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## Further In Vitro Evidence for a Cyclic Adenosine 3', 5'- Monophosphate Mediated Regulation of Renin Secretion by Dopamine in the Sodium Deficient Rat: Effect of Theophylline and Dopamine-Receptor Antagonistic Agents

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FURTHER IN VITRO EVIDENCE FOR A CYCLIC  
ADENOSINE 3',5'-MONOPHOSPHATE MEDIATED REGULATION  
OF RENIN SECRETION BY DOPAMINE IN THE SODIUM  
DEFICIENT RAT: EFFECT OF THEOPHYLLINE AND  
DOPAMINE-RECEPTOR ANTAGONISTIC AGENTS

by

Suzanne Marie Mottel

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## VITA

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## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS . . . . .	ii
VITA . . . . .	iii
LIST OF FIGURES . . . . .	iv
 Chapter	
I. INTRODUCTION . . . . .	1
II. LITERATURE REVIEW	
A. Control of Renin Secretion . . . . .	3
1. Baroreceptor Control of Renin Release . . . . .	4
2. Macula Densa Receptor Regulation of Renin Release . . . . .	5
3. Humoral Control of Renin Release . . . . .	6
4. Neural Control of Renin Release . . . . .	7
5. Dopaminergic Control of Renin Release . . . . .	10
III. MATERIALS AND METHODS . . . . .	14
A. Determination of Renin Concentration . . . . .	16
B. Tissue Renin Determination . . . . .	17
C. Determination of Tissue Cyclic Adenosine 3',5'-Monophosphate (cAMP) Content . . . . .	17
IV. RESULTS . . . . .	19
V. DISCUSSION . . . . .	34
REFERENCES . . . . .	38

## LIST OF FIGURES

Figure	Page
1. Resting renin release rate and tissue cyclic AMP content of unstimulated (control) renal cortical slices from sodium deficient rats at various times of incubation. . . . .	23
2. Effect of $10^{-3}$ M dopamine on renin release, tissue cyclic AMP content, and tissue renin content of renal cortical slices from sodium deficient rats . . . . .	25
3. Changes in renin release, tissue cyclic AMP content, and tissue renin content in response to $10^{-3}$ M dopamine and $10^{-3}$ M theophylline, added together or separately to renal cortical slices from sodium deficient rats . . . . .	27
4. Changes in renin release, tissue cyclic AMP content, and tissue renin content in response to $10^{-3}$ M dopamine and $10^{-6}$ M d-butacclamol, added together or separately to renal cortical slices from sodium deficient rats . . . . .	29
5. Changes in renin release, tissue cyclic AMP content, and tissue renin content in response to $10^{-3}$ M dopamine and $10^{-6}$ M l-butacclamol, added alone or together to renal cortical slices from sodium deficient rats. . . . .	31
6. Changes in renin release, tissue cyclic AMP content, and tissue renin content in response to dopamine ( $10^{-3}$ M) added alone or together with $10^{-3}$ M theophylline, $10^{-6}$ M d-butacclamol, or $10^{-6}$ M apomorphine to renal cortical slices from sodium deficient rats . . . . .	33

## CHAPTER I

### INTRODUCTION

Studies utilizing a rat renal cortical slice system (1) or the isolated, perfused rat kidney (2), suggest that peripheral dopamine directly stimulates renin release through a beta-adrenergic receptor mechanism similar to that postulated for norepinephrine by several investigators (3-8). Recent studies in this laboratory (9-10) utilizing the dopamine-beta-hydroxylase inhibitor FLA-63 which effectively prevents the conversion of dopamine to norepinephrine in in vitro tissue (11-12), have provided added support for this view as well as for the possibility that cyclic AMP may mediate the stimulatory effect of dopamine on renin release via this beta-receptor pathway. However, the results obtained in these studies have left unanswered the question as to whether or not dopamine may also influence renin secretion through an interaction with a specific dopamine receptor located in the membranes of the juxtaglomerular cells.

In order to effectively evaluate specific factors that may directly control renin release, various humoral and hemodynamic influences that also affect renin secretion in vivo must first be eliminated. Therefore, an in vitro rat kidney cortical slice preparation devoid of these influences was utilized in this study to further examine the type of mechanism involved in mediating a direct effect of dopamine on renin release. In addition, a number of in vivo and in vitro studies

have shown that the sensitivity of the renin-secreting cells to catecholamine stimulation is potentiated by dietary sodium deficiency (8,9,13,14). Thus, cortical tissue obtained from rats maintained on a sodium deficient diet was used in this investigation.

Since further characterization of the receptor type mediating the renin responses to dopamine administration is necessary, the present study was designed to 1) provide additional in vitro evidence regarding the involvement of a beta-adrenergic receptor mechanism in mediating the effect of dopamine on renin secretion, by utilizing the phosphodiesterase inhibiting agent, theophylline; 2) examine the possibility of dopamine-specific receptor participation, by evaluating the effect of the dopamine-receptor blocker, d-butacclamol; 3) determine if the renin secretory responses to these agents are coupled with tissue cyclic AMP content changes at various incubation times; and 4) evaluate if new synthesis of renin occurs as a result of administration of these agents to our cortical slice preparation.



## CHAPTER II

### LITERATURE REVIEW

#### A. Control of Renin Secretion

There is no question as to the importance of the glycoprotein hormone, renin (15), acting as a circulatory proteolytic enzyme, in the control of arterial blood pressure (16) and in the primary regulation of aldosterone secretion and thus of sodium and blood volume homeostasis (17-19). The mechanisms participating in the control of renin release have been the subject of intensive study for numerous years. Three major theories have been proposed to explain how a number of stimuli known to affect renin secretion activate the juxtaglomerular cells that produce it in the kidney; 1) a "Baroreceptor" or "Stretch Receptor" theory states that renin is regulated by changes in tone of the afferent arteriolar walls in response to changes in renal perfusion pressure (20), 2) a "Macula Densa" Theory holds that the rate of renin secretion is inversely related to sodium and/or chloride concentration in the distal tubular fluid, as sensed by specialized cells in that area (21), and 3) a "Neural" Theory postulates that there is a sympathetic nervous regulation of renin secretion either directly through nerve terminals synapsing with the juxtaglomerular cells or indirectly via circulating adrenal medullary catecholamines (20). Additional factors such as circulating angiotensin II, antidiuretic hormone (ADH), and intrarenal prostaglandins also appear to play a role in the regulation of renin

secretion (21) although not as importantly as the three major ones, and by mechanisms which are still largely unknown. It is clear, however, that in the live animal renin release depends to a large degree upon the simultaneous interaction of all of these various stimuli.

#### 1. Baroreceptor Control of Renin Release

The development of the baroreceptor concept came about as a result of studies on renal hypertension. Goldblatt found in the early 1930's that hypertension of renal origin could be induced by renal artery constriction and he, therefore, concluded that renal ischemia was an important factor stimulating the release of renin from the kidney (22). Subsequent studies have demonstrated that reduced renal perfusion pressure rather than an impaired renal blood flow is the primary stimulator of renin release (23-25), although they both are interrelated to a certain degree. This view led investigators to study the effect of changes in perfusion pressure on juxtaglomerular cell granulation as an index of juxtaglomerular cell synthetic activity and metabolism. Tobian (26) found an inverse correlation between renal perfusion pressure and juxtaglomerular cell granularity, which resulted in his support of the existence of an intrarenal stretch receptor in the renal afferent arteriole which responds to changes in blood pressure. Added evidence for the baroreceptor theory has been obtained through studies utilizing the denervated, non-filtering dog kidney model, which effectively isolates the afferent arteriolar mechanism of renin release regulation for those mediated by the renal nerves and macula densa. Utilizing this model, Blaine and co-workers (27-29) found that the increase

in renin release seen after hemorrhage occurred as a result of afferent arteriole constriction and decreased renal perfusion pressure despite the absence of macula densa and neural influences, supporting the concept of a renal vascular receptor involved in the regulation of renin secretion. Furthermore, the renin secretory responses to hemorrhage were abolished when this vascular receptor was blocked by administration of the smooth muscle relaxant, papaverine, to the denervated, non-filtering dog kidney (30). Some investigators, however, have shown an increased renin release rate associated with dilatation of the afferent arteriole during renal autoregulation (31,32).

## 2. Macula Densa Receptor Regulation of Renin Secretion

Electron microscopic studies have clearly shown that the macula densa cells in the renal distal tubule are in close anatomical association with the juxtaglomerular cells (33,34). The macula densa has therefore been implicated as an additional regulatory site participating in the overall control of renin secretion. Whether the stimulus to the macula densa to influence renin release is an increased (35) or decreased (24,36,37,38) sodium load to that area, remains unclear. Based on data from numerous distal tubule microinjection studies, Thurau and co-workers have suggested that it is an increase in sodium concentration rather than load, in this area which is the signal influencing renin secretion (39-41). Meyer et al. (42) and Cooke (43) using osmotic diuretics to increase sodium concentration to the macula densa have confirmed Thurau's conclusions. Conversely, Nash (35) has shown that an increase in sodium flux across the macula densa cells into the interstitium surrounding the

juxtaglomerular cells is responsible for the resulting increase in renin release. Increased chloride load to the macula densa has also been proposed as the possible signal for renin release (41,44). In contrast, Vander's experiments involving the use of diuretics during aortic constriction in order to vary distal tubular sodium load without altering arterial pressure (38) have led to the view that a decreased sodium load to the macula densa results in an increased release of renin. Vander (45) and DiBona (46) have conducted experiments in which decreases in both distal sodium load and concentration were produced by occluding the ureter during mannitol diuresis. Their results further support the concept of decreased sodium load to the macula densa as the stimulus for renin secretion.

### 3. Humoral Control of Renin Release

Angiotensin II and antidiuretic hormone (ADH) are two circulating factors known to exert an inhibitory effect on the secretion of renin (47,48). Shade (48), in experiments using dogs with a nonfiltering kidney found that renal artery infusion of angiotensin II or antidiuretic hormone inhibits renin secretion. He suggested that these agents exert their effects directly on the juxtaglomerular cells. A direct negative feedback action exerted by angiotensin II on renin release has been confirmed by others *in vitro* (8) and *in vivo* (49-51), although the mechanism involved remains unclear.

Bunag (47) and Vander (52) have confirmed Shade's findings concerning the inhibitory effect of ADH on renin release. Moreover, Tagawa (53) reports that increases in plasma ADH lowers plasma renin ac-

tivity in sodium deficient dogs. Even though the exact mechanism involved in the renin responses to ADH is not known, a direct effect is generally accepted. Vandongen's work in vitro supports this view (54).

Recently, intrarenal prostaglandins have been implicated in the control of renin secretion (61,62). In vitro studies have shown that PGE<sub>2</sub> increases release of renin whereas PGF<sub>2α</sub> has no effect on renin release in rabbit renal cortical cell suspensions (55). Gerber (56) has also demonstrated in vivo that administration of PGE<sub>2</sub> increases plasma renin activity. Conversely, Weber et al. (57,58) have shown that PGE<sub>2</sub> has no effect on the secretion of renin in vitro and that PGF<sub>2α</sub> actually decreased release in vitro and depressed plasma renin activity in vivo. Administration of prostaglandin precursors such as arachidonic acid and of prostaglandin synthetase inhibitors such as indomethacin are known to increase and decrease plasma renin activity, respectively, in the rabbit (59). Using filtering and nonfiltering kidneys in anesthetized dogs, Seymour and Zehr (60) have shown that various intrarenal prostaglandins have a direct effect on the juxtaglomerular cells to increase renin release. In view of these conflicting data, further investigation is in order to evaluate a possible regulatory action of prostaglandins on the release of renin.

#### 4. Neural Control of Renin Release; Action of Sympathetic Nerves and Catecholamines

The kidney cortex is innervated by the lesser splanchnic nerve, a branch of the sympathetic nervous system (63), and a great supply of sympathetic nerve terminals in close association with the juxtaglomeru-

lar cells has been demonstrated (64-67). Thus, approximately two decades ago, a direct neurogenic control of renin secretion was postulated and a large body of evidence supporting this concept has been provided since then (68-72). A significant decrease in renin release occurs when the kidney is denervated (69,70). Electrical stimulation of the renal nerves in the nonfiltering kidney results in an increase in the release of renin when the baroreceptors are inactivated (71). In some experiments, renal vasoconstriction has been observed in association with an increased renin activity after renal nerve stimulation (72).

Besides the peripheral neural control of renin release, central nervous system stimulation has also been shown to modify renin responses via the renal nerves (73-75). In these studies, electrical stimulation of the midbrain (73), pons (75), or medulla (74) increased renin release. In all cases, the responses were abolished by renal denervation. However, Ueda (73) observed a simultaneous increase in circulating catecholamines associated with diminution of the renin responses to midbrain stimulation after renal denervation. He thus suggested that the central nervous system regulates release of renin through a circulating catecholamine mechanism. Furthermore, the catecholamines epinephrine and norepinephrine (71,76) and tyramine (77), an amine capable of releasing norepinephrine from nerve endings (78), are known to stimulate renin release when infused directly into the renal artery. In a recent study by Johnson (79), epinephrine and isoproterenol infused into the inferior vena cava caused a marked increase in plasma renin activity, whereas renal artery infusion of epinephrine did not increase plasma renin activity.

L-propranolol blocked the increase in plasma renin activity caused by vena cava infusion of epinephrine, suggesting an extrarenal beta-receptor mediation.

On the other hand, norepinephrine administration induces release of renin when infused intravenously during aortic constriction (72) although Wathen et al. (76) disputes this. Intravenous infusion of norepinephrine and isoproterenol increases renin secretion according to Ueda (80), a response that is inhibited by administration of propranolol and not affected by denervation. Thus, sympathetic catecholamines may act either directly on the juxtaglomerular cells to stimulate renin release or indirectly by affecting the intrarenal receptors.

The direct effect of catecholamines on renin secretion has been studied extensively utilizing various agonistic and antagonistic agents in vitro to determine the mechanism mediating the renin release responses to these agents (3-10, 14, 81-86). Renal cortical slices (5-10,14), renal cortical suspensions (81), isolated glomeruli (84), or isolated perfused kidney preparations (85,86) have been used in these studies to evaluate a number of factors which may directly influence renin release, and Braverman (87), Hammersen (88), and Corsini (89) have shown that renin release in vitro is a metabolically active process.

It is generally agreed from these in vitro studies that a beta-adrenergic receptor mechanism is involved in the mediation of the stimulatory effect of norepinephrine on renin secretion (3-6,8,81-83) and that this mechanism appears to utilize cyclic AMP as the intracellular messenger (4-6,8-10,14-81). Support for cyclic AMP participation has

been obtained in in vivo (91-94) and in vitro (3,4,95,96) studies utilizing the phosphodiesterase inhibitor theophylline which effectively prevents the intracellularly generated nucleotide from being degraded, thus potentiating the renin secretory responses to catecholamine stimulation.

The type of beta-adrenergic receptor that is involved in the direct regulation of renin release is still under debate. Some studies have shown that renin release responses are mediated via a beta<sub>1</sub> or cardiac type receptor (97-99) while others (83,100) have demonstrated that a beta<sub>2</sub>-type (peripheral vascular type) is involved.

In addition to the stimulatory effect on renin release exerted by norepinephrine via a beta-adrenergic receptor mechanism, Lopez et al. (9) have shown that stimulation of renal alpha-adrenergic receptors by large concentrations of norepinephrine inhibits renin secretion and that this effect is coupled with decreases in tissue cyclic AMP. This concept of alpha-adrenergic receptor inhibition of renin release was clearly demonstrated by the fact that addition of alpha-adrenergic receptor antagonist agents result in removal of the renin release inhibition caused by norepinephrine. This view has been supported by others working with the isolated rat kidney (101), the cortical slice system (4,7,8), or the live animal (102).

##### 5. Dopaminergic Regulation of Renin Release

Dopamine is a catecholamine (3,4-dihydroxybenzene) (103) which is structurally similar to its beta-hydroxylated derivative, norepinephrine. Studies of the actions of dopamine indicate that many metabolic effects produced by this catecholamine are different from those caused by nor-



epinephrine (104-112), suggesting the existence of dopamine-specific receptors. For instance dopamine turnover rates are greater in certain areas of the brain than others even though norepinephrine concentration is the same (113). Also, despite the fact that over 50% of the total catecholamine content of the mammalian brain is dopamine, Carlsson (114-115), Sano (116), and others (117-118) have demonstrated that brain dopamine, unlike norepinephrine, is concentrated in a few specific areas, namely the corpus striatum of the basal ganglia and substantia nigra of the mesencephalon. Additionally, dopaminergic neurons are identifiable in the hypothalamus (119-120), possibly terminating at the origin of the hypophyseal portal system (119). These neurons are thought to play a role in the regulation of prolactin (121-122) and gonadotropin (123) release from the anterior pituitary gland.

In addition to its role as a neurotransmitter in the midbrain and hypothalamus, numerous studies have shown that dopamine can exert peripheral effects on organs controlled by the autonomic nervous system (124) and on the coronary (104-106) and renal (108-112) vasculature. Specifically, dopamine is known to 1) increase cardiac muscle force of contraction and heart rate by an action on beta-adrenergic receptors (104-106), 2) exert an effect on alpha-adrenergic receptors to cause vasoconstriction in peripheral vascular beds (104,107) and 3) produce vasodilatation in the kidney (108-110) via specific dopaminergic vasculature receptors (110-112). McDonald and Goldberg (125) have shown that intravenous infusion of dopamine stimulates renal blood flow, and increases glomerular filtration rate and sodium diuresis. Most of these

renal effects by dopamine are inhibited by haloperidol, a specific dopamine-receptor blocking agent (112,126). These findings are supportive of the existence of a specific vascular receptor by which dopamine causes renal vasodilatation and have recently been confirmed by Nakajima (127).

Dopamine has been identified in the kidney of several species (128-129), and the release of dopamine from sympathetic nerves (130) and the adrenal medulla (131) has been demonstrated. Because of these observations, and in view of the evidence Goldberg has produced in support of the existence of a specific dopamine receptor in the renal vasculature, a number of laboratories are currently evaluating the potential role which dopamine may play in the regulation of renin release from the renal juxtaglomerular cells.

Various in vivo studies have demonstrated that dopamine either increases (132-134) or decreases (135-136) renin secretion in the dog or in man. Such conflicting evidence is not unusual in view of the number of factors in the live animal which simultaneously interact to influence renin release. Thus, in vitro studies have been undertaken in which the direct effects of dopamine may be more effectively studied, in the absence of hemodynamic, humoral, and other in vivo influences (1,2,9,10).

Utilizing a rat renal cortical slice preparation, Henry et al. (1) found that addition of dopamine at concentrations of  $10^{-5}M$  or greater significantly increased renin release, and that the known beta-adrenergic receptor blocker, propranolol, prevented the stimulatory effect of dopamine on renin secretion. They concluded that dopamine directly stim-

ulated renin release by a mechanism involving a beta-adrenergic receptor, a view supported by recent observations of Quesada (2). Additionally, Lopez et al. (9,10) have shown that this direct stimulatory effect of dopamine on renin release is coupled with tissue cyclic AMP changes, which suggest the involvement of adenyl cyclase in the stimulatory pathway.

Data from studies by Nakajima et al. (127,137) utilizing spiroperidol, a dopamine receptor antagonist, in a rat kidney particulate preparation indicate that specific dopamine receptors mediate the elevation of cyclic AMP levels in renal tissue after addition of dopamine leading to renal vasodilation. These observations by Nakajima and those seen in our laboratory (9,10) constitute evidence for the existence of a cyclic-AMP-mediated receptor mechanism by which dopamine influences renal mechanisms. However, these data do not elucidate whether or not dopamine may influence renin secretion through an interaction with a specific dopamine receptor, as opposed to its clear beta-adrenergic receptor mediation on renin release. Furthermore, recent evidence by Keabian suggests that more than one type of dopamine receptor exists (138). In view of these conflicting results, further characterization of the receptor type mediating the renin responses to dopamine is in order.

## CHAPTER III

### MATERIALS AND METHODS

Thirty-six male Sprague-Dawley rats (Sprague-Dawley Co., Madison, Wisconsin) with initial weights of  $210 \pm 10$  g were used in this study. They were kept in a temperature-and-light-controlled room ( $23 \pm 2^{\circ}\text{C}$ ; 12 hour light-12 hour dark photoperiod), two animals per cage, with unlimited access to distilled, deionized water. The animals were fed a sodium-deficient diet (Teklad Test Diet Co., Madison, Wisconsin), providing less than 0.02 mEq of sodium per day for a period of 10-20 days. They were subsequently sacrificed by decapitation and their kidneys excised and placed in Robinson's buffer at  $4^{\circ}\text{C}$  (139). The isolated kidneys were then gassed again for 30 sec with the same gas mixture.

Slices of renal cortex, approximately 0.3 mm thick, were prepared from the decapsulated kidneys using a Stadie-Riggs microtome (A. Thomas Co.). Each slice was subsequently divided into six similarly-sized sections which were randomly distributed to incubation beakers containing 2.5 ml of Robinson's buffer media at  $4^{\circ}\text{C}$ . The procedure was repeated using slices from other cortical areas until each beaker contained  $50 \pm 30$  mg of homogeneously distributed cortical tissue. The sliced tissue was preincubated at  $37^{\circ}\text{C}$  for 15 min in a Shaking Dubnoff Metabolic Incubator (Precision Scientific Co.) under an atmosphere of 95%  $\text{O}_2$  - 5%  $\text{CO}_2$ . The preincubated tissue was then transferred to beakers containing 2.5 ml of fresh Robinson's buffer at  $37^{\circ}\text{C}$  and incubated for 5, 20 or 60

minutes under identical conditions as in the preincubation period.

One of each group of six tissue-containing beakers was used as an untreated, control sample, while the remaining tissue samples were treated with dopamine ( $10^{-3}\text{M}$ ; Sigma), added either alone or in conjunction with the dopamine-receptor blocking agent d-butacclamol ( $10^{-6}\text{M}$ ; Ayerst), its inactive isomeric form l-butacclamol ( $10^{-6}\text{M}$ ), or the phosphodiesterase inhibitor theophylline ( $10^{-3}\text{M}$ ). D- and l-butacclamol and theophylline were also administered alone to the tissue preparation. In a simultaneous study, the specific dopamine receptor agonist, apomorphine ( $10^{-6}\text{M}$ ), was added to the tissue preparation either alone or with dopamine ( $10^{-3}\text{M}$ ). The dopamine beta-hydroxylase inhibitor FLA-63 ( $10^{-4}\text{M}$ ; Regis) was added to all samples to prevent conversion of dopamine to norepinephrine in the tissue system (11,12).

Theophylline, the blocking agents, apomorphine, and FLA-63 were added to the tissue preparation prior to both the pre-incubation and incubation periods, while dopamine was added prior to the incubation period only. D- and l-butacclamol, dopamine, apomorphine, and FLA-63 were prepared in a 0.1% ascorbic acid solution (Sigma) to prevent oxidation (5), while theophylline was prepared in Robinson's buffer.

Following incubation, the supernatant medium was collected and stored at  $-20^{\circ}\text{C}$  until assayed for renin concentration by angiotensin I radioimmunoassay (142). The incubated tissue was immediately frozen on dry-ice, homogenized in 1 ml of 8% trichloroacetic acid, transferred to tubes containing 5 drops of 0.1 N HCl, vortexed for 5 sec, and stored at  $-20^{\circ}\text{C}$  until assayed for cyclic AMP content by a modification of the

protein-binding assay of Gilman (141). In some instances, the incubated tissue was collected for determination of its renin content. The tissue was purified by a series of dialysis steps and measured for renin concentration by radioimmunoassay of angiotensin I.

#### A. Determination of Renin Concentration

Renin concentration in incubated supernatant was estimated indirectly by measuring the amount of angiotensin I generated when aliquots of the renin-containing samples were allowed to react with dog renin substrate in the presence of an appropriate inhibitor of converting enzyme and angiotensinases. When these enzymes are inactivated, the accumulation of angiotensin I during a given generation period reflects renin activity. Blockade of converting enzyme and angiotensinase activity was achieved in this study by the use of a 1% solution of phenylmethylsulfonylfluoride (PMSF; 142).

To generate angiotensin I, the frozen samples were thawed in an ice bath at 4°C and vortexed for ten seconds. Aliquots of 0.025 ml from each sample were then transferred to tubes containing 0.1 ml of saturated NaCl. Six-hour nephrectomized dog plasma containing renin substrate (angiotensinogen) and previously treated with PMSF (0.05 ml/ml plasma) was then added to each tube (0.4 ml/tube). The mixture was incubated for one hour at 37°C and diluted with one ml of distilled, deionized water. The samples were vortexed for five sec, covered with aluminum foil, and placed in a boiling water bath for three min to prevent further angiotensin I generation. The samples were then allowed to cool to 25°C and stored at -20°C. The amount of angiotensin I gener-

ated was measured by a modification of Angiotensin I ( $^{125}\text{I}$ ) Radioimmunoassay (New England Nuclear). Renin concentration was expressed in ng/mg/hr -(ng of angiotensin I generated/ mg of tissue/ hour of generation).

#### B. Tissue Renin Determination

Tissue collected for determination of renin content was homogenized in 1 ml of 0.9% NaCl at 4°C and centrifuged for 15 min at 3500 g. The supernatant was subsequently dialyzed for 24 hours against an EDTA-acetic acid buffer (pH 3.3), followed by heating to 32°C for 1 hour to selectively denature endogenous renin substrate and angiotensinases, and dialyzed again for 24 hours against an EDTA-phosphate buffer (pH 7.5) as described by Skinner (143).

The purified tissue homogenates were then used to generate angiotensin I as described previously and subsequently measured for renin concentration by Angiotensin I ( $^{125}\text{I}$ ) Radioimmunoassay (New England Nuclear). The values were expressed in ng of angiotensin I generated/ mg wet tissue/ hour of generation.

#### C. Determination of Tissue Cyclic Adenosine 3',5'-Monophosphate (cAMP) Content

The frozen tissue homogenates collected for cAMP determination were thawed in an ice bath at 4°C, and centrifuged at 4000 g for ten min at 4°C. The supernatant was transferred, while the protein pellet was discarded. Two ml of water-saturated ether were added to each sample, vortexed for ten sec, and the ether phase aspirated and discarded. This extraction procedure was repeated three additional times.

The cAMP-containing water soluble phase was then decanted into vials previously placed on dry ice, and the frozen samples were subsequently lyophilized. The lyophilized material was then stored at  $-20^{\circ}\text{C}$  until assayed for cAMP content by a modification of the competitive protein binding assay of Gilman (141). Tissue cAMP concentration was expressed in pmol/mg (pmol of cAMP/mg wet tissue).

#### Calculations

Statistical significance of the data was evaluated by Student's paired and unpaired t tests using a programmable desk top calculator (Hewlett-Packard) at Argonne National Laboratory (Argonne, Illinois). A Linear Regression Analysis was performed on the control data (144).



## CHAPTER IV

### RESULTS

Resting renin release and tissue cyclic AMP content of untreated (control) renal cortical tissue from sodium deficient rats are shown in Fig. 1. Renin release increased linearly with time of incubation ( $r: 0.62; p < 0.001$ ). In contrast to renin, tissue cyclic AMP content values were maximal at 20 min of incubation and decreased by 60 min to levels seen at 5-min incubation.

The effect of  $10^{-3}M$  dopamine on three parameters, renin release, tissue renin content, and tissue cyclic AMP at various incubation times, is depicted in Fig. 2. Renin release responses to dopamine administration gradually increased over control by 5 and 20 min and were significantly greater by 60 min. In contrast, cyclic AMP content values in response to added dopamine appeared significantly greater than those of control samples as early as 5 min of incubation. Although mean tissue renin content of the dopamine-treated samples appeared higher than that of controls by about 60 nanograms at 60 min, this difference was not statistically significant.

The effect of the phosphodiesterase inhibitor theophylline ( $10^{-3}M$ ) added alone or together with dopamine ( $10^{-3}M$ ) on the same three parameters in relation to time of incubation, is demonstrated in Fig. 3. Theophylline appeared to increase the renin release responses to added dopamine at 20 and 60 min, while significantly potentiating the cyclic

AMP responses to dopamine administration at the same time periods. Theophylline by itself significantly increased renin release by 60 min, not only in relation to controls but in regard to dopamine alone ( $p < 0.02$ ). Cyclic AMP content changes in response to theophylline alone were significantly potentiated when compared to those exerted by dopamine alone at all times of incubation ( $p < 0.01$ ,  $p < 0.02$ ,  $p < 0.02$  for 5, 20, and 60 min, respectively), but were not different from those seen in response to theophylline plus dopamine treatment at corresponding times.

Mean tissue renin content at 60 min appeared increased by about 25 nanograms in response to theophylline plus dopamine and by an additional 85 nanograms in the presence of theophylline alone when compared to that observed in response to dopamine added alone, although these differences were not significant.

Addition of the specific dopamine-receptor blocker d-butacclamol ( $10^{-6}M$ ) to the cortical slice preparation increased the mean renin release levels in relation to those seen with dopamine alone at 60 min, but not significantly (Fig. 4). Also, d-butacclamol administration did not further alter the significant stimulation of tissue cyclic AMP levels seen with dopamine alone at the same time period. By itself, d-butacclamol had no effect on renin release or tissue cyclic AMP content. Mean tissue renin content in the presence of d-butacclamol added together with dopamine was increased by about 40 nanograms in relation to that seen in response to dopamine alone, but the difference was not significant.

L-butacclamol at  $10^{-6}M$ , added alone or with dopamine was ineffective in altering the renin release and cyclic AMP levels seen with

dopamine alone (Fig. 5). A 60 nanogram increase in tissue renin content occurred in response to dopamine plus l-butacclamol treatment relative to control at 60 min, but this difference was again not significant.

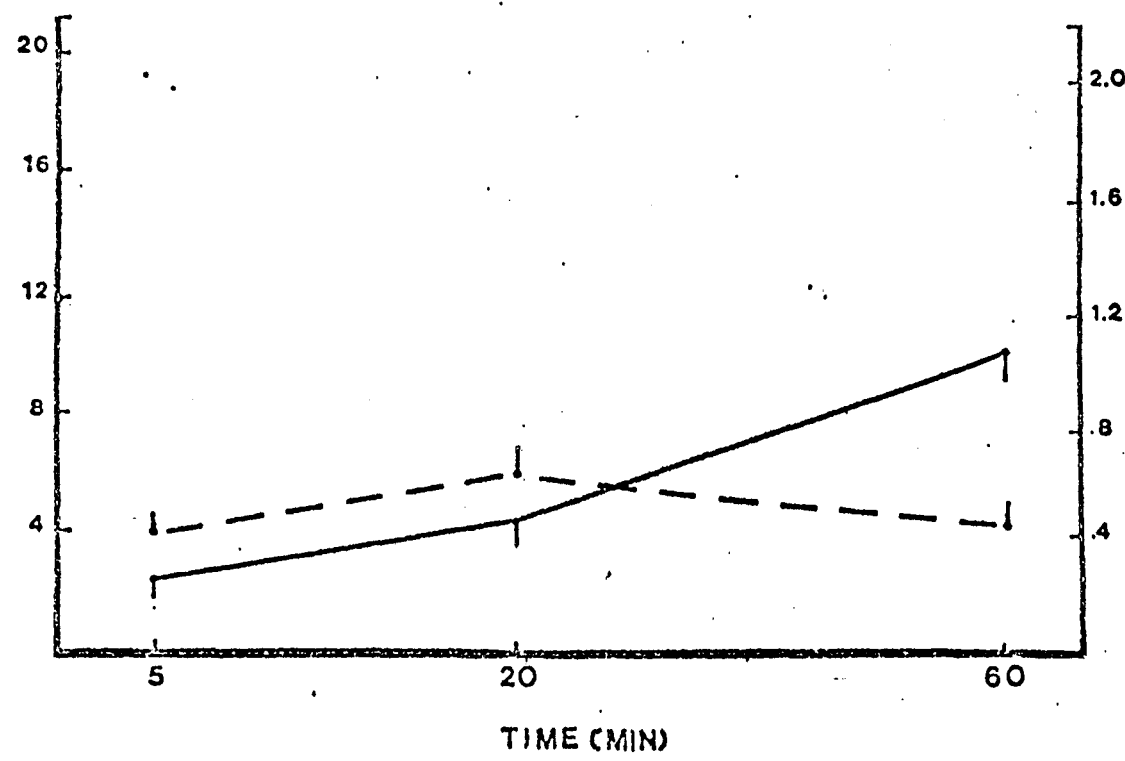
Figure 6 summarizes the results of this study utilizing theophylline and d-butacclamol, and those obtained in a parallel study with apomorphine, a dopamine-receptor agonist. From this figure it is clear that in addition to the data reported in this section, apomorphine ( $10^{-6}M$ ) prevented the stimulatory effect of  $10^{-3}M$  dopamine on renin release and cyclic AMP content at 60 min incubation. The overall significance of these observations will be discussed.

Figure 1. Renin release rate and tissue cyclic AMP content of unstimulated (control) renal cortical slices from sodium deficient rats at incubation times of 5, 20, and 60 min. The renin release data represent the mean control value  $\pm$  S.E. of 14-32 observations for each time period. Similarly, the cyclic AMP content data represent the mean control value  $\pm$  S.E. of 12-14 observations for each time period. The dopamine beta-hydroxylase inhibitor 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)

Figure 2. Effect of  $10^{-3}$ M dopamine on renin release, tissue cyclic AMP content, and tissue renin content of renal cortical slices from sodium deficient rats. The data represent the mean renin release rate  $\pm$  S.E. of 14-32 observations, and the mean tissue cyclic AMP content  $\pm$  S.E. of 12-14 nontreated (control) and dopamine-treated samples at various incubation times. The data also show that mean tissue renin content  $\pm$  S.E. of 23 observations in control and dopamine-treated samples at 60 min incubation.

 CONTROL  
 DOPAMINE 10<sup>-3</sup>M

\* significantly different from control (p<0.05)  
 \*\* significantly different from control (p<0.01)

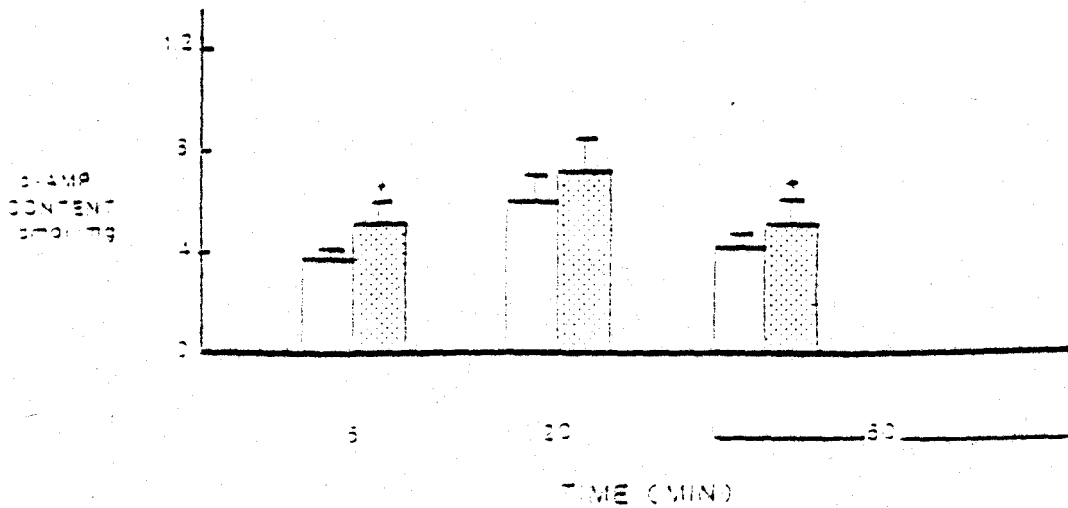
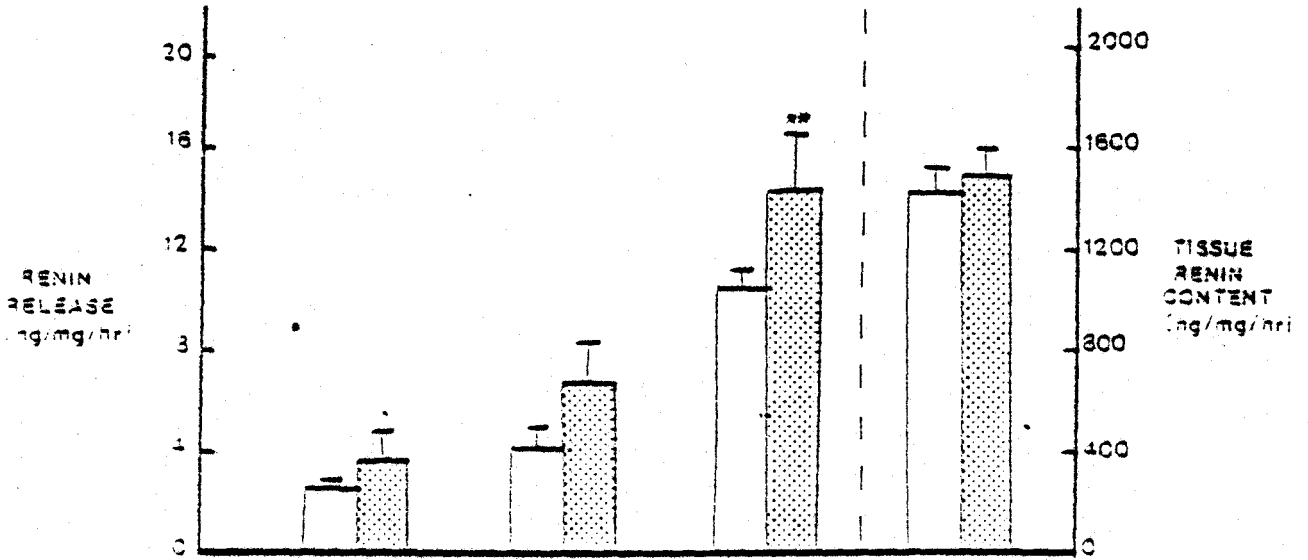


Figure 3. Changes in renin release, tissue cyclic AMP content, and tissue renin content, in response to  $10^{-3}$ M dopamine and  $10^{-3}$ M theophylline, added together or separately to renal cortical slices from sodium deficient rats. The data represent the mean renin release change  $\pm$  S.E. of 7-13 observations, the mean tissue cyclic AMP content change  $\pm$  S.E. of 9-11 observations for each treatment at 3 incubation times. The mean renin release rates for nontreated (control) tissue at 5, 20, and 60 min incubation were  $2.55 \pm .276$  ng/mg wet tissue/hr,  $3.86 \pm .801$  ng/mg/hr, and  $10.28 \pm 1.01$  respectively. The corresponding mean control cyclic AMP content values were, respectively,  $.397 \pm .032$  pmol/mg,  $.616 \pm .076$  pmol/mg, and  $.437 \pm .046$  pmol/mg. The mean control tissue renin content at 60 min was  $1419 \pm 103$  ng/mg/hr.



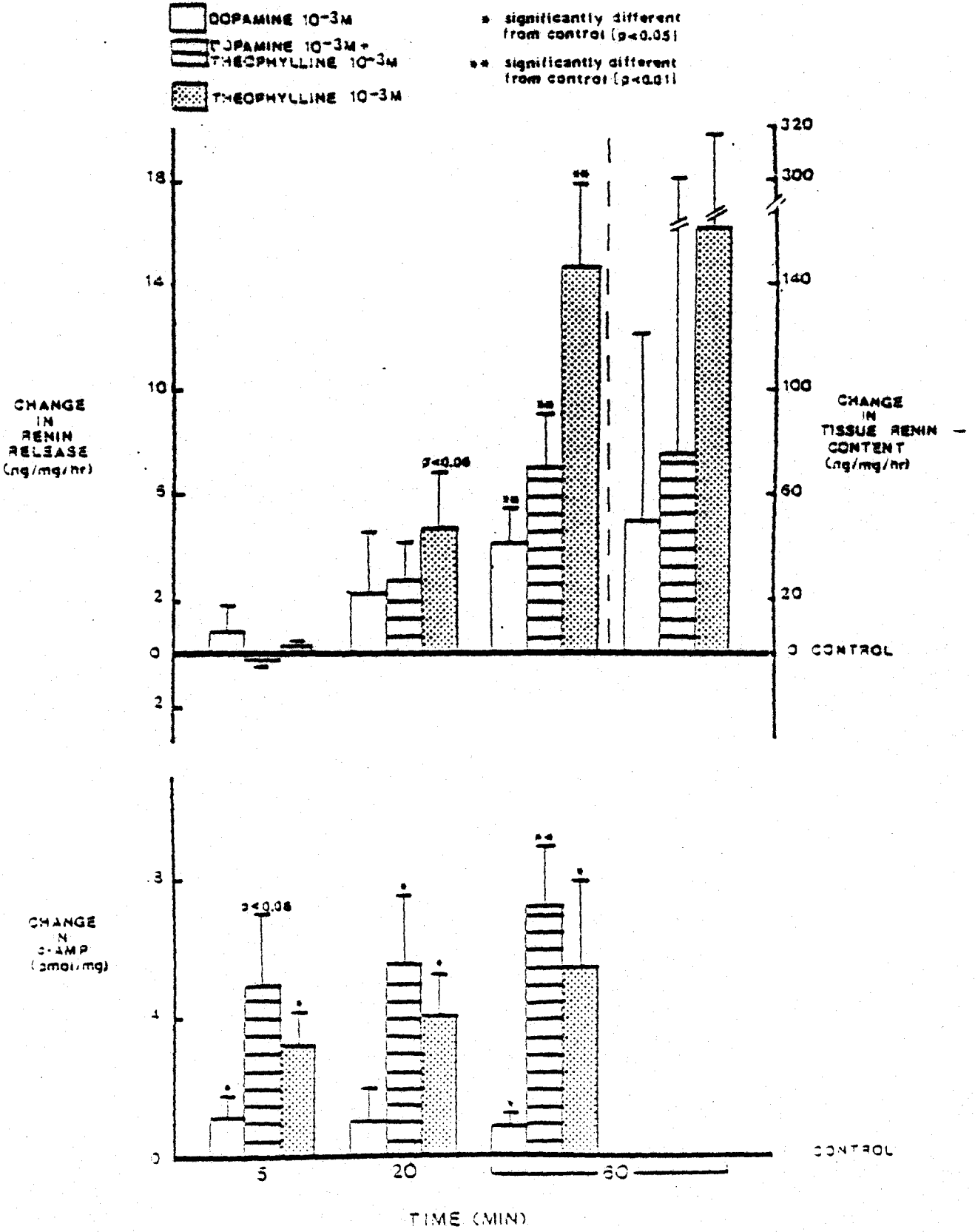


Figure 4. Changes in renin release, cyclic AMP content, and tissue renin content in response to  $10^{-3}$ M dopamine and  $10^{-6}$  d-butacclamol, added together or separately to renal cortical slices from sodium deficient rats. The data represent the mean renin release change  $\pm$  S.E. of 2-18 observations, the mean tissue cyclic AMP content change  $\pm$  S.E. of 2-12 observations, and the mean tissue renin content change  $\pm$  S.E. of 13 observations for each treatment at various incubation times. The mean control (nontreated) values for renin release rate, tissue cyclic AMP content, and tissue renin content, for each time period are the same as those in Figure 3.

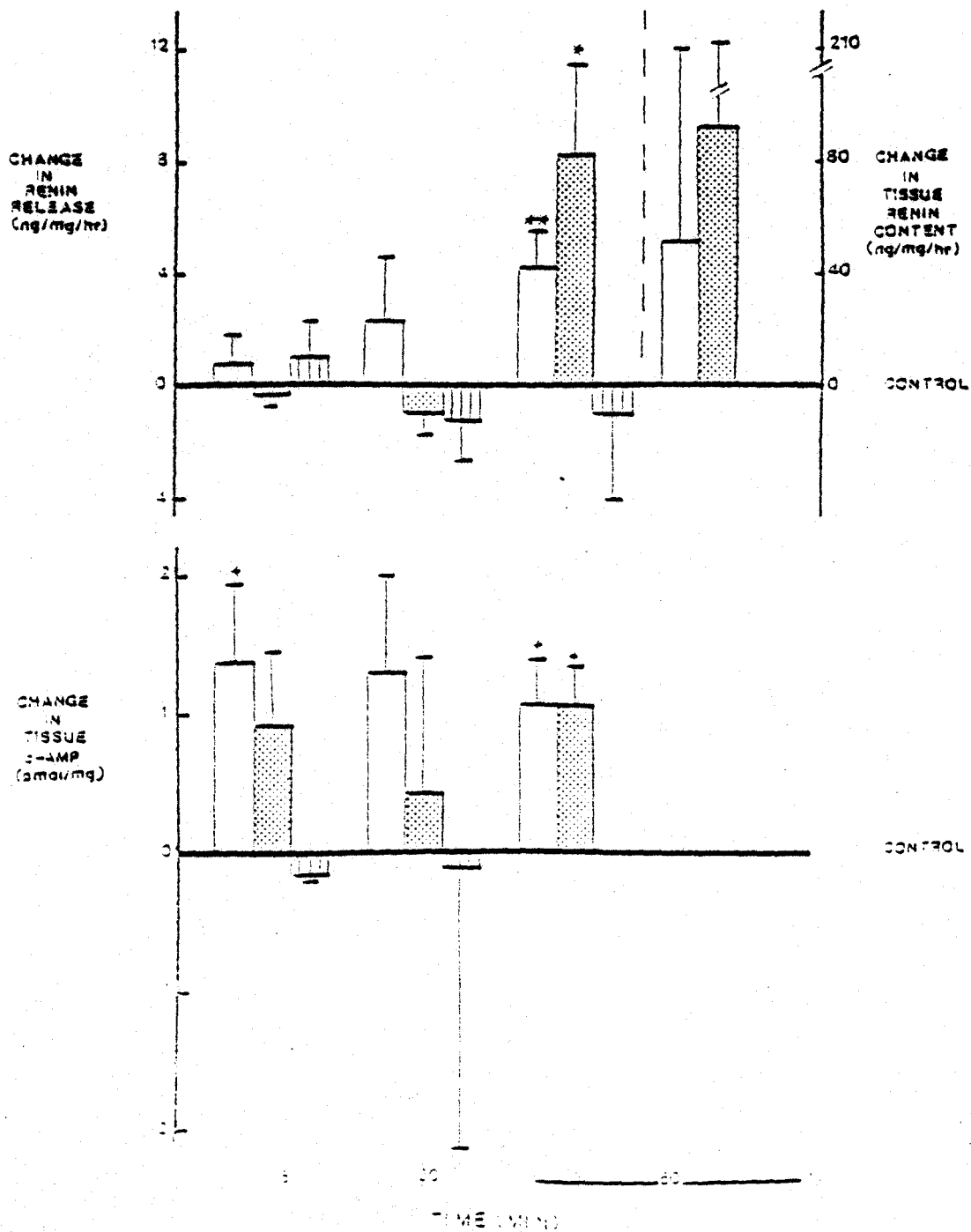
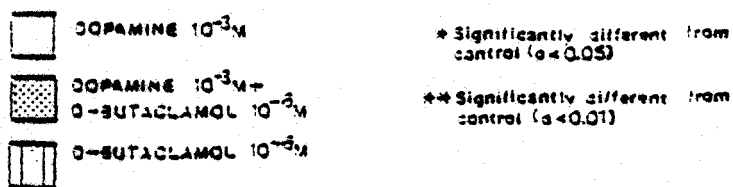
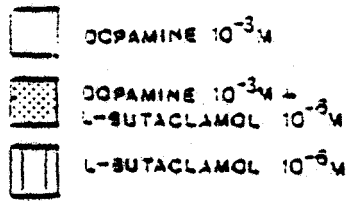


Figure 5. Changes in renin release, tissue cyclic AMP content, and tissue renin content in response to  $10^{-3}$ M dopamine and  $10^{-6}$ M 1-butacclamol, added alone or together to renal cortical slices from sodium deficient rats. The data represent the mean renin release change  $\pm$  S.E. of 4-14 observations, the mean tissue cyclic AMP content change  $\pm$  S.E. of 2-11 observations, and the mean tissue renin content change  $\pm$  S.E. of 10 observations for each treatment at various incubation times. The mean control (untreated) values for renin release rate, tissue cyclic AMP content, and tissue renin content, for each time-period are the same as those described in Figure 3.



\* Significantly different from control ( $p < 0.05$ )

\*\* Significantly different from control ( $p < 0.01$ )

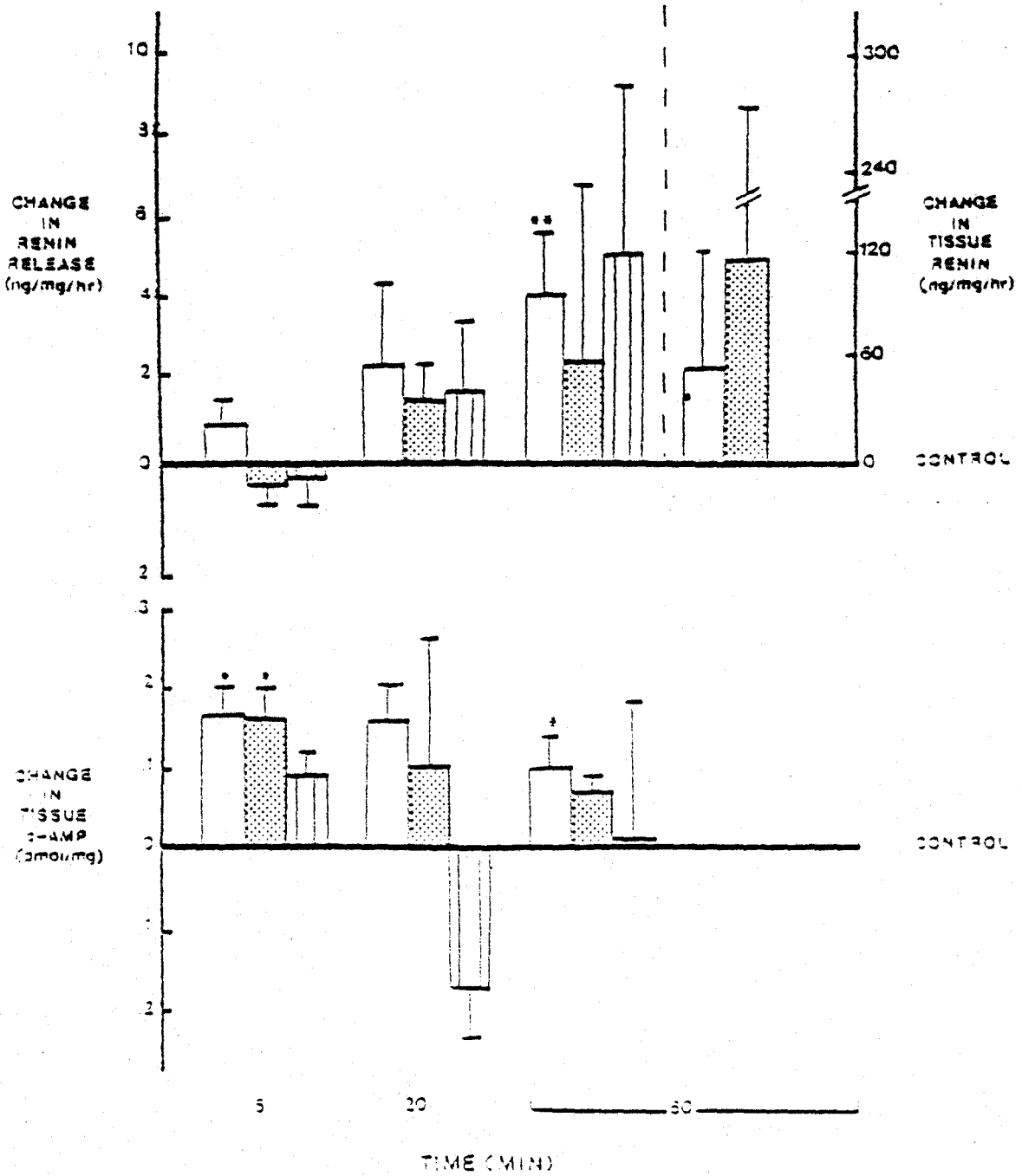




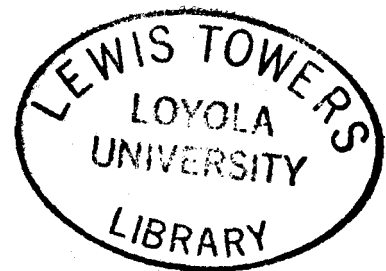
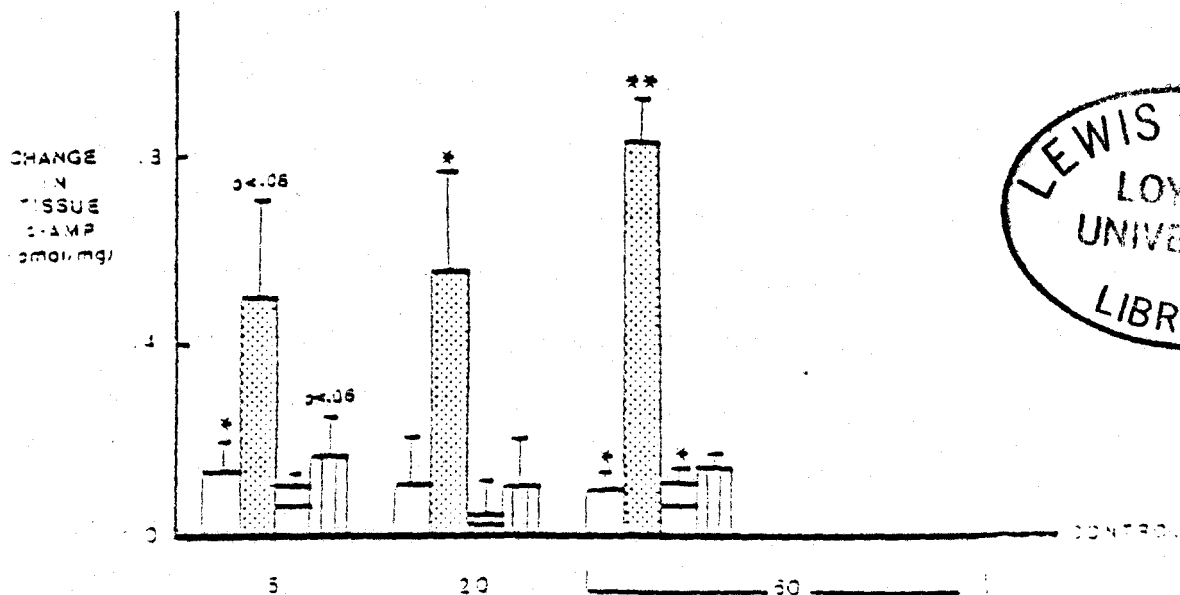
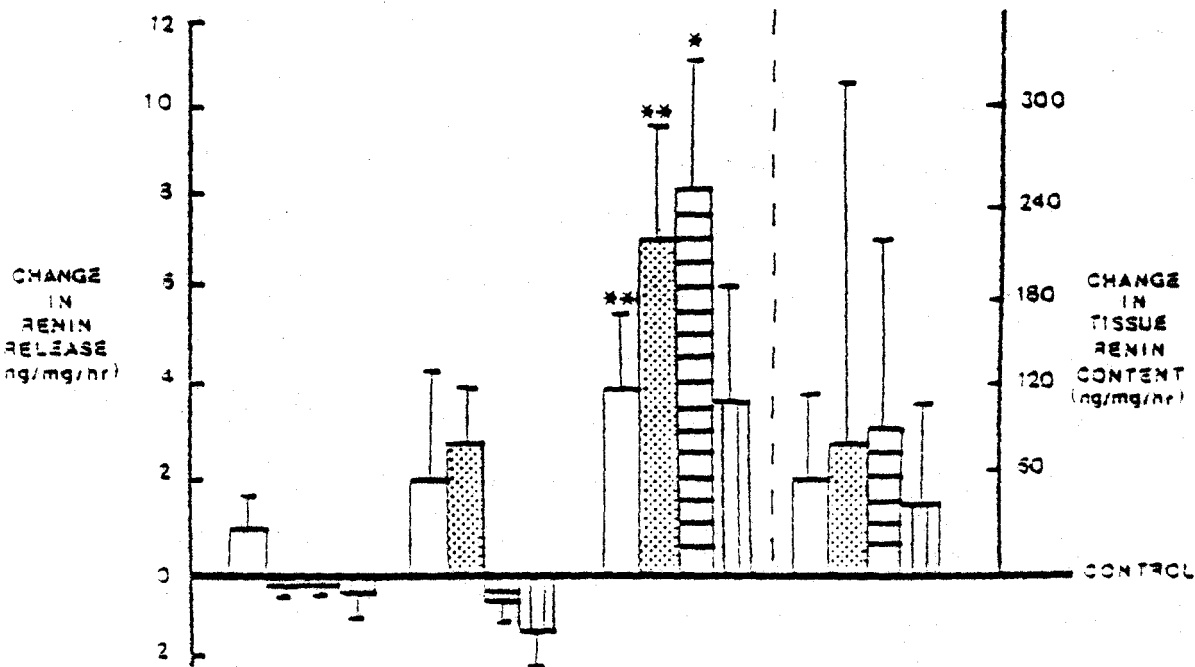


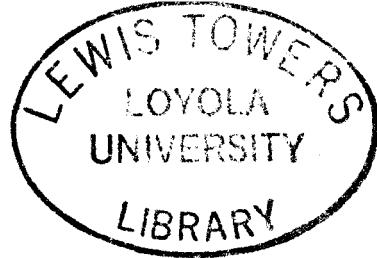
Figure 6. Changes in renin release, tissue cyclic AMP content, and tissue renin content in response to dopamine ( $10^{-3}\text{M}$ ) added alone or together with  $10^{-3}\text{M}$  theophylline,  $10^{-6}\text{M}$  d-butacclamol, or  $10^{-6}\text{M}$  apomorphine, to renal cortical slices from sodium deficient rats. The data represent the mean renin release change  $\pm$  S.E. of 2-18 observations, the mean tissue cyclic AMP content change  $\pm$  S.E. of 2-12 observations, and the mean tissue renin content change  $\pm$  S.E. of 9-24 observations for each treatment at various incubation times. The mean control (untreated) renin release rate, tissue cyclic AMP content, and tissue renin content at various times of incubation, are the same as those indicated in Figure 3. The corresponding mean control values at 3 incubation times, for treatments involving dopamine alone or together with apomorphine were, respectively,  $2.56 \pm 0.48$ ,  $4.69 \pm 1.51$ , and  $10.60 \pm 1.39$  ng/mg/hr for renin release;  $0.39 \pm 0.03$ ,  $0.53 \pm 0.09$ , and  $0.45 \pm 0.07$  pmol/mg for cyclic AMP content; and  $489 \pm 399$  ng/mg/hr for tissue renin content at 60 min.

-  DOPAMINE  $10^{-3}M$
-  DOPAMINE  $10^{-3}M$  + THEOPHYLLINE  $10^{-3}M$
-  DOPAMINE  $10^{-3}M$  + O-BUTACLAMOL  $10^{-5}M$
-  DOPAMINE  $10^{-3}M$  + APOMORPHINE  $10^{-5}M$

\* Significantly different from control ( $p < 0.05$ )

\*\* Significantly different from control ( $p < 0.01$ )





## CHAPTER V

### DISCUSSION

Renal cortical slice preparations have been successfully utilized in numerous studies to evaluate the direct effect of catecholamines and other agents on renin release (5-10, 14), or to examine renin secretory rates in unstimulated cortical tissue (6, 90, 145, 146). The results obtained in this study support previous observations in studies utilizing rat kidney slice systems (6, 90, 145, 146), that renin release in resting renal tissue is directly proportional to time of incubation which is to be expected of metabolically active, oxygen-consuming (147) endocrine tissue. Furthermore, these data provide added evidence for the postulation that resting renin secretory rates in renal slices from sodium deficient rats are comparably greater than those seen in sliced tissue obtained from rats maintained on sodium replete diets (6, 90, 145), which may reflect an increased responsiveness of the juxtaglomerular cells in the sodium deficient state.

Studies in this laboratory (9,10) have recently shown that dopamine at  $10^{-3}M$  can directly stimulate renin release through a beta-adrenergic receptor mechanism apparently mediated by tissue cyclic AMP changes, which confirm previous observations by Henry (1) and Quesada (2). The data generated in the present study offer further support for the concept of a cyclic AMP-mediated beta-receptor regulation of renin release by dopamine, by demonstrating that cyclic AMP content of the



incubated tissue in response to dopamine administration was significantly increased over that of controls as early as 5 min of incubation, whereas renin release appeared significantly stimulated only after 60 min of incubation. These observations are consistent with the model proposed by Sutherland (148) regarding mediation of stimulus-hormonal responses by adenylate cyclase/cyclic AMP, and they agree with the postulated beta-adrenergic receptor mechanism of action by which other catecholamines stimulate renin secretion (3,5,7-10). Furthermore, the results of this study utilizing the phosphodiesterase inhibitor theophylline, provide added evidence for this concept by showing that both tissue cyclic AMP and renin secretory rates were potentiated in the presence of this agent, as suggested in previous studies with other catecholamines (4,81). Although the data are not conclusive in this regard, the apparent increase in tissue renin content in response to dopamine and theophylline administration in this study, may be suggestive of new synthesis of renin in addition to increased release as suggested by Katz in studies with dog renal cortical slices (149). Effective binding of dopamine to receptors other than dopamine-specific ones is not unusual, since Goldberg (150) has demonstrated that this amine has the biochemical flexibility to bind to beta- and perhaps also to alpha-adrenergic receptors. Thus, it appears that dopamine stimulates renin release by a beta-adrenergic receptor mechanism, a view supported by other investigators (1,2,10) who have shown that the stimulatory effect exerted by dopamine on renin release can be suppressed by addition of the beta-receptor blocker, propranolol.

Recently, Quesada et al. (2) have produced evidence showing that the dopamine-receptor blocking agent, haloperidol, potentiates the renin release responses to dopamine administration. The results of the present study utilizing the dopamine-receptor blocker, d-butacclamol, agree with their observations and further suggest that dopamine-specific receptors may have no effect or may participate in an inhibitory fashion in the regulation of renin release. Whether or not tissue cyclic AMP is also involved in mediating this apparent inhibitory control of renin secretion by dopamine is not clear, since d-butacclamol administration did not further alter the content of the nucleotide in relation to that seen with dopamine alone. However, data from another study using the dopamine-receptor agonist agent, apomorphine, appear to indicate that decreases in tissue cyclic AMP content may mediate the action of the inhibitory component of dopamine, in view of the fact that specific stimulation of dopamine receptors by this agent prevented the increase in renin release and tissue cyclic AMP content observed with dopamine alone. Thus, dopamine may stimulate renin release via a beta-receptor mechanism involving cyclic AMP content changes and it may inhibit it by its interaction with a dopamine-specific receptor.

Although the possibility of a dual regulatory control exerted by dopamine on renin secretion is attractive and a similar concept has been postulated in regard to the regulation of renin release by norepinephrine (6,8,101,102), further work is needed to determine if the apparent inhibition mediated by a dopamine receptor is real, since the results seen in this study with the inactive isomer, l-butacclamol (151) are contradic-

tory and do not fit the proposed model. Therefore, additional doses of apomorphine and d,- and l-butacclamol need to be evaluated and other known dopamine-receptor agonist and antagonist agents must be examined, before this question can be effectively answered.

In summary, the data reported in this study suggest that, 1) dopamine is capable of directly stimulating the renal juxtaglomerular cells to influence renin release via a beta-adrenergic receptor mechanism involving tissue cyclic AMP changes; 2) new synthesis of renin, in addition to stimulation of its release, may result from the interaction of dopamine with this postulated beta-adrenergic receptor; and 3) dopamine may also exert a simultaneous inhibitory regulation of renin release via a dopaminergic-receptor mechanism, but further investigation is needed before this possibility can be properly evaluated.

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

*November 30, 1980*

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