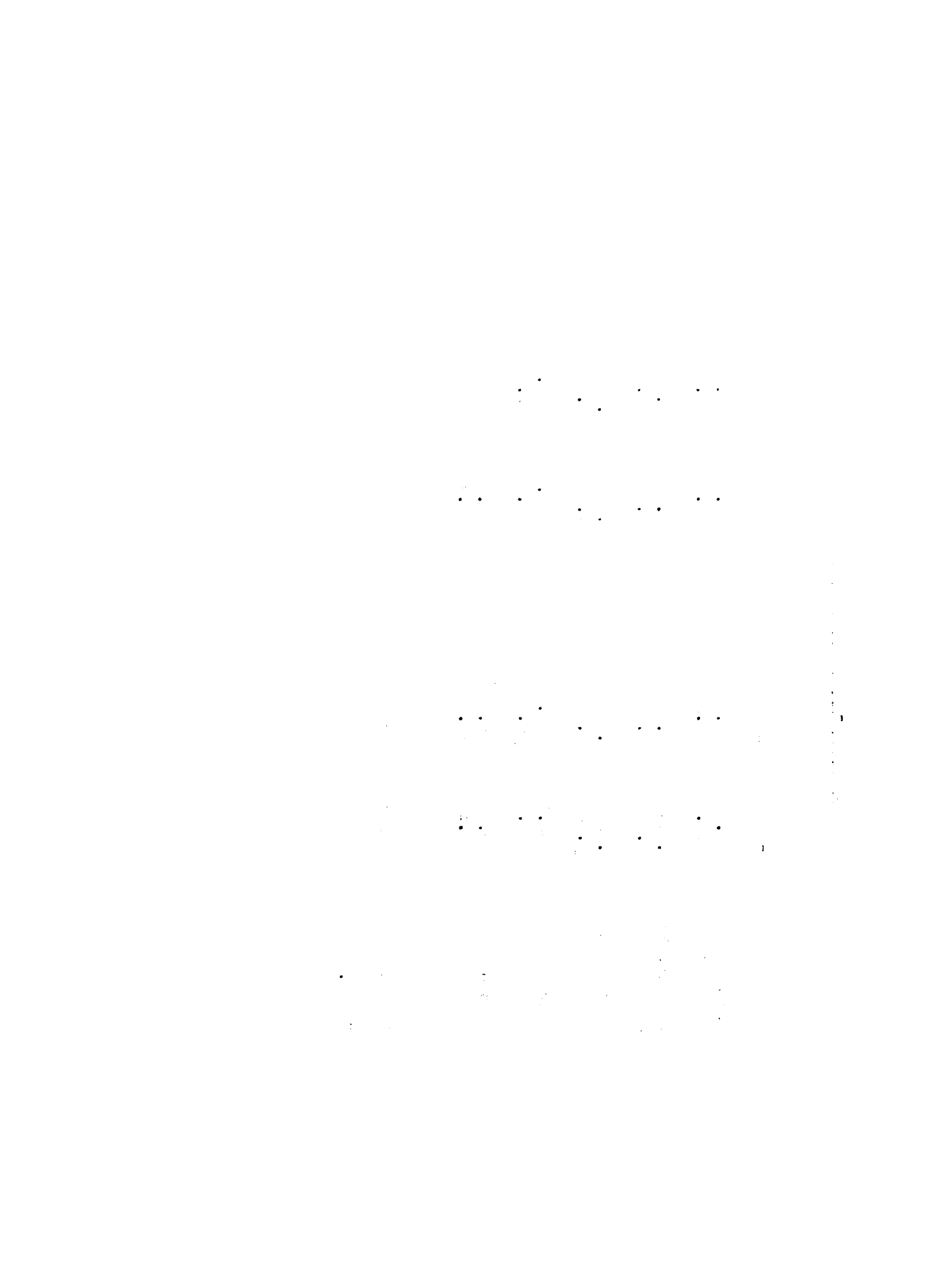
Table 5 - Volume expansion

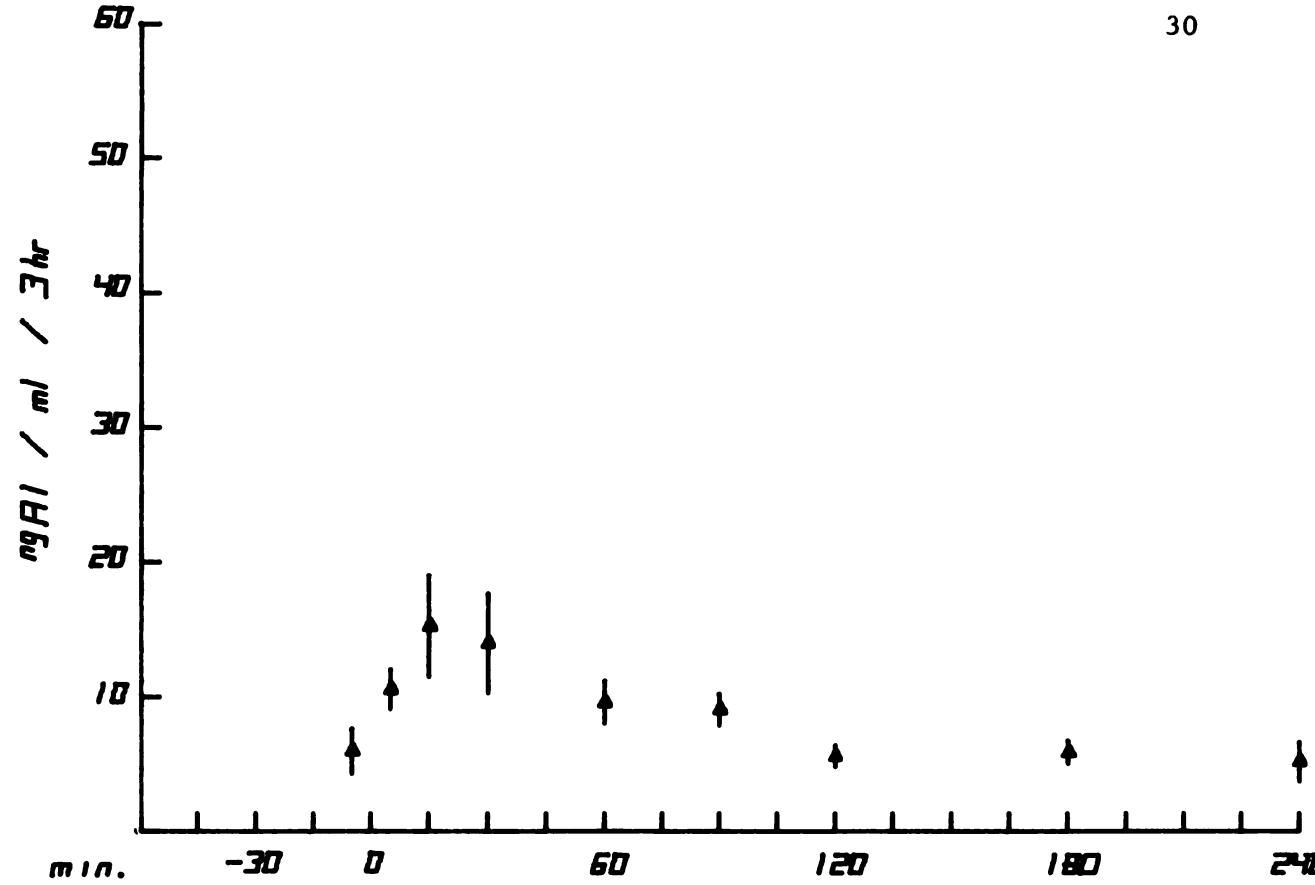
Time	-5	240	0	5	30
Renin ng AI/ml/3hr	43.0 ±19.0	28.9 ±5.2		15.5* ±2.6	20.9 ±1.9
Excreted Na^+ mEq/min	.025 ±.012	1.09 ±.47		4.82* ±.92	5.05* ±1.14
Excreted K^+ mEq/min	.005 ±.002	.050 ±.021		.062 ±.019	.061 ±.015
MABP mm Hg	102.1 ±14.0	103.8 ±4.6		120.9 ±7.0	125.0 ±7.8
Hematocrit	50.4 ±4.1	49.8 ±3.8		36.7* ±3.9	

All values ± one standard error

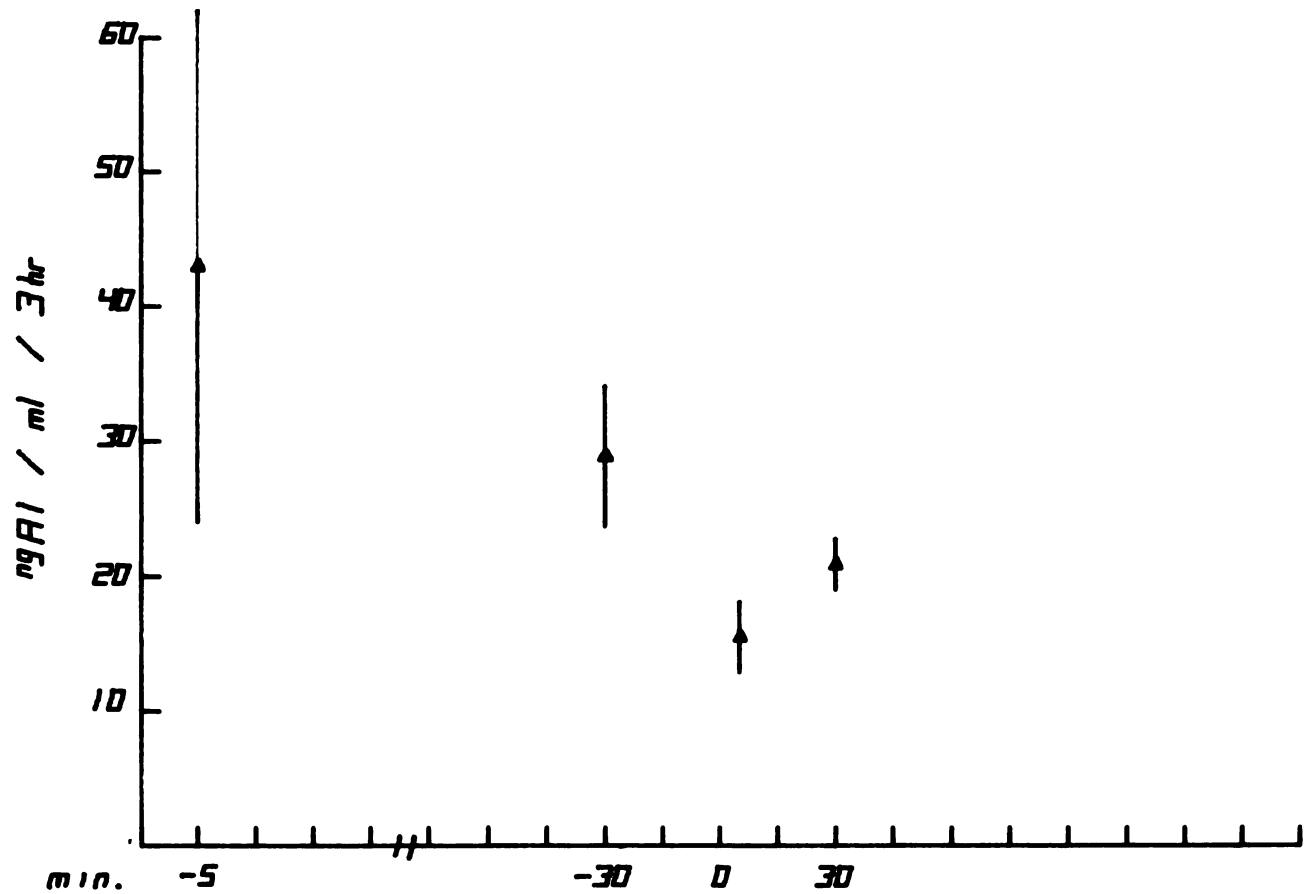
* $p < .05$

N = 4





GRAPH 4 - REPEAT STIMULATION



GRAPH 5 - VOLUME EXPANSION

As observed in Protocol 4, the depressor effect of furosemide immediately following injection was absent. Mean arterial blood pressure, in this case, increased from 103.8 $\pm$ 4.6 to 125.0 $\pm$ 7.8 thirty minutes following volume expansion and the injection of furosemide. This increase was not statistically significant, however. Again sodium excretion was significantly elevated, but potassium excretion remained about the same (see Table 5). Hematocrit remained relatively constant throughout the experiment, but fell significantly following volume expansion and furosemide.

DISCUSSION

These findings confirm previous reports showing furosemide to be an effective and rapidly acting agent in stimulating renin release in pentobarbital anesthetized dogs (Vander and Carlson, 1969). However, this response was not altered by the systemic administration of the beta-adrenergic blocking agent, propranolol. The response was modified by systemic alpha-adrenergic blockade using phenoxybenzamine. The effects, however, of the latter drug on the response were probably due to factors associated with the fall in mean arterial blood pressure following infusion. Although the drug was infused slowly over a period of an hour, a significant rise in PRA was observed which coincided with the marked decline in mean arterial blood pressure. Presumably, the latter effect involves inhibition of the tonic vasomotor discharge observed in pentobarbital anesthetized dogs (Olmsted and Page, 1966). Also it is possible that this decline in blood pressure contributed to the rise in renin that was observed, as Skinner et al. (1964a) have reported that small decreases in blood pressure may increase renin secretion. The depressor effect of phenoxybenzamine (in doses similar to those used in this study) followed by increased PRA has been shown by others to occur in rats (Alexandre et al., 1970) and in dogs (Loeffler and Ganong, unpublished observations; Assaykeen et al., 1970). Others, however, have been more successful in minimizing this effect of phenoxybenzamine (Passo et al., 1971a; Assaykeen et al., 1970). There is also a possibility that, since these animals were unable to eliminate excreted drugs and metabolites due to the ureteral venous shunting, the effects seen here were more pronounced. From examination of the renin response before and after adrenergic

blockade and the lack of significant changes in circulating epinephrine and norepinephrine it would appear that the mechanism controlling the rise in renin induced by furosemide is not directly mediated by the sympathetic nervous system or circulating catecholamines.

The possibility that the response can be explained in terms of the diuretic and saluretic effects of furosemide and subsequent changes in intratubular electrolyte concentrations, sodium transport, and flow rates deserve consideration.

Furosemide is a sulfonamide with potent natruretic and diuretic actions, and is rapidly absorbed. It is thought to have a locus of action similar to ethacrynic acid. Approximately two-thirds of the administered drug is excreted in the urine. This is accomplished both by glomerular filtration and proximal tubular secretion. Furosemide's main site of action is the ascending loop of Henle (Goodman and Gilman, 1970). Therefore, without volume changes, furosemide markedly increases the amount of sodium entering the early distal tubule.

It has been proposed by some (Thurau et al, 1967; Meyer, 1968b) that the increase in early distal tubular sodium concentration by furosemide is directly related to renin secretion. On the other hand, Vander (1967) and Nash et al. (1968) have suggested that the stimulus for renin secretion is inversely related to sodium load at the macula densa, and that the link between sodium load and renin release is the rate of transport by the macula densa cells and their intracellular sodium concentration.

Due to furosemide's action on the ascending limb, it has been suggested that possible direct inhibition of sodium reabsorption on macula densa cells would decrease the amount of sodium transported into these cells thereby

stimulating renin secretion despite the increases in intratubular sodium delivered to this area. This is the reverse of what is seen when tubular sodium is increased by other means (Vander and Miller, 1964; Tobian, 1967). There is data indicating that furosemide does act to reduce the initial entry of sodium into cells; and it is possible that this action may be present in the area of the macula densa as well as the ascending limb of the loop of Henle (Nagel and Karger, 1964).

Another consideration concerning the possible mechanism of this response involves renal hemodynamic changes induced by furosemide. Birtch et al. (1967) reported that the drug affects intrarenal blood flow. Flow in the juxtamedullary cortex was reduced, as was the blood flow in the peritubular capillaries of the outer medulla. Blood flow to the pars radiata of the cortex was increased. Also, as has been pointed out elsewhere, it is hard to understand how this effect would stimulate renin secretion since most renal renin is located in the outer cortex rather than the juxtamedullary cortex or medulla (Brown et al., 1965). In addition, it has been demonstrated that renal plasma flow is elevated even when plasma volume is maintained constant during the furosemide diuresis (Ludens et al., 1968). It would seem likely that in the present study any effects on renin due to renal hemodynamic changes induced by furosemide would be modulatory to some controlling mechanism rather that control the response in and of themselves. Perhaps the most adequate explanation for the changes in PRA that were observed in these experiments is found in a synthesis of the experimental findings.

First, as mentioned earlier, a significant transient depressor response was observed following the injection of furosemide. A fall in mean arterial

blood pressure of at least 10 mm Hg occurred within two to five minutes following the injection in control and blocked animals. Pulse pressure was diminished, but the animals returned to pre-injection levels within thirty minutes. This fall in mean arterial blood pressure corresponds to the initial rise in PRA that was observed in Protocols 1, 2, and 3. Although Vander and Carlson (1969) reported an increase in mean arterial blood pressure following administration of 2.5 mg/kg furosemide, their animals received this amount per hour as infusion whereas the dose used here was a single injection of 5 mg/kg. Interestingly enough in animals which were given a second injection of furosemide following the first experimental period, no significant changes in mean arterial blood pressure occurred, and the corresponding response in renin secretion was diminished and was only significant when compared to the second pre-injection controls. Likewise, the animals which were volume expanded responded inversely to the increase in mean arterial pressure with a significant decrease in PRA. It should be pointed out again, however, that these animals were alpha-blocked and that corresponding control values are not available for comparison. Therefore, the significance of this finding is uncertain at this time. It has been claimed that furosemide may have effects on smooth muscle (Blair-West, personal communication). It is therefore possible that these extrarenal effects are involved in the changes in blood pressure observed.

As discussed above, it is known (Skinner et al., 1964a) that even small decreases in blood pressure can stimulate a transient rise in renin secretion. A similar situation has been shown to occur following supplemental doses of sodium pentobarbital given periodically throughout an experiment in order to maintain a desired level of anesthesia (Olmsted and Page, 1966). It is

conceivable that this effect could have been contributory to the initial rise in PRA possibly via an intra- or extrarenal baroreceptor mechanism. It should be mentioned, however, that since the depressor effect of furosemide was present during adrenergic blockade as well as the control situation, the response is probably not reflexly (neurally) mediated. Additionally, the response (small decreases in mean arterial blood pressure) to supplemental doses of sodium pentobarbital were also noted during adrenergic blockade indicating a similarity to the former response. It has also been shown that a decrease in mean arterial blood pressure is a more important consideration in relation to changes in renin secretion than are changes in pulse pressure. Kolff showed in 1958 that a diminution of pulse pressure did not increase renin secretion in the dog, but a drop in mean arterial pressure did. Skinner et al. (1963, 1964b) confirmed this finding and were unable to lower renin secretion by increasing the pulse pressure in the face of a lowered mean arterial blood pressure. Therefore, the data presented here deals with changes observed in mean arterial blood pressure. It would seem incomplete to explain the magnitude and duration of the renin response to furosemide only in terms of a transient decrease in mean arterial blood pressure. However, it may be possible for such a fall in blood pressure to "trigger" a secondary autonomic response capable of sustaining the renin response.

Some have suggested that variations in central venous pressure rather than systemic blood pressure are more directly related to renin secretion (Meyer et al., 1968a). This hypothesis was supported by the findings of Hodge et al. (1966) who found that changes in angiotensin generation rate are inversely correlated with central venous pressure and not with systemic

blood pressure. In these experiments it was difficult to obtain reproducible results between and often within experiments due to difficulties in placing and maintaining the catheter in a stationary state. As a result it was impossible to define a relationship between central venous pressure and renin secretion.

Regarding the natruretic effects of furosemide, it has been demonstrated in micropunture studies in the dog that this effect of furosemide is due to decreased reabsorption of sodium by the ascending limb of the loop of Henle (Clapp and Robinson, 1968). This was shown without increased delivery of fluid from the proximal tubule (Dirks et al., 1966). There is also additional evidence, some of it conflicting, that the drug may not only act in the proximal (Knox et al., 1969; Brenner et al., 1969), but distal convoluted tubule as well (Fraser et al., 1967). Therefore, as discussed previously and hypothesized by Vander (1967, 1969), it would appear reasonable that furosemide could have an inhibitory effect on sodium reabsorption at the level of the macula densa. Therefore, with the tremendous increase in excreted sodium that was observed immediately after injection of furosemide, it could be assumed that sodium reabsorption was inhibited in the area of the macula densa to such an extent that even with the increase of sodium delivered to the early distal tubule, the rate of sodium transport by the macula densa cells, their intracellular sodium concentration, and perhaps the rate of transport across the macula densa into the interstitium would decrease; resulting in an increase in renin secretion.

In the present study kidney function studies were not performed. However, due to the nature of the increase of excreted electrolytes and

the action of the drug, it could be safely assumed that the levels of excreted sodium and potassium reflected corresponding intratubular increases in the area of the macula densa and early distal tubule. Therefore, for the duration of an inhibitory effect of furosemide on sodium transport at the macula densa, the cells would experience a decrease in transport of sodium and this by some mechanism would stimulate renin release. As either the increase in intratubular sodium became such to override the inhibitory effects of furosemide or the inhibition of transport declined or some combination of both increased delivery of sodium to the macula densa, then inhibition of renin release would occur and PRA would decline. Comparison of sodium excretion in control, beta-blocked, and alpha-blocked dogs to that of the repeat stimulation and volume expansion animals shows a significant ($P < .005$) increase in excreted sodium five and fifteen minutes following furosemide (see Tables 1,2,3, and 4) in the latter cases. Since the rise in renin was diminished or absent in these animals, it might be assumed that the increase in intratubular sodium was sufficient to overcome to some extent the inhibitory effects of furosemide. Of course, the actual mechanisms whereby sodium stimulates renin release are still unknown. In addition to the possible effects of furosemide on the macula densa cells as described above, intratubular sodium concentration could also play a role. However, evidence indicates that, regardless of the mechanism of action, the renin response observed in this study was dependent upon the presence of tubular urine flow (Cooke et al., 1970). In the case of volume expansion, where a decrease in PRA was noted, proximal tubular reabsorption of sodium is known to decrease. Therefore, the reabsorption stimulating effect of this additional increase in sodium delivery to the early distal tubule

might overcome the furosemide-induced inhibition of sodium transport to such an extent as to decrease renin secretion. However, the assumption must be made that these animals are capable of a renin response similar to animals in Protocols 1 and 2.

The decrease in PRA seen at the end of the experimental periods in Protocols 1 and 2 could also be explained by a decrease in the inhibitory effect of furosemide on sodium transport. In addition, the significant increase in plasma potassium concentration seen at the end of these experiments can also cause a decrease in PRA and an increase in aldosterone secretion (Vander, 1970; Blair-West et al., 1962; Davis et al., 1963). Therefore, as plasma potassium concentration increased throughout the experiment, one would expect further suppression of renin secretion by changes perhaps secondary to aldosterone secretion and sodium balance (Maebashi et al., 1968), or by direct action on the kidney to inhibit renin release and/or sodium reabsorption (Vander, 1970; Dluhy et al., 1968). These latter data further explain the observed decline in PRA by the end of the experiments to or below control levels. It should also be pointed out that this explanation concerning the decline in PRA following the initial rise could account for the observation that while PRA had fallen to control values or below at the end of the experimental period, sodium excretion remained significantly elevated.

Although it was initially felt at the onset of these experiments that the renin response to furosemide was mediated via the sympathetic nervous system, the evidence presented here indicates that it is not. As previously discussed, stimuli that increase renin secretion and are related to sympathetic activity include: catecholamines, stimulation of renal nerves,

stimulation of sympathetic areas in brain, constriction of carotid arteries, and hypoglycemia. Other stimuli that may be mediated via the sympathetic nervous system are hemorrhage, assumption of the upright posture, and sodium depletion. However, the negative nature of the findings in this study demonstrate that in the case of furosemide-induced renin release, where volume depletion is prevented, the sympathetic nervous system does not play a measurable role.

Of course, due to the wide range of stimuli to renin secretion factors inherent to the manipulation of the animals and technical procedures should not be overlooked as factors contributing to the outcome of such experiments. Diet (Bunag et al., 1966b), pathology, anesthesia, stress, and biological variation are only a few of many factors which may affect the experimental results. It was noted in these experiments that occasionally following cannulation of ureters an increase in PRA was seen ($P > .10$). Irritation of the ureters during cannulation may have caused spasmodic contraction of the ureters effectively reducing urine flow and possibly increasing renal interstitial pressure due to the increased ureteral pressure. It has been shown that such an increase in pressure will cause an increase in PRA (Vander and Miller, 1964; Strand, Assaykeen, Otsuka, and Ganong, unpublished observations). However, prior to the injection of furosemide urine flow and PRA were stable.

In summary it appears that the sympathetic nervous system is not directly involved in the renin response to furosemide in anesthetized dogs where volume loss is prevented, and thus this is one stimulus to renin secretion which appears to function independently of the sympathetic nervous system. From examination of the experimental data, it seems likely that

the responses observed were due to one or more of the following: (1) the transient depressor effects of furosemide, (2) inhibition of sodium uptake and transport at the macula densa, (3) increases in intratubular sodium load, and (4) increases in plasma potassium concentration.

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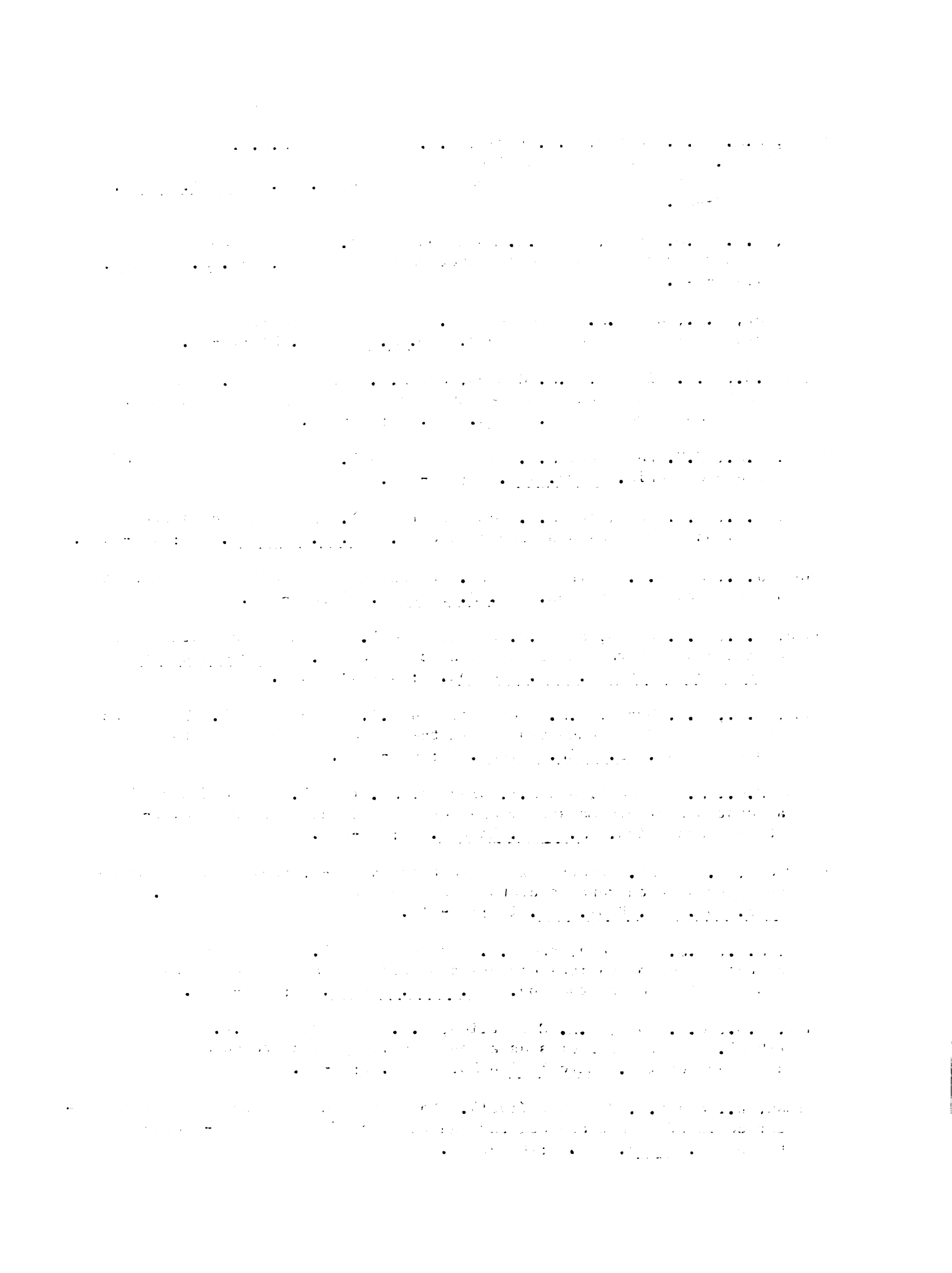

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