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Effect of Dopamine and Dietary Sodium Deficiency on in Vitro Renin Release and Cyclic 3' -5' Adenosine Monophosphate Content of Male Rat Renal Cortical Slices; Modification by Dopamine-Receptor and Beta-Adregenic Receptor Antagonists

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EFFECT OF DOPAMINE AND DIETARY SODIUM DEFICIENCY ON IN VITRO RENIN RELEASE AND CYCLIC 3'-5' ADENOSINE MONOPHOSPHATE CONTENT OF MALE RAT RENAL CORTICAL SLICES: MODIFICATION BY DOPAMINE-RECEPTOR AND BETA-ADRENERGIC RECEPTOR ANTAGONISTS

by

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VITA

The author, Fred Daniel Romano, is the son of Vincent John Romano and Carmela (Arena) Romano. He was born March 3, 1951, in Brooklyn, New York.

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In September, 1968, he entered Loyola University of Chicago, and in June, 1977, received the degree of Bachelor of Science with a major in biology.

In September, 1977, he entered the master's degree program in biology at Loyola University of Chicago and was granted a graduate teaching assistantship in January, 1978. His research findings throughout his graduate program of study in physiology in the Department of Biology, have been presented at regional meetings (Illinois Academy of Science meetings, Normal, Illinois, April, 1978) and national meetings (American Physiology Society, St. Louis, Missouri, October, 1978) and have been subsequently published (The Physiologist, 21 (4) 73, 1978; Proceedings of the Illinois Academy of Science, In Press).

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CHAPTER I

INTRODUCTION

The role of dopamine (3,4, dihydroxyphenethylamine) as an important central neurotransmitter agent has been acknowledged for some time (27). It has only been in recent years, however, that the possibility of a peripheral action of this catecholamine in the functional regulation of various organ systems has been given serious consideration, and Goldberg (15) has proposed a specific role for this agent in the control of the vascular dynamics of the kidney. A number of electron microscopic and fluorescent studies (2, 3, 28, 44) in this organ have shown that there is a direct secretomotor innervation by the sympathetic nervous system of the juxtaglomerular cells of the afferent arteriole. These cells are involved in the synthesis and secretion of the peptide hormone, renin. Norepinephrine is the neurotransmitter secreted by these sympathetic nerve terminals, and various studies have indicated that this catecholamine may exert a direct stimulatory control on renin secretion through a beta-adrenergic receptor mechanism, which utilizes cAMP as the intracellular mediator (8, 13, 31). Some investigators (8, 23) have also recently postulated an inhibitory role for norepinephrine on renin secretion, which appears to be mediated by alpha-adrenergic receptors. The significance of the apparent dual regulation of renin secretion by this catecholamine is unclear.

Despite the known importance of dopamine as a central nervous system neurotransmitter, no attempts have been made to investigate the possibility of renal dopaminergic nerve terminals participating in the regulation of renin secretion, as well as to examine whether or

not dopamine itself may be released from adrenergic nerve terminals to exert its effect, since it is a direct precursor of norepinephrine. Only two *in vitro* studies (18, 24) have addressed this question, and the data suggests that dopamine may directly stimulate renin secretion from rat renal cortical slices although the nature of the receptors or mechanisms involved remain unclear.

In a review by Goldberg (15) a number of physiological actions mediated by dopamine have been examined. This investigator suggests that the diversity of effects of this catecholamine on various organ tissues may be explained on the basis of the structure of the molecule itself, namely that the catecholamine head and flexible side chain have the structural capability of binding to various types of membrane receptors. This would certainly support the view that dopamine is capable of exerting a direct action on a given organ cell. The data of Henry and co-workers (18) appear indicative of such a direct influence of dopamine on the renal renin-secretory cells. Furthermore, preliminary studies in our own lab (23) suggest not only that dopamine directly stimulates renin secretion from the juxtaglomeurlar cells, but that this effect may be mediated by intracellular cAMP changes.

Although the previously cited studies implicate dopamine itself as having a direct effect on renin secretion, the possible conversion of dopamine to norepinephrine in the tissue preparation constitutes a factor which can not be overlooked. The use of a dopamine beta-hydroxylase inhibiting agent such as FLA-63 (5, 37) in *in vitro* studies would eliminate this possibility. A number of *in vivo* and *in vitro* studies have shown FLA-63 to be an effective inhibitor of

dopamine beta-hydroxylase at a range of concentrations in various tissues (5, 7).

In order to eliminate *in vivo* humoral and hemodynamic influences on renin secretion this investigation will utilize a hypersensitive renal cortical slice preparation from rats maintained on a sodium-deficient diet for 1.5 to 2.5 weeks. Lopez and co-workers (23) have confirmed previous *in vivo* work indicating that dietary sodium deficiency potentiates the renin secretory response to sympathetic stimuli. The hypersensitive *in vitro* kidney slice preparation is an ideal tool to more effectively evaluate not only positive, but as importantly negative renin release responses to dopamine and various receptor blockers, respectively.

Therefore, the purpose of the present study is: (1) to evaluate, utilizing an *in vitro* rat renal cortical slice preparation, a possible direct stimulatory role of dopamine itself on the renin-secreting juxtaglomerular cells of the kidney, when added either alone or in conjunction with the specific dopamine receptor blocking agent, pimozide; (2) to examine the possibility that dopamine may partially exert its influence on renin secretion through a beta-adrenergic receptor mechanism, by the use of the beta-receptor blocker propranolol; and (3) to elucidate whether or not cAMP is involved as an intracellular mediator of the renin secretory response to added dopamine.

CHAPTER II

REVIEW OF LITERATURE

During the past ten years a number of important effects of dopamine on peripheral organ tissue function have been proposed. Dopamine has been shown to exert a number of effects in the cardiovascular system (17, 34, 35, 40), some of which appear to be mediated by a beta-adrenergic receptor mechanism since the specific beta-adrenergic blocking agent propranolol inhibits the stimulatory effect of dopamine on heart rate and cardiac contractility. As suggested by Goldberg (15), these observations support the concept that dopamine can effectively bind to membrane receptors other than specific dopamine-sensitive receptors. This possibility is further supported by examining the effects of dopamine on blood vessels. Goldberg (17) and others (26, 35) have shown that dopamine infusion can stimulate the contractility of both arteries and veins. These actions can be effectively blocked by high concentrations of phenoxybenzamine, an alpha-adrenergic receptor blocker, which suggests that dopamine influences vascular contractility through an alpha-adrenergic receptor mechanism.

Dopamine also appears to be involved in the relaxation and vasodilation of vascular tissue through reflex and neurogenic mechanisms. This influence appears to be exerted by an action on beta-adrenergic receptors. In this regard, McNay and Goldberg (26) have demonstrated that after phenoxybenzamine administration dopamine infusion causes vasodilation in the skeletal muscle vascular bed of the dog. Furthermore, Kohli (22) has shown that relaxation occurs in the isolated aortic strip under similar conditions. Propranolol antagonism of these

actions suggests a dopamine-induced vasodilating mechanism exerted through a beta-adrenergic receptor. Conversely, propranolol antagonism may not be due to beta-adrenergic receptor involvement but rather to alpha vasoconstricting activity (32). This view has been supported by Goldberg and colleagues, who have shown that high concentrations of propranolol do not alter dopamine-induced relaxation of isolated canine renal, mesenteric, coronary or femoral arteries (39).

These conflicting observations have led to the postulation of an alternative mechanism of action of dopamine on smooth muscle vasculature which involves a direct interaction with specific dopamine vascular receptors (25). In one such study, administration of phenoxybenzamine to an anesthesized dog, followed by an intra-arterial infusion of dopamine resulted in vasodilation of the renal and mesenteric vascular beds in a dose-related fashion (17). This vasodilating effect could not be decreased by propranolol, atropine, anti-histamine or by pretreatment with reserpine, 48/80, or monoamine oxidase inhibitors (17). Similar results have been observed by investigators examining other vascular structures (34, 43).

The observations that dopamine causes renovascular dilation through an apparent specific dopaminergic receptor has stimulated the study of the role of this agent in blood pressure control and renin secretion. Most data in this regard have been obtained from studies performed in vivo utilizing dogs or human subjects. Ayers and co-workers (1) have shown that dogs, made hypertensive by right nephrectomy and partial occlusion of the left renal artery, exhibit chronic elevation of blood pressure but renin secretion remained normal. They demonstrated that infusion of the vasodepressor drugs

reserpine, hydralazine, and trimethophan did not produce significant increases in renin release. Conversely, infusion of isoproterenol lowered the blood pressure and produced a marked stimulation of renin release. On the other hand, the same investigators observed a marked increase in renin secretion following dopamine infusion in reno-vascular hypertension, in the presence of decreased, normal, or elevated arterial blood pressures. They concluded that it was not a blood pressure change but rather vasodilation that was important in stimulating renin release.

A number of other investigators, working in vivo have also shown that dopamine stimulates renin release. In a series of experiments, Bell and Lang (4) selectively initiated hemmorhage or acute suprarenal aortic stenosis in mongrel dogs, and measured plasma angiotensin II levels as an index of changes in renin secretion. Under these conditions, infusion of dopamine resulted in increased angiotensin II levels in both the hemmorhaging and ischemic groups. This increase was effectively blocked by the dopamine receptor antagonist, ergometrine. From these data, they concluded that stimulation of renin release is exerted through dopaminergic nerves, although the mechanisms involved are unclear.

Cuche and co-workers (11) have examined the involvement of dopamine in regard to the effects of posture changes in humans. It has already been established that changing from the supine to upright posture in humans stimulates the sympathetic nervous system (21). In their studies, they found that upon changing from the supine to the upright position, plasma renin activity increased, urinary dopamine concentration decreased, urinary norepinephrine concentration increased, and

urinary sodium concentration decreased. From these data Cuche and his group proposed a number of possible effects of dopamine on renin secretion. Since dopamine possesses some alpha-adrenergic action but little or no beta-adrenergic activity its inhibitory effect on renin release may be mediated by either a specific dopaminergic receptor or alternatively by competing with norepinephrine at the receptors of the juxtaglomerular cells. Therefore, each catecholamine could conceivably have an affect on renin-secretion through a direct action on the renin secreting cells. Cuche and co-workers (11) have alternatively suggested that dopamine may affect renin secretion through its effect on sodium excretion. This appears to be an indirect inhibitory effect on renin release through increases in sodium load at the macula densa. Furthermore, they have indicated that dopamine action could also be mediated by renal blood flow-dependent pressure changes at the juxtaglomerular cells, since dopamine inhibits plasma renin activity under conditions of low renal plasma flow (11). This action may be mediated by the afferent arteriole baroreceptors. Wilcox and his group (46) have also compared the effects of dopamine and norepinephrine on renin release in human subjects. They observed that plasma renin activity was increased with dopamine infusion. Plasma renin activity decreased with equipressor doses of norepinephrine. These data, although in disagreement with previous data suggesting an inhibitory role of dopamine on renin release, may actually support the view that it is the activation of specific dopaminergic receptors which mediate the effect of dopamine on renin secretion.

Additional studies by Chokshi and colleagues (9), have done little

to further clarify whether or not dopamine is directly stimulatory or inhibitory to renin secretion. In these studies dopamine and isoproterenol were alternately infused into the renal artery of adult dogs. Under these conditions, it was found that dopamine increased renal blood flow and decreased renin secretion, while isoproterenol increased both parameters. Propranolol treatment prior to dopamine infusion did not alter the response in renal blood flow or renin secretion. Infusion of propranolol prior to isoproterenol blocked the increase in renin secretion with no change in renal blood flow. The results seen with dopamine in these studies can be explained by the known vasodilating properties of dopamine on the renal vasculature which would cause a decrease in renin release. This would be considered an indirect effect rather than a direct one on the juxtaglomerular cells.

Most in vivo studies evaluating the possibility of a direct effect of dopamine on renin release have produced inconclusive data, perhaps because of the variety of factors interrelating in the live animal to influence secretion of this hormone. The possible mechanism and site of action of dopamine have therefore, remained largely unclear. Recently, however, a few in vitro studies have been more promising in this regard, primarily because this type of preparation effectively eliminates humoral, hemodynamic and other in vivo influences which are known to affect renin release.

Nakajima and colleagues (29) have shown that addition of dopamine to an in vitro rat kidney particulate preparation produced significant increases in the cAMP content of the tissue. This increase in the tissue nucleotide concentration was not affected by simultaneous addition of either the adrenergic blocker, phentolamine, or the beta-

adrenergic blocker, propranolol. This suggests the involvement of an alternative type of receptor. Addition of spiroperidol, a specific dopamine-receptor antagonist, effectively blocked the increase in tissue cAMP by dopamine, whereas, apomorphine, an analog of dopamine, increased tissue cAMP content when added to the preparation. From these observations, Nakajima and co-workers concluded that the increase in cAMP produced by the addition of dopamine constitutes evidence for the existence of a cAMP mediated dopamine-receptor mechanism in renal vascular tissue. This view is consistent with the existence of a dopamine-sensitive adenylate cyclase mechanism in the mammalian brain and retina (6, 10, 20).

In a follow-up study, Nakajima and co-workers (30) perfused an isolated rat kidney with various reagents. Perfusion with dopamine resulted in a significant increase in cAMP concentration in the perfusate. The increase in the concentration of this nucleotide was potentiated by theophylline, a phosphodiesterase inhibitor, and consistently blocked by spiroperidol, a dopamine-receptor blocking agent. They also observed that the stimulatory effect of dopamine on cAMP concentration was very rapid, an observation which is consistent with the proposed mechanism of action of receptors which utilize cAMP as an intracellular mediator. Nakajima and colleagues (29) have suggested that dopamine acts through a specific dopamine receptor located on the vascular tissue of the rat kidney to produce vasodilation. This view agrees with the concept of a renal vascular receptor proposed by Yeh, McNay and Goldberg (42).

In contrast, Henry and co-workers (18) utilizing an <u>in vitro</u> rat

renal cortical slice preparation, have recently evaluated the possibility of a direct effect of dopamine on renin secretion. Addition of dopamine at concentrations greater than 10^{-5} M resulted in significant increases in renin release, an observation which suggests a direct effect of this amine on the juxtaglomerular cells. Moreover, pheniprazine, a monoamine oxidase inhibitor, potentiated the rate of renin release. In the same studies, simultaneous addition of phentolamine (an alpha-adrenergic receptor blocker), haloperidol (a dopamine-receptor blocker), or cocaine (a catecholamine uptake inhibitor) did not affect the stimulatory effect of dopamine on renin release. However, the addition of propranolol, a beta-adrenergic receptor blocker, significantly inhbited the stimulatory effect of dopamine on renin secretion. From these observations, Henry and colleagues concluded that dopamine may directly influence renin release from the juxtaglomerular cells via a beta-adrenergic receptor mechanism most likely located on the membranes of those cells, rather than by a dopamine-sensitive receptor pathway.

Recent studies in our laboratory (24) have supported Henry's observation of a direct stimulatory effect of dopamine on renin secretion. We further extended their findings by clearly showing a positive correlation between the renin secretory responses and the changes in tissue cAMP content. This constitutes additional evidence for the existence of a cAMP-mediated receptor mechanism by which dopamine may directly influence renin release. Additionally, it has been shown (24) that pimozide, a dopamine receptor blocking agent, effectively prevents the stimulatory effect of dopamine on renin release.

This observation is not in agreement with Henry's, but supports the concept of a dopamine-sensitive receptor mechanism mediating the stimulation of renin secretion by dopamine.

It is obvious from the data reviewed here, that additional studies are needed to further examine the possibility that this important central nervous system neurotransmitter may play a significant regulatory role in the control of renin secretion, and thus, may participate in the regulation of extracellular fluid volume, electrolyte, and blood pressure homeostasis.

CHAPTER III

MATERIALS AND METHODS

Thirty-six male, Sprague-Dawley rats (Madison, Wisconsin), with initial weights of 210 ± 10 gm were fed a sodium-deficient diet, (Teklad Test Diets, Madison, Wisconsin), which provided less than 0.02 µg of sodium per day, for a period of 10 to 17 days. The rats were kept two animals per cage, in a temperature-controlled room (23 ± 2° C) with a 12 hour light/12 hour dark photoperiod. The rats had access to distilled, deionized water ad libitum. After 10 to 17 days on the diet, the animals were decapitated and their kidneys rapidly excised. The kidneys were then placed in Robinson's buffer medium at 4° C (12), gassed for 30 seconds with a mixture of 95% oxygen - 5% carbon dioxide, decapsulated, and gassed a second time. Renal cortical slices approximately 0.3 mm thick and weighing 50 ± 20 mg were subsequently prepared using a Stadie-Rigg's microtome (A.H. Thomas, Co.).

Each slice was divided into several similar sections, which were randomly assigned to seven incubating vessels containing 2.5 ml Robinson's buffer medium at 4° C. Renal slices from other areas of the cortex were similarly prepared until each incubating vessel contained 50 ± 20 mg of renal cortical tissue. The slices were then preincubated for 15 minutes at 37° C in a shaking Dubnoff Metabolic Incubator (Precision Scientific Co.), in a humidified atmosphere of 95% oxygen - 5% carbon dioxide. After preincubation, the slices were transferred to matching vessels containing fresh Robinson's buffer medium at 37° C and were subsequently incubated for one hour under conditions identical

To those during preincubation. One of each group of seven paired vessels served as an untreated control. The other six vessels containing the tissue slices received 10^{-3} M dopamine (Sigma Chemical Co.) added alone or in conjunction with 10^{-4} M of the beta-receptor blocking agent, propranolol (Sigma Chemical Co.), or one of three doses (10^{-7} M, 10^{-6} M, 10^{-5} M), of the dopaminergic receptor blocking agent, pimozide (Janssen Pharmaceutical). Propranolol and pimozide were also added alone to the slice preparation. The dopamine beta-hydroxylase inhibitor, FLA-63 (10^{-4} M) (Regis Chemical Co.) was added in conjunction with the experimental agents in all instances in order to inhibit the conversion of dopamine to norepinephrine. Control tissue was incubated in the presence or absence of FLA-63. The antagonistic agents and FLA-63 were added to the slice preparation in both the preincubation and incubation periods, whereas dopamine was added only in the incubation media. Dopamine and FLA-63 were prepared in a 0.1% ascorbic acid solution (Sigma Chemical Co.) to prevent oxidation (45). Pimozide was prepared in 1.2% tartaric acid (Fisher), also to prevent oxidation. Propranolol was dissolved in Robinson's buffer medium. After incubation, the supernatant medium was collected and stored at -20° C until assayed for renin concentration by radioimmunoassay of angiotensin I. The renal tissue was immediately frozen on dry-ice and homogenized in 1 ml of 8% trichloracetic acid (Mallinkrodt). The tissue homogenates were transferred to 12 x 75 mm pyrex tubes (Scientific Products), containing five drops of 0.1 N HCl, vortexed, and frozen at -20° C until assayed for cAMP content.

A. Renin Concentration Determination by Radioimmunoassay Angiotensin I

1. Angiotensin I Generation

Since no direct assay exists for renin, renin concentration was measured by determining the rate of Angiotensin I formation in the presence of exogenous renin substrate (angiotensinogen). This was followed by radioimmunoassay measurement of the Angiotensin I generated, which is proportional to the amount of renin present. To generate Angiotensin I, the supernatant samples were thawed, vortexed for ten seconds and maintained in a water bath at 4° C. Aliquots of 25 µl from each sample were transferred to 12 x 75 mm tubes and kept at 4° C. Five drops of saturated NaCl, and 0.5 ml of 24-hour nephrectomized dog plasma, containing renin substrate (angiotensinogen) were then added to each tube. The nephrectomized plasma had previously been treated with a 1% phenylmethylsulfonylflouride (PMSF) solution (0.05 ml of PMSF/ml of plasma) to inhibit the action of converting enzyme and other angiotensinases present in plasma. Thus, the degradation of generated Angiotensin I or the conversion of Angiotensin I to Angiotensin II was prevented. The PMSF treated nephrectomized dog plasma was then incubated for two hours at 37° C, followed by a 1:2 dilution with distilled, deionized water. The samples were then vortexed, covered with aluminum foil to prevent evaporation, and placed in a boiling water bath for 2-3 minutes to stop further Angiotensin I generation. The samples were allowed to cool to room temperature, covered with parafilm, and stored at -20° C until assayed for Angiotensin I content by radioimmunoassay.

2. Radioimmunoassay of Angiotensin I

Renin concentration in the unknown samples was determined by

radioimmunoassay of the generated Angiotensin I, utilizing as trace, radioiodinated Angiotensin I (^{125}I-Angiotensin I) and buffer solutions from the Squibb Company (Rolling Meadows, IL) and an Angiotensin I antibody (38) generously donated by Dr. Ian Reid (University of California at San Francisco). The results were expressed as nanograms of Angiotensin I generated per mg of wet tissue per hour.

B. Determination of Cyclic 3'-5' Adenosine Monophosphate (cAMP) by Competitive Protein Binding Assay

1. cAMP Extraction

Frozen tissue homogenates were allowed to thaw and then centrifuged at 4000 rpm for ten minutes at 4° C. The supernatant was transferred to conical centrifuge tubes using Pasteur pipets (Sargent-Welch) and the protein precipitate discarded. Two ml of water-saturated ether (Mallinkrodt) were then added to each sample, vortexed, and the ether phase was aspirated and discarded using Pasteur pipets. This procedure was repeated three times. After the fourth wash, the cAMP-containing water phase was decanted into glass vials and immediately frozen on dry-ice. The frozen samples containing the water-soluble cAMP were subsequntly lyophilized and the freeze-dried cAMP was then stored at -20° C until assayed.

2. Competitive Protein Binding Assay for cAMP

Cyclic AMP content of the tissue slices was determined by a modification of the protein-binding assay of Gilman (14). The results were expressed as picomoles of cAMP per mg of wet tissue.

C. Calculations

Statistical significance of the various parameters studied was evaluated by modified paired and unpaired students "t" tests (36) using a programmable desk top calculator (Hewlett-Packard 9100B) at Argonne National Laboratory (Argonne, IL).

CHAPTER IV

RESULTS

In an initial study the effect of the dopamine-beta-hydroxylase inhibitor, FLA-63 (10^{-4} M) on the basal renin release and cAMP content of rat renal cortical slices from sodium-deficient rats was examined. There were no significant differences in either renin release or cAMP content between non-treated (control) or FLA-63 treated tissue. The mean change in renin release from control for tissue treated with FLA-63 was -0.023 ± 0.25 ng/mg wet tissue/hour ($P > 0.05$). The mean changes in cAMP content from control was 0.0009 ± 0.009 pmole/mg wet tissue ($P > 0.05$). Thus, FLA-63 was utilized in conjunction with other experimental agents in subsequent studies.

Figure 1 shows the renin release and tissue cAMP content changes seen in renal cortical slices treated with 10^{-3} M dopamine alone or 10^{-3} M dopamine added together with FLA-63. In both instances, dopamine signficantly increased both renin release and the cAMP content of the tissue during a one hour incubation period.

In another study the effect of a stimulatory dopamine dose (10^{-3} M) on renin release was evaluated when the specific dopamine-receptor blocking agent, pimozide (10^{-6} M), was added to the slice preparation in the presence or absence of FLA-63 (10^{-4} M). These data can be seen in Figure 2. In the absence of FLA-63, pimozide blocked the stimulatory effect of 10^{-3} M dopamine on renin release in rat renal cortical slices from sodium-deficient rats. Pimozide added by itself also significantly inhibited renin release. In contrast, when pimozide

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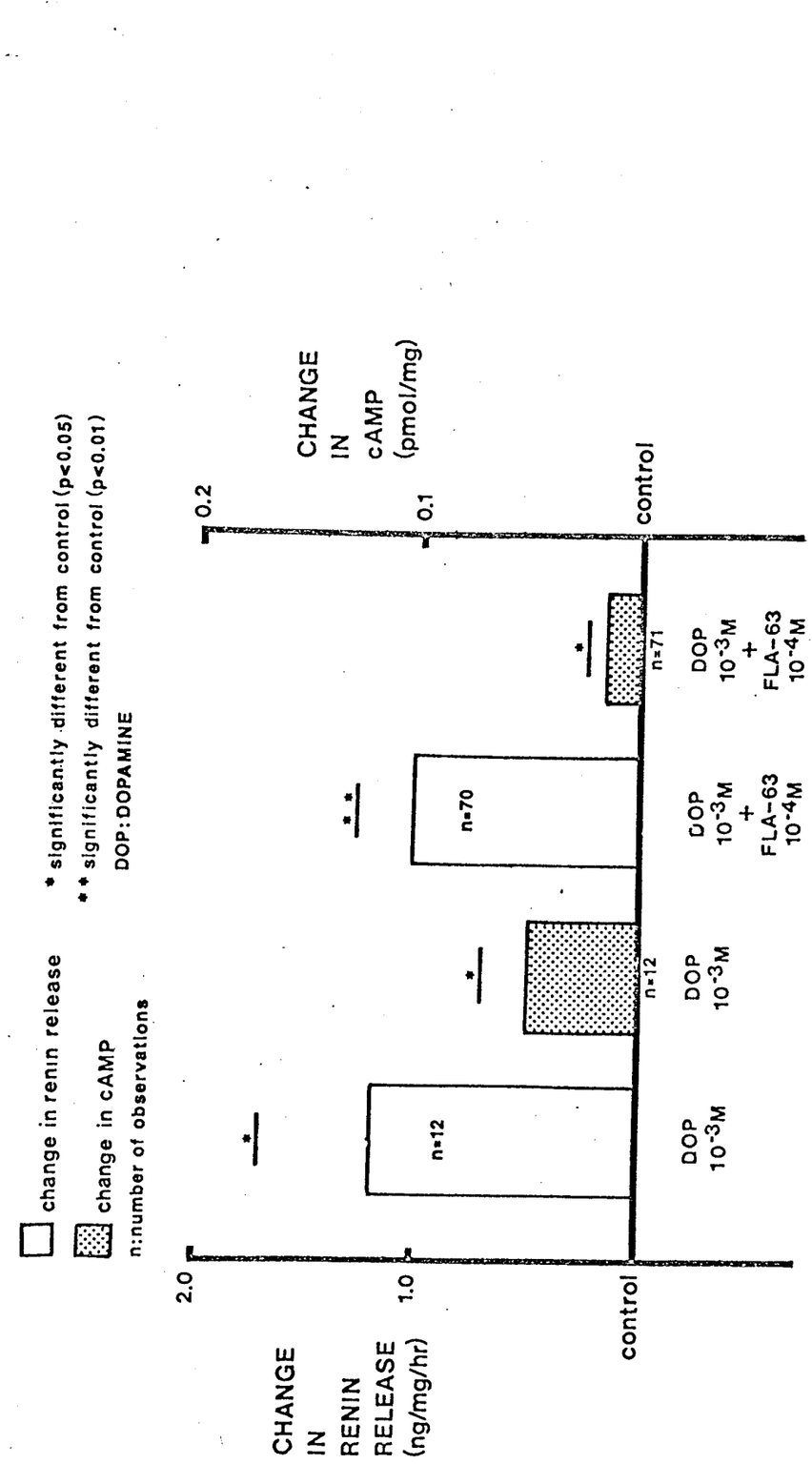
Figure 1.

Effect of dopamine hydrochloride (10^{-3} M) on renin release and cAMP content of renal cortical slices from sodium-deficient rats, added to the preparation either alone or in the presence of FLA-63. The data represent the mean renin release change ± S.E. and the mean cAMP content change ± S.E., respectively. The mean control (non-treated) rate of renin release was 5.43 ± 0.19 ng/mg/hr wet tissue in preparations without FLA-63 and 7.04 ± 0.32 in preparations with FLA-63. The mean control cAMP content was 0.42 ± 0.02 pmole/mg wet tissue in preparations without FLA-63 and 0.32 ± 0.01 pmole/mg wet tissue in preparations with FLA-63 present. Incubation time was 60 minutes.



was added together with dopamine in the presence of FLA-63, it potentiated the stimulatory effect of dopamine on renin release, an effect which was completely opposite to the inhibition on this parameter exerted by pimozide, when FLA-63 was absent from the preparation. Furthermore, when pimozide alone was added to the slice preparation in the presence of FLA-63, renin secretion was significantly stimulated in relation to control.

When the data on renin release (Figure 2) are compared to those in cAMP content in response to the same doses of dopamine and pimozide, in the presence of added FLA-63 (Figure 3), a clear dissociation between renin release responses and cAMP content changes was apparent. Previously it had been observed that without FLA-63 in the incubation medium, pimozide added together with dopamine significantly decreased both renin release and tissue cAMP content below control levels. The inhibitory effect of pimozide on tissue cAMP content was maintained when FLA-63 was added to the tissue preparation (Figure 3), whereas it was significantly reversed in regard to renin release (Figure 2). Moreover, the tissue cAMP content was significantly depressed in response to pimozide added alone (Figure 3) whether or not FLA-63 was present.

Since pimozide added alone to the renal cortical preparation significantly influenced both renin release and tissue cAMP, further experiments were undertaken to find a pimozide dose which by itself would be ineffective in influencing these parameters. Various doses of pimozide (10^{-7} M, 10^{-6} M, 10^{-5} M) were added to the slice preparation either alone or in conjunction with a stimulatory dopamine dose

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Figure 2

Comparison of changes in renin release in response to dopamine hydrochloride (10^{-3} M) added alone or together with 10^{-6} M pimozide to renal cortical slices of sodium deficient rats, with or without FLA-63 present in the incubating medium. The data represent the mean renin release change ± S.E.. The mean control (non-treated) rate of renin release was 5.43 ± 0.19 ng/mg/hr wet tissue in preparations without FLA-63 present and 7.04 ± 0.32 ng/mg/hr wet tissue in preparations with FLA-63. Incubation time was 60 minutes.

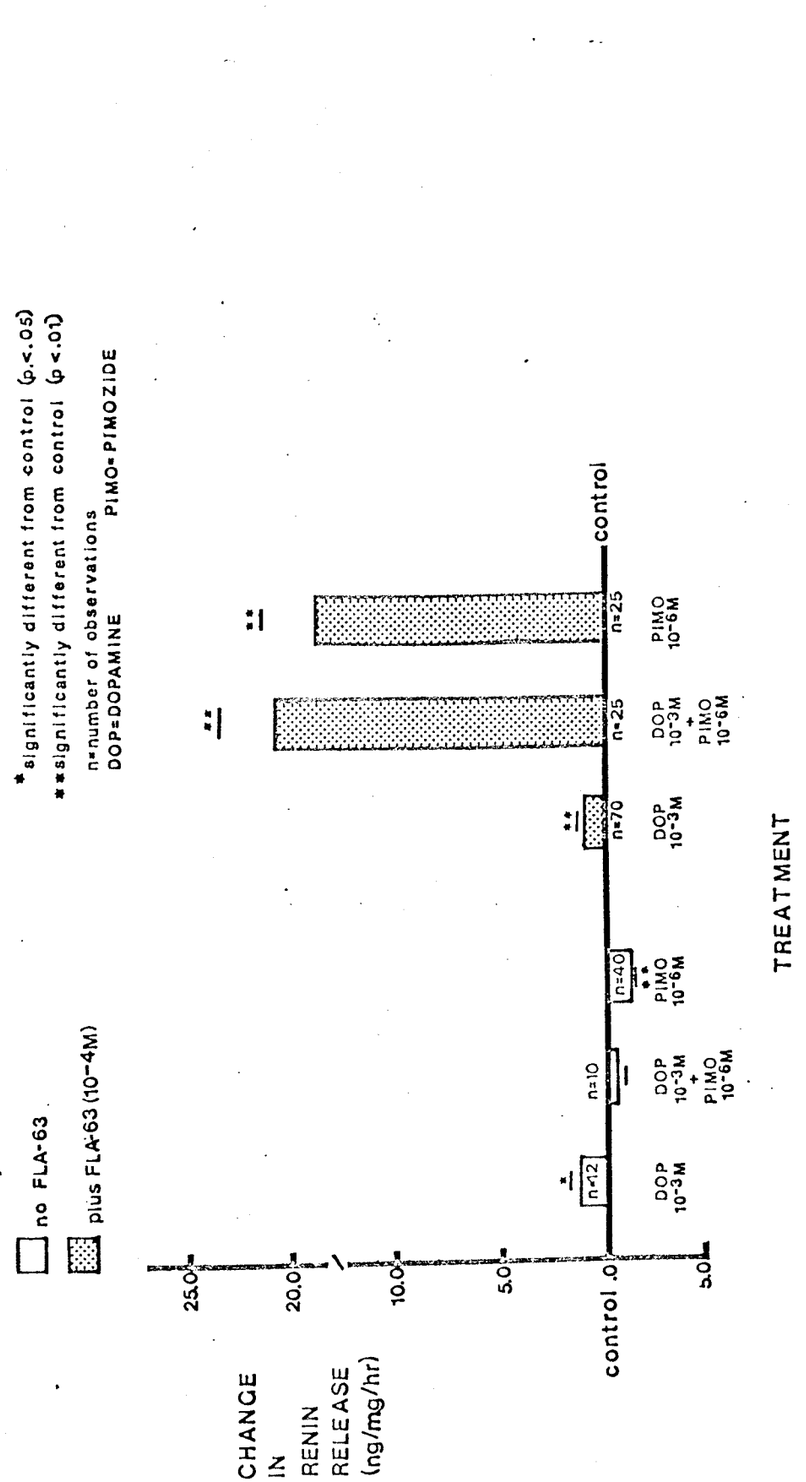

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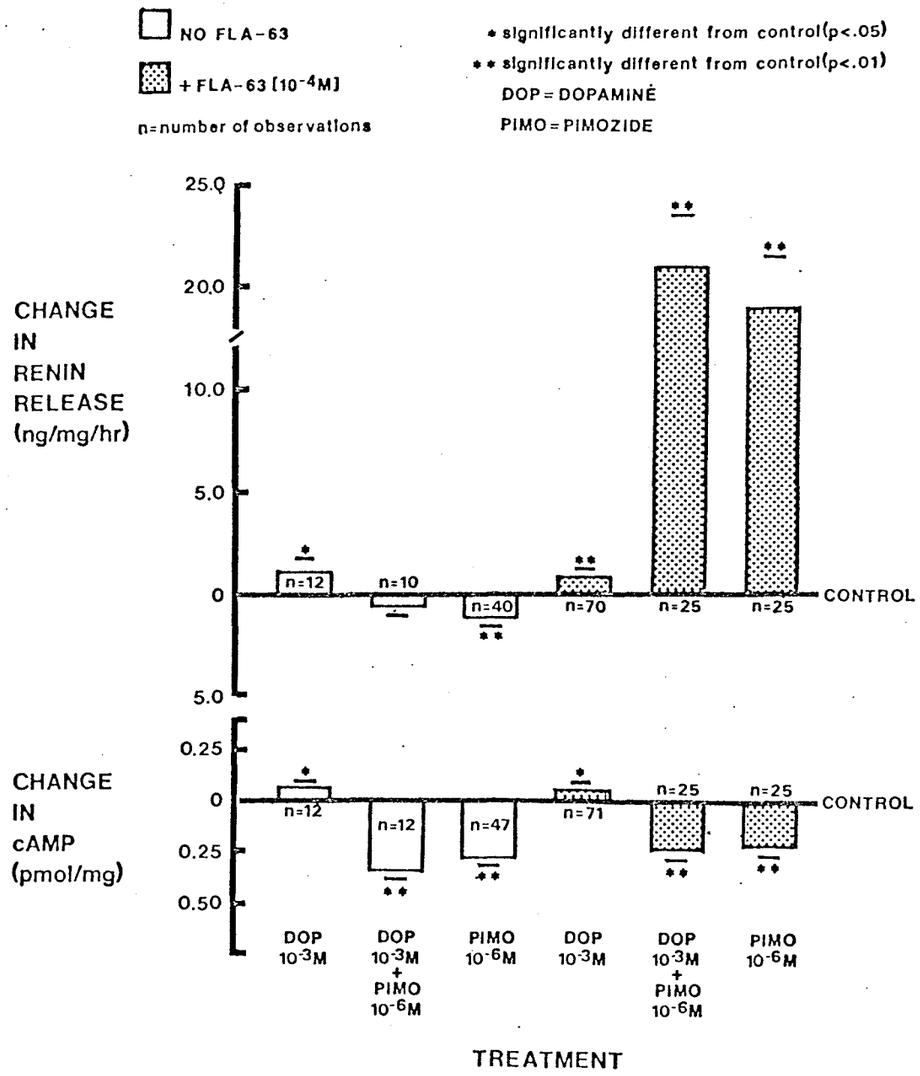
Figure 3

Changes in renin release and cAMP content in response to dopamine hydrochloride (10^{-3} M) added alone or together with 10^{-6} M pimozide to renal cortical slices of sodium-deficient rats, with or without FLA-63 present in the incubating medium. The data represent the mean renin release change ± S.E.. The mean control rate of renin release was 5.43 ± 0.19 ng/mg/hr wet tissue in preparations without FLA-63 and 7.04 ± 0.32 in preparations with FLA-63. The mean control cAMP content was 0.42 ± 0.02 pmole/mg wet tissue in preparations without FLA-63 and 0.32 ± 0.01 in preparations with FLA-63 present. Incubation time was 60 minutes.



(10^{-3} M). FLA-63 (10^{-4} M) was present in all instances. As seen previously with 10^{-6} M pimozide (Figure 3), additional doses of this agent in the presence of FLA-63 significantly potentiated the stimulatory effect of 10^{-3} M dopamine on renin secretion (Figure 4). The three pimozide doses added by themselves were also capable of significantly stimulating renin release. The stimulatory effect of each dose was not statistically different from the other two, an observation which suggests maximal potentiation at this range of pimozide concentrations.

At the three concentrations used, pimozide significantly decreased cAMP content of the tissue preparation when added either alone or in conjunction with dopamine. These data on cAMP content are again similar to those observed in previous studies in the absence of FLA-63. Also as previously observed with 10^{-6} M pimozide in the presence of FLA-63, the additional pimozide doses, either alone or together with dopamine, produced a marked dissociation of the renin release and tissue cAMP responses. Furthermore, the inhibitory effect of all three pimozide doses on tissue cAMP content when added alone appeared maximal.

Since dopamine may structurally be capable of binding to other types of receptors, an additional study was performed to explore this possibility. In these experiments (Figure 6), a stimulatory dose of dopamine (10^{-3} M) was added alone or together with the beta-adrenergic receptor blocker propranolol (10^{-4} M) to renal cortical slices from sodium-deficient rats. FLA-63 (10^{-4} M) was also added in conjunction with all treatments. At the concentration used, propranolol

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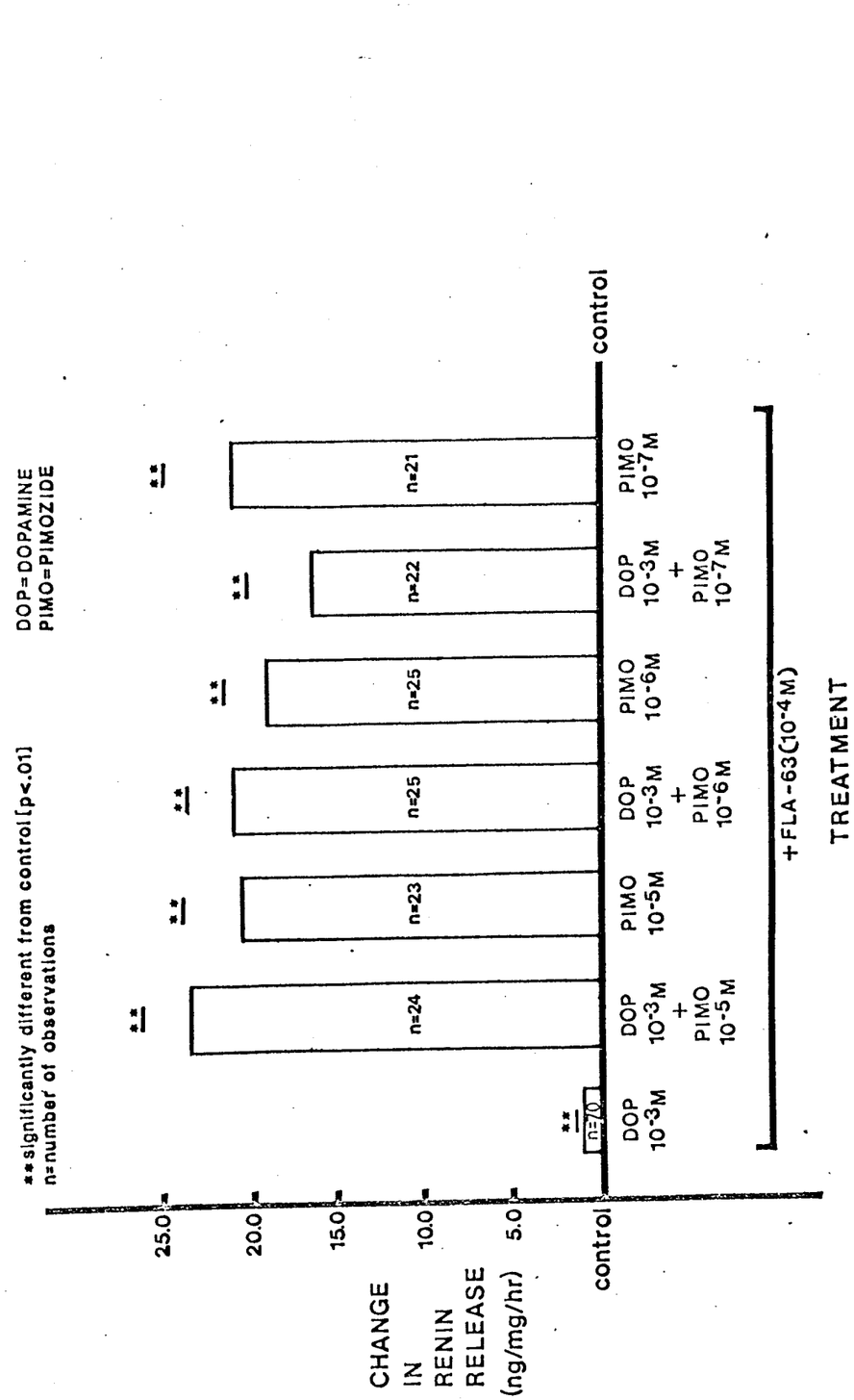
Figure 4

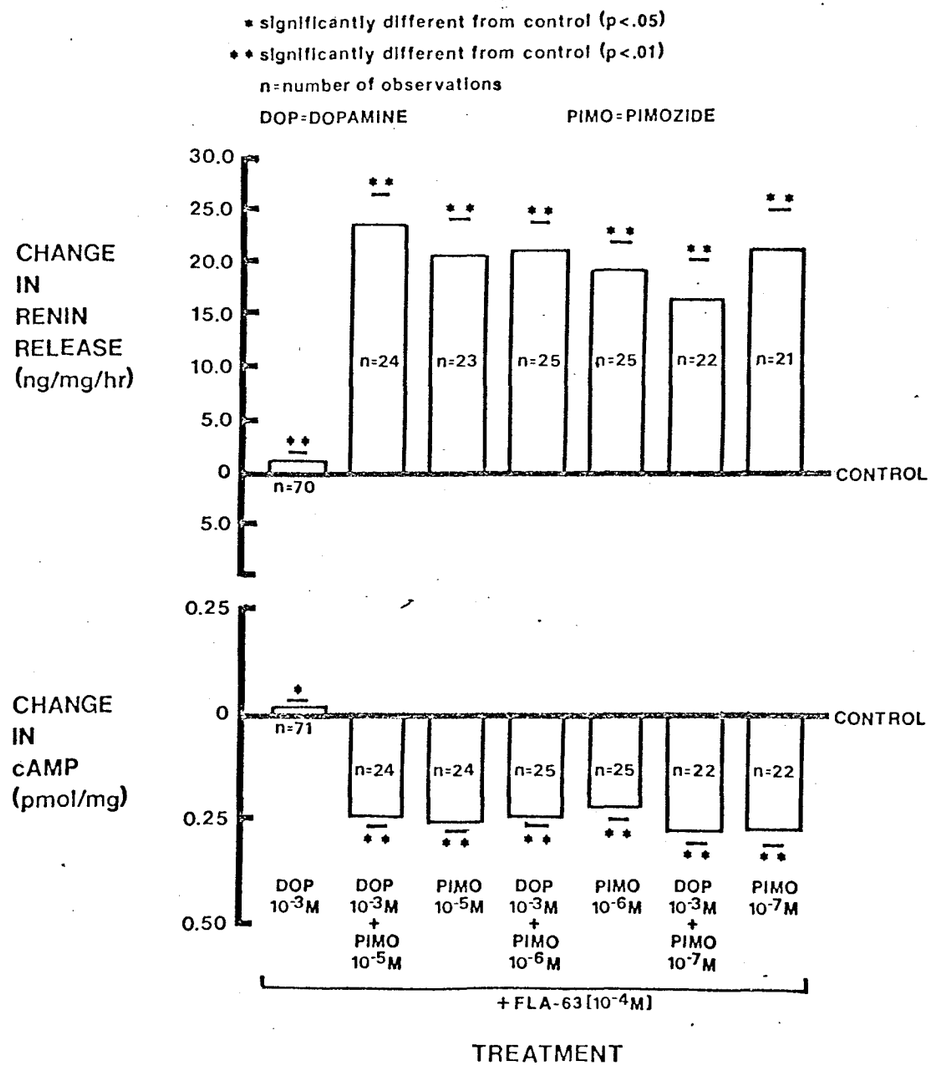
The effect of dopamine hydrochloride (10^{-3} M) when added alone or together with various doses of pimozide, on renin release from renal cortical slices from rats fed a sodium-deficient diet for 10 to 17 days. FLA-63 was present in all preparations. The data represent the mean renin release change ± S.E.. The mean control rate or renin release was 7.04 ± 0.32 ng/mg/hr wet tissue and the mean control content of cAMP was 0.32 ± 0.01 pmole/mg wet tissue. Incubation time was 60 minutes.



LEGEND

Figure 5

Changes in renin release and cAMP content in response to dopamine hydrochloride (10^{-3} M) when added either alone or together with various pimozide concentrations to renal cortical slices of sodium-deficient rats. FLA-63 (10^{-4} M) was present in all preparations. The data represent the mean renin release change ± S.E. and the mean cAMP content change ± S.E.. The mean control rate of renin release was 7.04 ± 0.32 ng/mg/hr wet tissue and the mean control content of cAMP was 0.32 ± 0.01 pmole/mg wet tissue. Incubation times was 60 minutes.

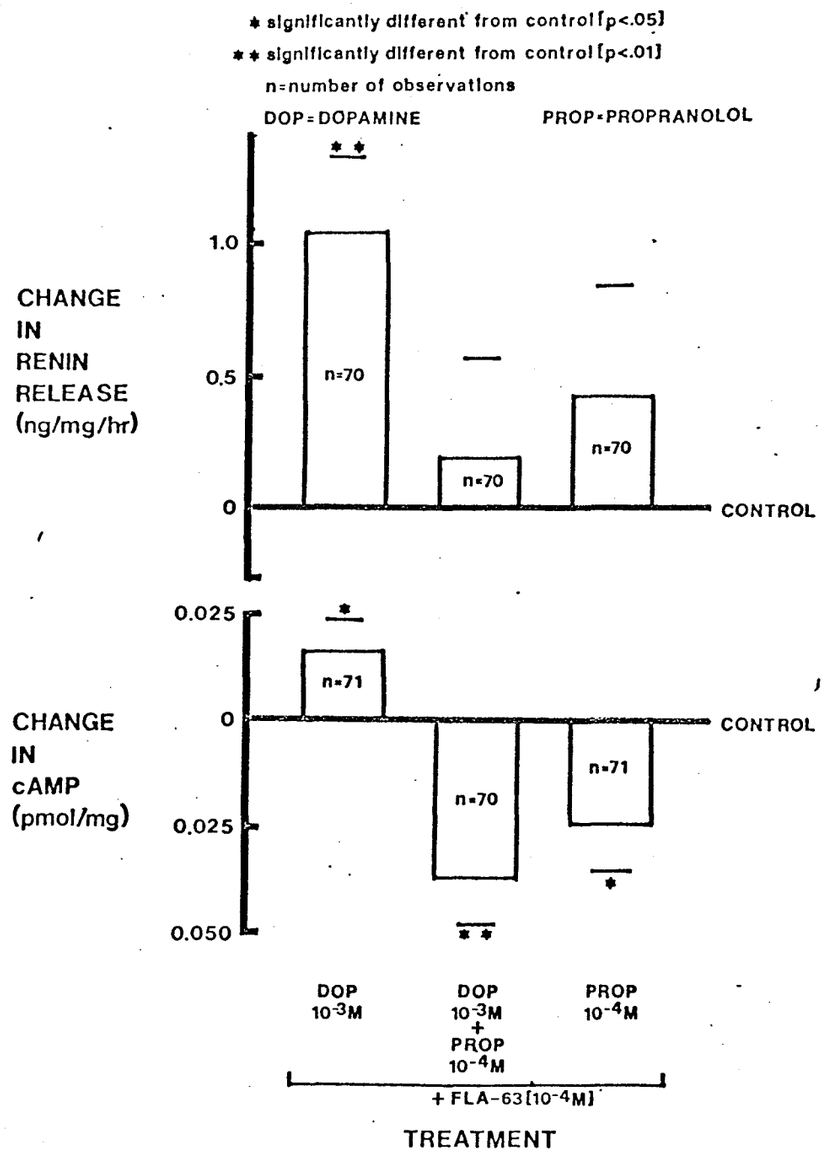


effectively prevented the stimulatory effect of dopamine on renin secretion. Propranolol added by itself was ineffective. Similarly, propranolol effectively blocked the stimulatory effect of dopamine on tissue cAMP content seen previously. By itself, however, propranolol was also capable of inhibiting tissue cAMP content.

LEGEND

Figure 6

Effect of dopamine hydrochloride (10^{-3} M) on renin release and cAMP content, when added either alone or together with propranolol (10^{-4} M) to renal cortical slices of sodium-deficient rats. FLA-63 (10^{-4} M) was present in all preparations. The data represent the mean renin release change ± S.E. and the mean cAMP content change ± S.E.. The mean control rate of renin release was 7.04 ± 0.32 ng/mg/hr wet tissue and the mean control cAMP content was 0.32 ± 0.01 pmole/mg wet tissue. Incubation was 60 minutes.



CHAPTER V

DISCUSSION

The *in vivo* studies of Goldberg and colleagues (16, 26, 47) and the *in vitro* observations of Nakajima and co-workers (29, 30) have provided strong evidence pointing to the existence of specific dopaminergic receptors involved in the control of renal vascular dynamics. The postulated receptors appear to be located on the walls of the renal blood vessels. These vascular receptors may well exist, however, their existence does not rule out the possibility that a specific dopamine receptor may be located elsewhere in the kidney, subserving other functions, and perhaps renin secretion. Data from *in vitro* studies of Henry and his group (18) support the view that renal dopamine receptors may be located on the renin-secreting juxtaglomerular cells. They propose that the stimulatory effect of dopamine is mediated by a beta-adrenergic receptor mechanism, similar to that involved in mediating the action of other catecholamines, such as norepinephrine. Additional observations by Lopez and co-workers (23) support the concept that the effect of dopamine on renin secretion may be directly exerted through a specific dopamine receptor located on the juxtaglomerular cells and that this receptor mechanism may be intracellularly mediated by cAMP changes.

The present study further attempts to evaluate the possibility of a direct regulation of renin release by dopamine. FLA-63 (10^{-4} M) by itself had no significant effect on resting renin release or cAMP content of the tissue. This is important since it essentially

eliminates the possibility that the added FLA-63 affects either renin release or tissue cAMP content. Furthermore, in the presence of FLA-63, dopamine (10^{-3} M) stimulates renin release and cAMP content simultaneously (Figure 1). Values for renin secretion and cAMP content were not statistically different from preparations in which dopamine was added without FLA-63. These data suggest that a dopamine-sensitive membrane receptor exists and that the stimulatory effect of dopamine on renin release may be mediated by increasing tissue cAMP content.

In the presence of FLA-63, pimozide markedly potentiated the dopamine stimulatory effect on release (Figure 2). This is in direct contrast to previous observations (23) that pimzoide blocked the stimulatory effect of dopamine on renin secretion when FLA-63 was absent from the incubation media. Pimzoide added alone to the slice preparation in the presence of FLA-63 also significantly increased renin release as opposed to its inhibitory effects when FLA-63 was not present (23). Conversely, pimozide added either alone or in conjunction with a stimulatory dose of dopamine significantly inhibited tissue cAMP generation in relation to controls whether FLA-63 was present or not (Figure 3). This dissociation of the effects of pimozide on renin release and cAMP content of the tissue when FLA-63 is added to the preparation is not easily explained. One possible explanation is that dopamine, itself, may actually control renin secretion in an inhibitory manner through a specific dopamine receptor mechanism and that the addition of pimozide releases this inhibition. However, the simultaneous significant decrease in tissue cAMP by pimozide is not

consistent with this view. Alternatively, since pimozide by itself was capable of causing marked increases in renin release and decreases in tissue cAMP content it is possible that the observed responses are strictly due to a generalized membrane effect of this agent. The effect being uncontrolled renin release from the juxtaglomerular cells, despite inhibition of secretory mechanisms mediated by cAMP. This generalized membrane effect concept has previously been postulated by Johns and co-workers (19) to explain the stimulatory influence of the synthetic catecholamine, isoproterenol, on renin secretion. It is also possible that pimozide and FLA-63 may interact in some manner to initiate these responses although the mechanism is unclear.

Subsequent studies utilizing additional doses of pimozide, in the presence of FLA-63, largely confirmed the initial observations that pimozide added alone or together with dopamine, is capable of significantly potentiating renin release. At the three doses examined, pimozide appeared to exert maximal effects on both renin release and cAMP content since no significant differences were detected when the various responses were statistically compared.

It is obvious from these data, that the question regarding the involvement of a dopamine-sensitive receptor in mediating the effect of dopamine on renin release can not be properly evaluated. It is necessary to find a dose of pimozide which, by itself, is ineffective in influencing both renin release and tissue cAMP content, as well as to examine other specific dopamine receptor blocking agents such as butaclamol. These data should be of great value in determine whether or not dopamine directly affects renin secretion through a specific dopamine-receptor pathway.

The observations of this study indicate that dopamine, by itself, is capable of significantly and simulatenously stimulating both renin release and tissue cAMP content, while the report of Henry and co-workers (18) suggests that this stimulatory effect may be mediated by a beta-adrenergic mechanism. To investigate the possibility that the renin-secretory responses to dopamine may be mediated by a beta-adrenergic mechanism a subsequent experiment utilized the specific beta-adrenergic receptor blocker, propranolol. In the presence of FLA-63 (10^{-4} M), propranolol (10^{-4} M) effectively prevented the stimulatory effect of dopamine (10^{-3} M) on renin secretion and cAMP content. Propranolol by itself had no effect on renin secretion, but it did inhibit tissue cAMP content. These data are consistent with the concept that the stimulatory effects of dopamine on renin secretion may be at least partially mediated by a beta-adrenergic receptor mechanism. Goldberg (15) has reported that dopamine is capable of binding to receptors other than specific dopamine ones, due to its catechloamine head and flexible side chain. Additional studies are being designed to examine the possiblity of a partial dopaminergic regulation of renin secretion through renal alpha-adrenergic receptors different from those known to mediate blood pressure changes in the renal vasculature.

In summary, the data present here support the view that dopamine itself exerts a direct regulation of renin secretion from the juxtaglomerular cells of the sodium-deficient rat. These results also support the view that this control of renin release by dopamine is partially exerted through a beta-adrenergic receptor mechanism which utilizes cAMP as the intracellular mediator.

However, the data have left unanswered the question as to whether or not the regulatory influence of dopamine on renin release is mediated by a specific dopamine-sensitive receptor mechanism. Studies designed to further evaluate this possibility, as well as the possibility that dopamine may partially regulate renin release through other receptor types, are clearly needed.

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LOYOLA UNIVERSITY

Spring, 1979

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY FRED DANIEL ROMANO ENTITLED EFFECT OF DOPAMINE AND DIETARY SODIUM DEFICIENCY ON IN VITRO RELEASE AND CYCLIC 3'-5' ADENOSINE MONOPHOSPHATE CONTENT OF MALE RAT RENAL CORTICAL SLICES: MODIFICATION BY DOPAMINE-RECEPTOR AND BETA-ADRENERGIC RECEPTOR ANTAGONISTS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

Advisor

Chairman of Department