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Effect of Dopamine and Dietary Sodium Deficiency on in Vitro Renin Release and Cyclic 3' 5'-Adenosine Monophosphate Content in Male Rat Kidney Slices: Modification by Dopaminergic-Receptor Agonistic and Antagonistic Agents

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EFFECT OF DOPAMINE AND DIETARY SODIUM DEFICIENCY ON IN VITRO
RENIN RELEASE AND CYCLIC 3' 5' - ADENOSINE MONOPHOSPHATE CONTENT
IN MALE RAT KIDNEY SLICES: MODIFICATION BY DOPAMINERGIC-RECEPTOR
AGONISTIC AND ANTAGONISTIC AGENTS.

by

Victor Allyn Aletich

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VITA

The author, Victor Allyn Aletich, is the son of Victor Andrew Aletich and Wilma (Reit) Aletich. He was born November 28, 1954, in Chicago, Illinois.

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TABLE OF CONTENTS

	Page
Acknowledgements.....	ii
Vita.....	iv
List of Tables.....	vii
List of Figures.....	viii
Abstract.....	ix
 Chapter	
I. Introduction.....	1
II. Review of Related Literature.....	5
A) The Renin-Angiotensin Aldosterone System.....	5
B) Factors Regulating Renin Secretion.....	6
1) Intrarenal Receptors.....	7
a) The Afferent Arteriole Baroreceptor.....	7
b) The Macula Densa Natrioreceptor.....	8
2) Humoral Factors.....	9
3) The Renal Sympathetic Nerves and Catecholamines.....	10
a) Juxtaglomerular Apparatus Innervation.....	10
b) Effect of Catecholamines on Renin Release.....	11
c) Effects of Dopamine on Renin Secretion.....	15
III. Materials and Methods.....	19
A) Renin Concentration Determination by RIA of Angiotensin I.....	22
1) Angiotensin I Generation.....	22
2) Radioimmunoassay Procedure of Angiotensin I.....	23

B) Determination of Cyclic 3' 5' Adenosine Monophosphate (c-AMP) By Competitive Protein Binding Assay.....	24
C) Statistical Calculations.....	25
IV. Results.....	26
V. Discussion.....	42
Summary.....	48
References.....	50
Appendix.....	57

LIST OF TABLES

- Table 1: Effect of Various Doses of Dopamine in the Presence and Absence of Apomorphine on Renin Secretion and c-AMP Content of Renal Cortical Slices from Sodium Deficient Rats.

LIST OF FIGURES

Figure

- 1) Changes in Renin Release and c-AMP Content in Renal Cortical Slices from Sodium-deficient Rats Compared to Rats Maintained on a Sodium-replete Diet (control)
- 2) Effect of Various Concentrations of Dopamine Hydrochloride on Renin Release by Renal Cortical Slices from Sodium Deficient Rats.
- 3) Effect of Various Concentrations of Dopamine Hydrochloride on Renin Release and c-AMP Content of Renal Cortical Slices from Sodium-Deficient Rats.
- 4) Changes in Renin Release in Response to Various Dopamine Hydrochloride Concentrations, Added Either Alone or Together With 10^{-6} M Pimozide to Renal Cortical Slices of Rats Fed a Sodium-deficient Diet for two to three Weeks.
- 5) Changes in Renin Release and c-AMP Content in Response to Various Concentrations of Dopamine Hydrochloride, Added Either Alone or Together With 10^{-6} M Pimozide to Renal Cortical Slices of Rats Fed a Sodium Deficient Diet for two to three Weeks.

ABSTRACT

The effect of four doses of dopamine (10^{-9}M , 10^{-7}M , 10^{-5}M and 10^{-3}M) and dopamine-receptor agonistic and antagonistic agents on renin release and 3' 5' - adenosine monophosphate (c-AMP) content of renal cortical tissue slices from sodium-deficient rats was studied in vitro. Male, Sprague-Dawley rats weighing 210 ± 10 g were fed a sodium deficient diet (less than 0.02 mEq/day) for periods of time of 2-3 weeks. Following decapitation, renal cortical slices weighing 50 ± 20 mg were prepared using a Stadie-Riggs microtome. After a 15 minute preincubation period, they were incubated for 1 hr at 37°C in Robinson's buffer medium with continuous 95% O_2 - 5% CO_2 gassing, in the presence or absence (control) of the pharmacological agents. Addition of 10^{-5}M and 10^{-3}M dopamine doses to the kidney slice preparation significantly stimulated renin release, and the c-AMP content of the renal cortical tissue slices was also significantly greater than controls at 10^{-3}M dopamine concentration. Dopamine doses lower than 10^{-5}M were ineffective in stimulating either renin release or tissue c-AMP content. Addition of the dopamine-receptor blocking agent pimozide (10^{-6}M) effectively inhibited the stimulatory effect of two dopamine concentrations (10^{-5}M

and 10^{-3}M) on renin secretion, while significantly decreasing tissue c-AMP content levels below those of control in slices treated with all four dopamine concentrations. Pimozide added alone to the tissue slice system significantly decreased both renin release and tissue c-AMP content levels below those seen in control slices. The dopamine-receptor agonist apomorphine (10^{-6}M) added to the in vitro slice preparation did not potentiate but actually prevented stimulation of renin release by dopamine (10^{-5}M and 10^{-3}M), and its action on the c-AMP content of slices treated with these two doses of dopamine was variable. Apomorphine added alone to the slice system did not change either renin release rate or tissue c-AMP content.

These data indicate that: 1) dopamine exerts a direct stimulatory effect on renin release which may be mediated by a dopamine-sensitive receptor mechanism utilizing c-AMP as the intracellular mediator; 2) in the sodium-deficient state and perhaps under sodium-replete conditions, dopamine may participate in the regulation of basal renin secretion through changes in intracellular c-AMP levels; and, 3) apomorphine at certain concentrations may have both dopamine-receptor agonistic and antagonistic properties.

CHAPTER I

INTRODUCTION

Our knowledge concerning the various mechanisms which control renin secretion from the juxtaglomerular cells of the kidney has increased at an accelerated pace during the last ten years. Davis (1) has classified these various factors or mechanisms into three major categories, 1) the intrarenal receptors, which refer to the renal vascular baroreceptors in the renal afferent arteriole sensitive to stretch due to changes in perfusing pressure (2-6), and the natrioreceptors of the macula densa in the distal convoluted tubule sensitive to changes in flux of sodium (7-13) or chloride (14-16); 2) the humoral factors, which include angiotensin II, vasopressin (17, 18), and several other factors such as the circulating catecholamines, sodium, potassium, calcium ions, and the steroid hormones (1); and 3) the neural factors which appear to exert their regulatory control over renin secretion by a direct effect on the juxtaglomerular cells via norepinephrine or its precursors released from postganglionic sympathetic nerve terminals in the kidney (19). Renin release, in most situations, occurs as a result of a complex interaction and interplay of these basic

mechanisms rather than by a preferential activity of any these individually.

It is now clear that the classic intrarenal mechanisms (i.e. afferent arteriole baroreceptors and macula densa natrioreceptors) play a substantial role in the control of renin secretion, and that the various other humoral and hemodynamic factors are also intimately involved. In recent years, however, evidence has accumulated pointing to the sympathetic nervous system as a very important regulator of renin release, through effects which appear to be directly exerted on the juxtaglomerular cells by catecholamines released from sympathetic nerve terminals. It is interesting to speculate that this sympathetic control may turn out to be an effective tool for fine regulation of renin secretion according to homeostatic needs, and possibly also the underlying cause of various pathophysiological alterations found in a number of hypertensive conditions.

In addition to norepinephrine, a catecholamine released from renal sympathetic nerve endings, the possibility of a direct action of norepinephrine precursors such as dopamine on renin secretion has been examined by several investigators. In vivo studies by Anton and Sayer (20) in 1964, have shown that the kidney contains large quantities of dopamine, although the intrarenal location

is not clear. Other in vivo studies (21-23) have presented evidence in favor of the existence of specific renal dopaminergic receptors, stimulation of which induces vascular vasodilation. Additional in vivo (23, 24) and in vitro studies (25) have suggested that these dopaminergic receptors may regulate renin release in addition to modulating vascular hemodynamics. Further evidence in this regard has come from in vitro studies by Henry et al (26), which suggest that dopamine-sensitive receptors are present in renal tissue but these receptors are probably not dopamine specific. Thus, further experimentation of a possible role of dopamine in the regulation of renin secretion is clearly in order. Therefore, the purpose of the present study is: 1) to evaluate, using an in vitro renal cortical slice preparation, a possible direct stimulatory effect of dopamine on the renin-secreting juxtaglomerular cells of the kidney, when added either alone or in conjunction with specific dopamine receptor stimulating (apomorphine) or blocking (pimozide) agents; 2) to examine the possibility of a partial dopaminergic control of basal renin secretion, by the addition of dopamine-receptor agonistic and antagonistic agents to nontreated control samples; 3) to elucidate a possible involvement of cyclic-AMP as an intracellular mediator of the renin secretory responses to added dopamine and other agents; and 4) to evaluate the effect of sodium

deficiency as a potentiating agent of the renin release responses to natural catecholamine precursors, as suggested for endogenous catecholamines (norepinephrine). This research is important in terms of defining a possible contributory role of catecholamine precursors (dopamine) released from renal nerve terminals on the minute-to-minute control of renin secretion, and would provide additional evidence toward characterizing the type of adrenergic receptor partly mediating the renin secretory responses to sympathetic stimuli.

CHAPTER II

REVIEW OF LITERATURE

To better understand the factors that affect renin secretion, the role of renin as a vital component of the renin-angiotensin system must first be reviewed. This system, plays a major role in blood pressure control by regulating plasma angiotensin II, as well as aldosterone synthesis and release.

A. The Renin-Angiotensin-Aldosterone System

Renin, a glycoprotein (27, 28), is secreted into the systemic circulation from the juxtaglomerular cells of the afferent arteriole of the kidney by various mechanisms (1, 19, 29, 30). The juxtaglomerular cells form part of a larger juxtaglomerular apparatus, which also includes the macula densa cells of the distal tubules (1). It is the juxtaglomerular cells of this juxtaglomerular apparatus that are responsible for the synthesis and release of renin into the afferent arteriole of the glomerulus (1, 29). The juxtaglomerular cells are modified vascular smooth muscle cells (31) that have evolved into endocrine cells. Once renin is released, it enzymatically cleaves four amino acids from the carboxyl

end of angiotensinogen, a tetradecapeptide plasma alpha globulin, which is produced in the liver. The resulting decapeptide, angiotensin I, is further hydrolyzed to an octopeptide, angiotensin II by a "converting enzyme" (32, 33) which is present in high concentrations in the pulmonary circulation. Angiotensin II is considered to be the most potent vasopressor substance in the body (34), and thus plays a major role in blood pressure regulation. Davis (1), and Laragh (32) also suggest that angiotensin II is the major stimulus for the synthesis and secretion of aldosterone by the adrenal glomerulosa cells. Vaughan et al (35), however, have recently indicated that the primary stimulus for aldosterone biosynthesis is angiotensin III, a heptapeptide which is formed by the action of plasma and tissue aminopeptidases on the octopeptide angiotensin II and perhaps also by hydrolysis of the carboxy-terminal end tripeptide of angiotensin I. In turn, aldosterone plays a vital role in the regulation of extracellular fluid homeostasis.

B. Factors Regulating Renin Secretion

As previously indicated, the major interacting factors involved in the regulation of renin secretion are classified as follows (1): 1) intrarenal receptors, which include the baroreceptors of the afferent arteriole and the natrioreceptors of the macula densa in the distal

tubule; 2) humoral factors, which include locally generated angiotensin II, vasopressin, circulating catecholamines, a number of plasma ions and estrogen, among other steroids; and, 3) neural factors, which primarily include norepinephrine secreted from renal sympathetic nerve endings in close association with the juxtaglomerular cells, and perhaps catecholamine precursors such as dopamine as well. Renal parasympathetic nerve endings appear to play no role in the regulation of renin secretion at this time (1).

1. Intrarenal Receptors

a) The Afferent Arteriole Baroreceptor

Evidence for the presence of a renal vascular receptor partially controlling renin release has existed for a number of years. Early studies by Kohlsteadt and Page (2) on the isolated kidney showed that a decrease in blood pressure stimulated renin secretion. It was not until 1958, however, that studies by Kolff (3) clearly pointed out that renin or a renin-like substance was released in response to renal afferent arteriolar constriction, independent of pulsatile flow. These evidence, added together with his own investigations, allowed Tobian (5) to propose the baroreceptor hypothesis which postulates that a decreased stretch of the renal afferent arteriole as a result of decreased blood pressure

or blood volume, is sensed by specialized baroreceptor cells on the walls of the afferent arteriole near the glomerulus, resulting in an increase in renin secretion, which will eventually lead to generation of angiotensin II and restoration of blood pressure.

b) The Macula Densa Natrioreceptor

In 1945 a functional relationship between the macula densa cells and the juxtaglomerular cells was suggested by Goormachtigh (7). Macula densa cells are modified epithelial cells of the distal convoluted tubules of the kidney, which appear sensitive to sodium and/or chloride changes (7-16). In 1964, Vander and Miller (8) provided the first experimental evidence that the macula densa is an intrarenal sensor. They suggested that a decreased sodium load or concentration is sensed by the macula densa cells which then convey this message to the juxtaglomerular cells to stimulate renin release. Nash et al (9), have provided additional evidence for an intrarenal sodium-sensitive mechanism in the regulation of renin secretion. They suggested that the signal for renin release is mediated by an altered sodium flux across the macula densa cells. However, studies by Burg and Green (14), and Kokko (15), have indicated that the macula densa cells respond to a chloride load signal, either along with a sodium signal or preferentially to a sodium signal.

2. Humoral Factors

Among the various humoral factors involved in the partial regulation of renin release, angiotensin II and vasopressin (ADH) are the most important. However, the mechanisms mediating the effects of these two agents on renin release are not clear and, moreover, they do not appear to play as important a role in regulating renin secretion as the intrarenal receptors and neural factors. In vivo studies by Shade (18), have shown that intrarenal infusion of ADH and angiotensin II inhibit renin secretion. Furthermore Bunag et al (36), and Ganong and Lopez (30), have confirmed these findings in regard to a possible local inhibitory effect of angiotensin II on renin secretion. Shade et al (18), have further suggested that angiotensin II may exert its inhibitory effect directly on the juxtaglomerular cells by a short loop feed-back system rather than by an indirect action. This proposed short loop feedback system mediating the action of angiotensin II on renin release is consistent with evidence provided by the in vivo studies of De Champlain et al (37) in man, Vander et al (17) in dogs, and Blair-West et al (38) in sheep. Recent studies by Ganong and Lopez (30), have additionally suggested that the inhibitory effect of angiotensin II on renin secretion may be mediated by a decrease in intracellularly generated cyclic 3' 5' adenosine

monophosphate, which is consistent with a direct action of angiotensin II on the juxtaglomerular cells's membranes.

3. The Renal Sympathetic Nerves and Catecholamines

a) Juxtaglomerular Apparatus Innervation

The early studies of De Muylder (39), first described a rich nervous system innervation of the renal afferent arterioles in the region of the juxtaglomerular cells. This initial study was followed by a number of light and electronmicroscopic studies (31, 40, 41) which confirmed the early observations, and showed the presence of numerous nonmyelinated nerve fibers in close association with the renal afferent and efferent arterioles. Subsequent electronmicroscopic (41) and histochemical fluorometric studies (42), have demonstrated the presence of a dense network of sympathetic nerve terminals in the region of the renal juxtaglomerular cells. In addition, the presence of nerve terminals in close proximity to the macula densa cells (43) suggests the macula densa is also under neural modulation; but, subsequent fluorometric studies (42), have been unable to confirm this. Recent studies by Muller and Barajas (44) have characterized the nerve terminals innervating the juxtaglomerular cells as being primarily adrenergic in nature with perhaps some cholinergic contributions as well.

b) Effect of Catecholamines on Renin Release

The morphological evidence regarding the existence of a dense sympathetic innervation of the juxtaglomerular cells, triggered the undertaking of numerous in vivo (45-48) and in vitro studies (30, 49-51) designed to evaluate the possibility of a direct sympathetic nervous system control of renin secretion. These and related data have been evaluated in various review papers (1, 19, 29, 52). These studies have, for the most part, favored the view that catecholamines, specifically epinephrine and norepinephrine, stimulate renin release by 1) affecting the intrarenal receptors (circulating catecholamines from the adrenal medulla) or 2) by a direct stimulatory effect exerted on the juxtaglomerular cells themselves by both circulating catecholamines and those released from renal sympathetic nerve endings in close association with the renin-secreting cells.

The mechanisms by which catecholamines stimulate renin secretion have been extensively studied in vivo by a number of investigators (37, 45-48, 53). In one study, Johnson et al (48) showed that in the nonfiltering kidney, deprived of macula densa effects, the increase in renin secretion during intrarenal epinephrine infusion was blocked by simultaneous intrarenal papaverine administration,

whereas the response to norepinephrine was sustained during papaverine infusion. Since these observations were made in the nonfiltering kidney with the renal baroreceptor blocked by papaverine and a nonfunctional macula densa, Johnson et al (48) suggested that the effect of norepinephrine was directly exerted on the juxtaglomerular cells, whereas the failure of epinephrine to act during papaverine infusion suggests that the renin release responses to epinephrine are primarily due to an effect of this catecholamine on the renal arteriolar baroreceptors. Studies by Vandongen et al (54, 55) have provided further evidence suggesting that norepinephrine directly stimulates renin secretion, and that renin secretion in response to this catecholamine remains increased even when renal arteriolar vasoconstriction is blocked with the alpha-adrenergic blocker phenoxybenzamine. The validity of the data from these in vivo studies, can be questioned because of the interrelation of the many humoral, hemodynamic and neural mechanisms regulating renin secretion all of which may participate in the observed responses. Thus, in order to evaluate the possibility of a direct regulatory control of the sympathetic nervous system on renin release, the effects of the various humoral and hemodynamic factors on this release must be effectively eliminated by utilizing in vitro systems. Utilizing an in vitro dog cell suspension system, Mickelakis et al (49) showed that renin secretion

was increased by both epinephrine and norepinephrine while Nolly et al (51) using a rat renal cortical slice preparation have produced data suggesting that the stimulatory effect of norepinephrine on renin release appear to be mediated by a beta-adrenergic receptor on the membranes of the juxtaglomerular cells themselves. The possibility of a direct stimulatory effect of catecholamines on renin secretion has been further supported by numerous other in vivo (45-48) and in vitro studies (30, 49-51).

In vivo studies by Otsuka et al (45) in the dog, have shown that the catecholamine-induced renin release from the juxtaglomerular cells is potentiated under sodium-deficient conditions, suggesting an enhanced sensitivity of the renin-secreting cells to adrenergic stimuli in the sodium-deficient state. This finding, which has been supported by others (30, 56) allows an evaluation of positive and negative effects of sympathetic stimuli on renin secretion. Previous evaluations of sub-control renin secretory responses to sympathetic stimuli were difficult in view of the already low basal control renin secretion levels observed in laboratory animals maintained on commercial chow diets which are rich in sodium. Thus, when renin secretory levels become significantly lower than control levels as a result of the effect of a given inhibitory agent, the low responses can not be clearly

quantitated even by radioimmunoassay. Commercial rat chow diets provide approximately 12 times (over 2.2 mEq Na/day) the amount of sodium recommended by the National Research Council for growing animals, and thus basal renin secretion is markedly suppressed. Conversely, animals maintained on low sodium diets, receive less than 0.02 mEq of sodium per day, and they exhibit a much higher basal renin release levels, which is an ideal starting point in the evaluation of positive and negative stimulating and inhibitory factors which affect renin secretion. More importantly, however, is the fact that the sodium-deficient animal exhibits a marked potentiation (hypersensitivity) of its renin secretory responses to sympathetic stimuli, thus making renal sodium-deficient in vitro system an ideal tool for evaluation of sympathetic stimuli on renin secretion.

Utilizing a hypersensitive in vitro renal cortical slice preparation from sodium-deficient rats, Ganong and Lopez (30) have examined further some of the mechanisms by which a variety of sympathetic agents may directly regulate renin secretion. Their data are consistent with those of Ostuka et al (45) in regard to the potentiation of the renin secretory responses to added catecholamines in the sodium-deficient animal, and they additionally indicate that sodium deficiency enhances the sensitivity of the juxtaglomerular cells to sympathetic stimuli.

Furthermore, their data (30) suggest that renin secretion is mediated by a beta-adrenergic receptor (45-47, 50) mechanism mediated by the generation of intracellular cyclic AMP, a view that is shared by a number of other investigators (30, 50, 51, 57). These data (30) clearly show that norepinephrine, in addition to its stimulatory effect on renin secretion when added to kidney slices in physiological or subphysiological concentrations, also exerts an inhibitory effect on renin secretion when added in large concentrations. This inhibitory effect of large norepinephrine concentrations on renin secretion, appears to be mediated by an alpha-adrenergic receptor mechanism since the alpha-adrenergic blocking agent phentolamine reversed the inhibition. Also, this alpha-inhibitory effect appears to be correlated to a significant decrease in c-AMP generation (30). Additional support for this concept has come from the recent in vivo studies of Pettinger (58) and the in vitro studies of Capponi and Volloton (59).

c) Effects of Dopamine on Renin Secretion

Dopamine is an obligatory precursor in the biosynthesis of catecholamines in the brain (60), adrenal medulla (61), and sympathetic nerve terminals (62). The possibility of a direct action of this catecholamine precursor on renin secretion has been examined with inconclusive results, primarily because most of the studies

were done in vivo and other factors could likely have contributed to the observed responses. Thus, in vitro renal preparations, which effectively remove humoral, hemodynamic, and other in vivo influences, clearly need to be used as the best alternative tools. The in vivo studies of Anton and Sayer (20) have shown that the kidney contains large quantities of dopamine, although the source whether circulating and/or nerve terminal-secreted is not clear. Data from various in vivo (23, 24, 63-65) and in vitro (26) investigations have suggested that dopamine stimulates renin release, but the mechanisms involved in this release are not clearly defined. Other in vivo studies (66) have suggested that dopamine stimulates renin release indirectly by stimulating the intrarenal baroreceptors, rather than by a direct effect on the juxtaglomerular cells. Conversely, Ayers et al (65) found that dopamine infusion instead of causing a decrease in renin secretion which one may expect as a result of renal arteriolar vasodilation actually caused the opposite effect. Goldberg (21) and Toda and Goldberg (22) have suggested the existence of renal dopaminergic receptors, stimulation of which induces renal vasodilation, but they did not attempt to correlate these observations with changes in release rates of renin. Further support for the existence of renal dopaminergic receptors has come from the in vivo studies of Imbs et al (23), in which renal perfusion with haloperidol (specific

dopamine-receptor blocker) rather than with propranolol (specific beta-adrenergic blocker) suppressed the renin hypersecretion induced by dopamine. Recent in vitro studies by Nakajima et al (25) have shown that dopamine significantly increased the concentration of c-AMP in a rat kidney particulate preparation which consisted of tubules, glomeruli, and blood vessels. In their work, addition of either the alpha-adrenergic blocker, phentolamine, or the beta-adrenergic blocker, propranolol, failed to significantly reduce the effect of dopamine on c-AMP concentration. Conversely the stimulatory effect of dopamine on c-AMP was selectively blocked by spiroperidol, a dopamine receptor antagonist. In addition, Imbs et al (23, 24) and Nakajima et al (25, 67) have data further supporting the existence of renal dopaminergic receptors which appear to differ from renal beta-adrenergic receptors, and which behave in a manner similar to that of the intrarenal receptors, thereby indirectly regulating renin secretion. In contrast, in vitro studies by Henry et al (26) suggest that dopamine-sensitive receptors may be located on the membranes of the juxtaglomerular cells themselves, but these receptors may not be specific for dopamine alone but rather of the beta-type, capable of binding norepinephrine and the other catecholamines as well.

In view of the importance of dopamine as a central neurotransmitter, and the fact that the kidney appears to contain large amounts of this catecholamine precursor, further studies are clearly in order to examine a possible regulatory role of this compound on renin secretion. Thus, a series of studies were designed to evaluate such a role, by examining a possible direct effect of dopamine on renin release, as well as the possibility that c-AMP may be involved as a mediator in this process. In addition the effects of specific dopamine-receptor agonistic and antagonistic agents are used to further characterize the type of receptors mediating the renin secretory responses to dopamine.

CHAPTER III

MATERIALS AND METHODS

Twenty-four male, Sprague-Dawley rats (Sprague Dawley Co. Madison, Wisconsin) with initial weights of 210 ± 10 grams were fed a sodium-deficient diet (Teklad Test Diets, Madison, Wisconsin) which provided less than 0.02 milliequivalents of sodium per day, for periods of time of 2-3 weeks. The rats were kept two animals per cage, in a temperature-controlled room ($23 \pm 2^\circ\text{C}$), with a 12 hr light/12 hr dark photoperiod, and had access to distilled, deionized water ad libitum. After 2-3 weeks on this diet the animals were sacrificed by decapitation and their kidneys were rapidly excised, decapsulated, and gassed with a mixture of 95% oxygen-5% carbon dioxide for 30 seconds, while being maintained in Robinson's buffer medium at 4°C (68). Slices of renal cortex approximately 0.3 millimeters thick and weighing 50 ± 20 milligrams were subsequently prepared using a Stadie-Riggs microtome (A. Thomas, Co.). Each cortical slice was divided into six similar portions which were randomly assigned to six separate incubating vessels containing 2.5 ml of Robinson's buffer. The procedure was repeated using slices from other areas of the renal cortex until each vessel contained 50

± 20 milligrams of renal cortical tissue. This tissue distribution insured a homogeneous population of renal cortical cells in each incubating vessel. The slices were then pre-incubated for 15 minutes at 37°C in a shaking Dubnoff Metabolic Incubator (Precision Scientific Co.), in an atmosphere saturated with 95% oxygen-5% carbon dioxide. The pre-incubated slices were subsequently transferred to similarly numbered vessels containing fresh Robinson's buffer maintained at 37°C and were then incubated for one hour under conditions identical to those during pre-incubation. One of each group of six vessels served as a nontreated control, while the other five vessels received respectively, one of various dopamine doses (Sigma Chemical Co.) ranging from 10^{-9} Molar to 10^{-3} Molar, which were added either alone or in conjunction with the dopamine-receptor agonist agent apomorphine (10^{-6} M) or the dopamine-receptor blocking agent pimozide (10^{-6} M). Apomorphine (10^{-6} M) and pimozide (10^{-6} M) were also added alone to the remaining vessels. Both agonistic and antagonistic agents were added to the slice preparation before the pre-incubation as well as incubation periods, whereas dopamine was only added at the beginning of the incubation period. Dopamine and apomorphine solutions were prepared in 0.1% ascorbic acid (Sigma Chemical Co.) to prevent oxidation of the catecholamine and the dopamine-receptor agonist. (Free, Lopez, Ganong, unpublished observations). Pimozide

was prepared in 0.1% tartaric acid (Janssen Pharmaceutica) which also prevented oxidation of the dopamine-receptor antagonist agent. After incubation, the incubated supernatant medium was collected in 12 X 75 mm tubes (Scientific Products) and stored at -20°C until assayed for renin concentration by radioimmunoassay (RIA) of angiotensin I (Squibb). The slices were rapidly frozen on dry ice and subsequently homogenated in 1 ml of 8% trichloroacetic acid (TCA). Homogenates were then transferred to 12 X 75 mm tubes containing 5 drops of 0.1 N HCl, vortexed, and immediately centrifuged at 4000 rpm for 10 min at 4°C. The protein-containing precipitate was discarded and the supernatant was transferred to conical centrifuge tubes using Pasteur pipets. The samples were then washed 4 times with water-saturated ether (Mallinckrodt), vortexed for 10 sec each time, and the upper, ether-containing phase, aspirated using Pasteur pipets and discarded after each wash. In turn, the lower, c-AMP-containing water phase, was decanted after each wash into appropriate glass vials which had been set on dry ice for immediate freezing. The frozen vials containing the water-soluble c-AMP were subsequently lyophilized (Virtix lyophilizer) for 4-5 hours to remove the water. The freeze-dried cyclic AMP was then stored at -20°C until assayed by a modification of the competitive protein binding assay of Gilman (69).

A. Renin Concentration Determination of Radioimmunoassay of Angiotensin I

1. Angiotensin I Generation

Since purified renin is not available, there is no direct radioimmunoassay of renin. In order to determine renin concentration, a method has been developed for the estimation of renin concentration through the radioimmunoassay of angiotensin I generated by the action of renin on incubated supernatant samples in the presence of renin substrate (angiotensinogen). To generate angiotensin I, the frozen incubated supernatant samples which had been kept at -20°C were allowed to thaw at room temperature and subsequently maintained at 4°C in an ice-water bath. Defrosted supernatant samples were vortexed for 15 seconds and 0.025 ml aliquots of each unknown were transferred to labeled 12 X 75 mm pyrex tubes also kept at 4°C . To each tube, 1 ml of 24-hr nephrectomized dog plasma, which has been dialyzed against phosphate-EDTA buffer was added. Prior to its addition to the incubated supernatant samples, the 1 ml nephrectomized dog plasma aliquots containing endogenous renin substrate (angiotensinogen) were treated with 0.05 ml of a 1% solution of phenylmethanesulfonyl fluoride (Sigma Chemical Co.) an inhibitor of plasma converting enzyme and other plasma

angiotensinases, to prevent degradation of generated angiotensin I or conversion of angiotensin I to angiotensin II. The inhibitor-treated nephrectomized dog plasma aliquots were then adjusted to a pH of 5.4 - 5.7 with 1% HCl to insure an optimum renin enzymatic activity. The unknown supernatant samples to which the nephrectomized plasma substrate had been added, were then incubated in a water bath at 37°C for two hours to allow generation of angiotensin I. Following this 2-hr generation period, the samples were removed from the water bath and 1 ml of distilled, deionized water was added. The angiotensin I containing samples were then vortexed, covered with aluminum foil to prevent evaporation, and immersed in boiling water for 2-3 minutes, to stop any further reaction by precipitation of most proteins except angiotensin I which is heat stable. At the end of the boiling period, the angiotensin I containing samples were allowed to cool, covered with parafilm (Fischer Co.), and stored at -20°C until assayed for angiotensin I content by radioimmunoassay.

2. Radioimmunoassay Procedure of Angiotensin I

Renin concentration was determined by RIA of angiotensin I utilizing materials purchased from the Squibb Co. (Rolling Meadows). This procedure used a specific antibody for angiotensin I ¹²⁵Iodine-labeled angiotensin I as the radioactive tracer and synthetic angiotensin I

as a reference standard. Duplicate unknown sample aliquots used for the RIA procedure were either 0.025 ml, 0.05 ml or 0.1 ml depending on the concentration of angiotensin I present in the samples. Following charcoal separation, the samples were centrifuged at 4500 rpm for 10 min at 4°C using a sorvall RC2-B refrigerated centrifuge and the supernatant decanted into 12 X 75 mm tubes for counting. Counting time was 1 minute. The various steps in this RIA procedure have been outlined in detail (Squibb).

B. Determination of Cyclic 3' 5' Adenosine Monophosphate (c-AMP) By Competitive Protein Binding Assay

Freeze-dried c-AMP samples obtained after lyophilization, were reconstituted with 0.25 ml of 4 mM Tris/EDTA buffer pH 7.5 (Amersham). Duplicate 0.05 ml aliquots of the reconstituted unknown samples were mixed in 12 X 75 mm tubes with 0.10 ml of binding-protein, and 0.05 ml of ³H-labeled c-AMP, to a total assay volume of 0.2 ml. C-AMP standards were prepared similarly. The samples were vortexed for 15 seconds and then allowed to incubate at 4°C for 90 minutes. At that time, 0.2 ml of a charcoal suspension (Amersham) were added to the cold-incubated samples to separate protein-bound from free c-AMP, the samples were vortexed for 5 seconds and immediately centrifuged at 4°C for 10 minutes at 3500 rpm. 0.2 ml of the supernatant were subsequently counted in a tri-carb

liquid scintillation spectrophotometer.

C. Statistical Calculations

Statistical significance of the c-AMP content and renin secretion was evaluated by a modified paired student's "t" test (70) using a programable desk top calculator (Hewlett-Packard, 1900B).

CHAPTER IV

RESULTS

In an initial study, the effect of dietary sodium changes on basal renin release and cyclic-AMP content of rat renal cortical slices was evaluated. These data can be seen in figure 1. As observed in this figure, the basal, non-stimulated, renin release rate in slices from sodium-deficient rats was significantly greater ($P < 0.01$) when compared to that seen in the sodium-replete animals. In turn, the cyclic-AMP content of slices from sodium-deficient rats was not significantly different from that of slices from the sodium-replete group of animals (figure 1).

Based upon the data obtained from the preliminary study, a renal cortical slice system prepared from sodium-deficient rats was utilized in all subsequent studies. Figure 2 depicts the changes in renin release in slices from sodium-deficient rats in response to various concentrations ($10^{-9}M$, $10^{-7}M$, $10^{-5}M$, and $10^{-3}M$) of dopamine hydrochloride. As shown in this figure, dopamine doses of $10^{-9}M$ and $10^{-7}M$ were ineffective in stimulating renin secretion. Conversely, higher concentrations of this catecholamine ($10^{-5}M$ and $10^{-3}M$), significantly stimulated

FIGURE 1

Legend:

Figure I.

Changes in renin release and cyclic AMP content in renal cortical slices from sodium-deficient rats compared to rats maintained on a sodium-replete diet (control). Length of high or low dietary sodium intake was 2-3 weeks. The sodium deficient renin data represents the mean change \pm S. E. of 124 observations (number of slices) compared to 20 observations for the sodium replete renin data. The corresponding c-AMP data are based on 121 observations (sodium-deficient) compared to 18 observations (sodium-replete). The sodium-replete (control) renin release rate was 1.38 ± 0.32 ng/mg/hr. and the c-AMP content was 0.64 ± 0.12 pmol/mg wet tissue, respectively. Incubation time was 60 minutes.

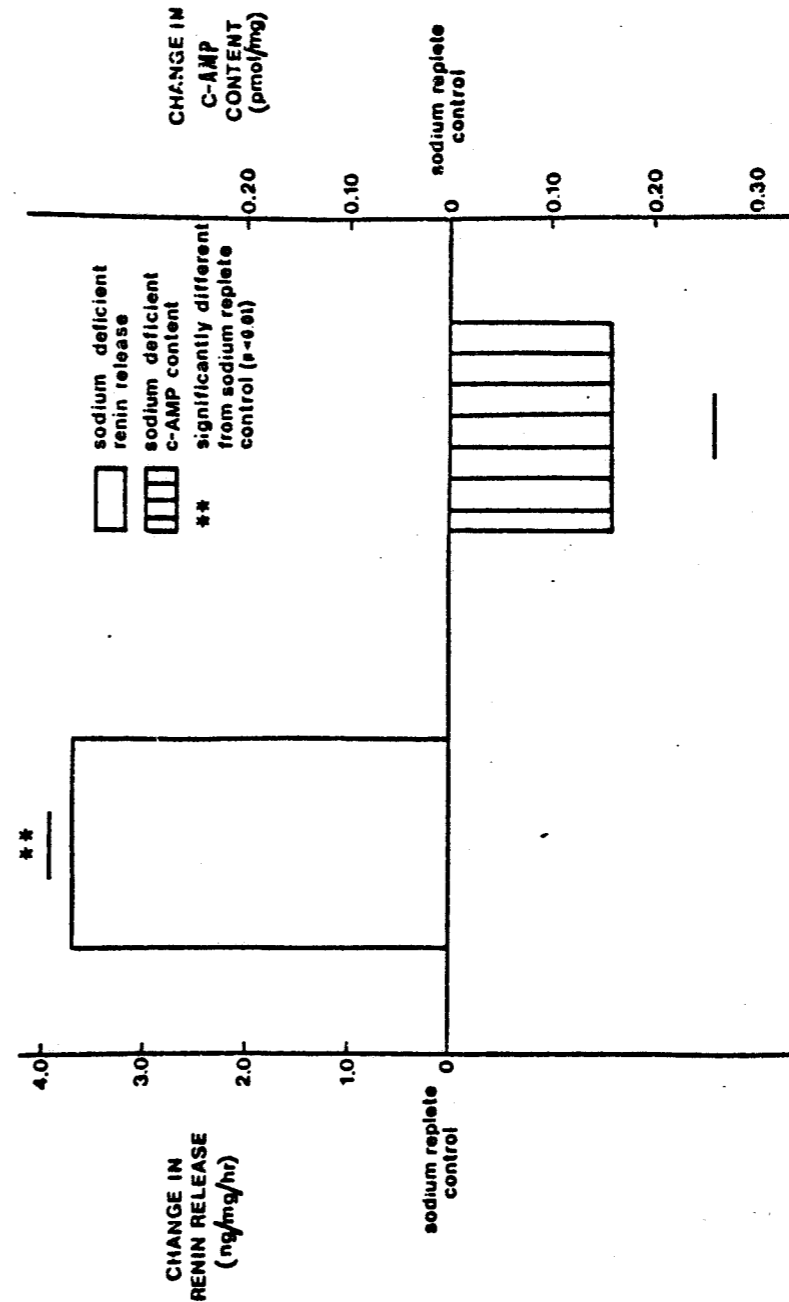
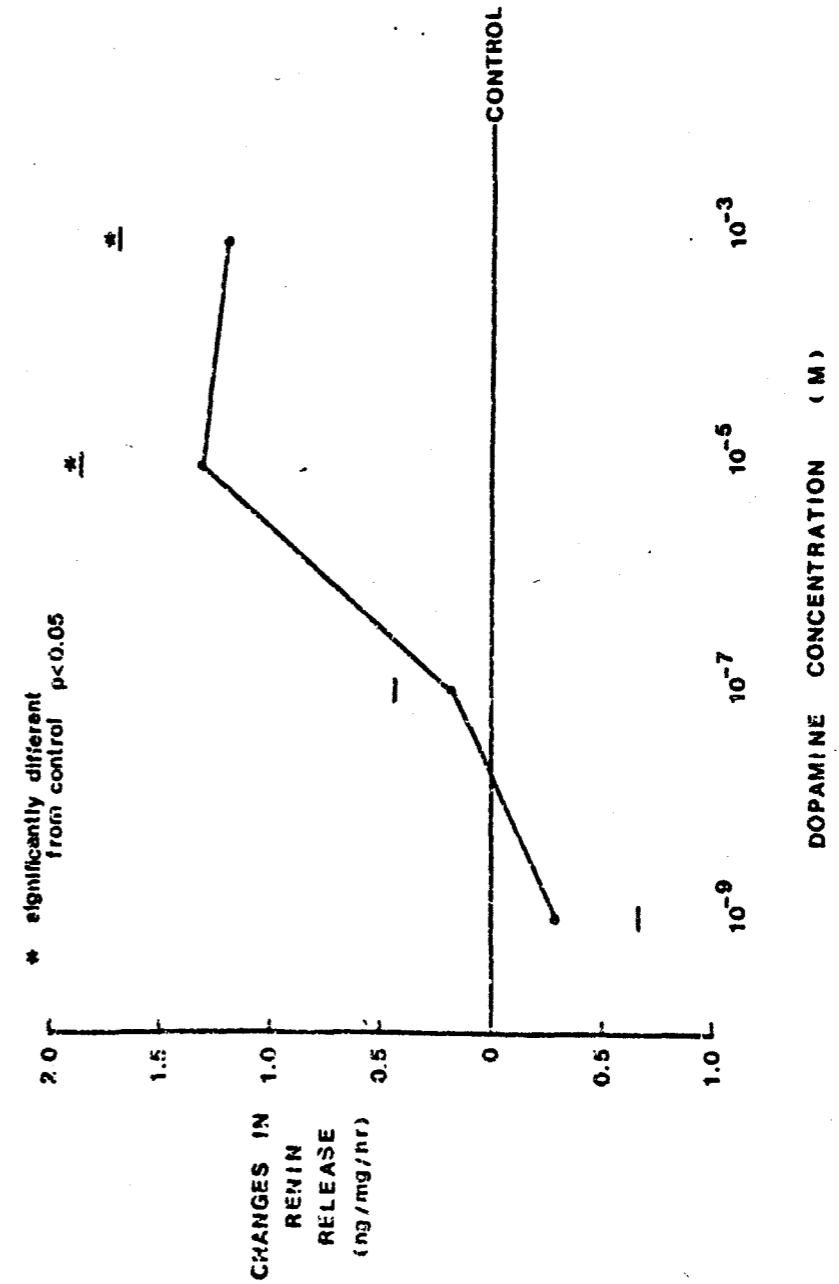


FIGURE 2

Legend:

Figure 2.

Effect of various concentrations of dopamine hydrochloride on renin release by renal cortical slices from sodium-deficient rats. The data represents the mean renin release change \pm S. E. of 12 observations (number of slice pairs) for each dopamine concentrations. The mean control (non-stimulated) rate of renin release was 5.43 ± 0.19 ng/mg/hr during a 60 minutes incubation period.



renin release, with the highest mean renin release values seen at a dopamine concentration of 10^{-5}M . However, a statistical comparison between the two stimulatory concentrations of dopamine showed no significant differences between them with respect to stimulation of renin secretion. When their stimulatory effects on renin release were compared to those exerted by lower dopamine doses (10^{-9}M and 10^{-7}M) a dose-response relationship was clear. Thus, these data (figure 2) indicate that threshold for maximal stimulation of renin release was reached with a dopamine concentration of 10^{-5}M , and that higher doses of this catecholamine were ineffective in further potentiating the renin secretory rate.

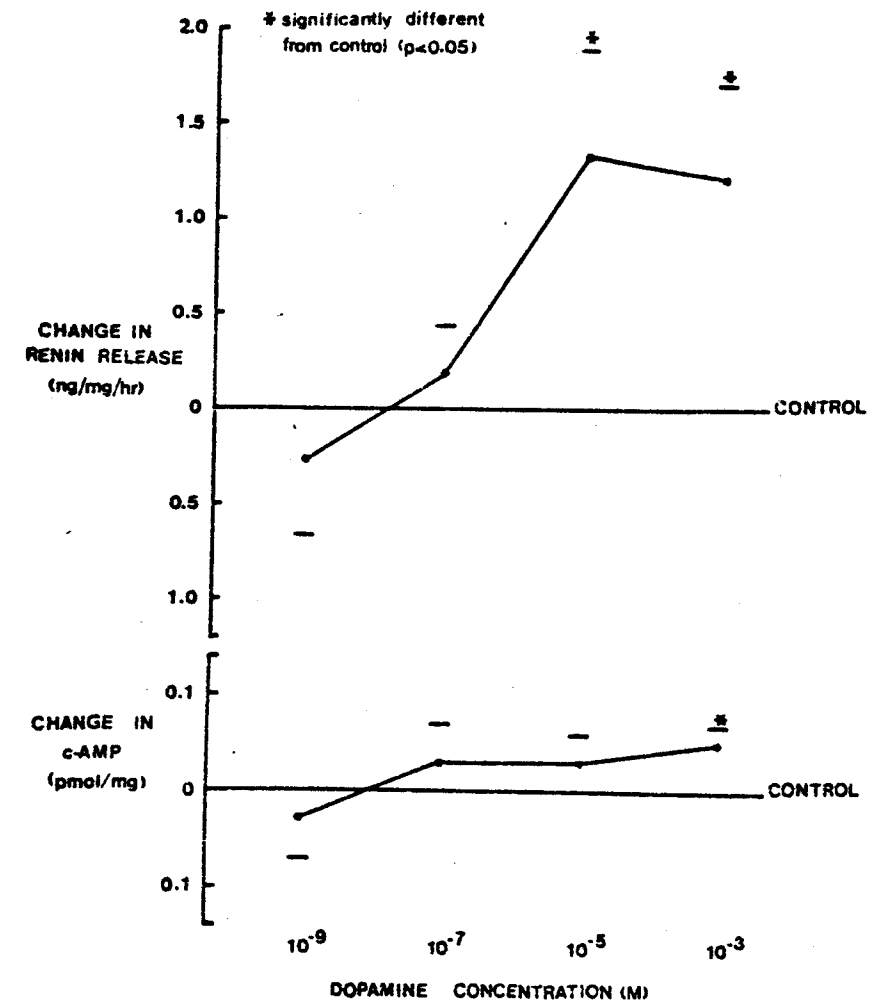
When the renin secretory responses to various dopamine doses (figure 2) are compared to those in c-AMP content in response to the same dopamine concentrations (figure 3) a partial correlation can be observed. These data (figure 3) show that at a concentration of 10^{-3}M , dopamine significantly stimulated both renin release and c-AMP content in cortical slices from sodium-deficient rats. At 10^{-5}M , however, dopamine significantly stimulated renin release, but did not affect c-AMP content. Dopamine concentrations of 10^{-7}M and 10^{-9}M did not change c-AMP content from that of controls, which is consistent with the changes seen in renin secretion in response to the

FIGURE 3

Legend:

Figure 3.

Effect of various concentrations of dopamine hydrochloride on renin release and c-AMP content of renal cortical slices from sodium deficient rats. The data represents the mean renin release change \pm S. E. and the mean c-AMP content change \pm S. E. respectively, of 12 observations (number of slice pairs) for each dopamine concentration. The mean control (non-treated) rata of renin release was 5.43 ± 0.19 ng/mg/hr and the mean control c-AMP content was 0.42 ± 0.02 pmol/mg wet tissue, respectively. Incubation time was 60 minutes.



same doses of this catecholamine (figure 3). As seen with the renin secretory responses to the four dopamine concentrations, a dose-response relationship is also apparent in regard to the c-AMP content changes (figure 3).

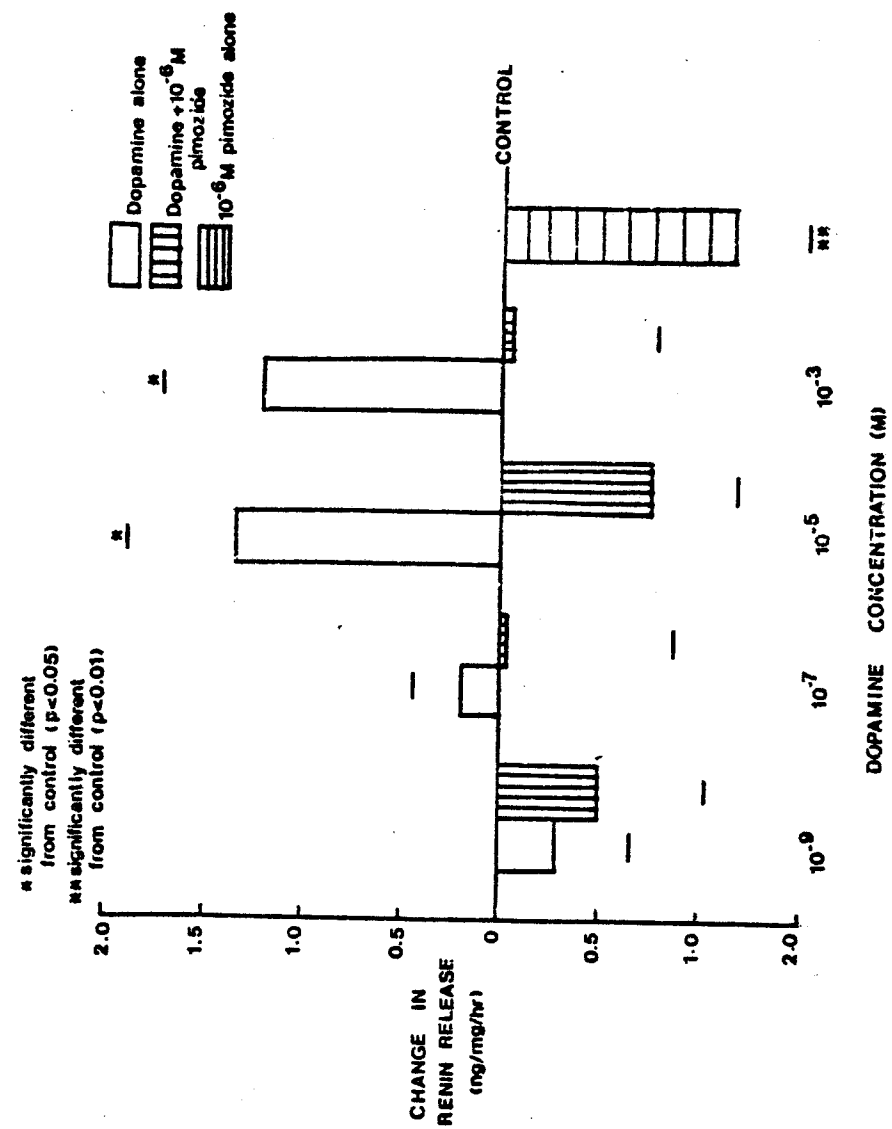
Data from a subsequent study designed to evaluate the possibility of a specific dopaminergic-receptor mechanism mediating the renin release and c-AMP content responses to added dopamine are shown in figure 4. This figure illustrates the renin secretory changes seen in renal cortical slices from sodium-deficient rats in response to four dopamine doses (10^{-9}M , 10^{-7}M , 10^{-5}M and 10^{-3}M), added either alone or in conjunction with a dopamine-receptor blocking agent, pimozide (10^{-6}M). As observed (figure 4), when added together with two dopamine doses (10^{-9}M and 10^{-7}M), which by themselves were ineffective in stimulating renin release, pimozide did not affect the renin release responses observed with dopamine alone although it did depress the mean renin release rates. Conversely, when added in conjunction with higher dopamine doses (10^{-5}M and 10^{-3}M) pimozide effectively prevented the significant stimulatory effect of these dopamine concentration on renin release. Furthermore, addition of pimozide alone to the renal cortical slice preparation significantly decreased renin secretion in relation to

FIGURE 4

Legend:

Figure 4

Changes in renin release in response to various dopamine hydrochloride concentrations, added either alone or together with 10^{-6} M pimozide to renal cortical slices of rats fed a sodium-deficient diet for 2-3 weeks. The data represents the mean renin release change \pm S. E. of 12 observations (number of slice pairs) for each dopamine concentration added alone, and 7-10 observations for each dopamine concentration added together with pimozide. The mean control (non-stimulated) rate of renin release was 5.43 ± 0.19 ng/mg/hr during a 60 minute incubation period.



the non-stimulated control samples.

When the renin release data on figure 4 are compared to those in c-AMP content in response to the same four doses of dopamine plus pimozide (figure 5), a clear correlation between renin release and c-AMP content changes can be observed. The inhibitory effect of pimozide was particularly striking in regard to cyclic-AMP content since it not only prevented the significant stimulatory effect of $10^{-3}M$ dopamine on c-AMP content but it significantly decreased the c-AMP content levels of tissue slices treated with all four dopamine doses to values far below those of non-treated control slices. Pimozide added alone significantly decreased both renin release as well as the c-AMP content of the tissue slices in relation to the non-stimulated control samples.

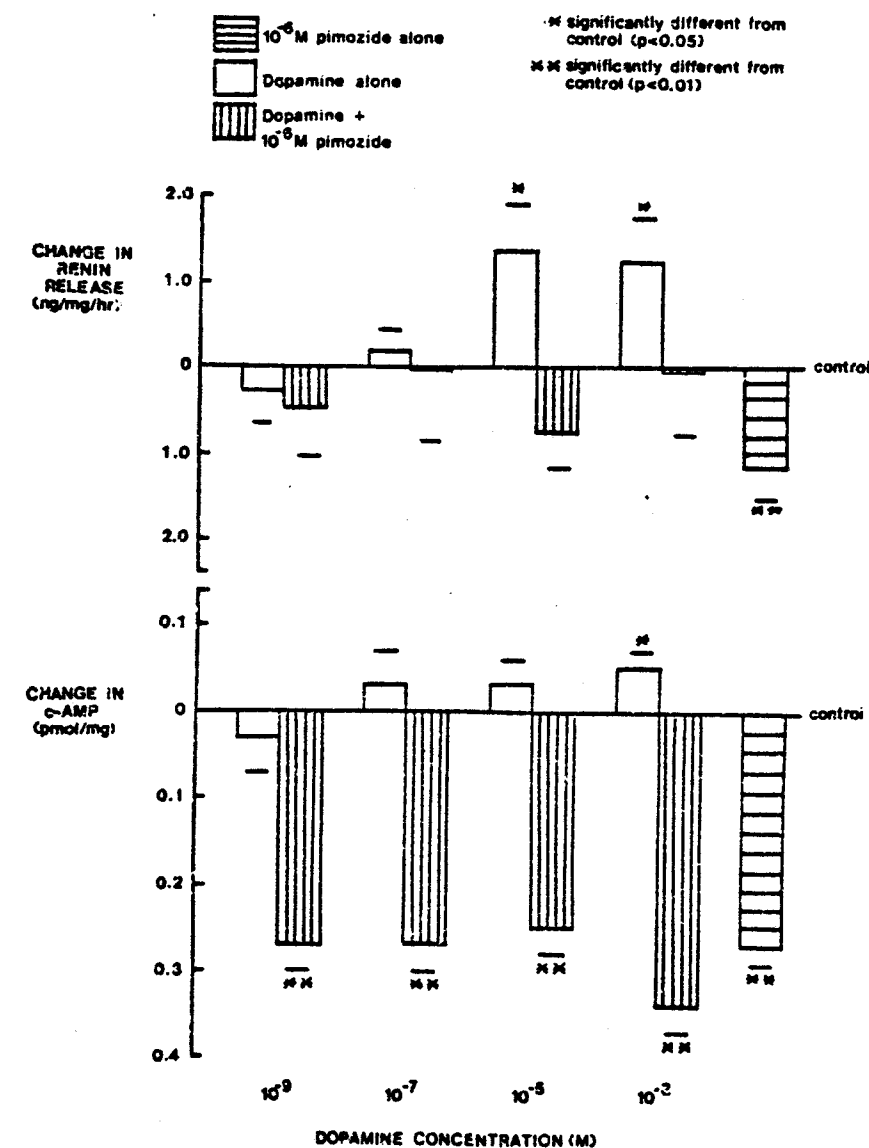
Table 1 illustrates the results of an additional study designed to evaluate the effect of the dopaminergic receptor agonist apomorphine ($10^{-6}M$), on the renin release and c-AMP content responses to four dopamine doses ($10^{-9}M$, $10^{-7}M$, $10^{-5}M$ and $10^{-3}M$) in renal cortical slices from sodium-deficient rats. As opposed to pimozide, when added alone the slice preparation, apomorphine did not alter renin secretion or c-AMP content levels in relation to those of controls. However, it did prevent the stimulatory effect of $10^{-5}M$ and $10^{-3}M$ dopamine concentrations on renin release

FIGURE 5

Legend:

Figure 5.

Changes in renin release and c-AMP content in response to various concentrations of dopamine hydrochloride, added either alone or together with 10^{-6} M pimozide to renal cortical slices of rats fed a sodium-deficient diet for 2-3 weeks. The data represents the mean renin release change \pm S. E. and the mean c-AMP content change \pm S. E., respectively, of 12 observations (number of slice pairs) for each dopamine concentration added together with pimozide. The mean control (non-treated) rate of renin release was 5.43 ± 0.19 ng/mg/hr. The mean control c-AMP content was 0.42 ± 0.02 pmol/mg wet tissue. Incubation time was 60 minutes.



Effect of various doses of dopamine (10^{-3} M, 10^{-5} M, 10^{-7} M, and 10^{-9} M) in the presence and absence of apomorphine (10^{-6} M) on Renin secretion and c-AMP content on renal cortical slices from sodium deficient rats.

	APO 10^{-6} M	DOP 10^{-3} M	DOP 10^{-3} M APO 10^{-6} M	DOP 10^{-5} M	DOP 10^{-5} M APO 10^{-6} M	DOP 10^{-7} M	DOP 10^{-7} M APO 10^{-6} M	DOP 10^{-9} M	DOP 10^{-9} M APO 10^{-6} M
Changes in Δ renin release ng/mg/hr from control	- Δ = 0.07 \pm 0.27	+ Δ = 1.21 \pm 0.51	+ Δ = 0.34 \pm 0.74	+ Δ = 1.34 \pm 0.55	+ Δ = 0.54 \pm 0.57	+ Δ = 0.19 \pm 0.25	+ Δ = 0.12 \pm 0.36	- Δ = 0.28 \pm 0.38	+ Δ = 0.10 \pm 0.48
P Value	0.74	0.03	0.66	0.03	0.32	0.45	0.77	0.48	0.84
N Value	46	12	10	12	12	12	12	12	12
Changes in Δ c-AMP content Pmole/Mg/wet tissue from control from control	- Δ = 0.01 \pm 0.01	+ Δ = 0.05 \pm 0.02	+ Δ = 0.01 \pm 0.02	+ Δ = 0.03 \pm 0.03	+ Δ = 0.06 \pm 0.03	+ Δ = 0.03 \pm 0.04	+ Δ = 0.03 \pm 0.02	- Δ = 0.03 \pm 0.04	+ Δ = 0.03 \pm 0.04
P Value	0.49	0.04	0.69	0.31	0.04	0.49	0.17	0.39	0.71
N Value	18	12	4	12	4	12	5	12	4

when added in conjunction with the catecholamine. Furthermore, apomorphine prevented the significant stimulatory effect of 10^{-3}M dopamine on c-AMP content although when compared to each other (10^{-3}M dopamine alone vs. 10^{-3}M dopamine plus apomorphine) there was no significant difference in the effect of both treatments on c-AMP content. Apomorphine did, however, potentiate the c-AMP content responses of renal cortical slices to 10^{-5}M dopamine, but again, comparison of the two values (10^{-5}M dopamine alone vs. 10^{-5}M dopamine plus apomorphine) showed no significant statistical differences. From these data (table 1) it is clear that at no time apomorphine potentiated the effects of any of the stimulatory dopamine doses on renin release and in fact it prevented the stimulation seen with 10^{-5}M and 10^{-3}M dopamine doses added alone to the slice preparation.

CHAPTER V

DISCUSSION

Studies utilizing in vitro kidney preparations (30, 49-51, 71) have provided evidence supporting the concept of a direct stimulatory effect of catecholamines on the juxtaglomerular cells of the kidney to affect renin release. In these preparations, the influence of various humoral, hemodynamic, and other factors which are known to influence renin release in the intact animal are eliminated, and although the various cell types are still present in the in vitro preparations, the effects of added catecholamines on renin release are most likely due to a direct effect on the renin-secreting cells. Furthermore, there is in vivo (45, 56) and in vitro (30, 72) evidence which indicates that sodium deficiency not only potentiates the renin secretory responses to sympathetic stimuli; but that it causes a chronic hypersecretion of renin resulting in elevated basal renin release levels (30, 72).

Thus, in an initial study utilizing as in vitro rat renal cortical slice preparation we evaluated the effect of dietary sodium changes on renin release and c-AMP content of kidney slices from animals which had been fed either

sodium-deficient or sodium-replete diets for 2-3 weeks. The results from this initial study (figure 1) confirmed previous findings (30, 72) indicating that the basal, non-stimulated renin release rate in renal cortical slices from sodium-deficient rats is significantly greater than that seen in slices from sodium-replete animals. Surprisingly, however, c-AMP content levels in slices from both sodium-deficient and sodium-replete rats were not different statistically, suggesting that the increased renin release rate seen in sodium-deficient animals may not involve changes in the intracellular concentration of this nucleotide. There are no previous data in this regard to allow for a comparison of our results on c-AMP content changes in response to dietary sodium manipulation.

In a subsequent study (figures 2 and 3) the effect of various concentrations of dopamine hydrochloride on renin release and c-AMP content in renal cortical slices from sodium-deficient rats was evaluated. As shown in these figures, two concentrations of dopamine ($10^{-5}M$ and $10^{-3}M$) significantly stimulated renin release, with threshold for a maximal renin secretory rate being observed at a dopamine concentration of $10^{-5}M$. Conversely, lower dopamine concentrations ($10^{-9}M$ and $10^{-7}M$) were ineffective in stimulating renin release. The results seen with the stimulatory concentrations of dopamine are consistent with

previous in vivo (23, 24, 63-65) and in vitro (26) observations which suggest that renin release may partially be under dopaminergic control. However, most of these studies above have not attempted to postulate a possible mechanism or mechanisms mediating the stimulatory action of dopamine or renin secretion. The in vitro renin release data (figures 2 and 3), on the other hand, suggest that the stimulatory effect of dopamine on renin release may be a direct one on the juxtaglomerular cells, a postulation which is in agreement with some (26). In partial support of this view are the data obtained in these studies (figure 3) in regard to the c-AMP content changes seen in kidney slices in response to the highest concentration of added dopamine ($10^{-3}M$) examined. The fact that both renin release rate and c-AMP content levels were significantly increased in response to this dopamine dose, clearly supports the possibility that dopamine may stimulate renin secretion by interacting with a membrane receptor which utilizes c-AMP as the intracellular mediator. Furthermore, even when the tissue c-AMP content levels were not significantly elevated in response to other dopamine doses (figure 3), they nevertheless paralleled at all times the changes seen in renin secretion in response to the same dopamine concentrations, further supporting a c-AMP-mediating action. It has been postulated that stimulation of renin release by other catecholamines is mediated by an increased

intracellular c-AMP generation and that dopamine may exert its action on renin secretion in a similar manner (25). The renin release and tissue c-AMP content data (figures 2 and 3) are consistent with this view, but do not clarify whether the membrane adenylate cyclase receptor complex activated by dopamine is specific for this catecholamine or whether it is of the beta-type which is responsive to all catecholamines as proposed by some investigators (26).

To further explore the possibility of a dopamine-specific receptor complex mediating the action of this catecholamine on the juxtaglomerular cells, changes in renin release and c-AMP content of renal cortical slices from sodium-deficient rats in response to various dopamine doses added either alone or in conjunction with a specific dopamine-receptor blocking agent pimozide ($10^{-6}M$) studied (figures 4 and 5). Addition of pimozide to the kidney slice system not only prevented the significant stimulatory effect of two dopamine doses ($10^{-5}M$ and $10^{-3}M$) but it significantly inhibited the basal renin secretion of the tissue slices when added alone (figures 4 and 5). Similarly, pimozide significantly decreased renal cortical tissue slice c-AMP content when added alone or in conjunction with all dopamine concentrations utilized (figure 5). Numerous studies (21-25) have proposed the existence of specific dopaminergic receptors, but other

(26) have indicated that these dopamine-sensitive receptors may not be specific for dopamine alone but rather of the beta type, capable of binding other catecholamines as well. These data (figures 4 and 5) support the concept that stimulation of renin release by dopamine is mediated by an increased intracellular c-AMP generation via a specific dopamine receptor mechanism. This view is shared by other investigators (25) but in their studies no attempt was made to measure renin release and c-AMP content changes simultaneously. Thus, the results obtained as a result of simultaneous evaluation of renin release and c-AMP content changes in these studies provide strong support for the existence of a specific dopamine-receptor mechanism mediating the action of this catecholamine on renin secretion. Furthermore, the significant inhibition seen in renin release and c-AMP content of slices treated with pimozide alone (figure 5), suggests that endogenous dopamine exerts a partial regulatory control over the basal renin secretory levels in the intact animal, and that this control may be mediated by intracellular c-AMP changes. The significance of this is not clear, but these observations certainly indicate that not only norepinephrine released from renal nerve endings but also dopamine, perhaps from the same source, may work in conjunction to modulate the minute-to-minute basal renin secretory rate in vivo. An alternative possibility may be proposed in view of the

fact that the basal renin release rate in the sodium-deficient animal is significantly greater than that seen in animals fed commercial chow diets. It is possible that the renin hypersecretion seen in sodium-deficient animals is the one under a partial dopaminergic control and that the basal renin secretory levels observed in sodium-replete animals are under a different type of control. Obviously, studies utilizing pimozide in slices from sodium-replete rats are clearly needed to evaluate this possibility and they are presently being conducted in our laboratory.

Potentiation of the stimulatory effects of two dopamine doses ($10^{-5}M$ and $10^{-3}M$) on renin secretion, by the simultaneous addition of a dopamine-receptor agonist apomorphine ($10^{-6}M$), was not observed in these studies as the concentration of apomorphine utilized (table 1). In fact, apomorphine actually prevented the stimulatory effect of dopamine ($10^{-5}M$ and $10^{-3}M$) on renin release. Although these results appear contradictory to what one may expect from the postulated action of apomorphine, they are nevertheless in agreement with another study (71) which has suggested that apomorphine at certain concentrations is antagonistic to dopamine action. The effect of apomorphine on tissue c-AMP content in our studies was not clear. It did inhibit the increase in c-AMP content seen in slices treated with $10^{-3}M$ dopamine, but it

significantly stimulated c-AMP content of slices treated with 10^{-5} M dopamine. These observations suggest that, whether stimulatory or inhibitory, the mechanism action of apomorphine on dopamine receptors may be independent of c-AMP changes. Further studies are clearly needed in this regard.

In summary, the data discussed in this thesis confirm previous observations which have been shown that the basal, non-stimulated renin release rate in renal cortical slices from sodium-deficient rats is significantly greater than that seen in sodium-replete animals. These data also indicate that dopamine exerts a direct stimulatory effect on renin release which appears to be mediated by a dopamine-sensitive receptor mechanism utilizing c-AMP as the intracellular mediator. Furthermore, the effect of dopamine on renin secretion and c-AMP content of slices from sodium-deficient rats appears to be concentrations dependent, ruling out leakage from the cells as a contributing factor in the results observed. Additionally, these results suggest that dopamine may be an important endogenous modulator of the basal renin secretion seen in non-stimulated, sodium-deficient juxtaglomerular cells, although similar results in slices from sodium-replete animals are needed to further support this view. Finally, these data are inconclusive as to the actual effects exerted

by apomorphine on renin release and tissue c-AMP content, and additional doses of this dopamine-receptor agonistic agent clearly need to be evaluated.

The data presented in this paper constitute additional evidence supporting the concept that the sympathetic nervous system exerts a direct regulatory control over the secretion of renin from the juxtaglomerular cells of the mammalian kidney, and support the view that dopamine-sensitive receptor mechanisms may participate in this control.

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APPENDIX A

APPENDIX A

1. Radioimmunoassay Calculations

To correct for dilution of the initial unknown sample aliquot (0.025) throughout the generation procedure and subsequent RIA, as well as to convert the RIA values expressed as picograms of A-I to the final values in nanograms of angiotensin I/mg wet tissue/hr, which is the conventional way of expressing renin concentration, the following formula was employed:

$$\begin{array}{l} \text{Renin} \\ \text{Concentration} \\ \text{expressed as} \\ \text{ng A-I/mg wet tissue/hr} \end{array} = \frac{\text{pg of A-I} \times 40 \times 2 \times (40, 20, \text{ or } 10) \times 2.5}{2 \times 100 \text{ mg wet tissue}}$$

In numerator:

- pg of A-I = picograms of angiotensin I determined for unknown samples by reading off the RIA standard curve for angiotensin I
- 40 = a factor obtained by diluting the initial 0.025 ml of incubated supernatant with 1 ml of 24-hr nephrectomized dog plasma plus inhibitor
- 2 = a factor obtained by diluting 1 ml of generated sample with 1 ml of distilled, deionized water following a 2-hr generation of angiotensin I
- 40, 20, or 10 = a factor which depends on the unknown generated sample aliquot size (0.025 ml, 0.05 ml, 0.1 ml) used for the RIA procedure, when diluted to a final assay volume of 1 ml

2.5 = a factor which represents the original volume of Robinson's buffer that was used to incubate the renal cortical tissue.

In denominator:

2 = a factor which converts the 2-hr generation time of angiotensin I to 1 hr.

1000 = a factor which converts picograms of angiotensin I to nanograms angiotensin I.

mg = milligrams of wet renal tissue weight. This factor converts nanograms angiotensin I per hour to nanograms angiotensin I per hour Per milligrams wet renal tissue weight.

2. Cyclic AMP Calculations

Since only 0.050 ml aliquots of the total 0.25 ml volume of tris/EDTA reconstituted unknown samples was utilized for the CPB assay, the value for the unknown, expressed as picograms of c-AMP were corrected for this by a 5.0 multiplication factor. Additionally, the final results were corrected for the wet tissue weight of the samples and expressed as picograms c-AMP per mg wet tissue.

APPROVAL SHEET

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

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

10/10/78
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