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STIMULATION OF RENIN SECRETION BY VASOACTIVE INTESTINAL PEPTIDE: MECHANISM AND PHYSIOLOGIC IMPORTANCE

by

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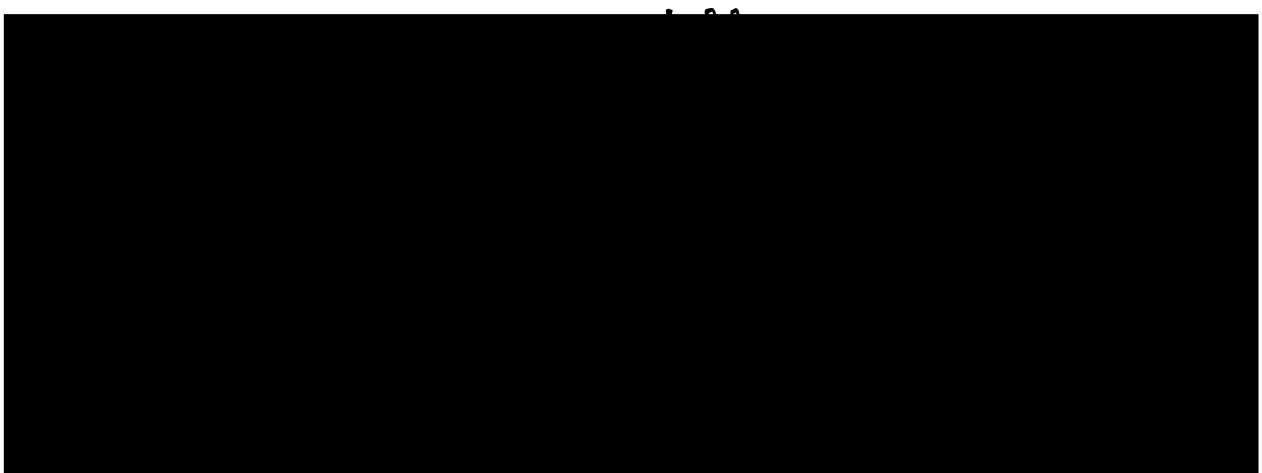

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ABSTRACT

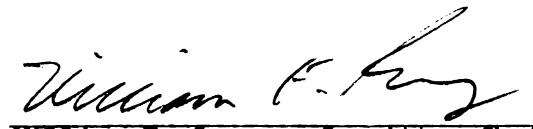
Stimulation of Renin Secretion by Vasoactive Intestinal Peptide: Mechanism and Physiologic Importance

by

James P. Porter

The effect of intrarenal and intravenous infusions of vasoactive intestinal peptide (VIP) on renin release were compared in anesthetized dogs. A 15-min infusion of VIP directly into the renal artery at a rate of 33 ng/kg/min increased PRA from 19.2 $\pm$ 2.3 to 29.2 $\pm$ 4.7 ng AI/ml/3h, and increased renin secretion rate from 1,461 $\pm$ 393 to 5,769 $\pm$ 1,794 ng AI/ml/3h/min. Renal blood flow and creatinine clearance were also increased, whereas plasma potassium concentration and diastolic blood pressure decreased. Sodium and potassium excretion did not change. When administered intravenously, 33 and 13 ng/kg/min VIP increased PRA. A dose of 3.3 ng/kg/min failed to increase PRA when given intravenously, but produced a significant increase in PRA when infused directly into the renal artery. This increase occurred without any change in plasma potassium concentration or blood pressure. A two to threefold increase in circulating VIP levels was sufficient to cause the increase in PRA. VIP in doses ranging from 10^{-9}M to 10^{-7}M significantly increased renin release from an isolated rat glomerular preparation in a dose-related manner. Using three different VIP antisera, all of which stained neural elements in the salivary gland, no specific VIP immunoreactivity could

be detected in canine renal cortex. Stimulation of the renal nerve in anesthetized dogs increased PRA significantly from 13.2 ± 2.5 to 21.2 ± 3.4 ng AI/ml/3h but did not affect renal venous levels of VIP. Intravenous infusion of neostigmine (0.07 mg/kg) in 6 anesthetized dogs caused an increase in circulating VIP in all dogs while PRA increased in 4 of the 6 dogs. Fourteen days of a low salt diet increased PRA significantly from 3.4 ± 0.8 to 8.0 ± 0.9 ng AI/ml/3h in 6 dogs without affecting circulating levels of VIP. These results suggest that VIP acts directly on the juxtaglomerular cells to increase renin secretion. However, VIP probably does not play a role in neurally mediated increases in PRA. It is also not likely to be a humoral factor involved in the renin response to a low salt diet. There may be other physiologic or pathophysiologic situations in which VIP functions as a renin-stimulating factor. Further work will be required to determine in which, if any, of these situations VIP plays a role in the regulation of renin secretion.

Approved by 

William F. Ganong, Committee Chairman

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INTRODUCTION

Vasoactive intestinal peptide (VIP) was first isolated from porcine duodenum in 1970 (Said and Mutt, 1970). It was originally thought to be a gastrointestinal hormone involved in the regulation of gastrointestinal blood flow or glucose homeostasis. In 1976, VIP was shown to be present in neurons of the central and peripheral nervous systems (Larsson et al., 1976b; Said and Rosenberg, 1976). The peptide probably functions as a neurotransmitter and is responsible for certain nonadrenergic, noncholinergic responses such as atropine-resistant vasodilation in the salivary gland and relaxation of gastrointestinal sphincters and airway smooth muscle (Goyal et al., 1980; Lundberg et al., 1980b; Matsuzaki et al., 1980). VIP is also present in hypophyseal portal blood and is thought to be involved in the regulation of prolactin secretion (Said and Porter, 1979; Rotsztejn et al., 1980a).

While investigating the effect of intravenously administered VIP on release of pituitary hormones in two anesthetized dogs, Ganong and associates made the observation that the peptide also caused an increase in plasma renin activity (PRA) (Porter and Ganong, 1982). This effect was accompanied by a decrease in blood pressure. Since a decrease in blood pressure is known to be one stimulus to renin secretion, the VIP infusions were repeated in two other dogs in which renal perfusion pressure was held constant. Again VIP increased PRA.

In the present work, this preliminary observation was followed up to determine if VIP actually is a renin-stimulating factor, to determine

by what mechanism it exerts this effect, and to determine if there are situations in which this peptide functions physiologically to influence renin secretion.

REVIEW OF LITERATURE

Renin

Renin is a proteolytic enzyme which is secreted into the blood stream by the juxtaglomerular cells of the kidney. It acts on a circulating alpha-2 globulin, angiotensinogen, to release the decapeptide, angiotensin I (AI). AI is converted to an octapeptide, angiotensin II (AII), by converting enzyme, a dipeptidyl carboxypeptidase, as the AI passes through the lungs and other organs. AII is the most potent vasoconstrictor known, and as such plays an important role in the regulation of blood pressure. It is also an important regulator of aldosterone secretion and therefore, indirectly, plays a role in the maintenance of salt and water homeostasis. It has numerous actions in the brain, probably by way of the circumventricular organs, including increased blood pressure, drinking, vasopressin secretion, and ACTH secretion (Ramsay, 1979). AII also increases sympathetic activity in the periphery by facilitating adrenergic transmitter release (Zimmerman, 1978). Investigations using different inhibitors of the renin-angiotensin system also point to a role for AII in renovascular and some forms of essential hypertension (Kotchen and Guthrie, 1980).

Regulation of Renin Secretion

The release of renin from the juxtaglomerular cells in the kidney is regulated by a number of different mechanisms. These include the intrarenal baroreceptor mechanism, the macula densa mechanism, and the sympathetic nervous system. Renin secretion is also influenced by several humoral agents including sodium, potassium, and peptides such as AII, vasopressin, glucagon, parathyroid hormone, substance P, and somatostatin.

The Intrarenal Baroreceptor Mechanism

Tobian et al. (1959) first postulated the presence of a mechanoreceptor within the arteriole of the kidney which regulates renin release. According to this hypothesis, a decrease in stretch in the arterioles leads to an increase in renin release and an increase in stretch leads to a decrease in renin secretion. The existence of the arteriolar baroreceptor was confirmed in a series of experiments by Blaine et al. (1970;1971). An animal preparation was devised in which the macula densa was eliminated by rendering the kidney nonfiltering and the sympathetic involvement eliminated by denervating the kidney and removing the adrenal glands. In these animals, a decrease in renal perfusion pressure resulted in an increase in renin secretion suggesting that there is an intrarenal baroreceptor capable of influencing renin secretion in the absence of other known regulating factors. This same effect was observed in an isolated perfused kidney preparation and increases in renal perfusion pressure were also shown to inhibit release of renin (Hofbauer et al., 1974).

Studies to determine the location of the baroreceptor were carried out using papaverine, a compound known to dilate the afferent arterioles. In the presence of papverine, the afferent arterioles are presumably maximally dilated and if the baroreceptor is located here it would be unable to respond to changes in stretch. Papaverine pretreatment blocked the increase in renin secretion with hemorrhage in dogs with denervated and nonfiltering kidneys suggesting that the baroreceptor is indeed in the afferent arteriole (Witty et al., 1971).

The nature of the stimulus perceived by the baroreceptor appears to be a decrease in vessel wall tension. According to the Law of Laplace, vessel wall tension is equal to the product of the vessel diameter and transmural pressure. Therefore, manipulations which lower intraluminal pressure, like renal artery constriction, or increase interstitial pressure, like ureteral occlusion, will activate the baroreceptor and increase renin secretion. Likewise, decreases in vessel diameter should also increase renin release. Manipulations which affect both vessel diameter and transmural pressure could increase, decrease, or have no effect on renin. For example, constriction of the renal artery results in an increase in renin secretion even though vasodilation associated with autoregulation occurs (Skinner et al., 1963). On the other hand, if vasodilation occurs without a change in perfusion pressure, renin release should be inhibited. This effect has been reported in an isolated perfused kidney preparation (Fray, 1976).

The Macula Densa Mechanism

The macula densa is a specialized segment of the distal convoluted tubule. The cells in this region take on a cuboidal or columnar shape

and are located in close proximity to the renin-containing cells in the afferent arteriole of the same nephron. Sometimes only an incomplete basement membrane separates the macula densa cells from the juxtaglomerular cells (Hartroft and Newmark, 1961). Because of the close anatomical relationship between the macula densa and the afferent arteriole, it was postulated that the composition of tubular fluid might influence the release of renin (Goormaghtigh, 1945). Vander and Miller (1964) provided the first evidence that such a relationship does exist. They showed that an inverse relationship existed between sodium excretion and renal venous PRA. This led them to postulate that decreases in sodium load (tubular sodium concentration X flow) to the macula densa resulted in increases in renin secretion. However, other investigators using diuretics which act on the Loop of Henle (loop diuretics), such as furosemide or ethacrynic acid, which lead to increased sodium excretion, showed that renin release increased and suggested a direct relationship between sodium load to the macula densa and renin secretion (Meyer et al., 1968; Woke et al., 1970). The discrepancy between these results and Vander's original hypothesis could be resolved if the loop diuretics also acted on the macula densa to decrease sodium reabsorption in that portion of the distal tubule. Unfortunately, the macula densa is inaccessable to micropuncture and sodium load to this region cannot be measured. Nevertheless, indirect evidence has been reported which suggests that loop diuretics do indeed act on the macula densa (Wright and Schnermann, 1974). Humphreys et al. (1975) used an isolated perfused kidney to provide further evidence in favor of Vander's hypothesis. The isolated kidney was perfused at constant pressure with blood from a donor dog. Hemodilution, without

volume expansion, increased sodium excretion in the perfused kidney which was accompanied by a decrease in renin release. In the donor dog, sodium excretion decreased due to the fall in mean arterial pressure and PRA increased significantly.

Recently, some controversy has arisen concerning the nature of the signal perceived by the macula densa cells. Kotchen et al. (1978) has presented evidence which suggests that a change in chloride load to the macula densa is more important than a change in sodium load. There is also preliminary data which suggest that prostaglandins are involved in the macula densa response (Francisco et al., 1980).

Neural Control of Renin Secretion

Innervation of the Juxtaglomerular Cell. Light and electron microscopic studies have demostrated the presence of adrenergic nerve terminals in contact with renin-containing juxtaglomerular cells (Barajas, 1964). These terminals contained the dense core vesicles that are typical of adrenergic neurons (Wolfe et al., 1962). Fluorescence histochemistry has demonstrated convincingly the presence of norepinephrine in these terminals. Reserpine, a drug which depletes catecholamines from nerve endings, led to a decrease in dense core vesicles and histofluorescence in these terminals and was accompanied by a decrease in PRA (Silverman and Barajas, 1974). There is also some evidence that the kidney receives parasympathetic innervation. Acetyl cholinesterase activity has been demonstrated in nerves which innervate the kidney (McKenna and Agelakos, 1968), but this could be contained in adrenergic neurons rather than cholinergic neurons (Barajas and Wang, 1975).

Effect of Direct Stimulation of the Renal Nerves. Direct electrical stimulation of the renal nerves is known to increase renin secretion. The early studies used loop electrodes placed around the renal artery so as to make contact with the renal nerves (Loeffler et al., 1972). While renin release did increase, the stimulation also may have caused a decrease in renal blood flow and sodium chloride delivery to the macula densa. Subsequently, other investigators found stimulation parameters which resulted in an increase in renin secretion without changing renal blood flow (La Grange et al., 1973). Renin also increased with renal nerve stimulation in animals with non-filtering kidneys (Johnson et al., 1971). This suggests that the norepinephrine released from the renal nerve terminals can act on the juxtaglomerular cells to stimulate renin release. In support of this hypothesis, it has been shown that norepinephrine causes renin release from kidney slices in vitro, again suggesting a direct action on the juxtaglomerular cells (Lopez et al., 1978).

Effect of Indirect Stimulation of the Renal Nerves. There is good evidence that the renin response to hemorrhage, upright posture or tilt, and dietary sodium restriction is mediated, in part, by the renal nerves.

The increase in renin with mild non-hypotensive hemorrhage can be blocked by renal nerve anesthesia (Bunag et al., 1966). Larger hypotensive hemorrhage results in other changes involving the intrarenal baroreceptor and the increase in renin in this case probably does not require the renal nerves (Blaine et al., 1970).

Upright posture in humans led to an increase in PRA and catecholamine excretion (Gordon et al., 1967). Patients with autonomic

insufficiency failed to show an increase in PRA or catecholamine excretion upon assumption of the upright posture. Direct evidence implicating the renal nerves in the renin response to upright tilt was obtained in anesthetized cats (Zanchetti and Stella, 1975). A sixty degree upright tilt led to an increase in renin secretion from innervated kidneys and only a partial increase in denervated kidneys. Additional bilateral adrenalectomy completely eliminated the response in the denervated kidneys.

The role of the renal nerves in the renin response to a low salt diet is not clear. Mogil et al. (1969) reported that surgical renal denervation completely blocked the increase in PRA during sodium deprivation. On the other hand, Gotshall et al. (1973) showed that PRA increased similarly with low salt diet in dogs with intact or denervated kidneys although the first renin measurement was not made until five days after the beginning of the diet. Also dogs with autotransplanted kidneys increase PRA with sodium restriction (Brennan et al., 1974). Brubacher and Vander (1968) reported that renal denervation slowed the renin response to a low salt diet, but by four days the response was normal. Propranolol, a beta-adrenergic receptor blocker, has been reported to decrease (Ganong, 1972a) or have no effect (Brubacher and Vander, 1968) on the elevated PRA due to a low salt diet. Since renal denervation can delay for four days the rise in PRA resulting from a low salt diet, it appears that the renal nerves may be involved in the initial renin response to sodium restriction but other release mechanisms are responsible for the chronic elevation of PRA.

Central Stimulation of Renin Release. Direct electrical stimulation of the midbrain central grey (Ueda et al., 1967), the dorsolateral pons (Richardson et al., 1974), the pressor region of the

medulla oblongata (Passo et al., 1971), and the lateral hypothalamus (Zanchetti and Stella, 1975) all resulted in an increase in PRA which could be blocked or partially blocked by renal denervation or beta-adrenergic blockade. Zehr and Feigl (1973) reported that stimulation of the pressor region of the anterior hypothalamus resulted in a decrease in PRA which was abolished by renal denervation.

Certain pharmacologic manipulations also point to a central involvement in the control of renin release. Clonidine, an alpha-2 adrenoreceptor agonist, has been shown to inhibit renin secretion by an action within the central nervous system (Reid et al., 1975). It is not yet clear exactly where or how clonidine acts to produce this effect. L-dopa, which can cross the blood-brain barrier and be converted to norepinephrine in adrenergic neurons, also inhibited renin release when peripheral decarboxylase inhibitor was given to insure only central conversion (Blair et al., 1977). Intraventricular infusions of norepinephrine or epinephrine or more potent alpha-1 adrenergic agonists led to an increase in PRA (Ganong and Barbieri, 1982).

While futher investigation is needed, these pharmacologic studies suggest the presence of a pathway in the central nervous system which inhibits renin secretion by an alpha-2 adrenergic mechanism. There is also some preliminary evidence which points to another pathway which stimulates renin release by an alpha-1 mechanism.

Recently, serotonin has been implicated as a central neurotransmitter involved in renin release. L-tryptophan, an amino acid which is taken up by serotonergic neurons and converted to serotonin, resulted in an increase in PRA when injected into anesthetized dogs (Zimmermann and Ganong, 1980). This response was blocked by renal

denervation. In rats, serotonergic agonists and serotonin releasing drugs increased PRA (Van de Kar et al., 1981). Injections of 5,7-dihydroxytryptamine, a drug toxic to serotonergic neurons, into the dorsal raphe nucleus of the midbrain lowered resting levels of PRA suggesting a central role for serotonin in maintaining normal levels of renin (Van de Kar et al., 1982). At present the pathways mediating these serotonergic effects are not entirelly known, but it appears that projections from the dorsal raphe nucleus to the mediobasal hypothalamus are involved (Karteszi et al., 1982).

Effect of Circulating Catecholamines. Intravenous infusions of norepinephrine or epinephrine in dogs resulted in an increase in PRA (Vander, 1965). The increased epinephrine associated with insulin-induced hypoglycemia also resulted in an increase in renin secretion (Otsuka et al., 1970). The response to hypoglycemia could be reduced by denervation of the adrenal gland but not the kidney. Therefore, stimuli which increase release of epinephrine from the adrenal medulla may also affect renin release.

There is some controversy regarding the location of the receptor which mediates the renin response to circulating catecholamines. Reid et al. (1972a) reported that intrarenal infusions of low doses of isoproterenol were ineffective in stimulating renin secretion while the same doses given intravenously did increase renin release. These authors suggested that extrarenal receptors were responsible for the response to isoproterenol. Similar results have been obtained with the renin response to intrarenal versus intravenous infusions of epinephrine and norepinephrine (Johnson et al., 1979). In an attempt to locate these extrarenal receptors, epinephrine was infused directly into

several vascular beds supplying the gut region but no renin response was obtained (Johnson, 1982).

Intrarenal Beta-adrenergic Receptor. While circulating catecholamines may act on extrarenal beta-adrenergic receptors which influence renin secretion, it seems likely that norepinephrine released from the renal nerves acts directly on a beta-adrenergic receptor within the kidney. Isoproterenol stimulated renin secretion from kidney slices and isolated glomerular preparations (Morris et al., 1979; Beierwaltes et al., 1980). When fluorescently labeled propranolol was injected in vivo, the fluorescence localized in the afferent arteriole of the kidney near the juxtaglomerular cells (Atlas et al., 1977). Also the renin response to direct stimulation of the renal nerves could be abolished by pretreatment with propranolol (Loeffler et al., 1972). It seems probable that the beta-receptor is located on the cell membrane of the juxtaglomerular cell and norepinephrine released from the nerve terminals innervating this structure stimulates renin secretion.

There is some uncertainty about which subtype of beta-receptor is responsible for the renin-stimulating effect. Some investigators have reported that beta-1 agonists stimulated and beta-1 antagonists inhibited renin release (Campbell et al., 1979; Himori et al., 1980). However, others have reported that beta-1 antagonists did not affect PRA so further investigation is needed (Weber et al., 1974).

Alpha-Adrenergic Receptors. There is also evidence which suggests that alpha-adrenergic receptors can influence renin release. Stimulation of alpha-adrenergic receptors leads to vasoconstriction, a response that should increase renin secretion via the baroreceptor or macula densa. In addition, there is evidence that direct stimulation of

alpha-receptors inhibits renin secretion. Phenoxybenzamine, an alpha-receptor blocker, was shown to potentiate the stimulatory effect of hypoglycemia on renin release (Assaykeen et al, 1970). Phenoxybenzamine and another alpha-receptor antogonist, phentolamine, potentiated the stimulatory response to norepinerphrine in vitro (Nolly et al., 1974). While low doses of norepinerphrine increased renin release from kidney slices, higher doses have been reported to inhibit release (Lopez et al., 1978). Further investigation is needed to determine the physiologic importance of the alpha-receptor mediated increase in renin release. Because of the high concentration of norepinephrine required to see an inhibitory effect of alpha-receptor stimulation it seems unlikely that such a mechanism is physiologically significant.

Parasympathetic Regulation of Renin Secretion. As mentioned previously, there is some anatomical evidence for parasympathetic innervation of the kidney. Additional evidence favoring a parasympathetic innervation was provided by the observation that cholinergic transmission was required for the autoregulatory vasodilation in the kidney following renal arterial constriction (Stinson et al., 1968). However, anticholinesterases and cholinergic antagonists had no effect on basal renal function (Vander, 1964). Intrarenal infusions of acetylcholine have no effect on renin secretion (Abe et al., 1973). However, the infusions also caused renal vasodilation and increased sodium excretion, two factors which could inhibit renin release via the intrarenal baroreceptor or macula densa mechanisms. Therefore, suggestions that cholinergic transmission is unimportant in controlling renin secretion may be premature and further investigation seems to be in order.

Bilateral cervical vagal cooling or sectioning has been reported to increase resting levels of renin (Yun et al, 1976; Stella et al., 1978). Since this effect could be blocked by propranolol, it probably was due to an increase in sympathetic nervous activity rather than a direct inhibitory effect of vagal efferents. An increase in cardiopulmonary afferent vagal transmission led to decreases in renin secretion (Brennan et al., 1971). This too was probably mediated by a decrease in sympathetic output to the kidney. The role of parasympathetic efferent traffic in renin secretion needs to be further investigated by determining the effects of vagotomy and direct electrical stimulation of the distal portion of the sectioned vagus nerve somewhere below the level of the heart.

Humoral Factors Regulating Renin Secretion

Sodium. It has been recognized for almost twenty years that renin secretion is influenced by plasma sodium concentration. Brown et al. (1963) reported that in a group of patients suffering from hypertension, plasma sodium concentration was inversely related to PRA. In anesthetized dogs with isolated blood-perfused kidneys, reductions in plasma sodium concentration resulted in a significant increase in renin release (Yamamoto et al., 1969). Acute intrarenal infusions of hypertonic saline have been shown to block the renin response to suprarenal aortic constriction and thoracic caval constriction (Nash et al., 1968; Shade et al., 1972). The response to changes in sodium concentration *in vitro* is confusing. Some investigators reported the same inverse relationship between sodium concentration and renin release

(Michelakis, 1971), while others reported a direct relationship (Lyons and Churchill, 1975) or no relationship at all (Aoi et al., 1974). Therefore, while a direct action of sodium on the juxtaglomerular cells is not ruled out, it seems unlikely. The elevated PRA during thoracic caval constriction was not decreased by hypertonic saline infusion into non-filtering kidneys (Shade et al., 1972). This suggests that sodium has its effect on renin secretion by way of the macula densa.

Potassium. Chronic potassium loading has been shown to decrease resting levels of renin (Sealey et al., 1970). Likewise, dietary potassium restriction was associated with an increase in PRA (Abbrecht and Vander, 1970). It is also known that infusions of potassium into the renal artery acutely inhibit renin secretion (Vander, 1970) and an acute decrease in plasma potassium brought about by infusion of glucose resulted in a stimulation of renin secretion (Himathongkam et al., 1975). Since an increase in plasma potassium leads to an increase in sodium excretion, it has been postulated that the decrease in renin is mediated by the macula densa (Vander, 1970). This is supported by the report that hyperkalemia did not suppress PRA in thoracic caval constricted dogs with non-filtering kidneys (Shade et al., 1972). However, in vitro high levels of potassium inhibited renin release (Park et al., 1981) It was suggested that high levels of this electrolyte depolarized the juxtaglomerular cells resulting in an inhibition of renin release. Therefore, a direct action of potassium on the juxtaglomerular cells cannot be ruled out.

Angiotensin II. Vander and Geelhoed (1965) first postulated a negative feedback role for AII in the regulation of renin secretion. They showed that infusions of AII inhibited resting levels of renin even

when renal perfusion pressure was held constant. Since the infusion resulted in a decrease in sodium excretion, a stimulus that would be expected to increase renin secretion, the authors concluded that the macula densa was not responsible and postulated a direct inhibitory action of AII on the juxtaglomerular cells. AII infusions have been shown to inhibit the renin response to aortic constriction (Bunag et al, 1967), isoproterenol (Meyer et al., 1975) and sodium depletion (Blair-West et al., 1971). This occurred with plasma levels of AII within the physiologic range. Additional information regarding the negative feedback role of AII was obtained using inhibitors of the renin-angiotensin system. Both a converting enzyme inhibitor, which prevents conversion of AI to AII, and saralasin, an AII receptor antagonist, in animals fed a low salt diet caused an increase in renin secretion (Bing, 1973; Johnson and Davis, 1973).

The site of action of AII is probably the juxtaglomerular cell. As mentioned previously, the inhibition of renin secretion with AII can occur without changes in renal perfusion pressure. A functional macula densa is also no required since the inhibtion occurred in dogs with non-filtering kidneys (Shade et al., 1973). Additional evidence was provided using <u>in vitro</u> techniques. AII inhibited renin release from renal cortical slices, an effect which was blocked completely by saralasin (Naftilan and Oparil, 1978). Depletion of norepinephrine in the renal nerves with reserpine prior to obtaining the cortical slices did not prevent the inhibition of renin with AII. Small carboxy-terminal fragments of AII also inhibited renin release but with much lower potency.

It appears therefore, that AII can act directly on the

juxtaglomerular cells to inhibit renin secretion. Since blockade of the action of AII leads to an increase in renin levels, it is probable that renin secretion is regulated by a negative feedback action of this peptide.

Vasopressin. Vasopressin is another peptide which has an inhibitory effect on renin secretion. Infusions of vasopressin which resulted in plasma levels of the peptide within the physiologic range inhibited the renin response to renal arterial hypotension (Bunag et al., 1967), ureteral occlusion (Vander, 1968), and sodium depletion (Tagawa et al., 1971). Since this peptide inhibited the renin response to sodium depletion in dogs with non-filtering kidneys without affecting blood pressure it appears that vasopressin exerts its effect directly on the juxtaglomerular cells (Shade et al., 1973). This is supported by the recent reports that vasopressin inhibited release of renin from kidney slices in vitro (Brooks et al., 1980; Park et al., 1981).

It is interesting that Brattleboro rats, which are unable to synthesize vasopressin and thus are chronically volume depleted, have elevated levels of renin (Gutman and Benzakein, 1974). Infusion of vasopressin into these rats resulted in a decrease in PRA to a level comparable to control rats. It is not known if vasopressin decreased PRA by a direct action on the juxtaglomerular cells or by ameliorating the volume depletion.

It has been suggested that vasopressin mediated the decrease in renin secretion produced by intracerebroventricular infusions of AII (Malayan et al., 1979). Hypophysectomy prevented the increase in plasma levels of vasopressin during central infusion of AII as well as the decrease in PRA. The decrease in renin secretion with central AII is

also absent in Brattleboro rats (Ganong et al., 1981).

Since the effect of vasopressin on renin release can be seen with plasma levels of the peptide within the physiologic range it seems likely that it is an important factor involved in the regulation of renin secretion. Since the effect can be seen without changes in renal hemodynamics, in the absence of a functional macula densa, and *in vitro* it is probable that vasopressin acts directly on the juxtaglomerular cells.

ACTH. There is some evidence which suggests that ACTH increases renin release. Kidney slices taken from rats treated with ACTH released more renin than slices taken from control animals (Bozovic et al., 1969). Also injection of ACTH has been reported to increase PRA in rats (Hauger-Klevene et al., 1969). However, these reports have not been confirmed by other investigators in rats or dogs (Palkovits et al., 1970; Ganong and Reid, 1976). In fact, ACTH treatment for six days in dogs on a low salt diet resulted in a significant decrease in PRA (Ganong, 1972b). Since ACTH treatment leads to volume expansion and elevated blood pressure, two factors known to inhibit renin secretion, an excitatory effect of ACTH could easily be masked. Another factor to consider is the effect of ACTH on plasma levels of angiotensinogen. Since ACTH increases glucocorticoids which are known to stimulate synthesis of angiotensinogen (Nasjletti and Masson, 1969), an elevation in PRA with ACTH could be brought about without an increase in renin secretion.

Glucagon and Parathyroid Hormone. Glucagon and parathyroid hormone, both hormones known to increase cyclic AMP, increased renin release *in vivo* and in isolated perfused kidneys (Vandongen et al.,

1973; Powell et al., 1978). In the case of glucagon, only amounts 100 times greater than an effective dose of isoproterenol stimulated renin and in the case of parathyroid hormone, infusions of the hormone 100-200 times its normal secretion rate were required to increase PRA. It is therefore unlikely that either of these hormones play a significant role in the regulation of renin secretion.

Substance P. Infusion of substance P into dogs with denervated kidneys resulted in a decrease in renin secretion accompanied by an increase in renal blood flow (Gullner et al., 1979). Substance P immunoreactivity has been reported to be present in nerve fibers innervating the kidney (Hokfelt et al., 1978). This provides the possibility that the peptide is released from renal nerve terminals and influences renin release via an action on the juxtaglomerular cells. However, further investigation is required to determine the physiologic importance and mechanism of action of substance P in the regulation of renin release.

Somatostatin. Somatostatin has been reported to inhibit the renin response to furosemide and pentobarbital anesthesia in dogs (Izumi et al., 1979) and sodium depletion in humans (Gomez-Pan et al., 1976). The mechanism of action of somatostatin and its physiologic significance in regulating renin secretion is unknown.

Stimulus-Secretion Coupling of Renin Secretion

Cyclic AMP. Since cyclic AMP is known to mediate the effect of beta-adrenergic stimulation in many instances, it is not suprising that

investigations have been carried out to determine the effect of this cyclic nucleotide on renin secretion. The initial reports were conflicting. Tagawa and Vander (1970) reported an inhibitory effect of cyclic AMP infusions in sodium depleted dogs. On the other hand, Winer et al. (1971) showed that intrarenal infusion of cyclic AMP resulted in a significant elevation in renin secretion. They suggested that the reason that Tagawa and Vander failed to observe an increase in renin was because PRA was already elevated in their dogs due to the low sodium diet and that cyclic AMP could not increase renin any further. Allison et al. (1972) showed that intrarenal infusions of dibutryl cyclic AMP, a more soluble form of the nucleotide, resulted in an increase in renin release without any significant changes in renal hemodynamics. Theophylline, a drug which inhibits the enzyme which breaks down cyclic AMP, led to an increase in resting renin levels (Reid et al., 1972b). The effect of dibutryl cyclic AMP on renin has now been confirmed in dogs (Okahara et al., 1977) and rats (Hauger-Klevene, 1970). Cyclic AMP has also been shown to stimulate renin release from cortical cell suspensions and kidney slices (Michelakis et al., 1969; Rosset and Veyrat, 1971). Theophylline also potentiated the stimulatory effect of norepinephrine in vitro (Nolly et al., 1974). Since epinephrine infusions increase cyclic AMP levels in the kidney (Beck et al., 1972) it seems likely that the effect of beta-adrenergic stimulation on renin secretion is mediated by cyclic AMP. Glucagon and parathyroid hormone, two hormones known to stimulate renin secretion, also increased cyclic AMP in the kidney (Morel, 1981). AII, a renin-inhibitory factor, decreased cyclic AMP levels in glomeruli and tubules (Lopez et al., 1978; Torres et al., 1978).

Taken together the available evidence suggests that cyclic AMP or dibutryl cyclic AMP can stimulate renin secretion by a direct action on the juxtaglomerular cells. Increased intracellular levels of this cyclic nucleotide probably mediate the effect of beta-adrenergic stimulation and hormones such as glucagon and parathyroid hormone. AII probably inhibits renin by decreasing intracellular levels of cyclic AMP.

Prostaglandins. Prostaglandins stimulated renin secretion both in vivo and in vitro (Whorton et al., 1977; Gerber et al., 1979). There is some controversy concerning which prostaglandins mediated the effect, but it appeared that PGI and PGE_2 were the most important (Bolger et al., 1976). A physiologic role for prostaglandins in regulating renin secretion was suggested by the report that blockade of prostaglandin synthesis by indomethacin resulted in a decrease in resting levels of renin (Frolich et al., 1976).

There is also some evidence that prostaglandins mediate the renin response to beta-adrenergic stimulation, intrarenal baroreceptor stimulation and macula densa activation. Campbell et al. (1979) reported that indomethacin pretreatment blocked the renin response to isoproterenol and H133/22, a specific b-1 agonist, in conscious rats. However, others have failed to confirm this in dogs and humans (Berl et al., 1979; Frolich et al., 1979). Furthermore, Beierwaltes et al. (1980) showed that indomethacin pretreatment did not block the effect of isoproterenol on renin release from an isolated glomerular preparation. Therefore, it seems unlikely that prostaglandins are required for the renin response to beta-adrenergic stimulation.

The role of prostaglandins in mediating the response to intrarenal

baroreceptor activation is more clear cut. A decrease in renal perfusion pressure is known to result in an increase in prostaglandin production in the kidney (Herbaczynska-Cedro and Vane, 1973). Indomethacin pretreatment blocked the renin response to a decreased renal perfusion pressure (Data et al., 1978). Blackshear et al. (1979) reported that decreases in renal perfusion pressure within the autoregulatory range required prostaglandin synthesis while decreases in pressure below the autoregulatory range did not.

Macula densa mediated increases in renin secretion also appear to require prostaglandin synthesis. In renally denervated dogs treated with propranolol and papaverine, a decrease in renal perfusion pressure resulted in an increase in renin secretion which could be blocked by indomethacin (Olson et al., 1980). Further evidence that the macula densa mediated this effect was provided by the finding that the decrease in perfusion pressure did not increase renin if the kidneys were made non-filtering. Also Francisco et al. (1980) measured the sodium load reaching the distal tubule of rats using micropuncture techniques. They showed that the increase in renin associated with a decrease in sodium delivery to the distal tubule due to a low salt diet was prevented by indomethacin.

Thus, prostaglandins have a direct stimulatory effect on renin secretion. They may play a role in maintaining normal levels of renin since indomethacin lowers resting levels. The response to macula densa activation is probably mediated by prostaglandins. The response to decreases in renal perfusion pressure within the autoregulatory range also appears to require prostaglandin synthesis. On the other hand, beta-adrenergic stimulation of renin secretion is probably not mediated via prostaglandins.

Calcium. It is generally believed that in most secretory systems an increase in calcium ion influx is responsibile for bringing about the events that lead to secretion. The effect of calcium on renin release appears to be just the opposite, ie, increases in intracellular calcium inhibit renin secretion and decreases stimulate secretion. The best evidence for this hypothesis is provided by in vitro work with kidney slices or isolated glomeruli. A decrease in the concentration of calcium in the bathing medium led to increased renin release (Baumbach and Leyssac, 1977) and an increase in calcium inhibited secretion (Park et al., 1981). The calcium ionophore, A23187, which facilitates passive diffusion of calcium, inhibited renin release if high levels of calcium were present in the bathing medium (Fynn et al. 1977). This is presumably because of an increased influx of calcium into the juxtaglomerular cells. On the other hand, if calcium was absent in the bathing medium, A23187 caused calcium efflux and renin release was increased.

In vivo studies of the effect of calcium on renin release have been more confusing. It has been reported that infusions of calcium chloride stimulated (Iwao et al., 1974) or inhibited (Kotchen et al., 1974) the release of renin. These infusions also affected sodium excretion and blood pressure and are therefore difficult to interpret.

Fray (1980a) has recently hypothesized that changes in intracellular calcium in the juxtaglomerular cells mediate the renin response to intrarenal baroreceptor activation, beta-adrenergic stimulation, and AII. In the isolated perfused rat kidney, the renin response to renal hypotension and renal vasoconstriction was blocked by depolarization of the juxtaglomerular cells with high levels of

potassium (Fray, 1978). Depolarization presumably opened the voltage dependent calcium channels and led to calcium influx. The response to low perfusion pressure was decreased as extracellular levels of calcium were decreased (Fray and Park, 1979). High perfusion pressure inhibited renin only if calcium was present; in the absence of calcium, the inhibition was changed to a stimulation. High perfusion pressure would increase the stretch of the juxtaglomerular cells and presumably increase the calcium permeability which would increase intracellular calcium and inhibit renin secretion.

The stimulatory effect of catecholamines was blocked by lanthanum, a compound which prevents calcium efflux (Logan et al. 1977). Ouabain, which increases intracellular calcium, inhibited the renin response to catecholamines (Fray, 1980b). These data are all consistent with the hypothesis that catecholamines stimulate renin secretion by lowering intracellular calcium.

AII inhibited renin secretion from an isolated perfused kidney only when calcium was present in the extracellular fluid. The inhibitory effect was greatest when the extracellular calcium was highest (Vandongen and Peart, 1974).

In summary, the effect of calcium on renin secretion is opposite to that in most secretory systems. The release of renin appears to be inversly related to the intracellular concentration of calcium. Changes in calcium may be the final common pathway for some stimuli to renin secretion. For example, there is evidence that the renin responses mediated by the intrarenal baroreceptor, the beta-adrenergic receptor, and AII involve changes in intracellular calcium.

Thus, cyclic AMP, prostaglandins, and calcium all appear to mediate

certain renin responses. The effect of cyclic AMP is stimulatory while the effect of calcium is inhibitory. Prostaglandins may act by influencing these other two factors or it may act independently.

Vasoactive Intestinal Peptide

Isolation and Structure

Vasoactive intestinal peptide (VIP) was first isolated by Said and Mutt (1970) in 1970. The peptide was purified from a side fraction obtained during purification of secretin from porcine duodenum. Isolation was based on the ability of VIP to lower blood pressure in anesthetized dogs.

Amino acid analysis revealed that the peptide was made up of 28 amino acids (Said and Mutt, 1972) and the sequence was determined to be His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH2 (Mutt and Said, 1974). VIP has several amino acid sequences in common with glucagon and secretin. It is now known that VIP isolated from bovine and human intestines has the same amino acid sequence as porcine VIP; chicken VIP differs from the mammalian sequence at only four points (Mutt, 1982).

Based on elution profiles with ion exchange chromatography several variant forms of VIP have been identified (Dimaline and Dockray, 1978). In some cases, as much as 50% of the total VIP immunoreactivity of certain tissue extracts was made up of variant forms (Dimaline and Dockray, 1979). Since the biologic significance of these variants is unknown, caution should be used in interpreting studies which measure tissue VIP levels by radioimmunoassay using antisera which have not been

proven to be specific for authentic VIP.

Localization

Central Neurons. Although VIP was originally thought to be a gastrointestinal hormone, in 1976 several groups of investigators demonstrated that VIP immunoreactivity was present in neurons (Larsson et al., 1976b; Said and Rosenberg, 1976). Since then detailed immunocytochemical studies have revealed a widespread distribution of VIP-containing neurons throughout the brain (Hökfelt et al., 1982). Immunoreactivity was especially dense in cortical, limbic, and hypothalamic regions and a VIPergic pathway connecting the amygdala to the hypothalamus via the stria terminalis has been described (Roberts et al., 1980). VIP-containing fibers also appear to innervate cerebral blood vessels (Larsson et al, 1976a). The origin of these fibers is unclear since removal of the ptergopalatine or superior cervical ganglia did not affect the VIP immunoreactivity in brain vessels (Edvinsson, 1982). It may be that the fibers originate intracerebrally or in local ganglia in the blood vessels.

Peripheral Neurons. VIP-containing neurons have been identified by immunocytochemical techniques in several peripheral organs. In the gastrointestinal tract, VIP immunoreactive neuronal cell bodies are present in the submucous plexus (Schultzberg et al., 1980). Cell bodies are also present in the myenteric plexus, but to a lesser degree. VIP nerve fibers are present in all layers of all parts of the gastrointestinal tract, especially along blood vessels and in smooth muscle. VIP-containing fibers are also present in many exocrine glands

including salivary glands, sweat glands, the pancreas, lacrimal glands, and glands in the trachea and gastrointestinal tract (Hakanson et al., 1982). Reports mentioning the VIP innervation of the kidney are conflicting. One study reported no VIP nerve fibers in the kidney of three species (Alm et al., 1980), while Hökfelt et al. (1978) reported VIP-containing fibers to be present in the renal cortex following blood vessels in the guinea pig. Other regions of the urogenital tract including ureter, bladder, uterus, cervix, prostate, and seminal vesicle all contain VIP immunoreactive fibers (Alm et al., 1980). VIPergic fibers are also present in the upper respiratory tract, especially in nasal mucosa and tracheal smooth muscle (Hakanson et al., 1982). Endocrine glands such as the thyroid, adrenal cortex, and pancreas also contain nerve fibers which stain for VIP (Bishop et al., 1980; Hakanson et al., 1982). Finally, VIP immunoreactivty has been demonstrated on the proximal side of ligatures tied around large nerves such as the vagus and sciatic nerves (Lundberg et al., 1978).

Autonomic Ganglia. Autonomic ganglia appear to contain preganglionic nerve fibers and postganglionic cell bodies which stain positively for VIP. In the coeliac, inferior mesenteric, and superior mesenteric ganglia, preganglionic nerve fibers containing VIP immunoreactivity surround the principal ganglion cell bodies but are absent near VIP-containing cell bodies (Hökfelt et al., 1977). Since ligation of the mesenteric nerve resulted in an accumulation of VIP on the intestinal side of the tie, it appears that some of the prevertrebral ganglionic innervation is afferent and comes from the gut (Lundberg et al., 1979). The VIP innervation of the salivary glands, sweat glands, and nasal mucosa appears to be postganglionic since the

ganglia which innervate these organs have VIP-containing cell bodies and removal of the ganglia abolishes VIP staining in the fibers which innervate the organs (Lundberg et al., 1979; Uddman et al., 1980). In the case of the gut, pancreas, and urogenital tract, VIP-containing fibers also arise from small local ganglia (Hakanson et al., 1982).

Co-localization with Acetylcholine. VIP immunoreactivity has been shown to occur in neurons that also stain for acetylcholinesterase suggesting that VIP and acethylcholine coexist in the same neuron (Lundberg et al., 1979). However, caution should be used in interpreting these data. Cholinergic neurons were labeled using antibodies to acetylcholinesterase which makes identification difficult since there are so many nonspecific cholinesterases which could interact with the primary antibody. The best evidence for coexistence was obtained with the sphenopalatine ganglion. It is known that this ganglion contains cholinergic neurons which innervate the nasal mucosa, and 98-99% of the cell bodies in this ganglion also contained VIP immunoreactivity (Lundberg et al., 1980a). Electron microscopic immunocyctochemistry revealed that VIP is present in the large dense core vesicles which are known to be sparsely interspersed among the dominant agranular small diameter vesicles of cholinergic neurons (Johansson and Lundberg, 1981). Also, subcellular fractionation showed that most of the VIP was present in the heavy fraction which contained mostly dense core vesicles. Acetylcholine was present in lighter fractions, but a small amount was also present in the heavy fraction (Lundberg et al., 1981).

A possible role for the coexistence of VIP and acetylcholine in the same neuron was demonstrated in the submandibular salivary gland.

Stimulation of the chorda nerve caused an increase in salivary blood flow and secretion accompanied by an increase in VIP in the plasma leaving the salivary gland. Exogenously administered VIP or acetylcholine mimicked the effect of nerve stimulation on salivary blood flow. Doses of VIP and acetylcholine which were ineffective when given separately caused the increase in blood flow when given together suggesting an interaction between the two substances (Lundberg et al., 1980b). It has recently been shown that VIP increases muscarinic receptor binding in salivary glands (Lundberg et al., 1982b). Therefore, it is possible that both VIP and acetylcholine are released from the postganglionic nerves which innervate the salivary gland and VIP potentiates the effect of acetylcholine by enhancing its binding to the postsynaptic receptor.

The coexistence of VIP and acetylcholine does not appear to be a general phenomenon. So far, only neurons which innervate exocrine glands show the co-localization. For example, the cholinergic neurons in the ciliary ganglion and ganglia in the urinary bladder as well as motor fibers to skeletal muscle do not contain VIP. There are also many VIPergic systems which do not contain acetylcholine (Lundberg et al., 1982a).

VIP in Endocrine Cells. VIP was originally thought to be a gastrointestinal hormone and evidence was presented which localized VIP in endocrine-like cells of the gastrointestinal tract (Polak et al., 1974). However, certain antibodies only detected VIP in neurons and not in endocrine cells (Larsson et al., 1976b) and isolated intestinal epithelial cells did not contain any assayable VIP (Besson et al., 1978). It has been suggested that authentic VIP is present only in

neurons and that the staining in endocrine cells is due to variant forms of the peptide (Dimaline et al., 1980). There is also evidence which suggests that the VIP staining in endocrine cells is nonspecific. Only antibodies which recognized N-terminal portions of VIP stained endocrine cells. Some of these antisera contained subpopulations of antibodies which cross reacted with glucagon, secretin, and GIP, all hormones present in endocrine cells of the gut and pancreas (Larsson et al., 1979; Larsson, 1982). This matter remains unsettled, but it is probable that VIP is primarily located in neurons and that its main function is neurotransmission.

Circulating VIP

VIP is present in the circulation of all species studied. Depending on the species and radioimmunoassay used, resting levels range from 2-40 pmoles/L (see Table 1). The origin of the circulating peptide is unknown. Since VIP is probably located only in nerve cells, the circulating peptide probably represents spill over from nerve terminals. Stimulation of nerves which innervate certain organs such as the salivary glands and pancreas produce an increase in blood levels of VIP in the venous effluent (Fahrenkrug et al., 1979; Lundberg et al., 1980b). Circulating levels of VIP are also very high in a pathological condition called the watery diarrhea syndrome, in this case the VIP is of tumor origin (Said and Faloona, 1975). There are also some experimental manipulations which significantly increased systemic levels of VIP. These are summarized in Table 1. It is not known if these changes in circulating levels of VIP have any significant physiologic effect.

Table 1. Peripheral plasma concentration of VIP before and after various stimuli.

Stimulus	VIP (pmole/L) Before	After	Reference
Human			
HCl in duodenum	1.7	6.6	Bloom et al., 1978
HCl in duodenum	4.3	9.8	Schaffalitzky et al., 1977
Fat in duodenum	4.3	7.5	Schaffalitzky et al., 1977
Ethanol in duodenum	4.3	12.6	Schaffalitzky et al., 1977
Neostigmine (i.v.)	12.4	36.0	Ebeid et al., 1979
Test meal	0.7	2.3	Burhol et al., 1979
Oral water load	—*	6 pmoles/L increase	Christofides et al., 1979
Dog			
Neostigmine (i.v.)	37-40	134	Ebeid et al., 1979
Calcium (i.v.)	33-43	53-71	Ebeid et al., 1976
Oxytocin (i.v.)	15	37	Bitar et al., 1978
Endotoxin (i.v.)	15	76	Freund et al., 1981
Pig			
Stimulate vagus	23	36	Fahrenkrug et al., 1978
HCl in duodenum	9	19	Schaffalitzky et al., 1977
Intestinal ichemia	8	14	Modlin et al., 1978a
Cow			
Stimulate vagus	17	39	Bloom and Edwards, 1980

Values reported in pg/ml have been converted to pmoles/L for purposes of comparison.
*Not reported.

Biologic Actions of VIP

Effect on Smooth Muscle. VIP relaxes smooth muscle and has potent vasodilatory actions. It also causes relaxation of airway smooth muscle and smooth muscle in gastrointestinal sphincters.

Exogenously administered VIP causes vasodilation in most vascular beds, including the splanchnic, femoral, and extracranial circulation (Said, 1982). It may play a role in regulating cerebral blood flow. Strips of cerebral arteries have been shown to relax after VIP administration in vitro (Larsson et al., 1976a). Likewise, VIP applied to pial vessels in vivo caused a significant increase in vessel caliber (Wei et al., 1980). Intracarotid infusions of VIP increased cerebral blood flow in rabbits (Heistad et al., 1980). In baboons, if the blood-brain barrier was first opened with hypertonic urea, VIP again increased cerebral blood flow (McCulloch and Edvinsson, 1980). Intracerebroventricular infusion of VIP in anesthetized dogs also increased cerebral blood flow (Wilson et al., 1981). Because of the extensive innervation of cerebral vessels by VIPergic neurons it seems probable that the peptide plays a role in regulating cerebral blood flow.

Intravenous infusions of VIP also increased coronary blood flow (Smitherman et al., 1982). This resulted from an increase in coronary vessel diameter. It is interesting that cardiac output also increased, but to a lesser degree, suggesting a preferred action of VIP on coronary vessels.

VIP also relaxes tracheal smooth muscle. Strips of guinea pig trachea were relaxed in vitro by porcine and hen VIP (Wasserman et al., 1982). Electrical field stimulation of tracheal strips caused

relaxation accompanied by release of VIP into the bathing medium (Matsuzaki et al., 1980). Pretreatment with VIP antiserum blocked the relaxation. VIP administered as an aerosol to anesthetized dogs blocked the bronchoconstriction caused by histamine and prostaglandins (Said et al., 1982).

A rich supply of VIP-containing nerve fibers has been reported in the lower esophageal sphincter and other gastrointestinal sphincters (Alumets et al., 1979). VIP relaxed the lower esophageal sphincter in the opossum (Rattan et al., 1977). It has recently been shown that relaxation of the lower esophageal sphincter caused by stimulation of the vagus nerve could be blocked by pretreatment with VIP antisera (Goyal et al., 1980). Therefore, it is possible that VIP is a physiologic factor involved in relaxation of smooth muscle in gastrointestinal sphincters.

In summary, VIP causes relaxation of smooth muscle in blood vessels, airway tubes, and gastrointestinal sphincters. Because field stimulation of tracheal strips or electrical stimulation of the vagus nerve resulted in a release of VIP, and the relaxation of smooth muscle caused by this stimulation was blocked by VIP antiserum, it appears that VIP is physiologically important in mediating these effects.

Effect on Ion Transport. In general, VIP appears to inhibit sodium chloride influx into cells. In the gut, VIP has been shown to inhibit water and sodium absorption and stimulate chloride secretion (Krejs, 1982). VIP increased secretion of chloride by strips of colon in vitro, probably by inhibiting sodium chloride influx from the lumenal side of the cells (Racusen and Binder, 1977). High concentrations of VIP are required for an effect on electrolyte transport. It has been suggested

that the increased water and electrolyte secretion seen with the watery diarrhea syndrome is due to the high circulating levels of VIP which occur with this condition (Said and Faloona, 1975).

In the exocrine pancreas, VIP caused an increase in bicarbinate secretion both in vivo and in vitro (Konturek et al., 1976; Lindkaer Jensen et al., 1978). Stimulation of the vagus nerve caused an increase in bicarbinate secretion which was accompanied by an increase in VIP levels in venous blood leaving the pancreas (Fahrenkrug et al., 1979). The effect of VIP on enzyme secretion is not clear cut. In pancreatic fragments from guinea pigs and rats, VIP increased amylase secretion, but in fragments from mice, cats, and dogs it had no effect (Robberecht et al., 1977).

The rectal gland in sharks is responsible for maintaining sodium chloride homeostasis. VIP increased chloride secretion from this gland in vivo and in vitro (Stoff et al., 1982). Since high levels of VIP are present in circulating plasma of sharks, this peptide may be important in regulating chloride secretion in the rectal gland.

There is one report that VIP caused natriuresis in the isolated rat kidney (Rosa et al., 1977). This is consistent with the hypothesis that VIP inhibits sodium and chloride uptake in general.

Effect on Hormone Secretion. VIP may be involved in the regulation of prolactin secretion. The first studies reported that intraventricular or intravenous VIP in rats stimulated prolactin release, while the peptide had no effect in vitro on rat hemipituitaries or dispersed pituitary cells (Kayto et al., 1978; Vijayan et al., 1979).

However, subsequent studies demonstrated that VIP does stimulate prolactin secretion *in vitro*. VIP, in a dose range of 10^{-9}M to 10^{-7}M, stimulated prolactin release from hemipituitaries and dispersed mammotrophs (Ruberg et al., 1978; Rotsztejn et al., 1980a). The discrepancy in the *in vitro* data may be due to the long incubation times used in the initial studies which could have resulted in the breakdown of VIP. It has recently been reported that VIP stimulated prolactin release in rhesus monkeys *in vivo* (Frawley and Neill, 1981). It appears, therefore, that VIP may be an important regulator of prolactin secretion, probably by a direct action on the pituitary. It is interesting that the VIP levels in hypophyseal portal blood are 19 times higher than the levels in the general circulation (Said and Porter, 1979).

The effect of VIP on other hypophyseal hormones is unsettled. Intraventricular, but not intravenous VIP doubled growth hormone release in rats (Vijayan et al., 1979). VIP also inhibited the release of somatostatin from hypothalamic slices (Epelbaum et al., 1979). Thus, it is possible that VIP influences growth hormone release by an action in the hypothalamus. *In vitro*, VIP had no effect on baseline growth hormone release, but it did prevent the inhibition of growth hormone by somatostatin (Tapia-Arancibia et al., 1980) so an action on the pituitary must also be considered. Intraventricular VIP also stimulated LH release in rats (Vijayan et al., 1979), but it did not stimulate release of LHRH from hypothalamic slices (Besson et al., 1979) and the peptide did not stimulate LH release *in vitro* (Rotsztejn et al., 1980a). There are not enough data available to draw conclusions about the effect of VIP on LH secretion. The same is true for its effect on FSH and TSH.

So far, one report showed no effect of VIP on the release of these two hormones *in vitro* (Vijayan et al., 1979).

In one preliminary report, intravenous VIP resulted in a significant increase in plasma ACTH in anesthetized dogs (Porter and Ganong, 1982). VIP has also been shown to stimulate release of ACTH from human pituitary adenomas and AtT20 cells (Nicosia et al., 1982; Westendorf and Phillips, 1982). However, no such effect was found with dispersed rat corticotrophs (Rotsztejn et al., 1980a). Since tumor cells often show atypical responses to various agents it is hard to draw conclusions about the physiologic role of VIP in regulating the release of ACTH.

Nerve fibers which contain immunoreactive VIP have been shown to innervate the thyroid gland and exogenous VIP stimulated thyroid hormone secretion (Ahren et al., 1980). This suggests that VIP may be involved in the regulation of thyroid hormone secretion.

Glucocorticoid secretion by adrenal tumor cell lines was also stimulated by VIP *in vitro* (Kowal et al., 1977). VIP appeared to act through a receptor different than that for ACTH. Glucocorticoids, in turn, appear to affect VIP in certain situations. In rats, adrenalectomy resulted in an increase in VIP content in the adenohypophysis which was reversed by treatment with dexamethasone (Rotsztejn et al., 1980b). Dexamethasone also has been shown to cause a rapid inhibition of VIP-induced increases in pituitary cyclic AMP (Rotsztejn et al, 1981). The importance of these observations is not yet clear.

VIP infusions in dogs resulted in an increase in insulin output and an increase in glucagon in the venous plasma leaving the pancreas

(Kaneto et al., 1977). In the isolated perfused pancreas of pigs and cats, VIP also stimulated release of glucagon and insulin (Schebalin et al., 1977; Lindkaer Jensen et al., 1978). This response required the presence of glucose. There is also some evidence that somatostatin release is stimulated by VIP (Ipp et al., 1978). Physiologically, it is probably the VIP present in neurons innervating the pancreatic endocrine cells which is responsible for influencing release of these hormones.

In summary, VIP has been implicated in stimulating secretion of a variety of hormones. Most of these hormones are released from endocrine cells which are innervated by VIP-containing neurons. VIP is also present in hypophyseal portal blood and has effects on prolactin secretion. Other pituitary hormones are also effected by VIP but the physiologic importance of these effects is unknown.

VIP as a Neurotransmitter

A widely accepted set of criteria for establishing the identity of a neurotransmitter are as follows. 1) The substance should be present in presynaptic nerve terminals. 2) Synthesis of the substance should be demonstrated. 3) Stimulation of the presynaptic neuron should cause release of the substance. 4) Exogenous administration of the substance should mimic endogenous actions of the substance. 5) An inactivation mechanism should be present (Werman, 1966).

As mentioned previously, VIP immunoreactivity has been demonstrated in nerve terminals throughout the central and peripheral nervous systems. Subcellular fractionation revealed that VIP is present in synaptosomal fractions of several brain regions (Giachetti et al, 1977). Thus, the first criteron is probably met by VIP.

Synthesis of VIP within neurons has not be demonstrated conclusively. However, there is one preliminary report that intracerebroventricularly injected ^{35}S-methione was incorporated into a large precursor form of VIP (Fahrenkrug, 1980). Indirect evidence for synthesis and transport has been provided by two kinds of experiments. First, colchicine, a drug which prevents axonal transport of substances, resulted in an accumulation of VIP in neuronal cell bodies (Loren et al., 1979). Second, ligation of several peripheral nerves or sectioning of certain central neurons resulted in an accumulation of VIP on the proximal side of the ligature or cut (Lundberg et al., 1978; Roberts et al., 1980). Further research will be required to show conclusively that VIP meets this second criterion.

Release of VIP from neurons has been demonstrated in a number of ways. Direct electrical stimulation of the sciatic nerve in cats and rats resulted in an increase in VIP levels in perfusate of spinal cords perfused in situ (Go and Yaksh, 1980). Since dorsal rhizotomy reduced VIP immunoreactivity in the dorsal horn of the spinal cord, VIP is probably present in sensory afferents and it is stimulation of these neurons which caused release of VIP into the cord perfusate. High levels of potassium also caused release of VIP from rat hypothalamic slices and cortical synaptosomal fractions in a calcium dependent manner (Giachetti et al., 1977; Emson et al., 1978). Iontophoretic application of VIP to the cortex or hippocampus increased spontaneous neural activity in these regions (Phillis et al., 1978; Dodd et al., 1979). Release of VIP from peripheral neurons has been shown in a different way. Stimulation of the nerves which innervate organs such as salivary glands, the stomach, the intestine, and the pancreas resulted in an

increase in VIP levels in the blood leaving these organs (Fahrenkrug, 1982). This is presumably "spill over" of the peptide from the nerve terminals into the blood. Electrical field stimulation of tracheal strips also resulted in a release of VIP into the bathing medium (Matsuzaki et al, 1980). None of the studies mentioned above showed a direct release of VIP from presynaptic nerve terminals. However, the indirect evidence is probably strong enough to conclude that the third criterion for establishing a substance as a neurotransmitter has been met.

As mentioned in previous sections, exogenously administered VIP has many effects and in most cases these are similar to that reported during neural stimulation. Thus the fourth criterion has been met by VIP.

Neurotransmitters are inactivated either by specific uptake systems in pre- or postsynaptic neurons or by specific enzymes. Radioiodinated VIP was not taken up by rat brain slices (Fahrenkrug, 1980) so it appears that the peptide is not inactivated by this mechanism. On the other hand, enzymes which specifically degrade VIP have been localized in brain tissue and in liver and kidney extracts (Straus et al., 1982). It is not known if these enzymes have access to synaptic VIP. Thus the last criterion may be met by VIP, but further work is required to settle the point.

In summary, VIP meets several of the critera which must be met to establish a substance as a neurotransmitter. It is present in presynaptic nerve terminals and appears to be released upon stimulation. Synthesis of VIP within neurons has not been conclusively demonstrated, but axonal transport of VIP from nerve cell body to terminus occurs and this is consistent with synthesis in the cell body. Exogenously

administered VIP mimics the effects of neuronal stimulation in several organs. An enzyme which specifically degrades VIP has been demonstrated in extracts from several organs, but whether or not this enzyme affects synaptic VIP is unknown. While further work is required, it seems probable that VIP is indeed a neurotransmitter in the central and peripheral nervous systems.

Mechanism of Action

VIP has been shown to stimulate cyclic AMP production in every tissue investigated (Amiranoff and Rosselin, 1982). In most cases, the concentration range of VIP which stimulated cyclic AMP was similar to that which caused its biologic actions. It has therefore been postulated that cyclic AMP is the mediator of the effects of VIP.

Prostaglandins have also been implicated in mediating certain effects of VIP. The vasodilatory effect of VIP on pial blood vessels was inhibited by indomethacin pretreatment (Wei et al., 1980). The role of prostaglandin synthesis in other systems which invovle VIP should be examined in more detail.

It has recently been suggested that changes in intracellular calcium may also play a role in mediating the effect of VIP on smooth muscle cells. VIP is known to inhibit contractions of uterine smooth muscle *in vitro* (Ottesen et al., 1979). It was demonstrated that VIP prevented calcium influx in these cells and thus caused relaxation (Bolton et al., 1981). The mechanism by which VIP affected calcium influx was not determined, but it was suggested that membrane hyperpolarization by VIP was involved.

In summary, cyclic AMP appears to mediate most responses to VIP.

Prostaglandins and calcium may also be involved. It is not known if these two substances act distal to the action of cyclic AMP or if their action is independent of cyclase activation.

PURPOSE

Renin secretion is regulated by a number of different mechanisms and VIP could increase renin release by any of these mechanisms. It is a vasodilator and could therefore stimulate renin release by decreasing blood pressure. VIP also influences electrolyte transport and could affect renin release by changing the amount of sodium chloride delivered to or transported by the macula densa. VIP is probably a neurotransmitter and there is at least one report that VIP is present in nerve fibers innervating the kidney. Since the renal nerves play an important role in the regulation of renin release, it is possible that VIP is released from renal nerve terminals and influences renin release in this manner. VIP could also cause a generalized increase in sympathetic activity which might increase renin secretion via release of norepinephrine from the renal nerves or adrenal medulla. Large doses of VIP also cause an acute decrease in plasma potassium levels (Porter and Ganong, 1982) which also can lead to an increase in renin secretion. Finally, VIP could act directly on the juxtaglomerular cells to cause renin release.

In the present work, investigations were carried out to determine the mechanism by which VIP increases renin secretion. This was done by administering VIP intravenously or directly into the renal artery in anesthetized dogs and by determining the effect of VIP on renin release from a preparation of isolated glomeruli. The possibility that VIP is in neurons innervating the kidney was investigated with

immunoctyochemical techniques. The ability of such neurons to release VIP was determined by stimulating the renal nerves and measuring the plasma levels of VIP in the renal venous effluent. A physiologic role for VIP in regulating renin release was investigated by correlating plasma levels of VIP and renin under different situations where the level of either substance was increased experimentally.

METHODS

Infusions of Exogenous VIP in Anesthetized Dogs

General Methods. Mongrel dogs of either sex weighing 13-32 kg were anesthetized with sodium pentobarbital (30 mg/kg). In all dogs, catheters were inserted into one femoral artery and vein. Blood pressure in the femoral artery was measured with a Statham P-23 pressure transducer and Grass Model 5 polygraph.

In animals receiving intrarenal infusions, the left kidney was exposed through an incision in the flank and a curved 23 gauge needle attached to polyethylene tubing was inserted into the renal artery. The renal vein was usually catheterized in a similar manner, but in some dogs a cannula was inserted into the renal vein by way of the gonadal vein. A flow probe was placed around the renal artery and renal blood flow was monitored continuously using an electromagnetic flow meter (Biotronex Laboratory, Inc., Maryland). Zero flow was established during a brief (< 15 sec) occlusion of the renal artery.

Dogs used in the renal function studies were prepared in a similar manner except a catheter was also inserted into the left ureter to allow collection of urine.

In animals receiving intravenous infusions, a Blalock clamp was placed around the aorta proximal to the renal arteries in order to control renal perfusion pressure. The clamp was adjusted so that pressure below the clamp (renal perfusion pressure) was maintained at

approximately 110 mm of Hg. Pressure above the clamp (systemic pressure) was measured by inserting a catheter into one brachial artery. Upon administration of VIP, the clamp was loosened if systemic blood pressure decreased so that the renal perfusion pressure did not change.

Experimental Protocols. 1. In nine dogs, simultaneous arterial and renal venous blood samples were collected before, during, and after infusion of VIP into the renal artery. At least 45 min after the completion of the surgical preparation, two sets of control samples were withdrawn 15 min apart. Highly purified porcine VIP (GIH Laboratory, Karolinska Institute, Stockholm), dissolved in iotonic saline, was infused into the renal artery for 15 min at a rate of 33 ng/kg/min. Blood samples were collected at 7.5 and 15 min during the infusion and 15 min after the end of the infusion. Renin secretion rate was calculated by multiplying the renal venous-arterial difference in PRA by the renal plasma flow (renal blood flow X 1-hematocrit).

2. In four dogs, the above protocol was repeated except isotonic saline alone was infused during the 15 min period.

3. The first protocol was repeated in seven dogs except urine was also collected from a ureteral catheter. Creatinine was infused continuously at a rate of 6 mg/min. Urine was collected during 15 min periods before, during, and after intrarenal infusion of VIP (33 ng/kg/min). Blood samples were withdrawn for measurement of creatinine and electrolytes at the midpoint of each 15 min collection period. Creatinine clearance and sodium and potassium excretion were calculated using the observed urine flow rate.

4. The first protocol was repeated in four dogs. Frozen aliquots of all the plasma samples were sent to Dr. S.I. Said at the University

Oklahoma for determination of VIP levels.

5. Five dogs received three 15 min intravenous infusions of VIP started 1 hr apart at rates of 3.3, 13, and 33 ng/kg/min. Arterial blood samples were collected 20 and 1 min before and 10 min after the start of each infusion. Samples were also withdrawn 5 and 15 min after the end of each infusion. All dogs received all three infusions in the order given above.

6. Seven dogs received a 15 min infusion of VIP directly into the renal artery at a rate of 3.3 ng/kg/min. The time course was the same as that for protocol #5. In addition, renal venous blood was collected along with the arterial samples at all time points.

7. In six dogs, the effect of propranolol treatment on the renin-stimulating effect of VIP was investigated. Following collection of two control arterial blood samples 15 min apart, a bolus injection of d,l-propranolol HCl (Ayerst) was given intravenously at a dose of 0.6 mg/kg. This was followed immediately by a constant intravenous infusion of propranolol at a rate of 0.3 mg/kg/h for the duration of the experiment. Blood samples were withdrawn 15 and 30 min after the start of the propranolol infusion. VIP was then infused intravenously at a rate of 13 ng/kg/min for 15 min. Arterial blood was drawn 10 min after the start and 5, 15, and 45 min after the end of the VIP infusion. Renal perfusion pressure was controlled as mentioned previously.

Analytical Procedures. Blood samples (5 ml) were collected in chilled tubes containing ethylenediamine tetraacetic acid (EDTA) and plasma was promptly separated and frozen. PRA in these specimens was subsequently determined by measuring the rate of AI formation at 37°C as outlined below. Frozen aliquots of the plasma were sent to Dr. Said who

determined the VIP levels by radioimmunoassay (Pandian et al., 1982). Blood samples for electrolyte measurement were collected in a few drops of heparin, or in the case of the intravenous dose-response study, in EDTA. In this case the potassium values were corrected for the dilution caused by the EDTA. Plasma and urinary electrolytes were measured using flame photometry. Creatinine was determined using a standard colorimetric method (Owen et al., 1954).

PRA Assay. One milliliter aliquots of the thawed plasma were mixed with 25 µl of 2,3-dimercaptopropanol (BAL) (4 mg/ml) and 25 µl of 8-hydroxyquinoline (10 mg/ml), both compounds which inhibit conversion of AI to AII. The pH was then adjusted to 5.5 with 10% HCl and the samples were incubated for 3 hr in a 37°C water bath. During this incubation, the renin in the sample converted some of the angiotensinogen present to AI (Stockigt et al., 1971). The reaction was stopped by diluting each sample with 1 ml of distilled water and placing it in a boiling water bath for 2-3 min. At this point, the AI in each sample was measured by radioimmunoassay or the sample was frozen for later determination.

The AI radioimmunoassay was performed as follows. Duplicate 10 µl and 50 µl aliquots of each sample were mixed with enough assay buffer (0.1M Tris, 0.5% BSA, 0.01% Neomycin, and 0.01% methiolate) to make 0.5 ml in each tube. For the standard curve, aliquots of AI ranging from 0.015 to 2.0 ng were mixed with assay buffer to give the same final volume. Radioiodinated AI was added so that each tube received 10,000 to 13,000 cpm. Finally, AI antiserum was added to each tube to give a final dilution of 1:80,000. This whole mixture was vortexed and incubated at 4°C for 48 hrs. Bound ^{125}I-AI was separated from free by adding 0.5 ml of dextran-coated charcoal (2.5%) to each sample. This

mixture was vortexed and centrifuged for 15 min at 2,500 rpm (1,250g) and 4°C. The supernatant which contained bound ^{125}I-AI was aspirated and discarded. The radioactivity remaining in the charcoal pellet was then determined using a gamma counter (Beckman). The cpm for each standard tube were plotted against the amount of AI added on semi-log paper. This produced a sigmoidal curve over the range of AI used (Fig. 1). This standard curve was used to determine the amount of AI in each unknown sample which was then converted to ng AI/ml/h using the appropriate dilution factor. The interassay variability of this assay was 16% (n=20) and the intraassay variability was 8% (n=20).

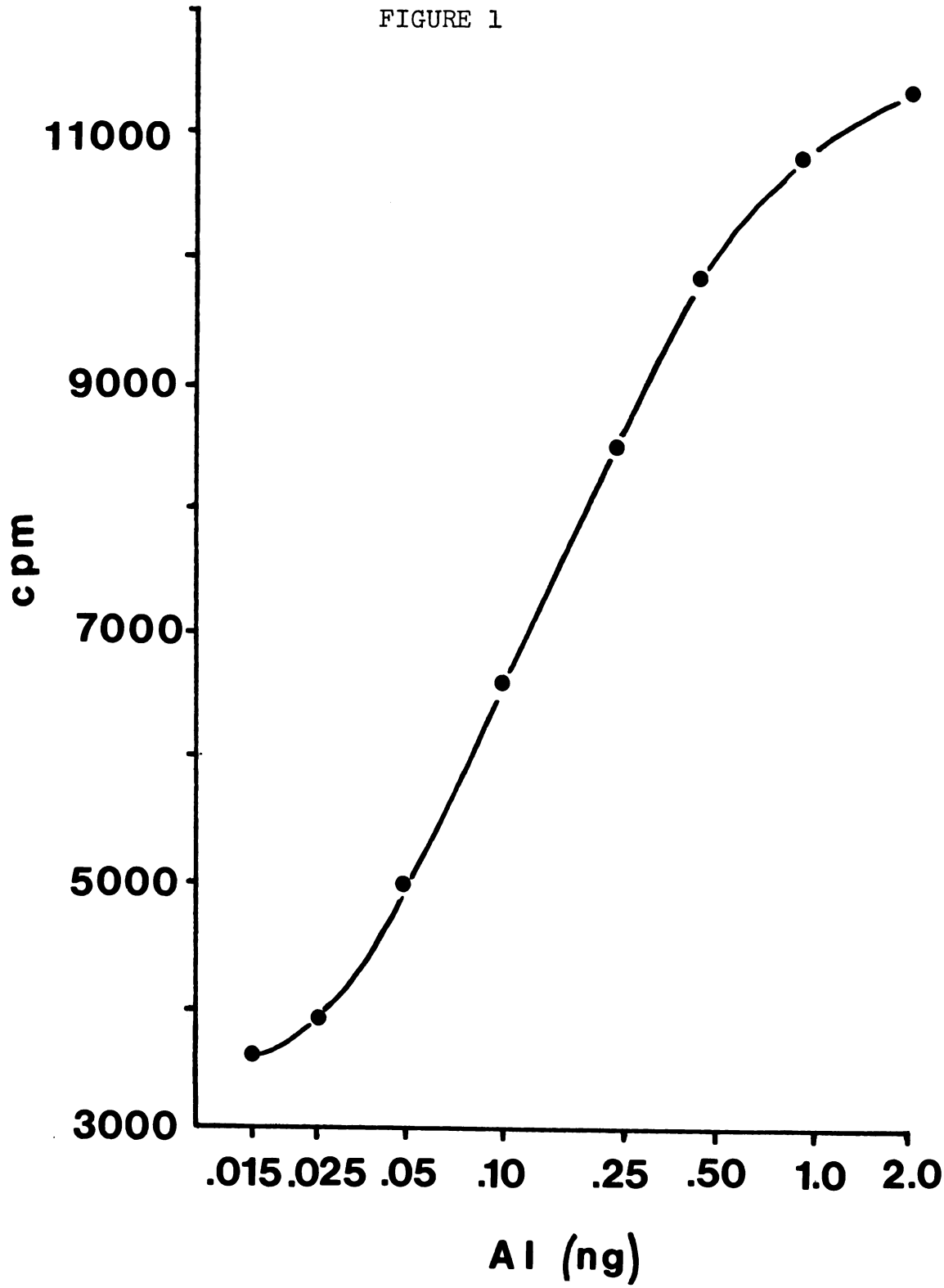
Statistical Analysis. Data were analyzed using analysis of variance (ANOVA) for repeated measures and Duncan's New Multiple Range Test, supplemented by Student's paired t test. Data obtained during intrarenal infusion of VIP at 33 and 3.3 ng/kg/min showed a significant ($p<0.05$) positive skewness. In these two cases, the skewness was eliminated by a log transformation prior to doing the ANOVA. A probability less then 0.05 was considered to be significant.

Isolation and Superfusion of Rat Glomeruli

Isolation. Male Sprague-Dawley rats weighing 200-350 gm were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The kidneys were exposed through a midline incision in the abdomen and the aorta was catheterized below the kidneys. The aorta was then clamped above the kidneys and a slit was made in the vena cava at the point where it is joined by the renal veins. Ten milliliters of an aerated (95% O_2-5% CO_2) modified Krebs-Ringer buffer containing 140 mM

FIGURE 1. A representative standard curve obtained in an AI radioimmunoassay.

FIGURE 1



NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 2 mM $CaCl_2$, 10 mM NaAc, 10 mM glucose, 20 mM Tris base, and 2 mM $NaPO_4$ (pH 7.4) was then infused into the aortic catheter. Since the aeration resulted in a significant acidification of the buffer over a several hour period, the solution was always used in 300 ml portions in which the pH had been adjusted to 7.4 prior to use. The flushing with buffer was repeated 3 to 4 times until the kidneys were free of blood. The kidneys were then decapsulated, excised, and demedullated. Minced cortical tissue was pressed through a stainless steel mesh (hole diameter 250 μm) with a spoon-like spatula. The resulting preparation was resuspended in buffer, drawn into a 20 ml syringe through a 21 gauge needle and divided into four 15 ml conical polyethylene centrifuge tubes. These were centrifuged for 3 min at 160 g. The supernatant was removed and discarded. The remaining pellet was resuspended in buffer. This whole procedure was repeated 2 more times until a clear supernatant was obtained. The pellet was again resuspended in 3 ml of buffer and poured through a series of nylon monofilament screens with hole diameters of 390 μm, 250 μm, and 212 μm respectively. These screens filtered out large tubular fragments and other debris and were discarded. The glomerular preparation was then collected on a Swiss bolting cloth with hole diameter of 60 μm. Fragments smaller than this passed through the bolting cloth and were discarded. The glomeruli recovered were rinsed again through a nylon screen with hole diameter of 212 μm to remove remaining tubular fragments. The glomeruli were allowed to stand for 10-15 min in 50 ml of the above buffer. The top 40 ml were then removed and discarded and the glomeruli were resuspended in clean buffer. This procedure was repeated two more times. The final preparation was then transferred to

a preweighed centrifuge tube and centrifuged for 5 min at 650 g. The supernatant was withdrawn and the inside wall of the tube was dried with a cotton swab. The tube and glomerular pellet were weighed to allow calculation of the wet weight of the preparation. The whole isolation procedure was carried out at room temperature and took approximately three hours.

Superfusion. The final glomerular pellet, which was 90% pure (9 glomeruli for every 1 tubular fragment), was resuspended and 25-40 mg were transferred to four 400 µl glass chambers. The glomeruli were held in the chamber between a sintered glass filter at the bottom and a small circular piece of filter paper (Scientific Products) placed over the opening at the top. A teflon O-ring joint was inserted into the top of the chamber to form a water-tight seal. The center of the O-ring was plugged with the rubber tip of a 1 ml syringe plunger and a 23 gauge needle was inserted into the rubber plug so that fluid could flow out. In this manner the glomeruli were encased in a chamber which allowed free flow of buffer (Fig. 2). The chambers were placed in a 37°C water bath and the glomeruli were superfused at a rate of 0.6 ml/min using a peristaltic pump (Sage Instruments). For the superfusion, 0.2% bovine serum albumin (Sigma) and 0.1% bacitracin (Sigma) were added to the above buffer. The superfusate was collected for 40 min and discarded. This first portion of superfusate always contained high levels of renin presumably resulting from cell damage during the isolation procedure. By 40 min the baseline renin release was relatively stable. Five-min fractions of the superfusate were collected before, during, and after addition of isoproterenol or VIP to the buffer. Four chambers were run simultaneously which allowed several different treatments at one time.

FIGURE 2. A schematic (cross-sectional) drawing of the glass chamber used for superfusion of isolated glomeruli. Arrow shows the direction of flow of superfusate.

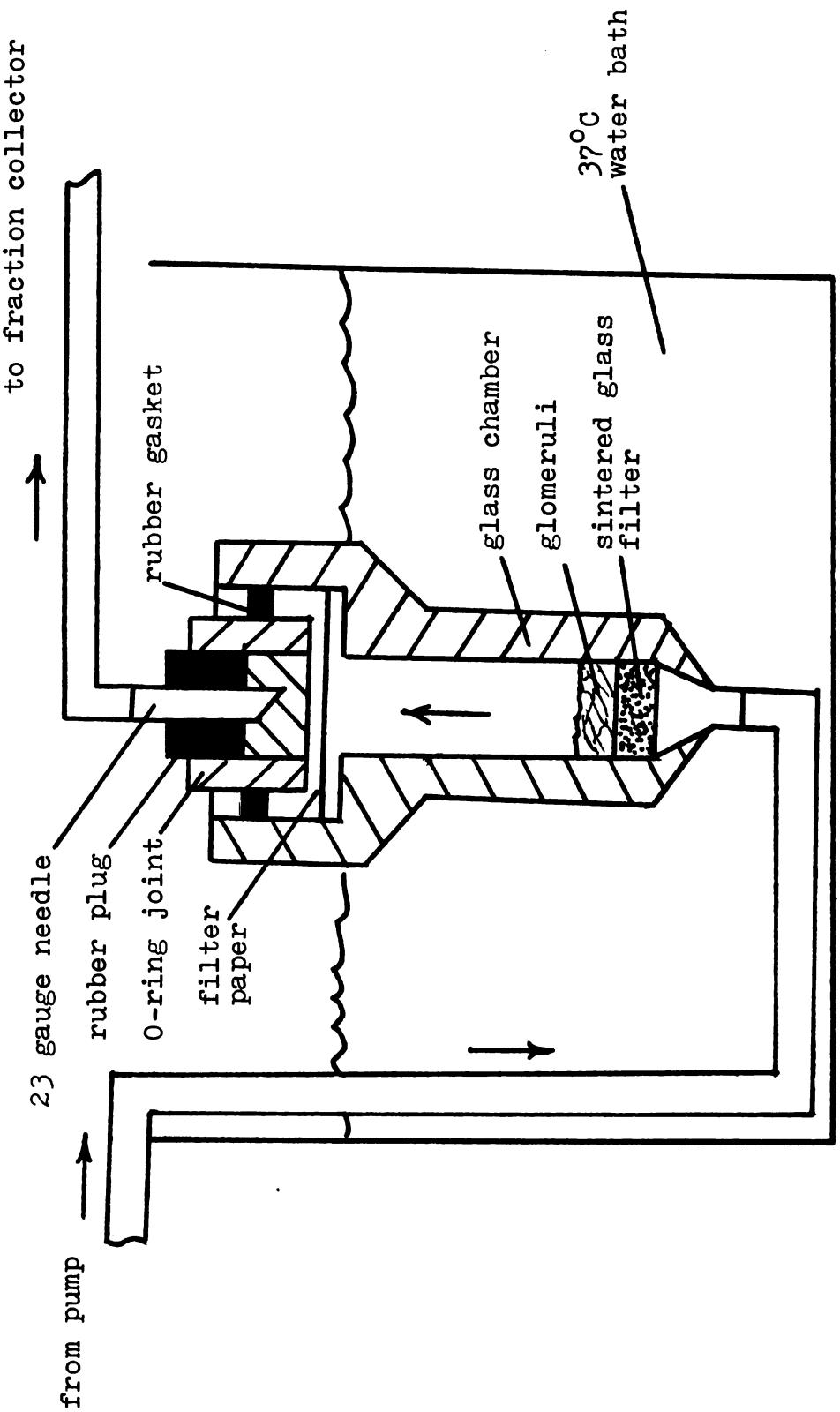

to fraction collector
37°C
water bath
rubber gasket
glass chamber
glomeruli
sintered glass
filter
23 gauge needle
rubber plug
O-ring joint
filter
paper
from pump

FIGURE 2

Kidneys from 3-4 rats resulted in sufficient glomeruli to fill four chambers. At the end of each experiment, the preparation was removed from one chamber and examined under a microscope to determine its purity.

Experimental Protocols. 1. Two 5-min control fractions of superfusate were collected. The inflow tube was then switched for 5 min to buffer containing isoproterenol at 10^{-4}M, 10^{-5}M, or 10^{-6}M or buffer alone. After 5 min the inflow tube was switched back to control buffer and two more 5-min samples were collected.

2. The above procedure was again carried out except that VIP at 10^{-7}M, 10^{-8}M, 10^{-9}M, or 10^{-10}M was used during the experimental period.

Renin Assay. Renin in the superfusate was determined by measuring the rate of AI formation from angiotensinogen at 37°C. Plasma from nephrectomized rats (100 µl), the source of angiotensinogen, was mixed with 25 µl of the superfusate, 5 µl of 4 mg/ml BAL, 5 µl of 10 mg/ml 8-hydroxyquinoline, 5 µl of 2.5% phenylmethylsulfonyl fluoride, and 60 µl of standard Tris assay buffer (see section on PRA) which contained an additional 0.38% EDTA. In each assay a blank tube was included which had 25 µl of buffer added rather that superfusate. This mixture was incubated for 1 hr at 37°C and then frozen. Two 50 µl duplicates of thawed incubate were subsequently added to the same AI radioimmunoassay described in the previous section. A small amount of AI was usually generated in the nephrectomized plasma blank (0.04 $\pm$ 0.02 ng, n=18). In each assay the amount of AI generated in the blank tube was subtracted from the amount of AI generated in each superfusate sample. The rate of AI formation was calculated as ng/ml/h. This value was then standardized using the wet weight of the glomeruli in each chamber to

ng AI/ml/h. This value was then standardized using the wet weight of the glomeruli in each chamber to ng AI/ml/h/40 mg. The interassay variability of this assay was 22% (n=14) and the intraassay varibility was 9.4% (n=7).

Statistical Analysis. All data were analyzed using analysis of variance for repeated measures and Duncan's New Multiple Range Test. A probability less than 0.05 was considered significant.

VIP in the Renal Nerve

The possiblity that VIP is present in the renal nerve was investigated using two different prodedures. First, the indirect peroxidase-antiperoxidase immunocytochemical technique was used to determine if VIP is present in dog kidneys. Second, the renal nerve was stimulated in four anesthetized dogs and VIP levels in the renal venous plasma were measured to see if release of the peptide occurred during the stimulation.

Immunocytochemistry. Kidneys were removed from anesthetized dogs and placed on ice. In one case, one kidney was surgically denervated 5 days prior to removal. Sterile saline was infused into the renal artery at a constant pressure of 100 mg Hg until all the blood was washed out of the kidney. Small pieces of renal cortex were cut using a razor blade and fixed for 3 hr in a modified Bouin's fluid containing 1% acetic acid. These pieces were embedded in paraffin and 10 μ m sections were cut. Submandibular salivary gland tissue was prepared in a similar manner and used as a positive control since VIP immunoreactivity is known to be present in this tissue (Lundberg et al., 1980b).

The kidney sections were later deparaffinized free-floating in test

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The kidney sections were later deparaffinized free-floating in test

tubes and taken through changes of 100%, 95%, and 75% ethyl alcohol to distilled water. The salivary tissue was mounted on slides and was not processed free-floating. The sections were then washed twice in a 0.05M phosphate buffer with 0.9% saline (PBS) containing 1% normal goat serum (GS). After the second washing the tissue was incubated on a shaker for 30 min in PBS containing 3% GS. This was replaced with one of three VIP antisera (Provided by S. Said, University of Oklahoma; E.Zimmerman, Columbia University; and J. Walsh, University of California Los Angeles) at dilutions ranging from 1:500 to 1:2,000 for 48 hr at 4°C. Control sections received either normal rabbit serum at the same dilution or VIP antisera which was preabsorbed overnight with 50 μg/ml VIP. After this incubation, the tissue was washed 3 times for ten min with 1%GS-PBS. Goat anti-rabbit IgG (Cappel Laboratories) was added at a dilution of 1:20 for 30 min. The sections were washed two more times with 1%GS-PBS and rabbit peroxidase-antiperoxidase was added at a dilution of 1:300 for 30 min. This was followed with two washings with PBS and one with a 0.05M Tris buffer (pH 7.6). Twenty milligrams of 3,3'-diaminobenzidine (DAB) (Sigma) was dissolved in 40 ml of the Tris buffer. The solution was filtered and the pH was readjusted to 7.6. Three percent hydrogen peroxide (41 μl) was then added to 12.5 ml of the DAB solution. This final mixture was applied to the sections for 5 min on ice and an additional 5 min at room temperature. The DAB solution was withdrawn and the reaction was stopped by adding Tris buffer. The sections were washed two more times and then placed on microscope slides and allowed to air dry.

Renal Nerve Stimulation. Four dogs were anesthetized with 30 mg/kg sodium pentobarbital. A femoral artery and vein were catheterized.

Blood pressure was monitored as described previously. The left kidney was exposed through a flank incision. A catheter was inserted into the renal vein via the gonadal vein. A bipolar stimulating electrode encased in a plexiglass block was placed around the renal artery and surrounding nerves. Two control renal venous and arterial blood samples were collected simultaneously 15 min apart. The renal nerves were stimulated for 30 min at 20 volts and 10 Hz (5.0 msec duration). Blood samples were withdrawn 1, 5, 15, and 30 min after the start of the stimulation and 30 min after it ended. In one dog, the stimulation procedure was repeated again one hour after the 30 min recovery, but in this case, the stimulating electrode was placed around only the renal nerve and did not touch the renal artery. Since the results from the second stimulation did not differ qualitatively from the first, the average of the two sets of data was used in the analysis.

Plasma was collected as described previously for measurement of PRA and VIP. In this experiment, VIP was measured using a commercial RIA kit (Immuno Nuclear). Briefly, 200 µl of plasma was incubated with 200 µl of rabbit anti-VIP antiserum for 24 hr at 4°C. ^{125}I-VIP (7,000 - 9,000 cpm) was then added for an additional 24 hr. Bound ^{125}I-VIP was separated from free using goat anti-rabbit immunoglobulin. Synthetic VIP ranging from 25 to 400 pg/ml was used to construct the standard curve. Addition of known amounts of either synthetic VIP (Penninsula Laboratories) or purified porcine VIP resulted in similar recoveries with a combined average recovery of 86% $\pm$ 8 (n=6). The baseline plasma levels of the peptide measured with the commercial kit were in the same range as those measured by Dr. Said.

Correlation of Plasma Levels of VIP and Renin

Neostigmine Infusions. Six dogs were anesthetized as described above. A femoral artery and vein were catheterized and blood pressure was monitored continuously. A Blalock clamp was placed around the aorta to control renal perfusion pressure. Two control blood samples were collected 20 min apart. Neostigmine (0.07 mg/kg) was infused over 1 min into the femoral vein. Blood samples for measurement of VIP and renin were collected 10, 20, 30, and 60 min after the injection. VIP was measured with the commercial kit.

Low Salt Diet. Six dogs were fed a low salt diet for 14 days. Four dogs received 350 ml/day of Lonalac, a low salt (< 1 mEq/L) milk substitute plus added salt (21-25 mEq/L) for 3 days, then the added salt was stopped for 14 days. Two dogs received a commercial low salt (1.5 mEq/day) canned dog food. Venous blood samples were withdrawn for 3 days before the diet and then every other day for 14 days during the diet. The dogs were housed in metabolism cages to allow daily collection of urine. Sodium and potassium levels in the urine were measured by flame photometry and daily excretion rates of the two electrolytes were calculated. PRA and VIP were measured as above.

RESULTS

Infusions of Exogenous VIP in Anesthetized Dogs

Protocol 1. The effect of a 15 min infusion of VIP into the renal artery at 33 ng/kg/min is shown in Fig. 3 and Fig. 4. PRA increased significantly during the infusion and was still elevated at 15 min. This was accompanied by a transient increase in renin secretion rate which returned to control levels by 15 min even though VIP was infused for the entire 15 min. Renal blood flow also increased by 33% during the infusion (Fig. 4). Systolic blood pressure did not change but there was a significant 5 mm Hg decrease in diastolic pressure.

Protocol 2. The effect of intrarenal infusion of 7.5 ml of 0.9% saline on the same variables is shown in Fig. 5 and Fig. 6. There was no significant effect on renin release, renal blood flow or blood pressure.

Protocol 3. Table 2 shows the effect of a 15 min intrarenal infusion of VIP (33 ng/kg/min) on renal function. Creatinine clearance increased significantly during the infusion (paired t test). Urine flow and sodium and potassium excretion all tended to increase but the changes were not statistically significant. There was also a significant decrease in plasma potassium which remained low during the recovery period. Plasma renin activity, renin secretion rate and renal blood flow increased as before (Fig. 7 and Fig. 8), but blood pressure was not significantly affected.

FIGURE 3. The effect of a 15 min intrarenal infusion of VIP at a rate of 33 ng/kg/min on plasma renin activity and renin secretion rate in 9 pentobarbital anesthetized dogs. * $p < 0.05$ compared to -15 min and -1 min value. For this and all subsequent figures, each point represents the mean value $\pm$ standard error.

FIGURE 3

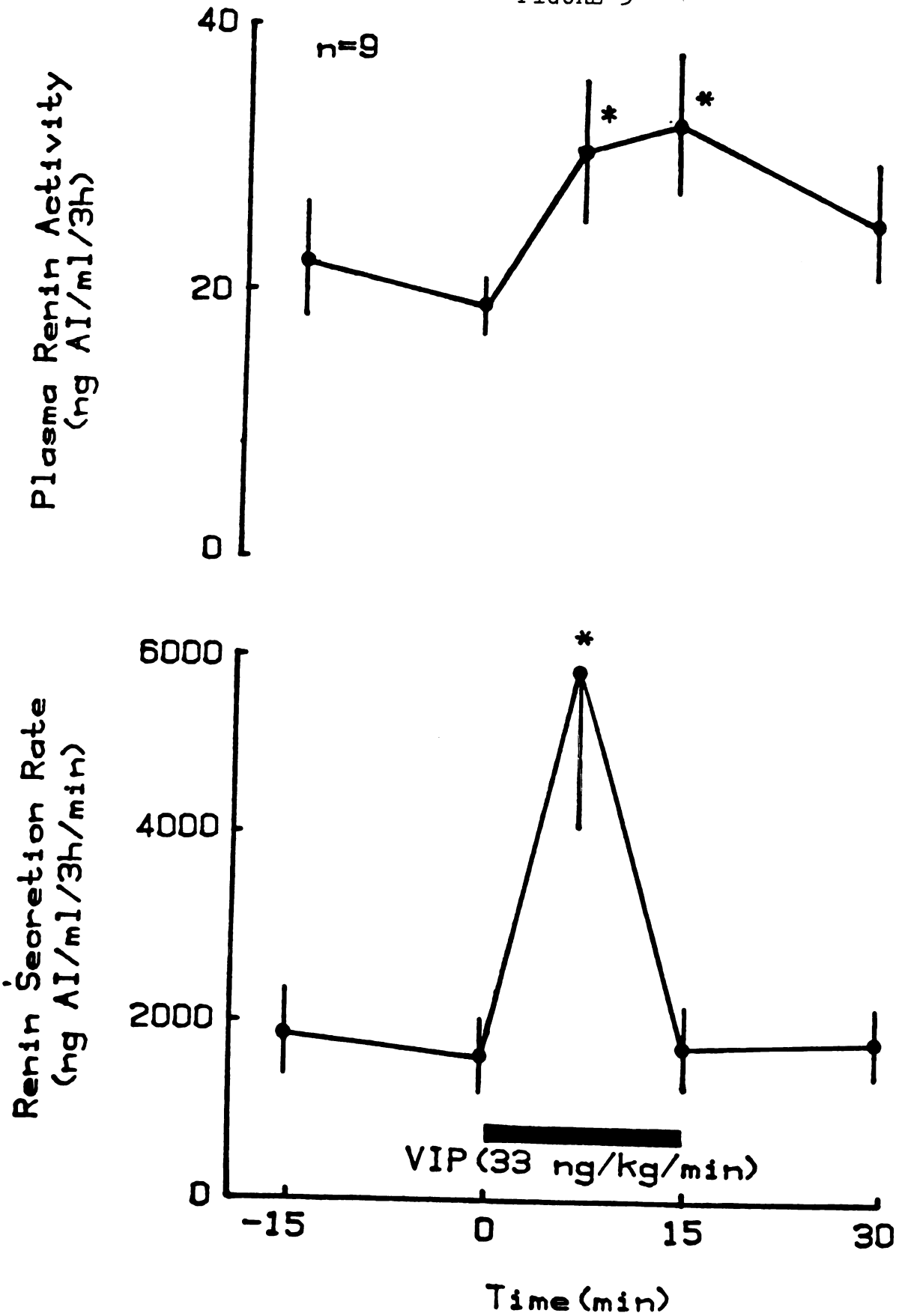

40
n=9
20
0
Plasma Renin Activity
(ng AI/ml/3h)
*
*
6000
4000
2000
0
Renin Secretion Rate
(ng AI/ml/3h/min)
*
VIP (33 ng/kg/min)
-15
0
15
30
Time (min)

FIGURE 4. The effect of a 15 min intrarenal infusion of VIP at a rate of 33 ng/kg/min on renal blood flow and blood pressure in 9 dogs. * $p < 0.05$ compared to -15 min and -1 min value.

FIGURE 4

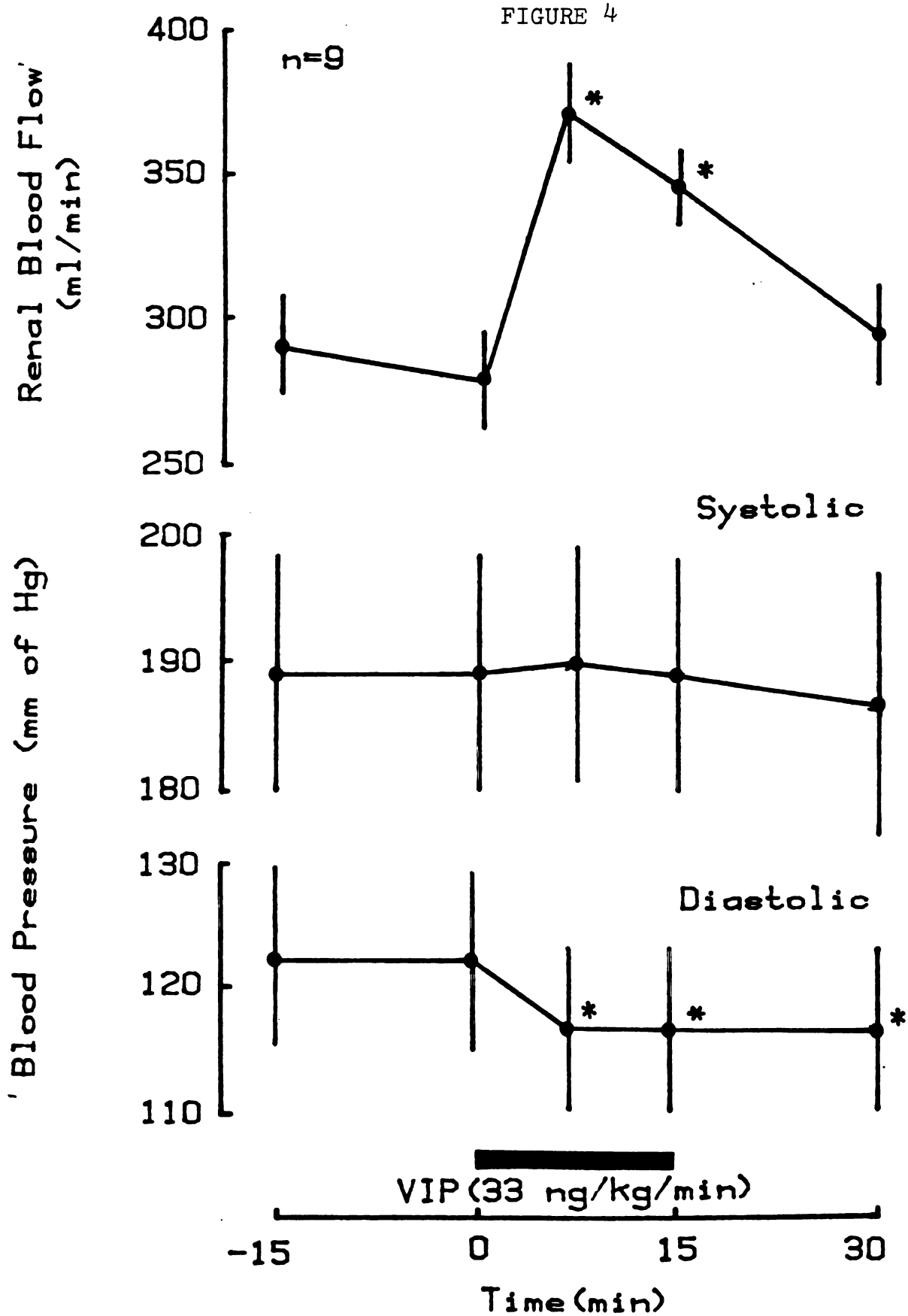

n=9
Renal Blood Flow (ml/min)
400
350
300
250
Systolic
200
190
180
Diastolic
130
120
110
Blood Pressure (mm of Hg)
VIP (33 ng/kg/min)
-15
0
15
30
Time (min)

FIGURE 5. The effect of a 15 min intrarenal infusion of 7.5 ml of 0.9% saline on plasma renin activity and renin secretion rate in 4 dogs.

FIGURE 5

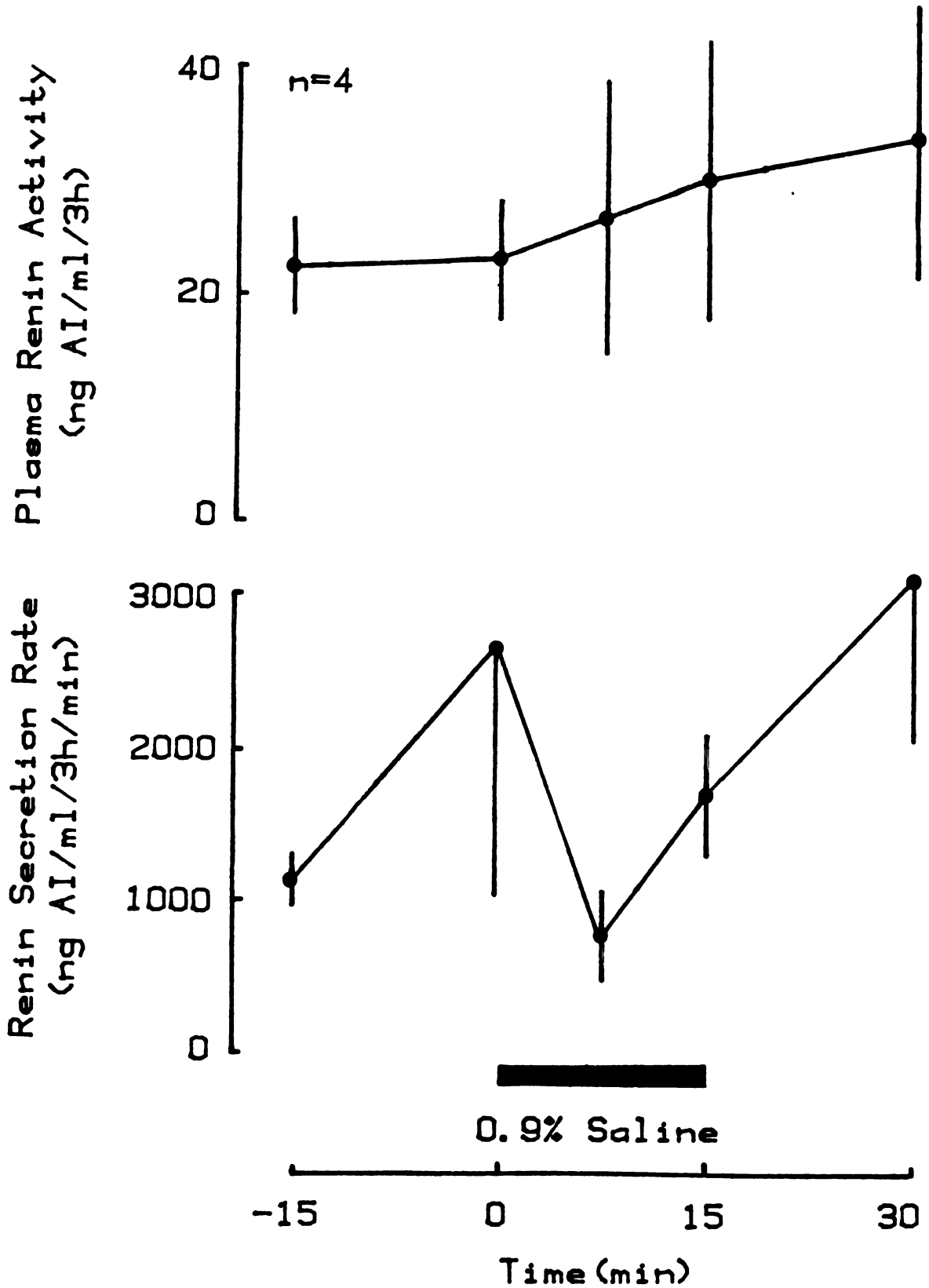

Plasma Renin Activity
(ng AI/ml/3h)
40
20
0
n=4
Renin Secretion Rate
(ng AI/ml/3h/min)
3000
2000
1000
0
0.9% Saline
-15
0
15
30
Time (min)

FIGURE 6. The effect of a 15 min intrarenal infusion of 7.5 ml of 0.9% saline on renal blood flow and blood pressure in 4 dogs.

FIGURE 6

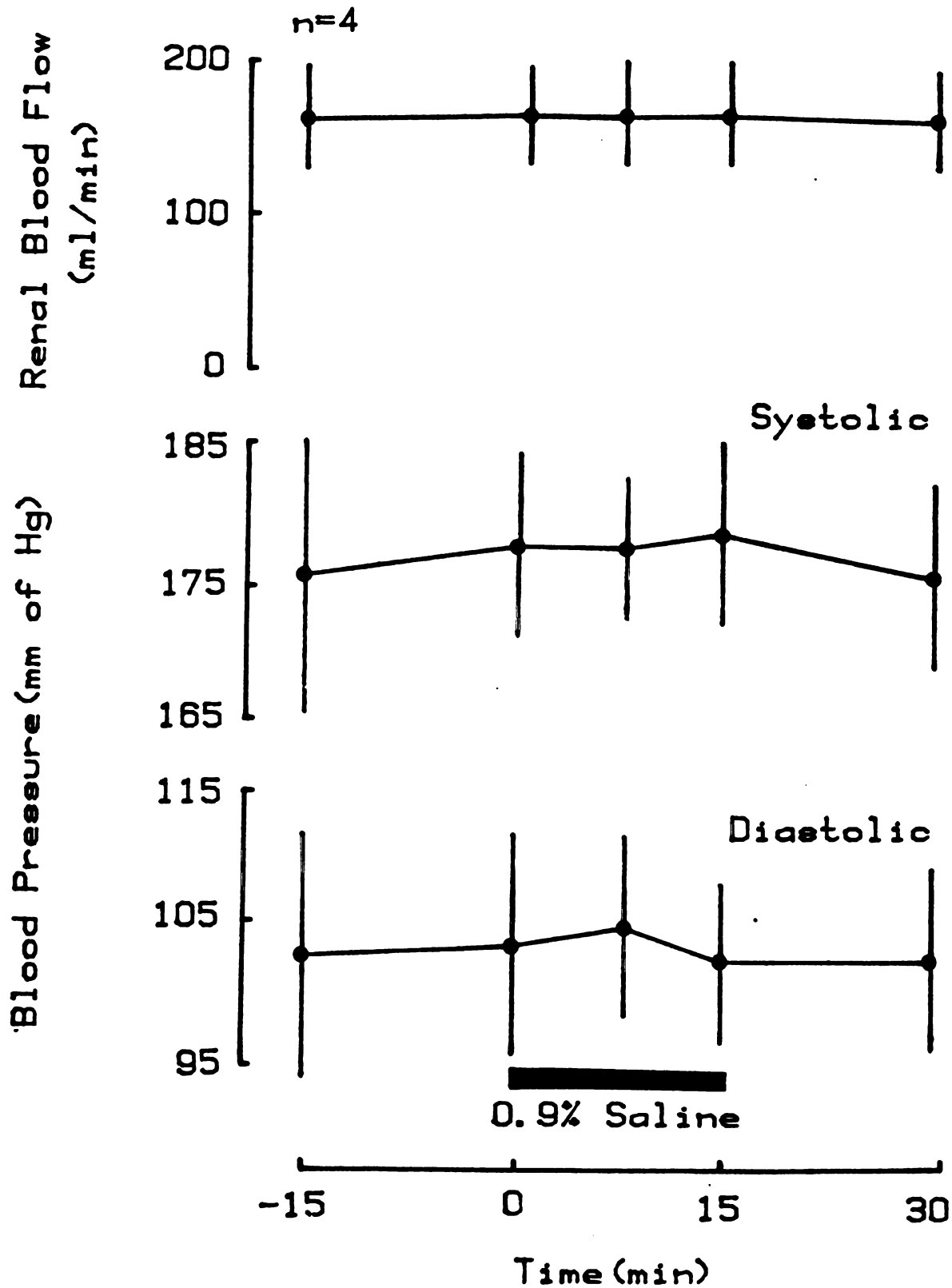

n=4
Renal Blood Flow (ml/min)
200
100
0
Blood Pressure (mm of Hg)
Syetolic
185
175
165
Diastolic
115
105
95
0.9% Saline
-15
0
15
30
Time (min)

Table 2. Effect of intrarenal VIP (33 ng/kg/min) on the function of the infused kidney in 7 dogs.

	Minutes before and after start of 15 min VIP infusion into the left renal artery		
	-15 - 0	0 - 5	15 - 30
Urine Volume (ml/min)	0.40 ± 0.14	0.65 ± 0.27	0.47 ± 0.20
Creatinine Clearance (ml/min)	20 ± 7	29 ± 7*	31 ± 10
Sodium Excretion (uEq/min)	60 ± 20	107 ± 43	70 ± 28
Potassium Excretion (uEq/min)	21 ± 5	31 ± 8	27 ± 8
Plasma Sodium (mEq/L)	141.3 ± 2.0	140.3 ± 2.0	139.9 ± 2.1
Plasma Potassium (mEq/L)	3.65 ± 0.19	2.91 ± 0.35**	2.85 ± 0.37**

*$p < 0.05$ compared to control, paired t test

**$p < 0.05$ compared to control, ANOVA

In this and all subsequent tables, each value is a mean ± standard error.

FIGURE 7. The effect of a 15 min intrarenal infusion of VIP at a rate 33 ng/kg/min on plasma renin activity and renin secretion rate in 7 dogs. ** $p < 0.05$ compared to -15-0 min value, paired t test.

FIGURE 7

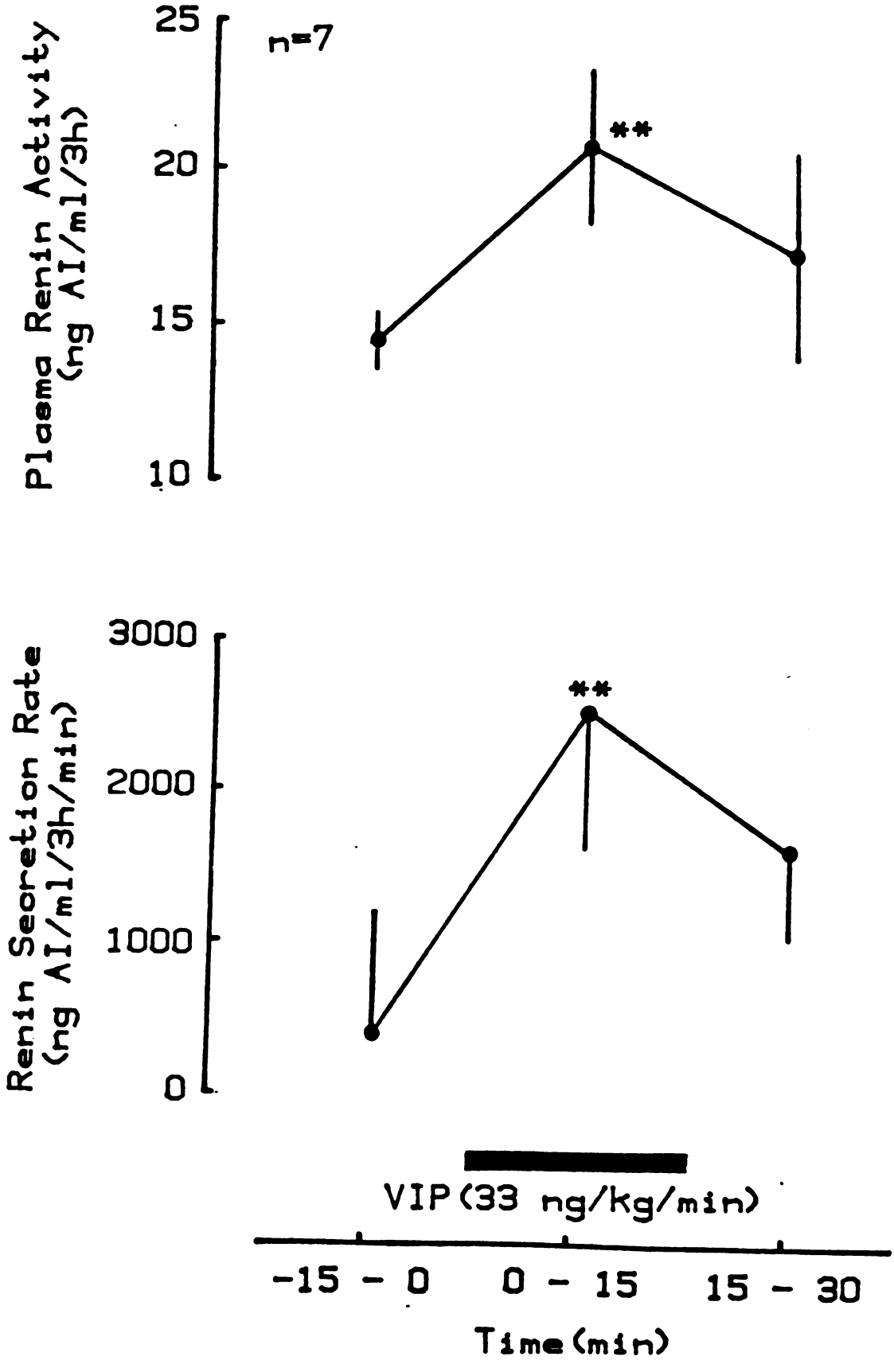
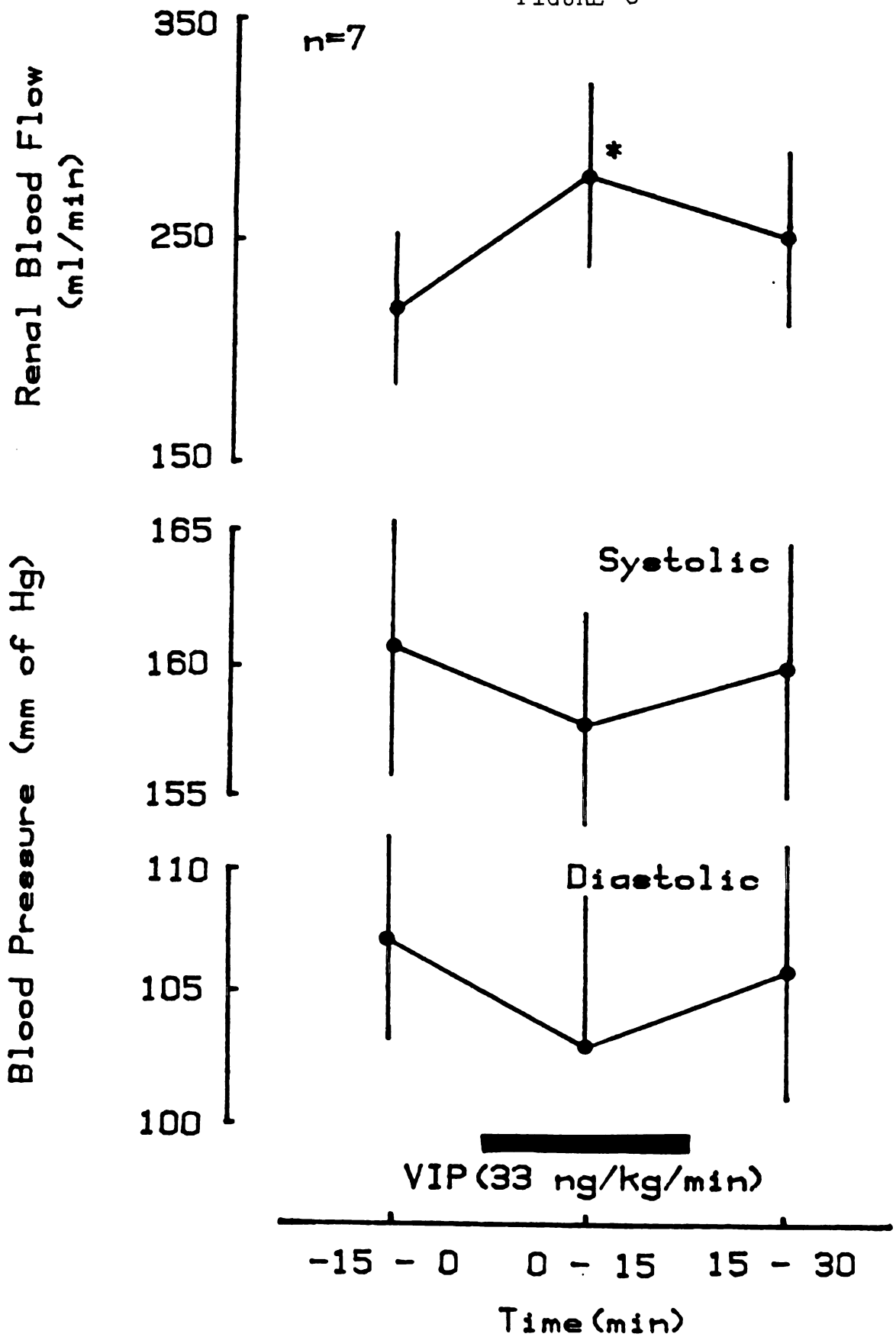

Plasma Renin Activity
(ng AI/ml/3h)
25
20
15
10
n=7
**
Renin Secretion Rate
(ng AI/ml/3h/min)
3000
2000
1000
0
**
VIP (33 ng/kg/min)
-15 - 0
0 - 15
15 - 30
Time (min)

FIGURE 8. The effect of a 15 min intrarenal infusion of VIP at a rate of 33 ng/kg/min on renal blood flow and blood pressure in 7 dogs. * $p < 0.05$ compared to -15-0 min value, ANOVA.

FIGURE 8


n=7
350
250
150
Renal Blood Flow (ml/min)
*
165
160
155
Syetolic
110
105
100
Diastolic
Blood Pressure (mm of Hg)
VIP (33 ng/kg/min)
-15 - 0
0 - 15
15 - 30
Time (min)

Protocol 4. The plasma levels of VIP produced by intrarenal infusion of the peptide at the same rate in four other dogs is shown in Table 3. Control renal venous levels of VIP were greater than arterial levels in 3 of the 4 dogs although the difference was not statistically significant. Infusion of VIP at a rate of 33 ng/kg/min elevated renal venous levels to 662 ± 102 pmole/L and arterial plasma levels to 264 ± 85 pmole/L. There was a prompt return to baseline levels after the end of the infusion. Renin secretion rate was not calculated in these dogs, but arterial and renal venous PRA increased significantly during the infusion.

Protocol 5. The effect of administering three consecutive doses of VIP intravenously is depicted in Fig. 9 and Table 4. A 15 min infusion at a rate of 3.3 ng/kg/min did not change PRA (Fig. 9). The 13 ng/kg/min dose increased PRA significantly at 10 and 20 min after the start of the infusion. Infusion of VIP at a rate of 33 ng/kg/min increased PRA by 10 min. Table 4 shows that the higher infusion rate significantly decreased distolic blood pressure and plasma potassium. The lower two infusion rates had no significant effect on these variables. Arterial plasma levels of VIP increased significantly to 41 ± 2 pmole/L during the 3.3 ng/kg/min infusion, 75 ± 3 during the 13 ng/kg/min infusion, and 130 ± 9 during the highest rate of infusion.

Protocol 6. Infusion of VIP directly into the renal artery at a rate of 3.3 ng/kg/min significantly increased PRA by 20 min (Fig. 10). Due to technical difficulties with the flow meter, renin secretion rate was calculated in only four of the seven dogs. While it increased in three of these four, the mean increase was not significant. Renal venous levels of VIP increased to 87 ± 10 pmole/L during the infusion

Table 3. Plasma levels of VIP resulting from an intrarenal infusion of the peptide (33 ng/kg/min) in 4 dogs.

	Minutes before and after start of 15 min VIP infusion into the left renal artery				
	-15	-1	7.5	15	30
Renal venous plasma VIP (pmole/L)	31 ± 8	32 ± 9	605 ± 95	662 ± 102	22 ± 2
Arterial plasma VIP (pmole/L)	18 ± 1	18 ± 1	264 ± 85	116 ± 26	25 ± 2

FIGURE 9. The effect of intravenous administration of VIP for 15 min at three different rates on PRA in 5 dogs. * $p < 0.05$ compared to -1 min value.

FIGURE 9

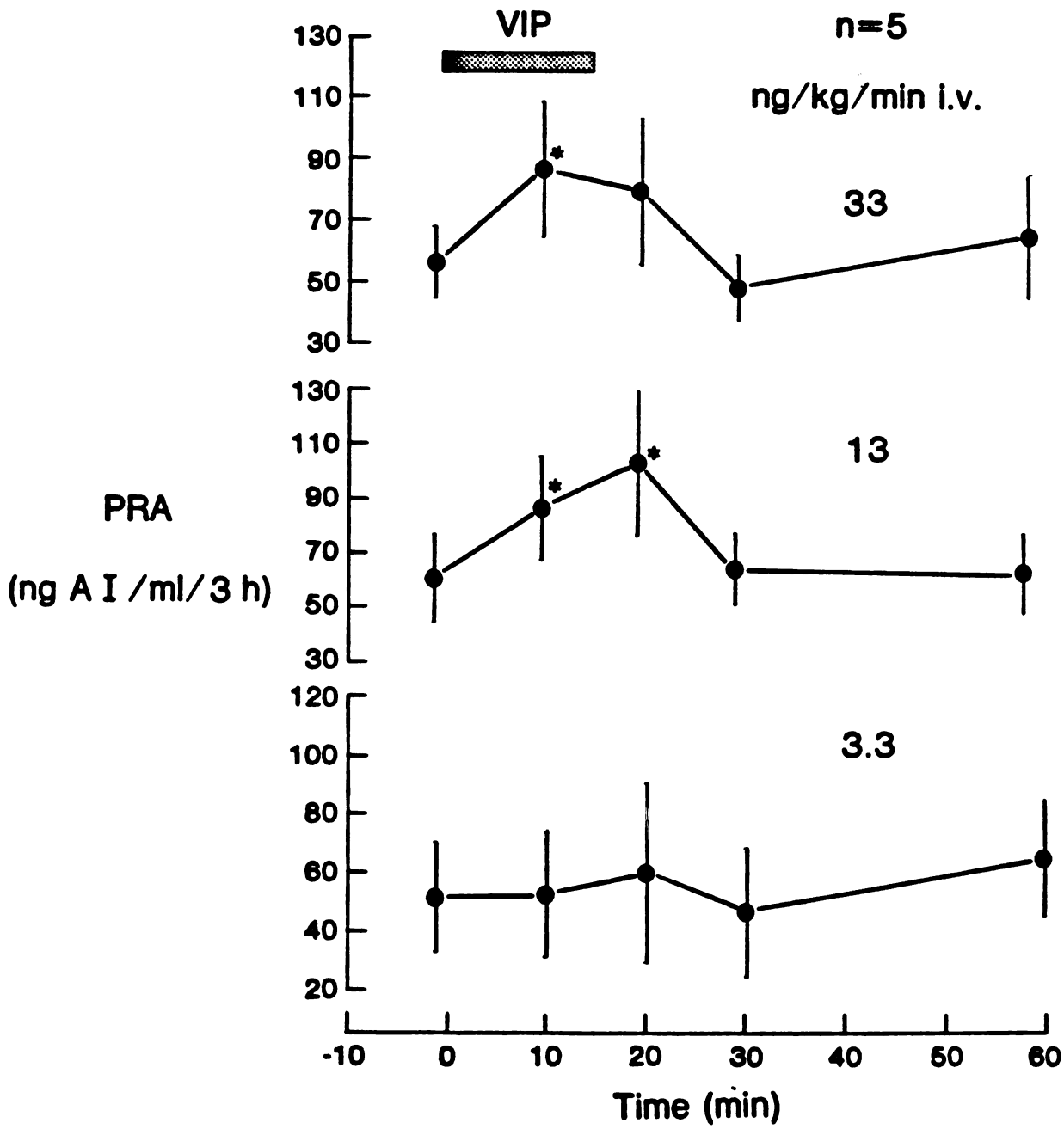

VIP
n=5
ng/kg/min i.v.
33
13
3.3
PRA
(ng A I /ml/ 3 h)
130
110
90
70
50
30
120
100
80
60
40
20
-10
0
10
20
30
40
50
60
Time (min)

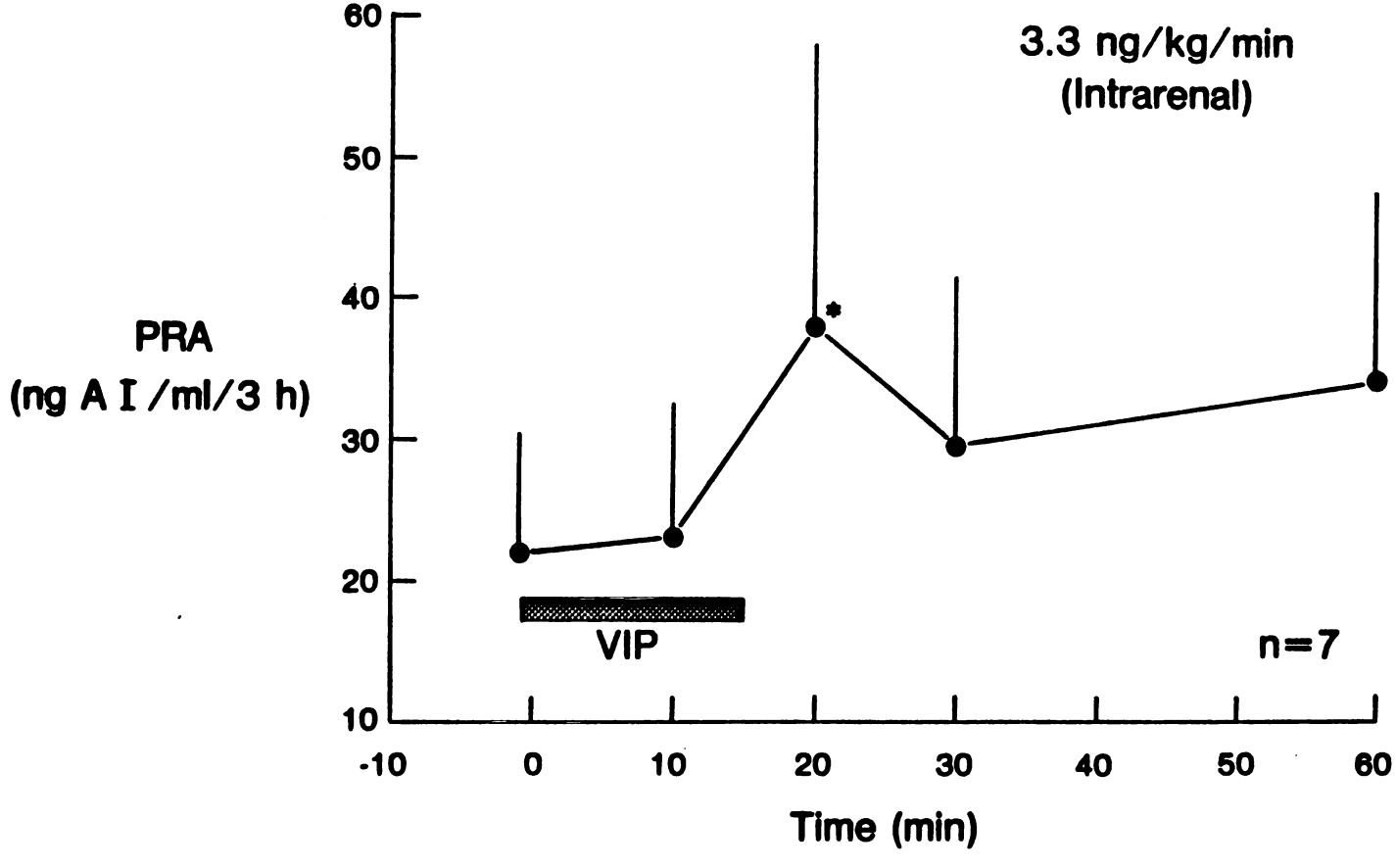
Table 4. Effect of intravenous administration of VIP at three different doses in 5 dogs.

Time (min)	Blood pressure systolic (mm Hg)	Blood pressure diastolic (mm Hg)	Plasma potassium (mEq/L)	Plasma VIP (pmol/L)
-20	164 ± 10	119 ± 6	3.39 ± 0.11	31 ± 1
-1	169 ± 9	118 ± 5	3.21 ± 0.11	32 ± 2
0	VIP, 3.3 ng/kg/min for 15 min			
10	171 ± 9	123 ± 4	3.19 ± 0.07	41 ± 2*
20	172 ± 8	124 ± 6	3.17 ± 0.07	33 ± 2
30	171 ± 10	127 ± 5	3.28 ± 0.07	32 ± 2
60	176 ± 8	130 ± 4	3.24 ± 0.07	30 ± 2
79	179 ± 10	132 ± 5	3.26 ± 0.11	33 ± 2
80	VIP, 13 ng/kg/min for 15 min			
90	176 ± 12	132 ± 8	3.25 ± 0.08	75 ± 3**
100	181 ± 10	137 ± 7	3.28 ± 0.18	37 ± 1
110	181 ± 10	136 ± 6	3.26 ± 0.08	30 ± 3
140	181 ± 9	135 ± 6	3.41 ± 0.18	30 ± 1
159	180 ± 8	136 ± 4	3.45 ± 0.16	29 ± 1
160	VIP, 33 ng/kg/min for 15 min			
170	173 ± 6	124 ± 6***	3.21 ± 0.11	130 ± 9***
180	174 ± 7	130 ± 5	3.16 ± 0.14***	46 ± 4***
190	172 ± 8	129 ± 5	3.54 ± 0.11	35 ± 1
200	173 ± 8	128 ± 6	3.40 ± 0.14	32 ± 2

*$p < 0.05$ compared to -1 min value, ANOVA
**$p < 0.05$ compared to 79 min value, ANOVA
***$p < 0.05$ compared to 159 min value, ANOVA

FIGURE 10. The effect of a 15 min intrarenal infusion of VIP at a rate of 3.3 ng/kg/min on PRA in 7 dogs. * $p < 0.05$ compared to -1 min value.

FIGURE 10


60
50
40
30
20
10
PRA
(ng A I /ml/3 h)
3.3 ng/kg/min
(Intrarenal)
*
VIP
n=7
-10
0
10
20
30
40
50
60
Time (min)

(Table 5). Blood pressure and plasma potassium did not change.

Protocol 7. Propranolol pretreatment significantly decreased PRA (Fig. 11). Subsequent infusion of VIP resulted in a significant increase in PRA. Diastolic pressure increased with propranolol but decreased significantly after infusion of VIP. Systolic pressure did not change .

Isolation and Superfusion of Rat Glomeruli

Photomicrographs of the isolated glomeruli are shown in Plate 1. The preparation consisted of glomeruli with and without intact Bowman's capsules. Occasionally a fragment of an afferent or efferent arteriole could be seen attached to the glomerulus. Plate 1(C-D) shows immunostaining in two glomeruli using an anti-hog renin antiserum (provided by T. Inagami, Vanderbilt Medical School) at a dilution of 1:1,000.

Protocol 1. The effect of isoproterenol at 10^{-4}M and buffer alone on renin release from the glomerular preparation is shown in Fig. 12(A). These data have been standardized by setting the value immediately before addition of isoproterenol equal to zero on the ordinate. The other points depict the change from this initial value. The absolute values at the zero time point are shown in parentheses in the upper left hand portion of the figure. Isoproterenol at 10^{-4}M significantly increased renin release at 5 and 10 min. Buffer alone had no effect. The effect of two lower doses of isoproterenol is shown in Fig. 12(B). Doses of 10^{-5}M and 10^{-6}M did not significantly affect renin release.

Protocol 2. The effect of VIP at doses ranging from 10^{-10}M to

Table 5. Effect of intrarenal infusion of VIP (3.3 ng/kg/min) in 7 dogs.

Time (min)	Blood pressure systolic (mm Hg)	Blood pressure diastolic (mm Hg)	Plasma potassium (mEq/L)	Plasma VIP arterial (pmol/L)	Plasma VIP renal venous (pmol/L)
-1	184 ± 10	115 ± 7	4.03 ± 0.12	34 ± 5	34 ± 4
0	VIP, 3.3 ng/kg/min for 15 min				
10	184 ± 10	114 ± 7	3.98 ± 0.11	40 ± 6*	87 ± 10*
20	184 ± 10	114 ± 7	4.00 ± 0.10	35 ± 5	37 ± 6
30	181 ± 9	115 ± 7	4.06 ± 0.12	34 ± 3	34 ± 5
60	181 ± 9	116 ± 8	4.04 ± 0.12	34 ± 5	33 ± 5

*$p < 0.05$ compared to -1 min value, ANOVA

FIGURE 11. The effect of propranolol pretreatment and subsequent intravenous infusion of VIP (13 ng/kg/min) on PRA and blood pressure in 6 dogs. * $p < 0.05$ compared to 30 min value. ** $p < 0.05$ compared to -1 min value.

FIGURE 11

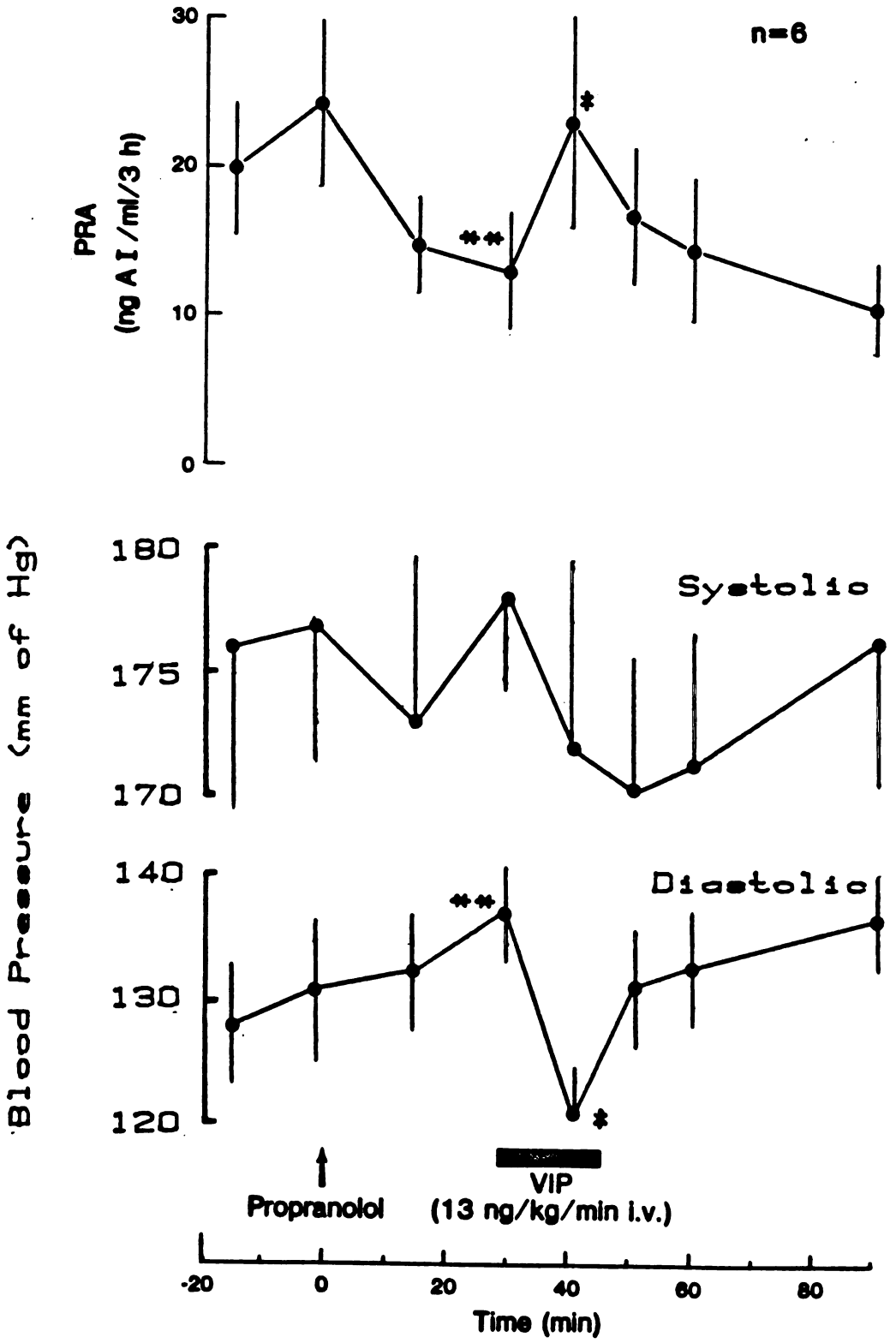
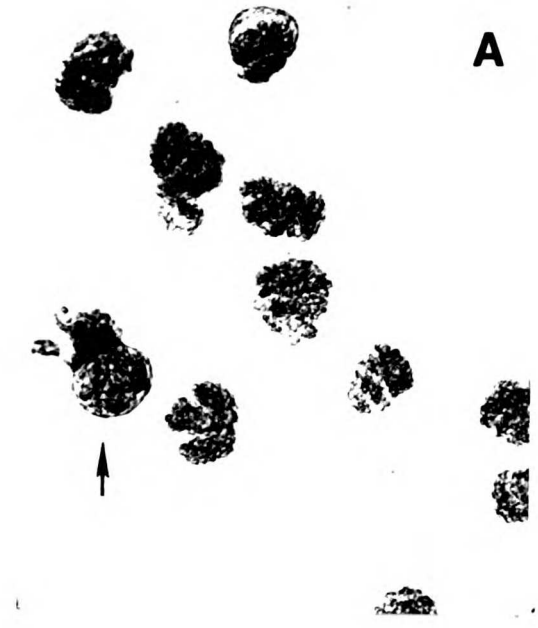

n=6
PRA
(ng A I /ml/3 h)
30
20
10
0
**
*
Blood Pressure (mm of Hg)
180
175
170
Systolic
140
130
120
Diastolic
**
*
Propranolol
VIP
(13 ng/kg/min i.v.)
-20
0
20
40
60
80
Time (min)

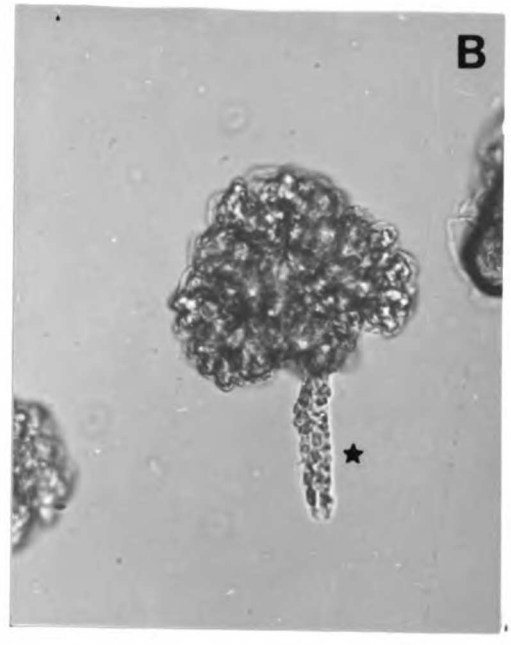
PLATE 1(A). Isolated rat glomeruli. Arrow points to intact Bowman's capsule. (X 115)

PLATE 1(B). Isolated rat glomerulus. Star indicates arteriolar fragment. (X 280)

PLATE 1(C-D). Isolated rat glomeruli stained with anti-hog renin antiserum (provided by T. Inagami, Vanderbilt Medical School) using the peroxidase-antiperoxidase technique. g = glomerulus, arrow points to immunoreaction. (X 520)

PLATE 1





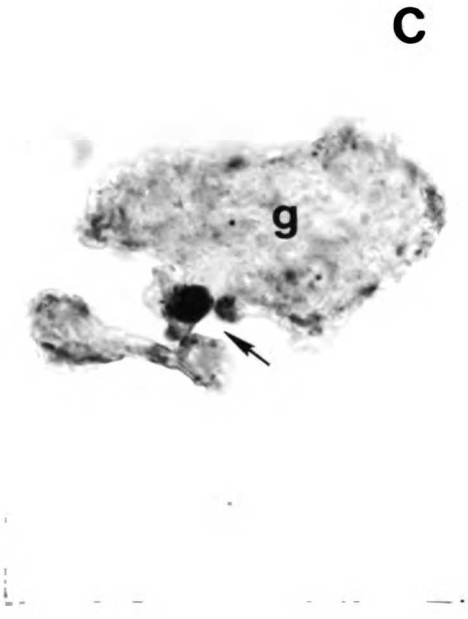
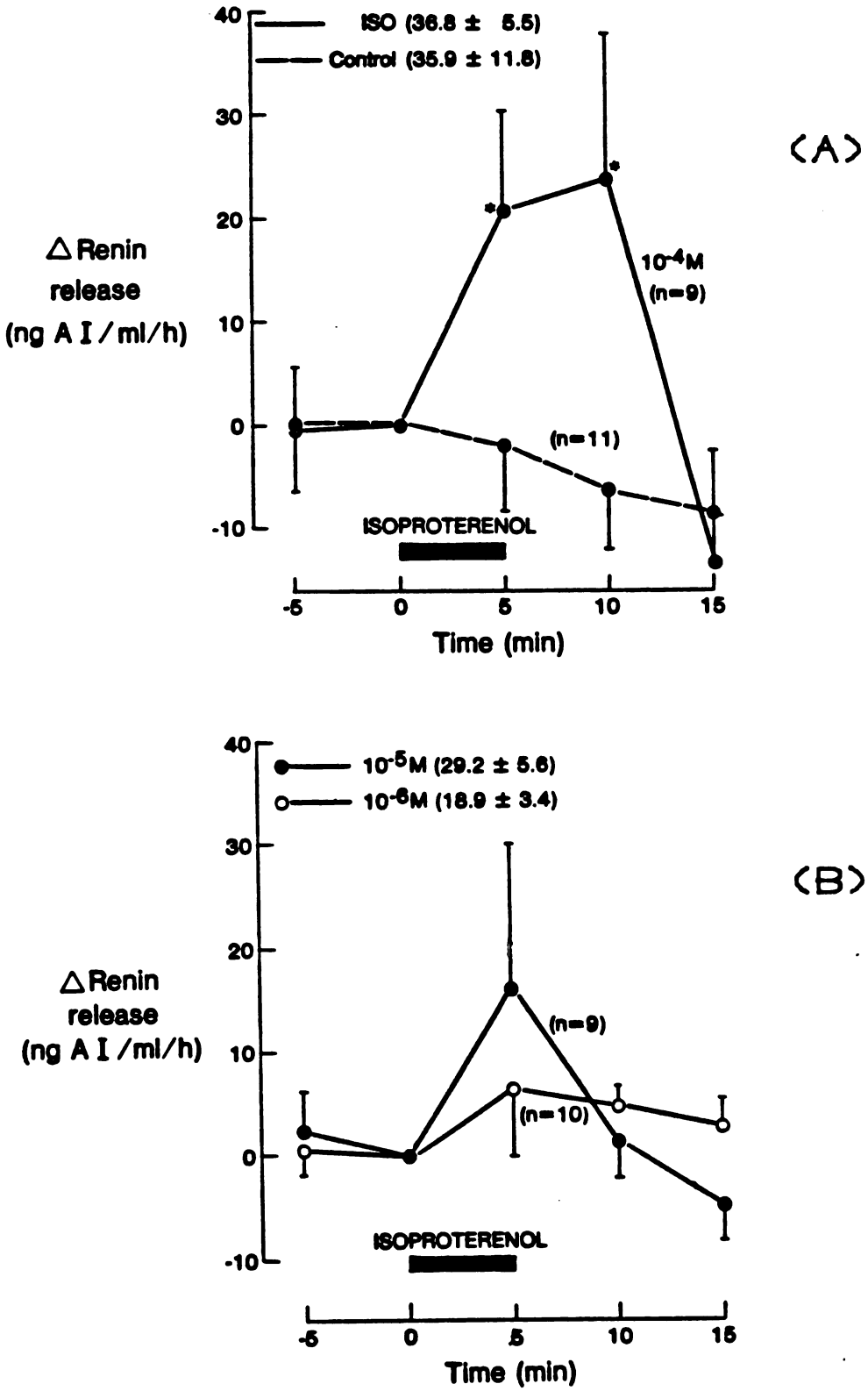


FIGURE 12(A). The effect of isoproterenol (10^{-4}M) and buffer alone on renin release from an isolated glomerular preparation. * $p < 0.05$ compared to -5 min and 0 min value.

FIGURE 12(B). The effect of isoproterenol (10^{-5}M and 10^{-6}M) on renin release from an isolated glomerular preparation.

FIGURE 12


ISO (36.8 ± 5.5)
Control (35.9 ± 11.8)
40
30
20
10
0
-10
△Renin release (ng A I / ml/h)
10-4M (n=9)
(n=11)
ISOPROTERENOL
-5
0
5
10
15
Time (min)
(A)
10-5M (29.2 ± 5.6)
10-6M (18.9 ± 3.4)
(n=9)
(n=10)
(B)

10^{-7}M is shown in Fig. 13. The three higher doses all increased renin release significantly at the 5 min time point. The lower dose was ineffective.

VIP in the Renal Nerve

Immunocytochemistry. The results of the immunocytochemical experiments are shown in Plates 2-5. The Zimmerman antiserum stained salivary tissue in a manner similar to that reported by others. VIP-like immunoreactive fibers could be seen along interlobar ducts and blood vessels although the walls of the blood vessels did not stain (Plate 2C). Local ganglion cell bodies stained intensly (Plate 2A) and preabsorption with 50 g/ml of VIP completely abolished this staining (Plate 2B). Plate 3 shows the results with innervated kidney sections processed at the same time as the salivary tissue. The Zimmerman antiserum showed a dark reaction in the walls of blood vessels including glomerular arterioles and juxtaglomerular cells (Plate 3A-C). This staining was distributed throughout the media layer of the arteriole and did not show the "beads-on-a-string" profile characteristic of neuronal staining. Preabsorption with VIP reduced the staining but did not abolish it. Surgical denervation did not affect this vascular staining (Plate 4A-C). The Said antiserum at a dilution of 1:500 and the Walsh antiserum at a dilution of 1:2,000 both stained salivary ganglion cells (Plate 5A,C). However, kidney tissue processed at the same time did not shown any immunoreactivity. Particularly, the blood vessels and juxtaglomerular cells did not stain (Plate 5B,D).

Renal Nerve Stimulation. Stimulation of the renal nerves resulted

FIGURE 13(A). The effect of VIP (10^{-7}M) on renin release from an isolated glomerular preparation. The dashed line is the control data redrawn from Fig. 12(A). * $p < 0.05$ compared to -5 min and 0 min value.

FIGURE 13(B). The effect of VIP (10^{-8}M, 10^{-9}M, and 10^{-10}M) on renin release from an isolated glomerular preparation. *$p < 0.05$ compared to -5 min and 0 min value.

FIGURE 13

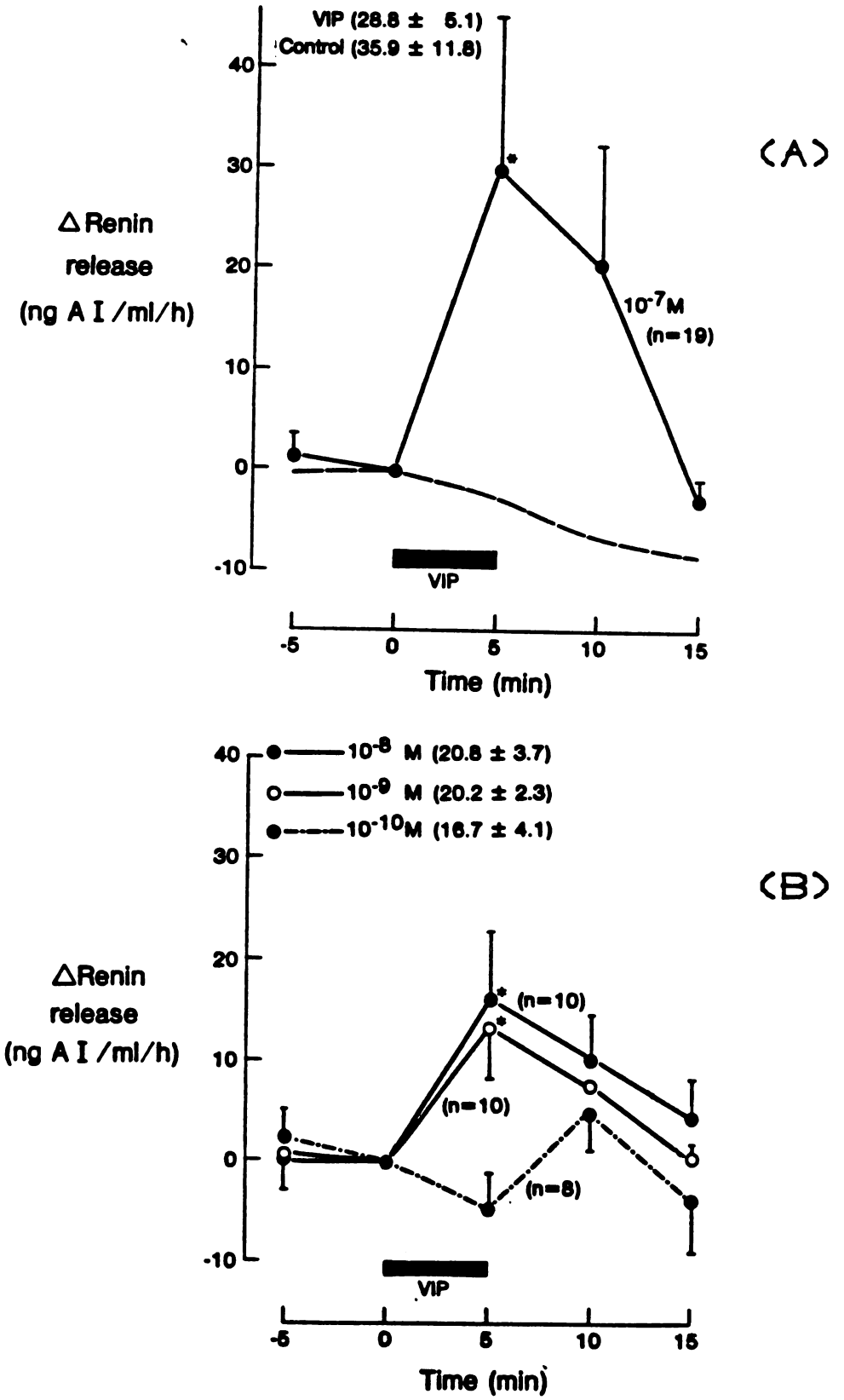
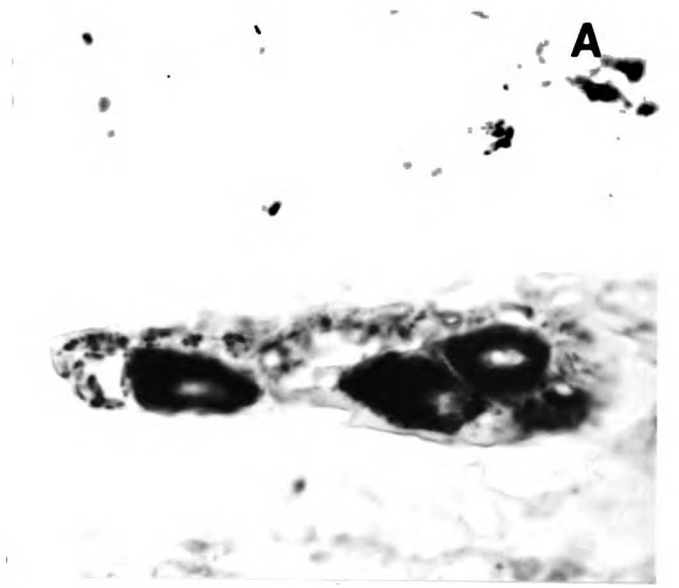

VIP (28.8 ± 5.1)
Control (35.9 ± 11.8)
(A)
△Renin release (ng A I /ml/h)
40
30
20
10
0
-10
10⁻⁷M (n=19)
VIP
-5
0
5
10
15
Time (min)
10⁻⁸ M (20.8 ± 3.7)
10⁻⁹ M (20.2 ± 2.3)
10⁻¹⁰M (16.7 ± 4.1)
(B)
△Renin release (ng A I /ml/h)
(n=10)
(n=10)
(n=8)
VIP
Time (min)

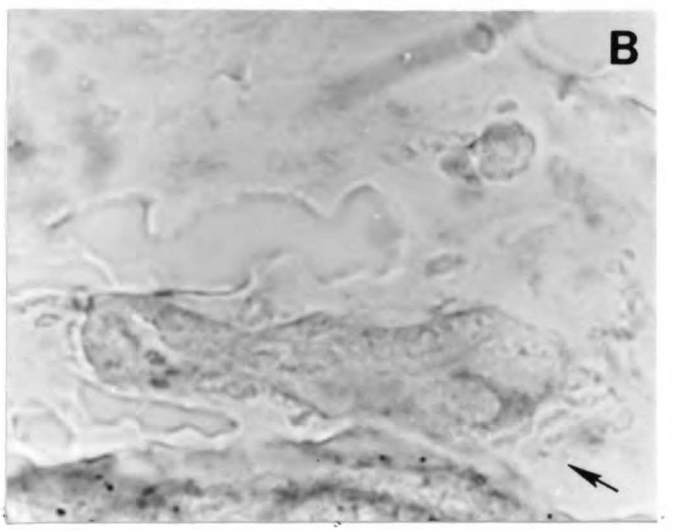
PLATE 2(A). Dog submandibular gland. Interlobar ganglion cells show intense immunoreactivity using Zimmerman VIP antiserum, 1:1800. (X 700)

PLATE 2(B). Adjacent section showing lack of immunostaining after preabsorption with 50 μg/ml VIP. Arrow points to ganglion.

PLATE 2(C). Arrow points to VIP-immunoreactive fiber running along blood vessel. star = blood vessel. (X 210).

PLATE 2





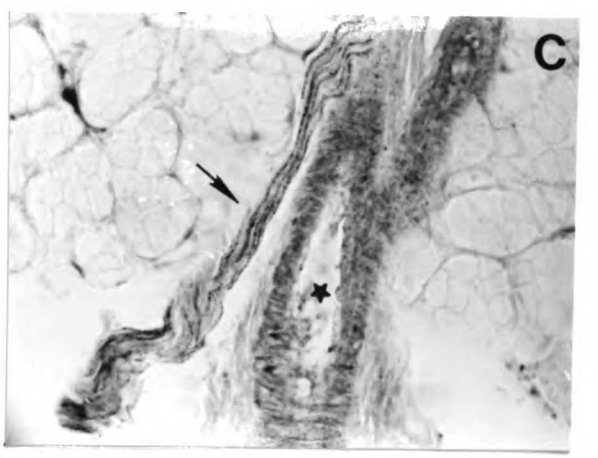
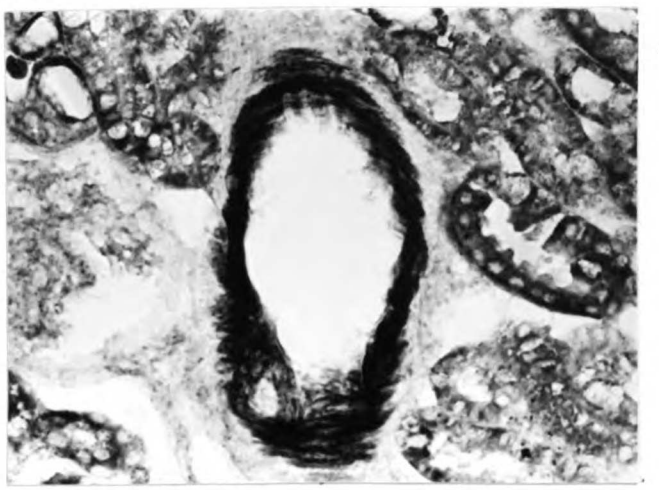


PLATE 3(A). Blood vessel from renal cortex stained with Zimmerman VIP antiserum, 1:1800. (X 250).

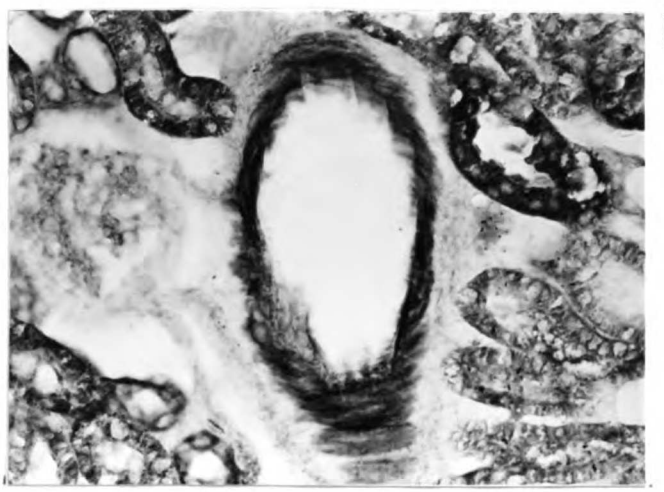
PLATE 3(B). Adjacent section treated with VIP antiserum which was preabsorbed with 50 μg/ml VIP.

PLATE 3(C). Adjacent section stained with normal rabbit serum, 1:1800.

PLATE 3



A



B

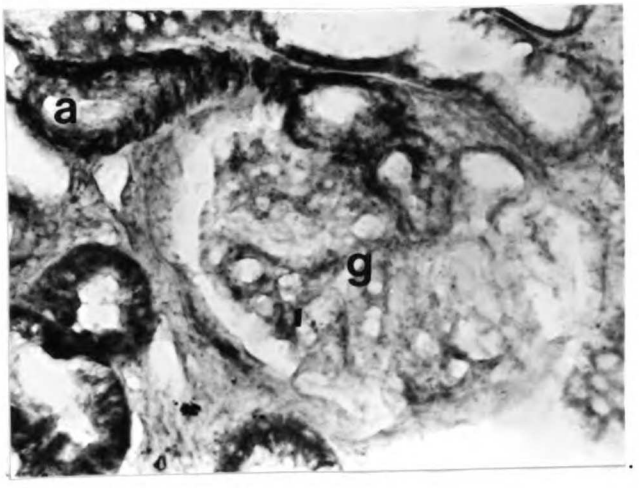
C

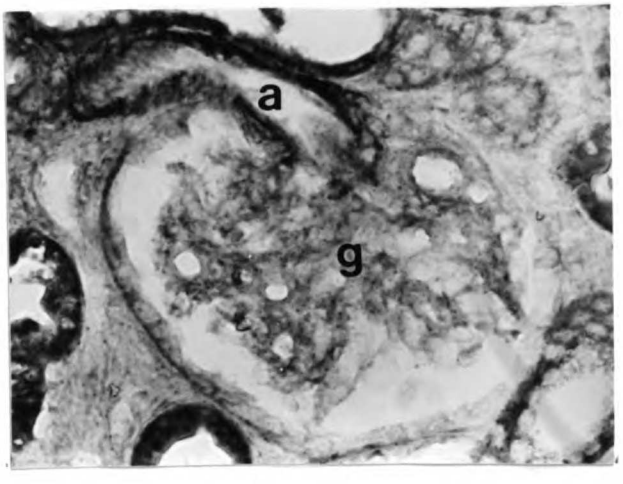
PLATE 4(A). Glomerular arteriole from denervated dog kidney stained with Zimmerman VIP antiserum, 1:1800. a = arteriole, g = glomerulus (X 340)

PLATE 4(B). Adjacent section treated with Zimmerman antiserum which was preabsorbed with 50 µg/ml VIP.

PLATE 4(C). Adjacent section stained with normal rabbit serum, 1:1800.

PLATE 4





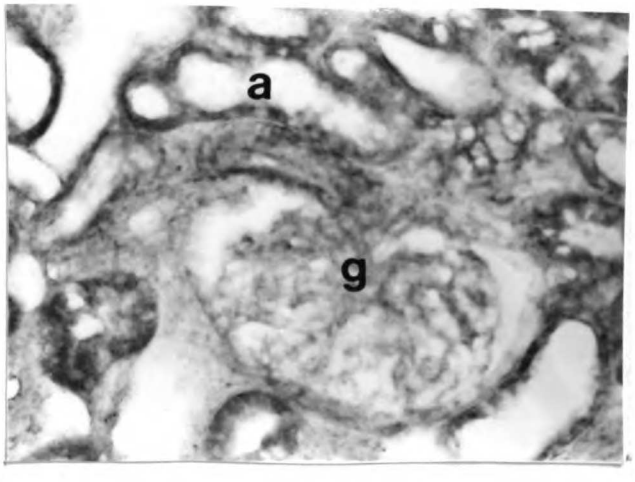


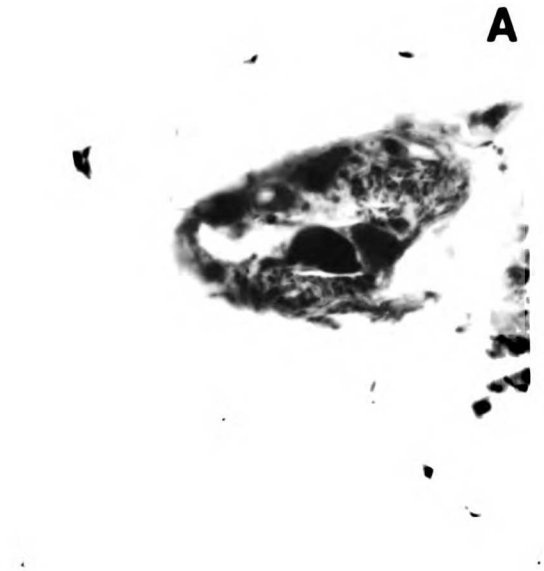
PLATE 5(A). Dog submandibular ganglion cells stained with Said VIP antiserum, 1:500. (X 400)

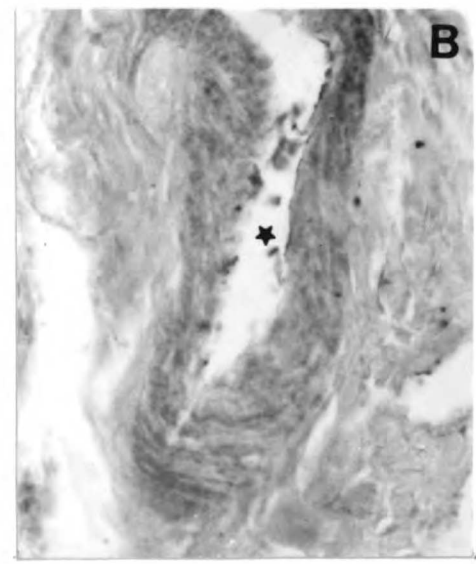
PLATE 5(B). Blood vessel from dog renal cortex treated with Said VIP antiserum, 1:500. star indicates blood vessel (X 240)

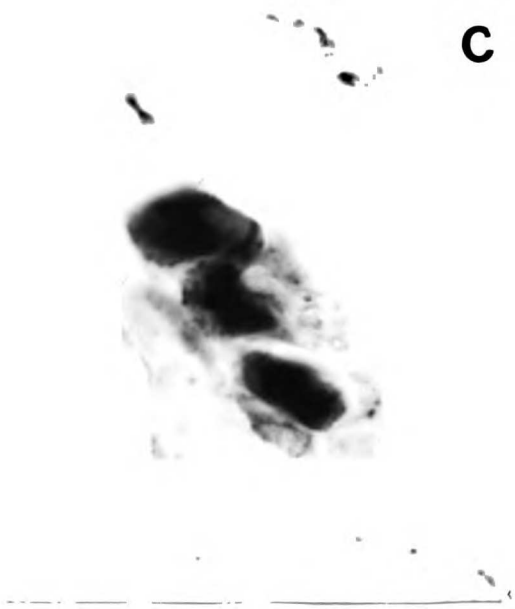
PLATE 5(C). Dog submandibular ganglion cells stained with Walsh VIP antiserum, 1:2000. (X 550)

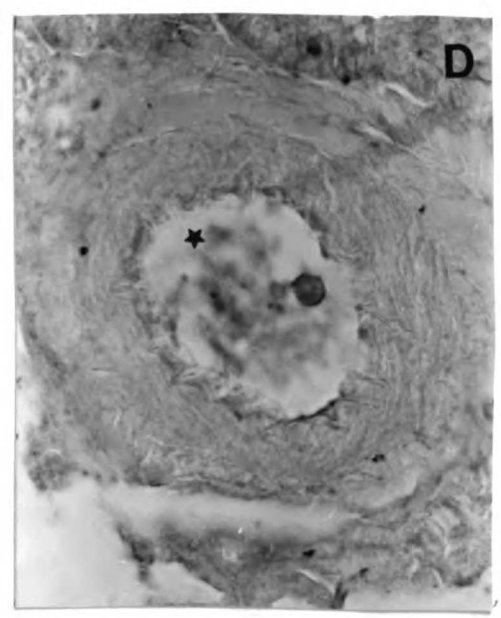
PLATE 5(D). Blood vessel from dog renal cortex treated with Walsh VIP antiserum, 1:2000. star indicates blood vessel (X 240)

PLATE 5









in a prompt increase in renal venous and arterial PRA (Fig. 14). The renal venous PRA was significantly elevated by 5 min and started to return toward control levels despite continued stimulation. The arterial PRA was also elevated by 5 min but stayed high for the duration of the stimulation period. Blood pressure increased transiently at the beginning of the stimulation. Neither renal venous or arterial levels of VIP were changed during the 30 min of stimulation.

Correlation of Plasma Levels of VIP and Renin

Neostigmine Infusion. Intravenous administration of neostigmine resulted in a prompt increase in systolic blood pressure which was maintained for 20 min (Table 6). Diastolic pressure was not significantly affected. PRA increased in four dogs but was unchanged in two, so the mean increase was not statistically significant. Plasma levels of VIP increased significantly by 10 min and remained elevated for 30 min. In the two dogs that did not show an increase in PRA, VIP levels increased from 14 pmole/L to 140 pmole/L and from 20 pmole/L to 43 pmole/L.

Low Salt Diet. Feeding dogs a low salt diet resulted in a significant decrease in sodium excretion by 24 hr which continued to decrease throughout the two weeks (Table 7). Potassium excretion did not change. PRA also began to rise by 24 hr but the increase was not significant until 5 days after the start of the low salt diet. The dogs which received the canned dog food, which contained 1.5 mEq of sodium, did not decrease their sodium excretion as much as those dogs on Lonalac, which contained less sodium, but the rise in renin was similar

FIGURE 14. The effect of renal nerve stimulation on PRA and plasma VIP in 4 anesthetized dogs. * $p < 0.05$ compared to -1 min value.

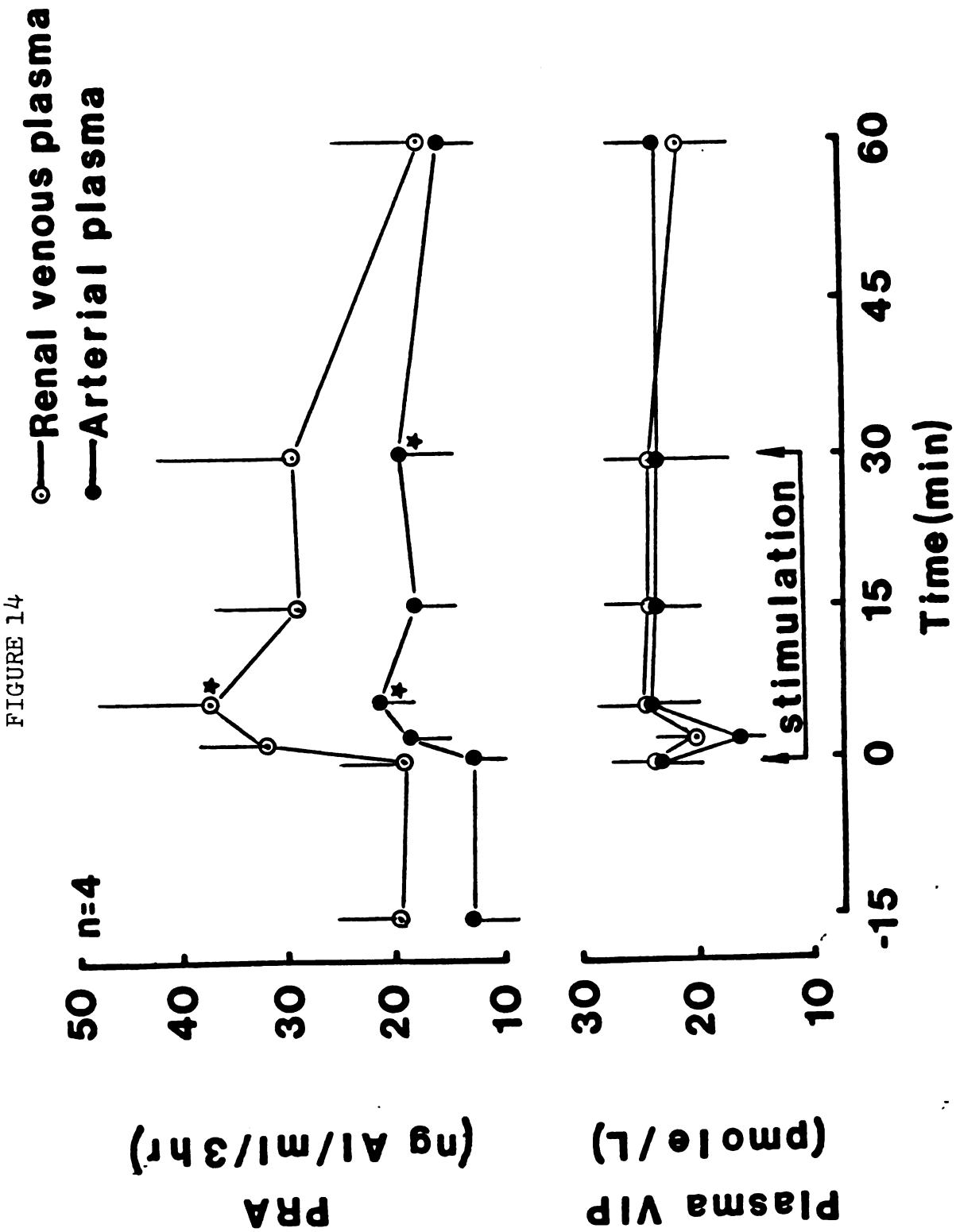

Renal venous plasma
Arterial plasma
n=4
PRA
(ng AI/ml/3hr)
50
40
30
20
10
Plasma VIP
(pmole/L)
30
20
10
stimulation
-15
0
15
30
45
60
Time(min)

FIGURE 14

Table 6. Effect of intravenous neostigmine (0.07 mg/kg) in 6 anesthetized dogs.

Time (min)	Blood pressure systolic (mm Hg)	Blood pressure diastolic (mm Hg)	Plasma renin activity (ng AI/ml/3h)	Plasma VIP (pmol/L)
-20	194 ± 4	135 ± 6	25.1 ± 5.1	--
-1	198 ± 4	142 ± 8	29.9 ± 1.9	15 ± 2
0				
10	223 ± 10*	142 ± 9	49.9 ± 12.9	63 ± 12*
20	219 ± 9*	141 ± 9	46.0 ± 10.4	88 ± 23*
30	210 ± 8	142 ± 10	47.5 ± 11.9	86 ± 33*
60	210 ± 8	143 ± 9	41.6 ± 11.8	51 ± 19

*$p < 0.05$ compared to -1 min value

Table 7. Effect of 14 days of dietary sodium restriction on 6 dogs.

Day	Sodium intake (mEq/day)	Sodium excretion (mEq/day)	Potassium excretion (mEq/day)	Plasma renin activity (ng AI/ml/3h)	Plasma VIP (pmol/L)
-4	28.6 ± 3.7	25.7 ± 8.3	25.9 ± 5.7	4.9 ± 1.2	29 ± 4
-2	28.6 ± 3.7	29.3 ± 3.0	29.5 ± 5.3	4.1 ± 1.0	33 ± 2
0	0 - 1.5	30.1 ± 3.4	28.4 ± 0.7	3.4 ± 0.8	38 ± 3
1	0 - 1.5	9.0 ± 3.8*	22.3 ± 7.8	5.2 ± 1.7	33 ± 6
3	0 - 1.5	3.2 ± 1.0*	22.2 ± 3.2	5.5 ± 1.2	34 ± 6
5	0 - 1.5	1.9 ± 0.5*	26.3 ± 3.2	8.2 ± 2.7*	38 ± 5
7	0 - 1.5	1.7 ± 0.7*	26.1 ± 4.4	8.6 ± 1.3*	33 ± 4
9	0 - 1.5	1.5 ± 0.6*	18.5 ± 5.7	5.2 ± 0.9	26 ± 2
11	0 - 1.5	1.4 ± 0.7*	27.8 ± 11.4	7.4 ± 1.5*	27 ± 2
14	0 - 1.5	0.9 ± 0.4*	22.9 ± 2.0	8.0 ± 0.9*	32 ± 3

*$p < 0.05$ compared to day 0, ANOVA

so all six dogs were combined for the statistical analysis. There was some day to day variation in resting levels of VIP, but sodium depletion did not produce a statistically significant change.

DISCUSSION

Mechanism by which VIP Stimulates Renin Secretion

In the present investigation the renin-stimulating effect of VIP has been confirmed and extended.

VIP could increase release of renin by a number of different mechanisms. Since the peptide is vasodilatory, it could decrease blood pressure and stimulate renin secretion via the intrarenal baroreceptor. Likewise, a fall in systemic blood pressure could lead to a reflex increase in sympathetic output which also could result in an increase in renin. VIP could also influence renin via the macula densa mechanism since it has effects on the transport of sodium and chloride. Finally, the peptide could act directly on the juxtaglomerular cells to increase renin secretion. In the present investigation the possibility that each of these mechanisms is responsible for the increase in renin with VIP was examined separately.

The preliminary observation that VIP increased PRA in anesthetized dogs provided the first evidence that the baroreceptor is not required. In two of the original four dogs, VIP injection increased PRA even though renal perfusion pressure was held constant. In the present work, an intrarenal infusion of VIP at 33 ng/kg/min stimulated renin secretion without changing systolic blood pressure and with only a 5 mm Hg decrease in distolic pressure (Fig. 2 and Fig. 4). It is unlikely that such a small decrease in diastolic pressure could lead to a significant

increase in renin secretion which would be mediated by the intrarenal baroreceptor. Also lower doses of VIP increased renin without affecting blood pressure (Fig. 9 and Fig. 10). VIP caused marked renal vasodilation (Fig. 4). As mentioned previously, an increase in arteriolar diameter in the presence of constant perfusion pressure should inhibit renin rather that stimulate it by increasing the wall tension in the baroreceptor (Fray, 1976). Substance P, another vasoactive peptide, has been reported to cause renal vasodilation but resulted in an inhibition of renin secretion (Gullner et al., 1980). It seems unlikely, therefore, that VIP has its effect on renin release via the intrarenal baroreceptor.

Several problems with the intrarenal infusion studies need to be considered further. Infusion of 0.9% saline into the renal artery did not significantly affect renin secretion rate (Fig. 5), but there is considerable variation in the data. Since renin secretion rate is a calculated value it is subject to compounding errors resulting from measurement of renal blood flow and PRA in both renal venous and arterial plasma. The large standard errors at the -1 min and 30 min time points are due to high values in one dog.

Comparison of renal blood flow values shows that the mean control levels in the VIP-infused dogs were much higher than the saline controls (Fig. 4 vs Fig. 6). The difference can partly be explained by the fact that the VIP-infused dogs were larger than the control animals, since renal blood flow increases with body size.

It is difficult to determine the role played by the macula densa in situations where renin secretion is increased since changes in sodium chloride delivery to that region of the distal tubule cannot be directly

measured. An indirect method for looking at the involvement of the macula densa is to measure urinary sodium excretion. An increase in sodium excretion is usually associated with a decrease in renin secretion (Nash et al., 1968). In the present investigation, intrarenal infusion of VIP caused a slight increase in glomerular filtration rate, but sodium and potassium excretion were not significantly affected (Table 2). It has been reported that in the isolated perfused rat kidney VIP caused a significant natriuresis (Rosa et al., 1977) and in other organs it prevents sodium chloride uptake (Krejs, 1982). It appears therefore, that if VIP does anything to sodium chloride handling in the kidney it would result in an increase in sodium delivery to the macula densa which should inhibit renin release rather than stimulate it. However, if VIP inhibits sodium chloride uptake in the macula densa itself, renin secretion could be stimulated.

A relfex increase in sympathetic activity probably occurred with the intravenous infusion of VIP at 13 ng/kg/min. This because diastolic blood pressure fell during the infusion of the peptide after, but not before beta-adrenergic blockade with propranolol (Fig. 11). Heart rate was not measured in these experiments, but a positive chronotrophic effect of VIP has been reported by others (Smitherman et al., 1982). However, this small increase in sympathetic output was not responsible for the increse in PRA via a beta-adrenergic mechanism since infusion of VIP after propranolol pretreatment still resulted in a significant increase in renin (Fig. 11).

It is difficult to compare the effect of intravenous infusion of VIP on PRA after propranolol pretreatment to VIP infusion alone. The levels of PRA before VIP in the dogs which did not receive propranolol

were four times higher than those in the propranolol-treated dogs (Fig. 9 and Fig. 11). This is partly due to the fact that propranolol decreased the resting levels of PRA, but even the PRA values before propranolol were 1/3 those of the non-pretreated dogs. The discrepancy in control PRA levels is unexplained, although we routinely see a wide variation in PRA in anesthetized dogs. Nevertheless, in both the propranolol pretreated and the untreated animals, intravenous infusion of VIP resulted in approximately a doubling in PRA.

An unexpected complicating observation made during the renal function study was the significant decrease in plasma potassium concentration (Table 2). The decrease seen with intrarenal infusion of VIP at a rate of 33 ng/kg/min was about 0.75 mEq/L. The mechanism by which VIP lowers plasma potssium is not known. VIP is known to stimulate insulin secretion (Kaneto et al., 1977) and insulin can produce hypokalemia (Cooke et al., 1973). Beta-adrenergic stimulation has also been shown to cause a decrease in plasma potassium, probably by increasing muscular uptake of the electrolyte (Todd and Vick, 1971; Clausen and Flatman, 1977). This effect is probably mediated by cyclic AMP because theophylline potentiated the epinephrine-stimulated uptake of potassium by rat soleus muscle (Clausen and Flatman, 1977). Since VIP is known to increase cyclic AMP in many situations, it is possible that the potassium-lowering effect of the peptide is mediated by this cyclic nucleotide. Others have reported that a prolonged infusion of VIP in pigs also decreased plasma potassium levels (Modlin et al., 1978b). It is known that an acute decrease in plasma potassium of only 0.3 mEq/L can stimulate renin secretion (Himathongkam et al., 1975). However, infusion of VIP at lower doses increased PRA without affecting

plasma potassium (Table 4 and 5), so it appears that the effect of VIP on renin is not solely mediated by changes in this electrolyte.

The studies in anesthetized dogs suggest that VIP has an action directly on the kidney. Intravenous infusion of the peptide at a rate of 3.3 ng/kg/min did not affect PRA (Fig. 9), but direct intrarenal infusion at the same dose did significantly increase PRA (Fig. 10). The renal venous level fo VIP produced during this infusion, 87 $\pm$ 10 pmole/L (Table 5) is similar to the systemic level of the peptide produced during intravenous infusion at a rate of 13 ng/kg/min (Table 4), which was the minimum effective intravenous dose. It appears that circulating VIP has to increase to about 2 1/2 times resting levels before renin release is significantly increased.

It is not clear why the intrarenal infusion at 3.3 ng/kg/min did not affect PRA until five minutes after the end of the infusion. However, examination of all the data shows that the time course of the effect of VIP on renin is not consistent. Intrarenal infusion at 33 ng/kg/min increased renin secretion rate only transiently (Fig. 3). The transient nature of the effect of VIP has been reported by others. For example, the salivary vasodilation resulting from VIP infusion is also transient (Lundberg, 1980b). Intravenous infusion of VIP at 13 ng/kg/min increased PRA at 10 and 20 min after the start of the infusion (Fig. 9) while infusion at 33 ng/kg/min increased renin only at the 10 min time point. This may have been due to the development of tachyphylaxis since the higher dose was always given after the lower dose.

The data with the isolated glomerular preparation suggest that VIP can act directly on the juxtaglomerular cells. In this preparation, the

intrarenal baroreceptor, the macula densa, and the sympathetic system are presumably nonfunctional. VIP, at a dose range similar to that reported for other effects of the peptide, significantly elevated renin release (Fig. 13). The effect was short lived, as it was seen only during the 5 min that VIP was in the superfusion buffer. Isoproterenol at a dose of 10^{-4}M was required to increase renin release in this preparation. It is not apparent why such a large concentration was required. However, others who have worked with isolated glomeruli report similar minimum effective doses (Morris, 1976; Beierwaltes et al, 1980). For some reason the beta-adrenergic receptor is not very sensitive after this isolation procedure. Nevertheless, the data with isoproterenol show that the glomerular preparation used in the present investigation is viable and capable of responding to a known stimulus.

In summary, VIP is a renin-stimulating factor. A 2 1/2 to 3 fold increase in circulating levels of the peptide can bring about this effect. Since renin increases with VIP when renal perfusion pressure is held constant, and without a change in blood pressure, it appears that the effect is not mediated via the intrarenal baroreceptor. Since renin increased with VIP without a change in sodium excretion the macula densa is probably not involved. Since VIP can stimulate renin secretion after pretreatment with propranolol, beta-adrenergic receptors do not mediate the effect of this peptide on renin secretion. The action of the peptide appears to be on the kidney, since intravenous infusion of VIP at a rate of 3.3 ng/kg/min did not affect PRA, but intrarenal infusion at the same dose significantly increased renin release. Finally, since VIP can stimulate release of renin from an isolated glomerular perparation which lacks a functional baroreceptor, macula densa, or

nervous system, it appears that one way the peptide acts to increase renin release is by a direct action on the juxtaglomerular cells.

Role of the Renal Nerves

Because VIP is present in neurons it was attractive to hypothesize that VIP was present in the renal nerves and involved in the neural regulation of renin secretion. While the data in the present study do not definitely rule out this possibility, they suggest that the nerves are not involved. Using three different VIP antisera, all of which stained neural elements in salivary glands, no definite VIP immunoreactivity could be detected in the renal cortex associated with the juxtaglomerular arterioles. The dark staining of the blood vessels with the Zimmerman antiserum was probably nonspecific. Preabsorption with VIP completely eliminated the staining in salivary tissue but only partially reduced the reaction seen in the renal vessels. Surgical denervation had no effect on this vascular staining, although if VIP is present in cell bodies in local renal ganglia, denervation would not be expected to abolish the staining. The absence of VIP immunoreactivity in the kidney of cats, rats, and guinea pigs has been reported by others (Alm et al., 1980), although Hokfelt et al. (1978) reported a sparse innervation of VIP-containing fibers in the guinea pig kidney.

It is presently impossible to measure direct release of VIP from nerve terminals and only indirect methods can be used. Simulation of VIPergic nerves that innervate several exocrine glands and gastrointestinal regions is known to result in an increase in VIP in the venous plasma leaving these regions suggesting neural release

(Fahrenkrug, 1982). In the case of the kidney, stimulation of the renal nerve has been shown to result in a prompt increase in renal venous norepinephrine levels (Oliver et al., 1980). In the present investigation, stimulation of the renal nerves with one set of parameters resulted in a clear increase in renin release but in no case did renal venous levels of VIP increase. It is unlikely that a transient increase was missed since the first blood sample was collected only one minute after the start of the stimulation. This suggests that VIP is not released during renal nerve stimulation. The possibility remains that stimulation with different parameters would release VIP although the parameters used in the present study were similar to those used in other stimulation studies where VIP release occurred (Fahrenkrug, 1982).

VIP levels were measured in this and subsequent experiments using a commercial radioimmunoassay kit. Since addition to plasma of different dilutions of purified porcine VIP and also synthetic VIP resulted in an average recovery of 86% it seems likely that the antiserum provided in the kit was indeed measuring VIP. The baseline levels of VIP measured with the kit were similar to levels measured in other dogs by Dr. Said. Likewise, circulating levels of VIP measured by the kit were increased by neostigmine to a comparable degree to those measured in other dogs with a different radioimmunoassay (Ebeid et al., 1979).

Taken together, the data in the present investigation suggest that VIP does not influence renin secretion via release from renal nerves. VIP does not appear to be present in neurons innervating the renal cortex and stimulation of the renal nerve does not result in an increase in renal venous VIP levels.

Correlation of Plasma Levels of VIP and Renin

One approach to discovering situations in which VIP might act physiologically as a renin-stimulating factor is to determine if plasma levels of renin are increased in cases where VIP secretion is stimulated and vice versa. While a positive correlation between VIP and renin cannot prove a cause-and-effect relationship it would strengthen the hypothesis that circulating VIP influences renin release.

One stimulus to VIP secretion is intravenous infusion of neostigmine, a drug that inhibits acetylcholinesterase. Neostigmine presumably increases cholinergic transmission to the gut and exocrine glands which results in release of VIP into the circulation. In the present study, neostigmine resulted in a marked increase in plasma levels of VIP. PRA was increased in 4 of 6 dogs but the increase was not significant. The elevated VIP could have contributed to the increase in PRA in these four dogs. Alternatively, a generalized increase in sympathetic output might have been responsible. Physostigmine, another anticholinesterase, has also been shown to result in an increase in PRA, but this was blocked by propranolol (Alexandre et al., 1970).

In the two dogs that did not show an increase in PRA, the circulating levels of VIP increased from 15-20 pmole/L to 43-140 pmole/L. It is interesting that in these two dogs blood pressure also failed to rise. The reason for the lack of a renin response in these dogs despite elevated VIP is unexplained but suggests that a cause-and-effect relationship between VIP and renin may not exist in this situation.

The renin response to a low salt diet is one situation in which an unknown renin-stimulating humoral factor could be involved. Brubacher and Vander (1968) found that renin increased with sodium restriction in renal denervated dogs before there was any change in glomerular filtration rate or blood pressure. This led Vander (1967) to hypothesize the existence of a renin-stimulating factor which he called "hormone X". In the present experiment, dietary sodium restriction resulted in an increase in plasma renin activity. However, plasma levels of VIP did not change significantly. Plasma VIP tended to be quite variable from day to day, but sodium depletion did not increase it significantly. A greater degree of sodium depletion brought about by addition of a diuretic, which is known to cause even greater increases in PRA, might elevate plasma levels of VIP significantly, but based on the present evidence it can be concluded that VIP is probably not "hormone X".

Physiologic Significance of the Renin-Stimulating Effect of VIP

A physiologic role for VIP in regulating renin secretion was not established in the present investigation. VIP does not appear to be present in neurons innervating the juxtaglomerular cells and is not released into the renal venous plasma during renal nerve stimulation. It therefore seems unlikely that VIP is involved in renin responses that are brought about by the renal nerves. VIP also does not appear to play a role in the increase in PRA during sodium depletion. When plasma VIP was elevated by neostigmine to levels comparable to those which stimulated renin release after infusions of exogenous VIP, PRA increased

in 4 of 6 dogs although the mean increase was not significant. Other factors could have been responsible for the increase in PRA in these four dogs.

Therefore, in situations investigated at so far, there does not appear to be a phsyiologic relationship between VIP and renin secretion. It may be that stimulation of renin secretion by VIP is a pharmacologic rather than a physiologic phenomenon. However, there are other situations in which renin secretion is elevated such as hemorrhage, upright tilt, and thoracic caval contriction where circulating levels of VIP have not been measured. It may be that VIP plays a role in mediating these effects. Endotoxin shock has been reported to increase circulating VIP to levels which should stimulate renin secretion (Freund et al., 1981). It would be interesting to see if renin secretion changes in this situation. Likewise, patients with the watery diarrhea syndrome have very high circulating levels of VIP, but PRA values have not been reported in these patients.

SUMMARY AND CONCLUSION

VIP stimulates renin secretion *in vivo* in anesthetized dogs and *in vitro* in a preparation of isolated superfused glomeruli. This effect may result from a direct action of the peptide on the juxtaglomerular cells and can occur independently of the intrarenal baroreceptor, the macula densa, and the sympathetic nervous system. A two to threefold increase in circulating levels of VIP is needed to bring about this increase in renin secretion.

Using three different antisera to VIP, no specific VIP immunoreactivity could be detected in the renal cortex even though all three antisera stained neural elements in the salivary gland. Stimulation of the renal nerves resulted in a prompt increase in renin secretion but the renal venous levels of VIP did not change. Taken together, these data do not support the hypothesis that VIP is present in the renal nerves and is released in situations where there are neurally mediated increases in renin secretion.

A reduced sodium intake for two weeks also resulted in an increase in PRA but circulating VIP did not change significantly. Therefore, VIP is not likely to be a humoral factor involved in the renin response to sodium restriction.

Neostigmine administration in anesthetized dogs produced a significant increase in circulating levels of VIP. PRA did not increase significantly, but four of the six dogs did show an appreciable rise. It is not known if the elevated levels of VIP contributed to the rise in

renin in these four dogs since other factors might have been involved. Since two dogs did not increase PRA despite elevated levels of VIP it is uncertain whether a cause-and-effect relationship exists between VIP and PRA in this situation.

There are other factors in which VIP could possibly function physiologically or pathophysiologically as a renin stimulating factor. Further work will be required to determine in which, if any, of these situations VIP plays a role in the regulation of renin secretion.

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