1980

The Effect of Pimozide and Apomorphine on Dopamine-Induced Renin Release, Tissue Renin Content, and 3'5'-Adenosine Monophosphate Levels in Rat Venal Cortical Slices

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by

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

April

1980

ACKNOWLEDGMENTS

I would like to express my heartfelt thanks to Dr. G.A. Lopez for his encouragement, advice and guidance during the course of my graduate research. Thanks are also due to the members of my Committee, Drs. A.J. Rotermund and J.J. Peluso for their valuable advice. My fellow graduate students, Suzanne M. Mottel and Brett Sheppard are also thanked for their support and friendship. My sincere appreciation is extended to Dr. N.J. Dun, Department of Pharmacology, Stritch School of Medicine of Loyola University for the donation of apomorphine, Dr. R. Gallo, Department of Physiology, University of California for supplying pimozide and Dr. A. Krause, Argonne National Labs for the use of Hewlett-Packard Programmable calculator.

Finally I would like to thank the members of my family for their love and understanding. For the technical assistance in the preparation of this manuscript, I would like to thank my sister, Usha.

VITA

The author, Visalakshi Rao, is the daughter of Yedavalli Shyamsunder Rao and Kameswari (Veluri) Rao. She was born on January 2, 1957 in Secunderabad, India.

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She is the author or co-author of a number of research papers:

Rao, V., Evidence for a Dopamine-Receptor Mediated Inhibition of Renin Release. Sigma Xi Graduate Student Research Forum, Loyola University Stritch School of Medicine, 1980.

Rao, V., S. Mottel, B. Sheppard, G.A. Lopez, Evidence for a Dopamine-Receptor Mediated Inhibition of Renin Release, Illinois State Academy of Science, 1980.

Lopez, G.A., S. Mottel, V. Rao, B. Sheppard, Further Evidence for Cyclic AMP Mediation of the Stimula-

tory Effect of Dopamine on Renin Release In Vitro, Fed. Proc., 39: 827, 1980.

Lopez, G.A., S. Mottel, V. Rao, B. Sheppard, Evidence for a Dual Regulatory Mechanism Mediating the Effect of Dopamine on Renin Release in the Sodium Deficient Rat, Proc. Soc. Exp. Biol. Med., (submitted for publication).

Lopez, G.A., V. Rao, S. Mottel, and B. Sheppard, Stimulation of Renin Release by Pimozide Via a Generalized Membrane Effect Involving Inactivation of Adenylate Cyclase in Rat Kidney Slices, Life Sciences (submitted for publication).

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CHAPTER I

INTRODUCTION

In vitro studies in our laboratory (1,2) and elsewhere (3,4) have shown that dopamine can directly stimulate renin release from the mammalian renal juxtaglomerular cells and that this effect appears to be mediated by a beta-adrenergic receptor mechanism involving increases in tissue cyclic AMP levels (1,2). We have also evaluated the possibility that dopamine-specific receptors may participate in the regulation of renin secretion (1,2). In these studies, the dopamine-receptor blocking agent, pimozide, significantly stimulated renin secretion from renal cortical slices of sodium deficient rats. This marked increase in renin secretion occured whether or not dopamine was added in conjunction with this agent, suggesting that the stimulatory effect is due to pimozide itself. Furthermore, the observed increase in renin secretion in response to various doses of pimozide was coupled with a significant decrease in tissue cyclic AMP content after a one hour incubation period. These data suggest an inhibitory action of dopamine or renin release which is prevented by administration of pimozide. Conversely, these results could reflect a generalized effect of pimozide

on the juxtaglomerular cell membrane resulting in uncontrolled release of pre-formed renin. Clearly, further studies are required in order to fully evaluate the type of mechanism by which pimozide itself influences renin secretion, and to further examine the possibility that dopaminergic receptors may participate in the regulation of renin release.

In vitro renal tissue preparations are particularly useful in the examination of specific factors which may directly affect renin release since they effectively eliminate the action of other factors known to influence renin release in the live animal. Furthermore, in vivo (5-8) and in vitro studies (1,2,9-11) have shown that both renin secretion and the renin secretory responses to sympathetic stimuli, are potentiated by dietary sodium deprivation. Thus, a renal cortical slice preparation from sodium deficient rats which maximizes juxtaglomerular cell responsiveness, is an effective tool for the evaluation of the mechanisms by which isolated factors regulate renin secretion.

The present study utilizes a hypersensitive renal cortical slice system from the sodium deficient rat to: 1) determine if the stimulatory effect of dopamine on renin secretion involves the release of pre-formed renin and/or de novo synthesis of renin, by examining both secretory and tissue renin content changes in response to dopamine administration at various time intervals; 2) further evaluate the possible role of dopaminergic receptors in the regulation of renin release by utilizing the dopamine-receptor

agonist, apomorphine; 3) provide additional evidence for a possible mechanism by which pimozide itself may affect renin release by examining the changes in tissue renin content, tissue cyclic AMP levels, and renin secretion rate with time, in response to administration of this agent to the renal cortical slice system and; 4) further investigate the role of cyclic AMP as an intracellular mediator by determining if tissue content responses of this nucleotide are coupled with changes in tissue renin content and renin release.

Portions of these data have been reported in preliminary form (12).

CHAPTER II

LITERATURE REVIEW

A. Renin-Angiotensin-Aldosterone Regulation of Electrolyte and Blood Pressure Homeostasis

Renin, a glycoprotein (13) synthesized in the juxtaglomerular cells of the kidney, is normally secreted into the systemic circulation where it cleaves a hepatic alpha$_2$-globulin angiotensinogen (renin substrate) to form the decapeptide angiotensin I (13). Angiotensin I is further split by the action of a circulating converting enzyme to yield the powerful vasoconstricting agent angiotensin II (13). Angiotensinase enzymes present in the systemic circulation in turn inactivate angiotensin II to produce metabolic by-products which possess little or no vasoconstrictor activity and, are normally excreted in the urine (13). In addition to its metabolic fragment angiotensin III, can exert an indirect regulatory effect on blood volume. Both angiotensin II and angiotensin III infusions stimulate synthesis and secretion of aldosterone and antidiuretic hormone (ADH), thereby effecting electrolyte and blood volume homeostasis (14-

16). Thus, renin, through its effect on angiotensin II and aldosterone production, is an important factor in the maintenance of electrolyte, blood pressure and blood volume homeostasis.

B. Control of Renin Secretion

It is well established that renin release in the live animal, is under the simultaneous influence of a variety of factors. These factors include: 1) intrarenal receptors, such as the afferent arteriole baroreceptor and the macula densa natrioreceptor; 2) humoral factors such as ADH, angiotensin II, estrogen, and intrarenal prostaglandins; 3) central nervous system pathways originating in the vasomotor centers of the medulla oblongata and the hypothalamus; and 4) peripheral sympathetic neural mechanisms which are mediated by the renal nerves and circulating catecholamines from the adrenal medulla.

1. Intrarenal Receptors

a. Afferent Arteriole Baroreceptor Control of Renin Release

The renin-secreting juxtaglomerular cells are modified smooth muscle cells and as such are sensitive to stretch. Tobian (17) and Skinner (18) first proposed the existence of a renal perfusion pressure, and a number of subsequent studies have confirmed their findings by demonstrating that al-

terations in renal blood flow and perfusion pressure can modify renin release. Utilizing the non-filtering, denervated kidney in the dog, Blaine and co-workers (19,20) have shown that the influence of the baroreceptor mechanism can be fully exerted in the absence of a functional macula densa and the renal nerves, suggesting that this pathway can operate independently of the other factors which affect renin secretion. Conversely, infusion of the smooth muscle relaxing agent, papaverine into the renal artery of the non-filtering dog kidney (21), have been shown to block the secretory responses to hemorrhage, further supporting the existence of a vascular baroreceptor mechanism, participating in the regulation of renin release.

b. Macula Densa Control of Renin Release

The close anatomical association between the macula densa cells of the distal convoluted tubule and the juxtaglomerular cells of the renal afferent arteriole, has led to the hypothesis that renin release may also be modified by the electrolyte composition of the filtrate in the distal tubule. However, the mechanism by which these changes ultimately affect renin release is still under debate. Studies by Vander and Miller (22) have suggested that a decreased sodium load to the macula densa area is the main stimulus leading to renin release. However, micropuncture studies by Thurau and associates (23) have shown that it is an increas-

ed delivery of sodium to the macula densa area which is responsible for the observed effects on renin release. Studies by Nash et. al. (24) and Humphrey et. al. (25) have supported this later view. These data, therefore, can be interpreted as being suggestive of participation of the macula densa mechanism primarily in those instances in which renin release is to be inhibited. Alternatively, Barajas (26) has proposed that macula densa influence on renin release is dependent on the degree of distal tubular and afferent arteriolar contact and that contact increases with increasing tubular sodium concentration. Recently, some studies have proposed that distal tubular chloride load rather than sodium load may be the stimulus for triggering the macula densa-dependent effect on renin secretion (27,28,110,111).

2. Humoral Factors

A large number of circulating factors are known to influence renin secretion. Most important among these angiotensin II and ADH, and to a lesser degree, ovarian estrogens and adrenal corticoids.

Infusions of angiotensin II into the renal artery of dogs have been shown to inhibit renin release (29-32), possibly a direct negative feedback effect on the juxtaglomerular cells or, indirectly through its effect on vascular contractility (31,32). Blair-West and associates (31) have demonstrated that chronic intravenous infusion of angiotensin

II prevents the rise in plasma renin activity associated with sodium depletion. Shade et. al. (32) have extended these findings by showing that the inhibitory effect of angiotensin II on renin secretion is not only seen in the non-filtering kidney, but it also appears to be independent of renal blood flow and arterial pressure changes, thus supporting the view of a direct feedback effect of this agent on renin release. This concept has received added support by investigators utilizing in vitro kidney slice systems (10,33) or the isolated, perfused rat kidney (34).

ADH has also been postulated to exert an inhibitory influence on renin secretion (30,32,35). Tagawa and associates (36) have confirmed these observations by showing that physiological increases in ADH cause significant decreases in renin secretion in the unanesthetized sodium-deprived dog. However, the mechanisms mediating this inhibitory control of renin release by ADH remains unclear.

The effects of ovarian estrogens and adrenal steroids on renin secretion have also been investigated. Oral estrogenic contraceptives are known to cause variable increases in plasma renin activity (37) perhaps by their stimulatory action of hepatic production of renin substrate. Conversely, adrenal glucocortocoids may influence renin release through their actions of renal hemodynamics and glomerular filtration rate (38). Aldosterone, however, appears to have no direct influence on renin secretion but it may indirectly

affect it by its known action on sodium reabsorption (39).

Recent studies in a number of laboratories have presented evidence supporting the view that prostaglandins participate in the control of renin release in normal and pathological states. Treatment of malignant hypertension with the cyclooxygenase inhibitor, indomethacin, has been shown to supress plasma renin activity (40). Administration of arachidonic acid, a prostaglandin precursor, stimulates renin release in renal cortical slices of the rabbit (41). PGI_2 has been found to be present in large concentrations in the renal cortex and to be capable of stimulating renin secretion in both the intact kidney and in renal cortical slices (41,42). However, the mechanism involved in the action of prostaglandins remains to be determined.

3. Central Nervous Control of Renin Release

It is now clear that a central nervous mechanism participates in the peripheral regulation of renin release. Stimulation of the pons results in dramatic increases in the rate of renin release in the intact kidney (43-44). Ueda et. al. (45) have observed similar increases following mesencephalic stimulation. Zehr and Feigel (46) have presented data suggesting the existence of a central inhibitory component as well. In their studies, they found that stimulation of the anterior hypothalamus resulted in the suppression of plasma renin activity in the unanesthetized dog. These

results are consistent with the antagonistic roles these areas play in the regulation of behavior and circulation (47).

In addition to the direct regulatory action of angiotensin II on renin secretion, recent evidence indicates that it also has a central regulatory role. Infusions of this agent into the third cerebral ventricle, are followed by a decrease in plasma renin activity (16,48). It is not known whether this effect may be exerted by angiotensin II generated locally in the brain or alternatively, by systemic angiotensin II which crosses the blood brain barrier. Regardless, the possible existence of a functional brain renin angiotensin system is currently the subject of much controversy. Philips et. al. (49) have shown that in addition to angiotensin II, other components of the system including renin, angiotensinogen, and converting enzyme are also present in brain tissue, thus supporting the existence of a central renin-angiotensin system. Conversely, Day and Reid (50) have suggested that brain "renin" is instead the lysosomal enzyme cathepsin D. Additionally, they indicate that the optimum pH for this so-called brain "renin" enzyme is outside the physiological range, and that the concentrations of the other components of the system are too small to be of functional significance.

4. Peripheral Sympathetic Neural Control of Renin Release

Numerous electron microscopic (51-53) and histo-chemi-

cal flourometric studies (54,55), have demonstrated the presence of a large network of nerve terminals in close association with the renal afferent and efferent arterioles and the juxtaglomerular cells. These studies have also demonstrated that these nerve terminals are primarily sympathetic in nature. These observations have led to the postulation that the renal sympathetic nerves may play an important role in the homeostatic regulation of renin secretion.

a. Effects of Renal Nerve Stimulation and Renal Denervation

It is generally accepted that the sympathetic nervous system can influence renin release by both circulating catecholamines from the adrenal medulla and by norepinephrine secreted by renal sympathetic nerve terminals (39,56). The effect of circulating epinephrine and norepinephrine on renin secretion may be indirectly mediated through their actions on the afferent arteriole baroreceptors, or the macula densa natrioceptors. On the other hand, norepinephrine released by renal sympathetic nerve terminals may directly stimulate renin release through an interaction with specific receptors located on the juxtaglomerular cell membrane (10,13).

Evidence for a direct sympathetic effect which is independent of vascular and macula densa influences, has been gathered from a number of studies. Johnson et. al. (57) showed that electrical stimulation of the renal nerves caused

an increase in renin release from the non-filtering dog kidney treated with papaverine to inactivate baroreceptor responses. Similarly, increases in renin release produced by the stimulation of the vasomotor area of the cat brain are abolished by renal denervation or treatment with the beta-adrenergic blocker, propranolol (44). Other studies have additionally demonstrated that renal nerve stimulation can directly affect renin release in the absence of other factors (58-60).

Data generated from studies which have utilized the denervated kidney have largely confirmed these findings. Passo et. al. (43) found that the increase in renin release associated with stimulation of the medulla oblongata, was abolished by renal denervation. Denervation has also been shown to prevent the effects of postural changes and furosemide infusion on renin secretion (61). Sympathetic effects on renin release are also blocked by administration of propranolol (44), by ganglionic blockade or by local anesthesia (30).

b. Adrenergic Receptor Mediation of Renin Secretion

Numerous in vivo and in vitro studies have shown that catecholamine administration results in an increase in renin release (1,2,6,9,10,58,62-67). Experiments by Otsuka et. al. (68) in the dog indicate that the marked increase in renin release caused by insulin-induced hypoglycemia, can be abolished by adrenal denervation and restored by catecholamine

infusion. Thus, these data are indicative of the direct effect of catecholamines on the renin secreting juxtaglomerular cells.

The stimulatory effect of catecholamines on renin secretion has been postulated to be mediated by a beta-adrenergic receptor-mechanism (1,2,9,10,69-72), involving intracellular generation of cyclic AMP (1,2,9,10,70-73).

Alternatively, Vandogen et. al. (74), Nolly et. al. (70), and Pettinger (75), have evaluated the role of alpha-adrenergic receptors in the control of renin secretion. Their data show that stimulation of renal alpha-adrenergic receptors results in a marked inhibition of renin secretion, which can be prevented by the addition of alpha-blocking agents. Moreover, this inhibitory effect on renin release produced by alpha-adrenergic receptor activation has been shown to be coupled with significant decreases in the cyclic AMP content of the renal tissue (9,10,76).

c. The Dopamine Vascular Receptor and Dopaminergic Control of Renin Release

It has been amply demonstrated that dopamine is an obligatory precursor of norepinephrine in the central nervous system and in peripheral sympathetic nerve terminals (77). It is also known that dopamine is an important central neurotransmitter (78) and its presence has been reported in renal tissue (79). In view of these findings, considerable

interest has arisen in recent years in regard to the possibility that this catecholamine precursor may participate in the regulation of renin secretion. This has been further strengthened by the findings of a number of investigators pointing to the existence of a renal dopaminergic receptor which may mediate renal vascular dynamics (80-89).

McDonald et. al. (80,81), have reported that intravenous infusion of dopamine in human subjects, results in marked reduction in renal arterial resistance coupled with a significant increase in renal plasma flow and sodium excretion. McNay et. al. (82), have observed a similar effect in the anesthetized dog. Since alpha- and beta-blocking agents did not alter these responses, they suggested that dopamine may exert its effect by an alternate receptor mechanism, possibly one that is dopamine-specific. The experiments of Yeh and co-workers (83), in which intrarenal injections of the dopamine-receptor blocker, haloperidol, attenuated the renal vasodilating effect of dopamine provided added support for the existence of a dopamine renal vascular receptor. Similar results have been obtained using additional dopamine-receptor blockers such as chloropromazine (84), and bulbocapnine (85,86). Furthermore, the dopamine-receptor agonist apomorphine and the N-methyl dopamine analog epinine, have been shown to cause dopamine-like effects on renal vasodilation (87).

Nakajima and collegues (88,89) have extended these

findings by suggesting that the action of this dopamine vascular receptor may depend on the activation of an adenylate cyclase system. In their studies, addition of dopamine to a rat kidney particulate preparation resulted in a significant increase in the cyclic AMP content of the medium, which was unaffected by alpha- (phentolamine) or beta- (propranolol) blockade. Conversely, spiroperidol an effective dopamine-receptor antagonist, blocked this stimulatory effect while the dopamine-receptor agonist apomorphine potentiated it. A subsequent study by the same investigators (89) confirmed their original observations and further demonstrated that, the increased cyclic nucleotide levels produced by addition of the phosphodiesterase inhibitor theophylline, were effectively blocked by spiroperidol. Taken together, these findings support the existence of a dopamine-specific, adenylate-sensitive, vascular receptor in the kidney. This is consistent with other evidence suggesting the presence of similar cyclic AMP-mediated dopamine receptors in the brain and retina (90-92). In view of the previously discussed data regarding the role of dopamine in the regulation of renal vascular dynamics, a number of studies are presently being directed toward determining whether dopamine may have a direct effect on the juxtaglomerular cells to increase renin secretion, and thus indirectly regulate electrolyte, blood volume, and blood pressure homeostasis.

Working <u>in vivo</u>, Ayers and collegues (62) have shown

that dopamine administration results in a marked increase in renin release. Bell and Lang (93) have also demonstrated that dopamine can stimulate renin secretion and that this effect is blocked by the dopamine-receptor antagonist, ergometrine. Wilcox et. al. (65), as well as Imbs and collegues (94,95), have confirmed these observations.

On the other hand, some studies have provided contradictory evidence. Cuche et. al. (96) found an inverse relationship between urinary dopamine and plasma renin activity, as a result of postural changes from the supine to the upright position. Since plasma renin activity increased while urinary dopamine decreased, they suggested a possible inhibitory role for dopamine on renin release, mediated by changes in renal plasma flow or sodium load to the macula densa. Chokshi and associates (97), have supported this concept by showing that renal artery infusion of dopamine causes an increase in renal plasma flow and a simultaneous decrease in renin secretion.

In vitro studies generally supported the view of a direct action of dopamine on renin release. Henry and co-workers (3) have demonstrated in a rat renal cortical preparation, that administration of dopamine doses of 10^{-7}M or greater results in significant increases in renin secretion. They also found that alpha-receptor blockade (phentolamine), dopamine-receptor blockade (haloperidol), or addition of cocaine (a catecholamine re-uptake inhibitor), did not alter

the stimulatory effect of dopamine, but that propranolol administration effectively prevented it. They concluded that renin release may be partially regulated by a beta-adrenergic mechanism, and that dopamine-specific receptors may not participate in this regulation. Their findings have been supported by Quesada et. al. (4). Similar in vitro studies in our laboratory (1,2) have confirmed Henry's and Quesada's observations in regard to the stimulatory effect of dopamine on renin release and the inhibition of this effect by propranolol. However, we have further extended their observations by demonstrating that both, the stimulatory effect on release and the blockade of this effect by propranolol, are coupled with corresponding changes in the cyclic AMP content of the sliced tissue. These data strongly support the view that the action of dopamine on renin secretion, similar to that exerted by norepinephrine, is mediated by the activation of an adenylate cyclase receptor complex in the membrane of the juxtaglomerular cells. Also, since the data regarding the possible participation of dopamine-specific receptors is controversial at best, it is clear that further studies designed to investigate this possibility are in order.

CHAPTER III

MATERIALS AND METHODS

Male, Sprague-Dawley rats (Sprague-Dawley Co.) with initial weights of 210 ± 10g, were fed a sodium deficient diet (Teklad Test Diet Co.) providing less than 0.02mEq of sodium per day for 1.5 to 2.5 weeks. Male animals were chosen to eliminate the influence of cyclic estrogenic variations, in view of reports that circulating estrogens can exert effect on renin release (37). They were kept in a temperature-controlled room, two animals per cage and had unlimited access to distilled-deionized water. At the end of the dietary intake period, the animals were sacrificed by decapitation and their kidneys rapidly excised, gassed for 30 sec with 95% O_2 - 5% CO_2 prior to and after decapsulation, and placed in Robinson's buffer medium (98) at 4°C. Renal cortical slices approximately 0.3mm thick were prepared using a Stadie-Riggs microtome (A. Thomas Co.). Each cortical slice was further divided into several portions according to the number of treatments, and randomly assigned to separate incubating vessels containing 2.5 ml of Robinson's buffer. This procedure was repeated using other areas of the renal cortex until each vessel contained 50 ± 30mg of cortical tissue.

This random distribution of sliced tissue ensured that all incubating vessels contained a homogeneous cell population from each cortical area, and thus allowed one of each group to be used as a common untreated control for each group of treatments. The tissue was then pre-incubated for 15 min at 37°C in a shaking Dubnoff Metabolic Incubator (Precision Scientific), in an atmosphere saturated with 95% O_2 - 5% CO_2. The pre-incubated slices were subsequently transferred to corresponding incubating vessels containing 2.5 ml of Robinson's buffer previously equilibrated to 37°C, and incubated for 5, 20 or 60 min, under conditions identical to those of the pre-incubation period.

A group of experimental vessels, having a common control sample, was treated with dopamine (10^{-3}M) added either alone or together with either of two doses of the dopamine-receptor blocker pimozide (10^{-8}M or 10^{-6}M). Pimozide at both concentrations was also added alone to the renal cortical preparation. Alternatively, a second group of vessels was similarly treated with 10^{-3}M dopamine, but in this instance the dopamine-receptor agonist apomorphine (10^{-6}M) was added to the slice system either alone or together with dopamine. All tissue samples, including controls, were also treated with the dopamine beta-hydroxylase inhibitor FLA-63 (10^{-4}M) to prevent conversion of dopamine to norepinephrine (99) in the slice preparation. FLA-63, apomorphine, and pimozide, were added to the tissue system prior to both the pre-incubation

and incubation periods whereas, dopamine was added prior to the incubation period only.

Dopamine (Sigma), apomorphine, and FLA-63 (Regis Chemical Co.) solutions were prepared in 0.1% ascorbic acid (Sigma) to prevent oxidation. Pimozide solution (Janssen Pharmaceutical) were prepared in 1.2% tartaric acid (Sigma) for the same reason.

Following incubation, the supernatant medium was collected and stored at -20°C until assayed for renin concentration. Tissue used for cyclic AMP determination was collected at the end of all incubation periods and rapidly frozen on dry ice. The frozen tissue was then homogenized in 1 ml of 8% trichloroacetic acid and stored at -20°C.

A. Tissue Cyclic AMP Extraction and Measurement of its Content

Frozen cyclic AMP tissue homogenates were allowed to thaw in an ice-water bath, followed by centrifugation at 3100g for 10 min at 4°C. The supernatant was transferred to conical centrifuge tubes using disposable Pasteur pipettes while the protein-containing precipitate was discarded. The supernantant samples were then washed four times with 2 ml of water-saturated ether, vortexed for 10 sec at each step, and the ether phase aspirated and discarded. After the final wash, the cyclic AMP-containing water phase was decanted into glass vials previously placed on dry ice for rapid

freezing. The frozen samples were lyophilized and the freeze-dried residue was stored at -20°C until assayed for cyclic AMP content.

Cyclic AMP content of the freeze-dried material was measured by a modification of the competitive protein-binding assay of Gilman (100). The results were expressed as picomoles of cyclic AMP per mg of wet tissue.

B. Purification of Tissue Renin

Samples collected for determination of tissue renin content were homogenized in 1 ml of 0.9% sodium chloride (physiological saline) at 4°C, and centrifuged for 15 min at 3500g. The supernatant was dialyzed for 24 hrs at 4°C against an EDTA-saline buffer at pH 3.3, followed by heating to 32°C for one hour to denature any endogenous renin substrate or angiotensinases, and then dialyzed again for 24 hrs at 4°C against an EDTA-phosphate buffer at pH 7.5 (101). The purified supernatant samples were then stored at -20°C until assayed for renin concentration by radio-immunoassay of angiotensin I.

C. Angiotensin I Generation and Measurement

Renin concentration in both the dialyzed purified and the incubated supernatant samples, was indirectly measured by determining the rate of angiotensin I generation produced by endogenous renin present in our samples upon exposure to

exogenous substrate (angiotensinogen) obtained from 4 hr nephrectomized dog plasma (102). An inhibitor of converting enzyme and angiotensinases was used to prevent any further conversion of the angiotensin I generated during a one hour incubation period at 37°C. Briefly, both the dialyzed and incubated supernatant samples were allowed to thaw, vortexed for ten seconds and maintained at 4°C in an ice-water bath. Aliquots of 0.025 ml from each sample were transferred to test tubes at 4°C. Five drops of saturated sodium chloride, and 0.5 ml of 4 hr nephrectomized dog plasma previously treated with the converting and angiotensinase inhibitor, phenyl methyl sulfonyl flouride (PMSF, Sigma, 0.05 ml per ml plasma), were added to each tube. The samples were then incubated for one hour at 37°C, diluted 1:2 with distilled-deionized water, vortexed, and then placed in a boiling water bath for 3 min to stop further generation of angiotensin I. The samples were allowed to cool and then stored at -20°C until assayed.

Renin concentration in all samples was then determined by radioimmunoassay of angiotensin I in the generated samples (New England Nuclear). The results were expressed as nanograms of angiotensin I generated per mg of wet tissue per hour.

Statistical significance of the data was evaluated by Student's paired and unpaired t-tests and Linear Regression

Analysis using a programmable desk-top calculator (Hewlett-Packard).

CHAPTER IV

RESULTS

Resting renin release rate and tissue cyclic AMP content of renal cortical slices from sodium deficient rats were initially evaluated in relation to the length of incubation time (Fig. 1). Renin release rate from these control tissue samples was linear with time of incubation ($p < 0.0005$), which would be expected of metabolically active endocrine tissue. In contrast, cyclic AMP content was maximal by 20 min of incubation and then decreased to a level similar to that seen at 5 min by the end of a one hour incubation period.

Renin release and tissue cyclic AMP content responses to 10^{-3}M dopamine administration were subsequently examined and compared to those of non-treated control samples (Fig. 2). These data show that the mean renin release rate of the dopamine-treated samples appeared to be higher than that of controls at 5 min and 20 min, and was significantly greater by 60 min. Similarly, corresponding tissue cyclic AMP levels were significantly greater than those of controls as early as 5 min. Tissue renin levels in dopamine-treated samples were not different from those of controls at 60 min.

To further evaluate the type of mechanism mediating the stimulatory effect of dopamine on renin release, an experiment was designed to examine the effect of the dopamine-receptor agonist apomorphine, added alone or together with dopamine, on renin secretion, tissue cyclic AMP content, and tissue renin content (Fig. 3). In the presence of 10^{-6}M apomorphine, dopamine was no longer capable of stimulating renin release or tissue cyclic AMP content levels in our cortical preparation. Apomorphine by itself did not affect renin release but it did stimulate cyclic AMP content at 60 min. Mean tissue renin content changes in the presence of dopamine plus apomorphine were consistent with those seen in renin release and cyclic AMP content.

An additional study was undertaken to provide further evidence regarding the mechanism by which the dopamine-receptor blocker pimozide stimulates renin secretion as previously reported (1,2). In this study, the effect of pimozide alone or together with dopamine, on renin release, tissue cyclic AMP content, and tissue renin content was examined in relation to the time of incubation (Fig. 4). The data show that two doses of pimozide (10^{-8}M and 10^{-6}M) added to the tissue preparation, caused a significant increase in renin release coupled with a significant decrease in tissue cyclic AMP content during a one hour incubation period. These changes were seen whether dopamine was present or not, suggesting the the effect was due to pimozide itself. In addition, the

mean tissue renin content appeared decreased by the administration of pimozide when compared to controls. When the renin release, tissue renin content, and tissue cyclic AMP content responses to both pimozide doses were evaluated in regard to time of incubation (Fig. 5), the significant stimulatory effect of this agent on renin release and its simultaneous marked inhibition of cyclic AMP content were evident as 5 min of incubation. At 60 min, mean tissue renin content of pimozide-treated samples appeared to be lower than that of controls. Furthermore, the renin release and tissue renin content changes caused by pimozide administration appeared dose-dependent, whereas those seen in tissue cyclic AMP content were similar in magnitude regardless of the dose used.

Fig. 6 depicts the results of our study with dopamine and apomorphine, and those generated in a parallel study utilizing the dopamine-receptor blocker d-butaclamol and the phosphodiesterase inhibitor theophylline. The implication of the significant stimulation of renin release and tissue cyclic AMP content seen in the presence of dopamine, theophylline or d-butaclamol, and the corresponding inhibition of the stimulatory effect of dopamine on these parameters caused by apomorphine administration is discussed in the next section.

LEGEND

Figure 1

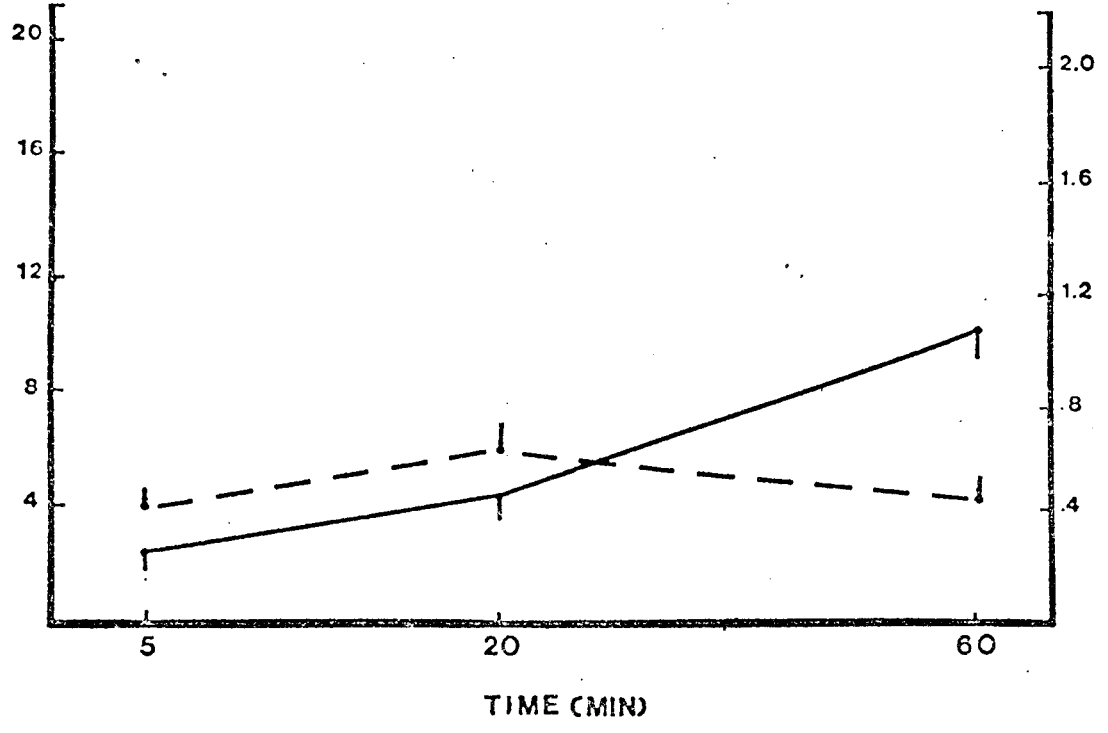
Resting renin release rate and tissue cyclic AMP levels in renal cortical slices from sodium deficient rats. The data represent the mean renin release rate ± S.E. of 14 - 32 observations and the mean tissue cyclic AMP level ± S.E. of 12 - 14 observations at 3 incubation times. The mean resting renin release rate at 5, 20, and 60 min of incubation was 2.55 ± 0.28ng/mg/hr, 3.86 ± 0.80ng/mg/hr, and 10.28 ± 1.01ng/mg/hr, respectively. The corresponding mean resting tissue cyclic AMP levels were, 0.397 ± 0.032pmol/mg, 0.616 ± 0.076pmol/mg, and 0.436 ± 0.046pmol/mg, respectively. The DBH inhibition FLA-63 (10^{-4}M) was added to all samples in this and subsequent experiments.

RENIN ——

c-AMP — —

SLOPE (Renin) = .1436 ng/mg/hr/min

r (Renin) = .6238



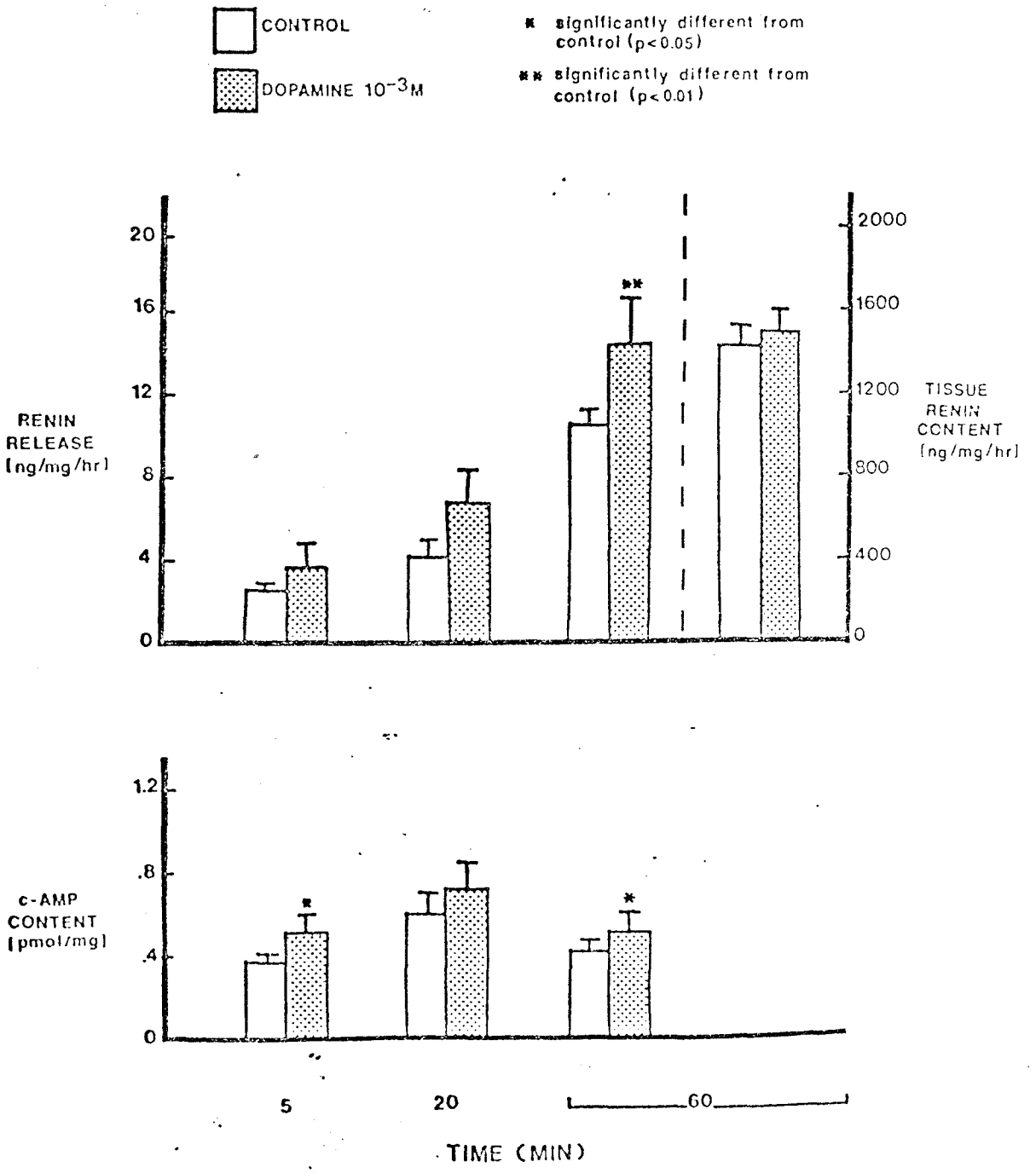
RENIN RELEASE (ng/mg/hr)

c-AMP CONTENT (pmol/mg)

LEGEND

Figure 2

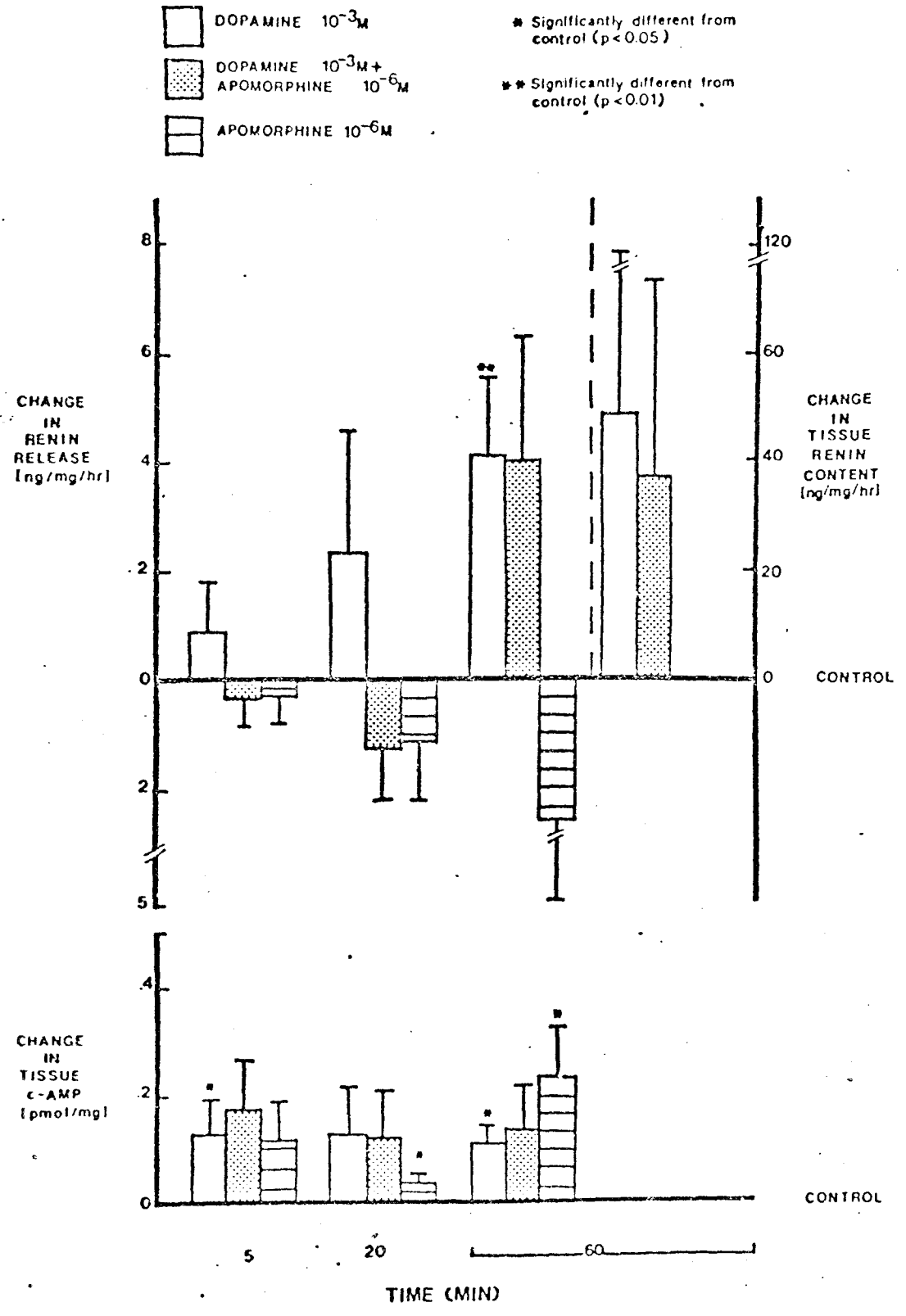
Renin release rate, tissue cyclic AMP content, and tissue renin content responses to 10^{-3}M dopamine added to renal cortical slices from sodium deficient rats. The data represent the mean renin release rate ± S.E. of 14 - 32 observations, the mean tissue cyclic AMP content ± S.E. of 12 - 14 observations, and the mean tissue renin content ± S.E. of 23 observations at 3 incubation times. The mean control (untreated) renin release rate and tissue cyclic AMP content values are the same as those described in Fig. 1. Mean control tissue renin content at 60 min was 1419 ± 103ng/mg/hr.


CONTROL
DOPAMINE 10^{-3}M
* significantly different from control ($p<0.05$)
** significantly different from control ($p<0.01$)
RENIN RELEASE (ng/mg/hr)
TISSUE RENIN CONTENT (ng/mg/hr)
20
16
12
8
4
0
2000
1600
1200
800
400
0
**
c-AMP CONTENT (pmol/mg)
1.2
.8
.4
0
*
*
5
20
60
TIME (MIN)

LEGEND

Figure 3

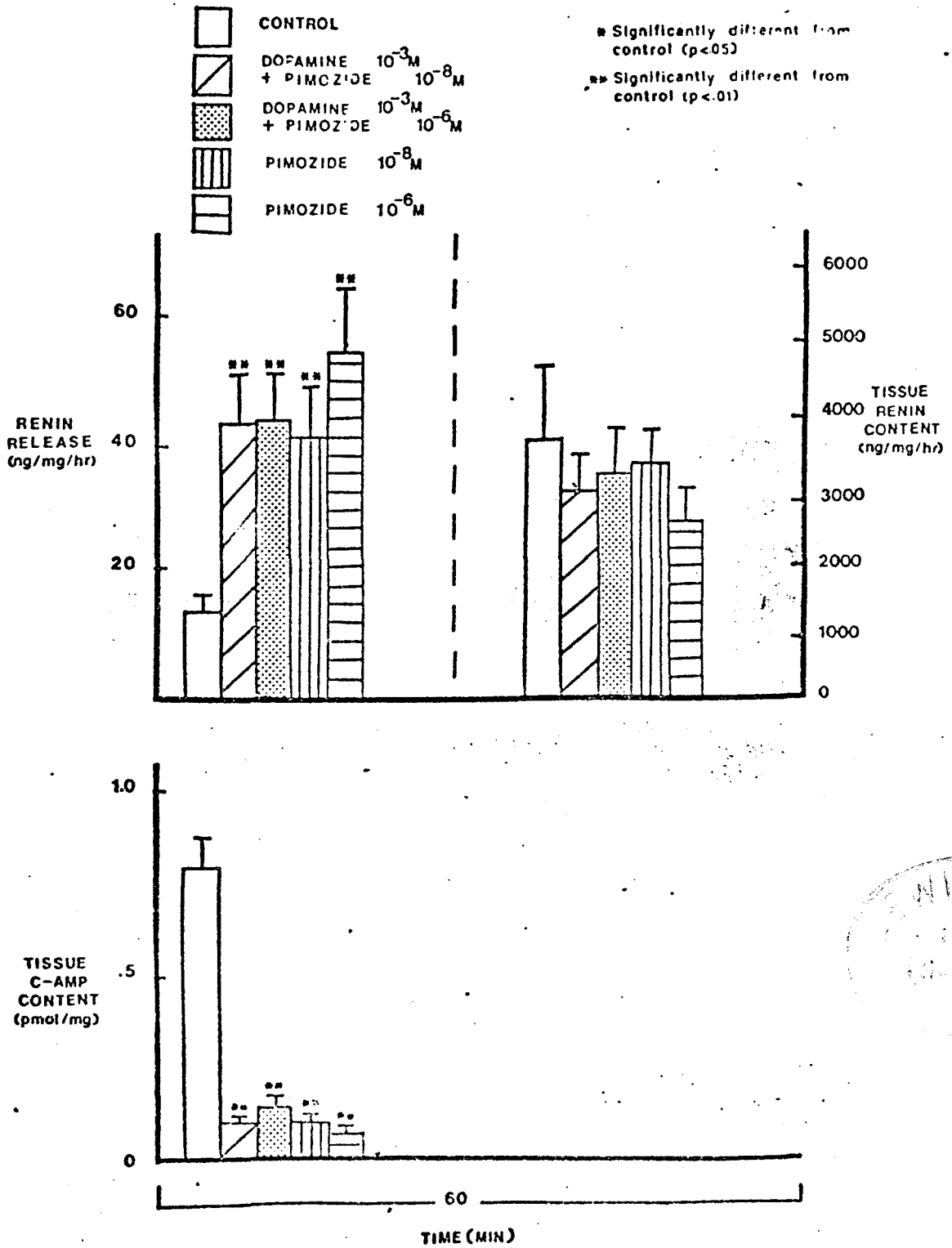
Changes in renin release, tissue cyclic AMP content, and tissue renin content, in response to 10^{-3}M dopamine and 10^{-6}M apomorphine, added separately or together to renal cortical slices from sodium deficient rats. The data represent the mean renin release change ± S.E. of 7 - 15 observations, the mean tissue cyclic AMP content change ± S.E. of 7 - 8 observations, and the mean tissue renin content change ± S.E. of 24 observations for each treatment at 3 incubation times. The mean control (untreated) renin release rate at 5, 20, and 60 min of incubation was 2.56 ± 0.48ng/mg/hr, 4.69 ± 1.51ng/mg/hr, and 10.60 ± 1.39ng/mg/hr, respectively. The corresponding mean control cyclic AMP content values were, respectively, 0.39 ± 0.033pmol/mg, 0.527 ± 0.094pmol/mg, and 0.453 ± 0.072pmol/mg. Mean control tissue renin content at 60 min was 489ng/mg/hr.


DOPAMINE 10^{-3}M
DOPAMINE 10^{-3}M + APOMORPHINE 10^{-6}M
APOMORPHINE 10^{-6}M
* Significantly different from control (p<0.05)
** Significantly different from control (p<0.01)
CHANGE IN RENIN RELEASE [ng/mg/hr]
CHANGE IN TISSUE RENIN CONTENT [ng/mg/hr]
CONTROL
CHANGE IN TISSUE c-AMP [pmol/mg]
CONTROL
5
20
60
TIME (MIN)

LEGEND

Figure 4

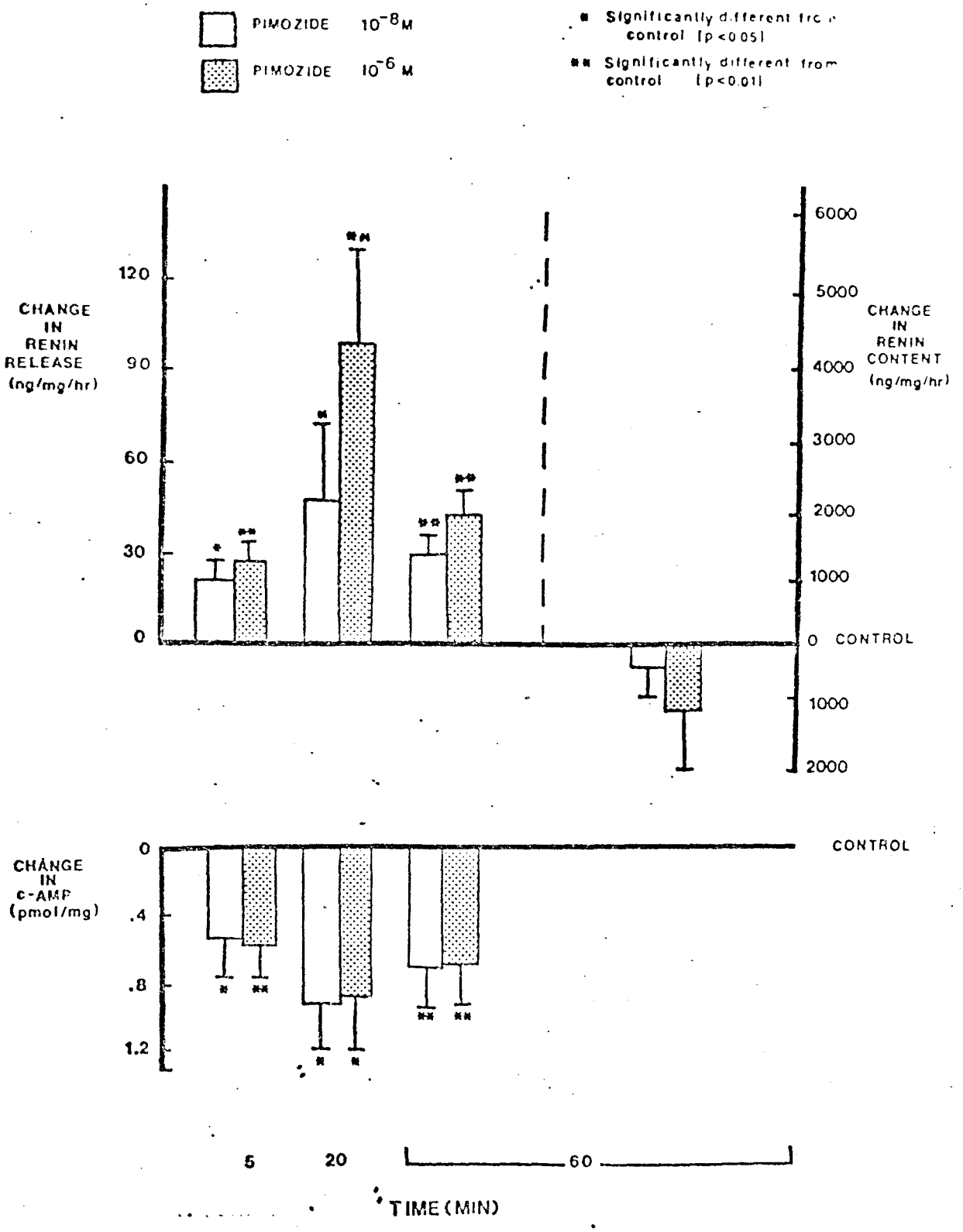
Renin release, tissue cyclic AMP content, and tissue renin content responses to 10^{-3}M dopamine, added along or together with 10^{-8}M or 10^{-6}M pimozide to renal cortical slices from sodium deficient rats. The data represent the mean renin release rate ± S.E. of 11 observations, the mean tissue cyclic AMP content ± S.E. of 9 observations, and the mean tissue renin content ± S.E. of 11 observations, for each treatment during a 60 min incubation period. The mean control (non-treated) renin release rate was 14.2 ± 3.61ng/mg/hr, mean control cyclic AMP content was 0.794 ± 0.194pmol/mg, and mean control tissue renin content was 3833 ± 866ng/mg/hr, respectively.



LEGEND

Figure 5

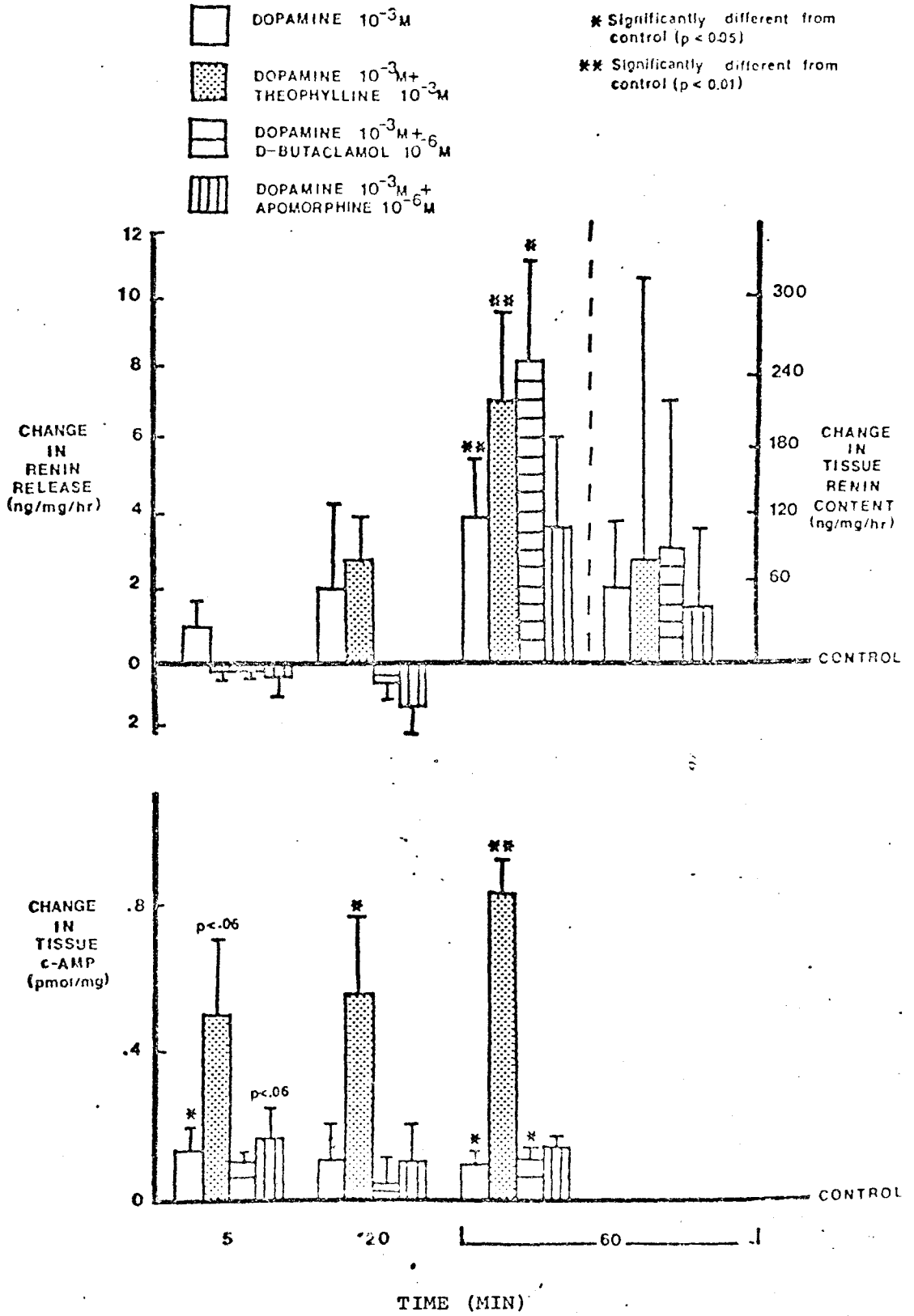
Changes in renin release, tissue cyclic AMP content, and tissue renin content, in response to two doses of pimozide added separately to a renal cortical slice preparation from sodium deficient rats. The data represent the mean renin release change ± S.E. of 11 observations, the mean tissue cyclic AMP content change ± S.E. of 6 - 9 observations, and the mean tissue renin content change ± S.E. of 11 observations for each treatment at 3 incubation times. The mean control (non-treated) renin release rate at 5, 20, and 60 min of incubation was 3.38 ± 0.88ng/mg/hr, 8.84 ± 1.97ng/mg/hr, and 14.2 ± 3.61ng/mg/hr, respectively. The corresponding mean control cyclic AMP content values were, respectively, 0.767 ± 0.176pmol/mg, 1.14 ± 0.33pmol/mg, and 0.794 ± 0.194pmol/mg. Mean control tissue renin content at 60 min was 3833 ± 866ng/mg/hr.


PIMOZIDE 10^{-8}M
PIMOZIDE 10^{-6} M
* Significantly different from control (p<0.05)
** Significantly different from control (p<0.01)
CHANGE IN RENIN RELEASE (ng/mg/hr)
120
90
60
30
0
6000
5000
4000
3000
2000
1000
0 CONTROL
1000
2000
CHANGE IN RENIN CONTENT (ng/mg/hr)
CHANGE IN c-AMP (pmol/mg)
0
.4
.8
1.2
CONTROL
5
20
60
TIME (MIN)

LEGEND

Figure 6

Changes in renin release, tissue cyclic AMP content, and tissue renin content in response to 10^{-3}M dopamine added alone or together with 10^{-3}M theophylline, 10^{-6}M d-butaclamol, or 10^{-6}M apomorphine, to renal cortical slices from sodium deficient rats. The data represent the mean renin release change ± S.E. of 2 - 18 observations, the mean tissue cyclic AMP content change ± S.E. of 2 - 12 observations, and the mean tissue renin content change ± S.E. of 9 - 24 observations for each treatment at 3 incubation times. The mean control (non-treated) values for treatments involving dopamine alone or together with theophylline and d-butaclamol at 5, 20, and 60 min of incubation were, respectively, 2.55 ± 0.276, 3.86 ± 0.801, and 10.28 ± 1.01ng/mg/hr for renin release; 0.397 ± 0.032, 0.616 ± 0.076, and 0.437 ± 0.046 pmol/mg for cyclic AMP content; and 1419 ± 303ng/mg/hr for tissue renin content at 60 min. The corresponding mean control values for treatments involving dopamine alone or together with apomorphine in regard to the same 3 parameters at 5, 20, and 60 min, are the the same as those described in Figure 3.


DOPAMINE 10^{-3}M
DOPAMINE 10^{-3}M+ THEOPHYLLINE 10^{-3}M
DOPAMINE 10^{-3}M+ D-BUTACLAMOL 10^{-6}M
DOPAMINE 10^{-3}M+ APOMORPHINE 10^{-6}M
* Significantly different from control (p < 0.05)
** Significantly different from control (p < 0.01)
CHANGE IN RENIN RELEASE (ng/mg/hr)
CHANGE IN TISSUE RENIN CONTENT (ng/mg/hr)
CONTROL
CHANGE IN TISSUE c-AMP (pmol/mg)
p<.06
p<.06
CONTROL
TIME (MIN)

CHAPTER V

DISCUSSION

The use of an *in vitro* renal cortical slice system in this study allowed an effective evaluation not only of the effect of isolated stimuli on renin release, but also of the dynamics of renin secretion in resting tissue in relation to time. Investigators utilizing this type of renal preparation have demonstrated that in resting renal tissue (11,103), renin secretion is an active process which is linear with time. The data generated in the present study, utilizing the sodium deficient rat (Fig. 1), clearly support this view.

Dopamine has been shown to stimulate *in vitro* renin secretion by a beta-adrenergic receptor mechanism (1,2,3,4) mediated by tissue cyclic AMP changes (1,2). Our data supports these observations by demonstrating that the significant stimulatory effects exerted by dopamine on renin release after one hour of incubation (Fig. 2), was preceded by a significant increase in tissue cyclic AMP content as early as 5 min of incubation. This view is further supported by data from a parallel study in our laboratory (12), which show that addition of the phosphodiesterase inhibitor, theophylline, to our cortical preparation, significantly poten-

tiates the renin release and tissue cyclic AMP content responses to dopamine (Fig. 6).

Although the mean tissue renin content of our dopamine-treated samples was not significantly different from that of controls (Fig. 2), it was greater in magnitude, suggesting the possibility of renin synthesis in response to dopamine administration. Data from parallel studies utilizing theophylline (Fig. 6) are consistent with this possibility since the mean tissue renin content responses to dopamine administration were potentiated when theophylline was simultaneously added to our in vitro system. This agrees with a number of previous studies reporting that catecholamine administration stimulates renin synthesis as well as renin secretion. For instance, addition of norepinephrine or epinephrine has been reported to increase tissue renin content in renal preparations from cats (73) and that this effect is prevented by administration of the protein inhibitor, cyclohexamide. The use of radioactively labeled amino acids in monitoring new renin synthesis in response to sympathetic stimuli, has recently confirmed these findings (104).

The concept of a specific renal dopaminergic receptor mediating the stimulatory effect of dopamine has been previously proposed (105,106). The results of our study utilizing the dopamine-receptor agonist, apomorphine (Fig. 3), do not agree with this view. Instead, they suggest that dopamine may exert an inhibitory effect on renin release via

a cyclic AMP-mediated-dopamine-specific receptor mechanism, as indicated by the fact that apomorphine prevented the stimulatory effect of dopamine on renin release and tissue cyclic AMP content. The observation in a parallel study (Fig. 6) on the potentiation of dopamine-induced renin release by the dopamine-receptor blocker, d-butaclamol, offers further support for the concept of dopamine-receptor mediated inhibition of renin release. Thus, our data suggest that dopamine may exert a dual regulatory control on renin secretion, namely a stimulatory one through a beta-adrenergic receptor mechanism and an inhibitory one via a dopamine-receptor pathway. This is not surprising, since dopamine has been reported to have the molecular flexibility required to effectively bind to structurally different receptors (107). However, the physiological significance of this dual regulation of renin secretion by dopamine is unclear. A similar type of control has been previously proposed in regard to the effect of norepinepherine on renin secretion (9,10,74,75). Recently, Quesada et. al. (4), have reported results similar to those seen in our study, although the significance of the potentiation of renin release observed upon administration of the dopamine-receptor blocker, haloperidol, was not discussed. In hibitory effects by dopamine, which appear to be mediated by dopamine-specific receptors, have also been demonstrated in regard to aldosterone (108), episodic luteinizing hormone (109), and prolactin re-

lease (112).

Data from the present study (Fig. 4) confirm previous in vitro observations in our laboratory (1,2) in regard to the significant effect exerted by the dopamine-receptor blocker, pimozide, on renin release and tissue cyclic AMP content during a one our incubation period. The significant stimulatory effect on renin release and the simultaneous depression of cyclic AMP content caused by the administration of this agent in previous studies, was confirmed in this study utilizing two additional doses. Furthermore, this dissociation of the renin release and tissue cyclic AMP responses to pimozide administration, occurred whether or not dopamine was simultaneously added, suggesting that the effect was due to pimozide itself. Our results (Fig. 4) have extended our previous observations by showing that tissue renin content at 60 min appeared decreased in relation to controls, suggesting an inhibition of de novo synthesis. The results of a subsequent experiment (Fig. 5), which examined the effects of pimozide on renin release, cyclic AMP content, and tissue renin content in relation to time, suggest that the action of this agent on these parameters is not mediated by a physiological mechanism, but rather by a toxic effect exerted on the membrane of the renin-secreting cells. This view is supported by our observation that the rate of renin release in response to pimozide was markedly increased as early as 5 min, suggesting that pre-formed renin was being prefer-

entially released, perhaps due to the alterations in juxtaglomerular cell membrane permeability. Furthermore, the decreased levels in tissue cyclic AMP and tissue renin content seen in our study, are consistent with the view that pharmacological toxicity will inactivate membrane-bound adenylate cyclase systems. Pharmacological changes in membrane structure and permeability leading to uncontrolled renin release, have also been postulated to be important in the stimulatory effect exerted by the beta-receptor agonist isoproterenol (66), perhaps involving changes in tissue cyclic AMP and tissue renin content similar to those seen in our study. This possibility however, cannot be confirmed, since these investigators only evaluated changes in renin release. In view of reports (95) questioning the specificity of pimozide as a dopamine receptor blocker in peripheral organ systems, it is clear that specific dopamine-receptor antagonists need to be used in order to effectively evaluate the possibility that dopamine receptors participate in the regulation of renin secretion from the mammalian kidney.

In summary, the data reported in this paper support the concept that dopamine may regulate renin secretion by two distinct receptor mechanisms involving changes in tissue cyclic AMP levels. Activation of beta-adrenergic receptors mediates its stimulatory effect (2), while its inhibitory action may be exerted through dopamine-specific receptors (12). Additionally, these results clearly suggest that pim-

ozide-stimulated renin release may be the consequence of adenyl cyclase inactivation induced by a generalized toxic effect exerted by pimozide on the juxtaglomerular cell membrane, leading to alterations in its permeability.

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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Biology.

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