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Cyclic Adenosine 3′:5′-Monophosphate Mediation of the Effect of Dopamine on Renin Release by Renal Cortical Slices from Sodium-Deficient Rats: Modification by Dopaminergic and β-Adrenergic Receptor Blockade (40707)[1, 2]

G. A. LOPEZ,[3] F. D. ROMANO, V. A. ALETICH, AND L. M. LISSUZZO

Department of Biology, Loyola University of Chicago, Chicago, Illinois 60626

Abundant *in vivo* (1–4) and *in vitro* data (5–9) have clearly determined that the sympathetic nervous system can directly influence renin secretion by the juxtaglomerular cells of the mammalian kidney. This direct effect is primarily exerted by circulating catecholamines of adrenal medullary origin as well as by norepinephrine released by renal sympathetic nerve terminals (10, 11). It is also clear that the direct stimulatory effect of norepinephrine on renin release is mediated by a β-adrenergic receptor mechanism (5–9, 12–14), which utilizes cyclic AMP as the intracellular mediator (15, 16). Dopamine has also been postulated as exerting a regulatory control of renin secretion, either directly (17) or indirectly (18–21), but the data are conflicting and the mechanism involved in direct regulation remains unclear. In view of the importance of dopamine as a central neurotransmitter (22, 23), and reports that dopamine is present in renal tissue (24), further studies are clearly in order to examine a possible regulatory role of this catecholamine on renin secretion. The evaluation of a direct effect of dopamine on renin release in the live animal is difficult due to the interaction of humoral, hemodynamic, and neural factors, known to affect renin secretion. The utilization of *in vitro* renal cortical preparations has effectively eliminated the contribution of humoral and hemodynamic influences, allowing the study of stimuli which may directly affect renin release. Furthermore, *in vitro* studies in our laboratory (25) and elsewhere (9, 15, 16) using a rat renal cortical slice system, have shown that the renin secretory responses to catecholamine stimulation are potentiated by dietary sodium deficiency, thus making an *in vitro* kidney slice system from sodium-deficient animals a more effective and sensitive tool for the evaluation of catecholamine effects on renin secretion.

In the present study, a hypersensitive renal cortical slice preparation from sodium-deficient rats has been employed to examine the possibility of a direct action of dopamine on renin release. Since dopamine-sensitive adenylate cyclase systems have been reported in the brain (26, 27) and kidney (28, 29), changes in renin secretion and tissue cyclic AMP content were simultaneously measured in this study, in an attempt to identify the type of mechanism mediating the renin release responses to added dopamine. Additionally, the effect of dopaminergic and β-adrenergic receptor blocking agents on renin release and tissue cyclic AMP content was examined, in an effort to characterize the type of receptor involved in these responses.

Materials and methods. Male Sprague–Dawley rats (Sprague–Dawley Co.) with initial weights of 210 ± 10 g were used in these studies. They were kept in temperature-controlled rooms (23 ± 2°C), two animals per cage, and had unlimited access to distilled, deionized water. The animals were fed a sodium-deficient chow diet (Teklad Mills Co.), which provided less than 0.02 meq of sodium per day, for 15 days. They were then sacrificed by decapitation and their kidneys rapidly excised, decapsulated, placed in Robinson's buffer medium (30), and gassed with 95% oxygen–5% carbon dioxide for 30 sec. Slices of renal cortex approximately 0.3 mm thick were subsequently prepared using a Stadie–Riggs microtome (A. Thomas Co.). Each cortical slice was further divided into various portions according to the number of treatments, and randomly assigned to separate incubating vessels containing 2.5 ml of

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[2] Sponsor: James P. Filkins, Ph.D., Professor and Chairman, Department of Physiology, Loyola University Stritch School of Medicine, Maywood, Ill. 60153.

[3] To whom requests for reprints should be sent.

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Robinson's buffer medium. This procedure was repeated using slices from other areas of the renal cortex, until each vessel contained 50 ± 20 mg of renal cortical tissue. The tissue distribution ensured a homogeneous cell population in the incubating vessels, and it allowed the simultaneous evaluation and comparison of various tissue treatments having a common control sample. Thus, one of each group of incubating vessels served as an untreated control. The cortical tissue was then preincubated for 15 min at 37°C in a shaking Dubnoff metabolic incubator (Precision Scientific), in an atmosphere saturated with 95% O_2–5% CO_2. The preincubated slices were subsequently transferred to vessels containing fresh Robinson's buffer at 37°C, and incubated for 1 hr under conditions identical to those of the preincubation period.

Dopamine (Sigma) solutions, prepared in 0.1% ascorbic acid (Sigma) to prevent oxidation, were added to the slice preparation before the 1-hr incubation period. The dopamine-receptor blocking agent pimozide (Janssen Pharmaceutical), similarly prepared in 1.2% tartaric acid (Sigma) to prevent oxidation, and the β-adrenergic receptor blocker propranolol (Sigma), prepared in Robinson's buffer, were in turn added to the tissue preparation before both the preincubation and incubation periods. In some studies, the dopamine-β-hydroxylase enzyme inhibitor FLA-63 (Regis Chemical Co.), prepared in 0.1% ascorbic acid, was added to the slice system before both the preincubation and incubation periods in order to prevent conversion of dopamine to norepinephrine in the slice tissue (31). Following incubation, the supernatant medium was collected and stored at −20°C until assayed for renin concentration. In turn, the tissue slices were rapidly frozen on dry ice, homogenated in 1 ml of 8% trichloroacetic acid, and extracted with water-saturated ether. The ether phase was then discarded while the cyclic AMP-containing water phase was lyophilized. Cyclic AMP content in the lyophilized samples was determined by a modification of the protein binding assay of Gilman (32), and the results were expressed as picomoles cyclic AMP/milligram wet tissue. Renin concentration in the incubated supernatant medium was measured by radioimmunoassay of angiotensin I (33), and the values were expressed as nanograms angiotensin I generated/milligram wet tissue/hour. The data were evaluated for statistical significance by modified paired and unpaired *t* tests (34).

Results. In an initial study, the effect of various dopamine concentrations on renin release and cyclic AMP content in renal cortical slices from sodium-deficient rats was examined. At the highest dose tested (10^{-3} *M*), dopamine significantly stimulated both renin release and tissue cyclic AMP content, but a lower dopamine dose (10^{-5} *M*) was only effective in significantly increasing renin release (Fig. 1). Maximal stimulation of renin secretion was observed at 10^{-5} *M* dopamine since a higher 10^{-3} *M* dose did not statistically potentiate the renin secretory rate. Although dopamine concentrations of 10^{-9} and 10^{-7} *M* did not significantly affect renin secretion, a dose–response relationship was observed at a dopamine range of 10^{-9} to 10^{-5} *M* when the renin release responses to these doses were compared to each other.

In a subsequent study, the specific dopamine-receptor blocker pimozide (10^{-6} *M*) was added to the tissue slice preparation either alone or in conjunction with various dopamine concentrations (Fig. 2). In the presence of pimozide, two previously stimulatory dopamine doses (10^{-5} and 10^{-3} *M*) were no longer effective in stimulating either renin release or tissue cyclic AMP content, and in fact a marked inhibition of the latter parameter was now apparent. Pimozide added alone, however, significantly inhibited both resting renin secretion and the cyclic AMP content in the incubated slice tissue.

Additional studies were designed to evaluate the possibility that the stimulatory effect of dopamine on renin release and tissue cyclic AMP content we had observed (Fig. 1) may have been due to the conversion of dopamine to norepinephrine in our tissue preparation rather than to a direct action of dopamine itself. In these studies, we utilized a specific dopamine-β-hydroxylase enzyme inhibitor, FLA-63, which effectively prevented dopamine from being converted to norepinephrine (31). In the presence of FLA-63 (10^{-4} *M*), a 10^{-3} *M* dopamine concentration significantly stimulated both renin release and tissue cyclic AMP content (Fig. 3) in a manner similar to

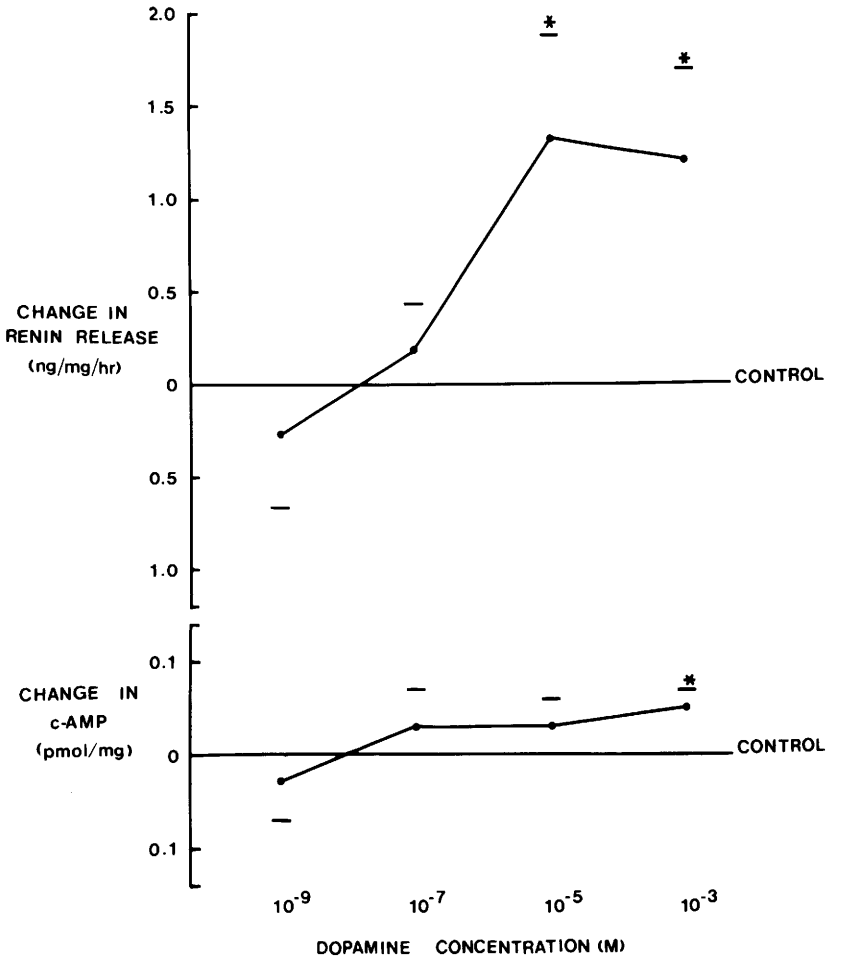

FIG. 1. Effect of various concentrations of dopamine on renin release and cyclic AMP content in renal cortical slices from sodium-deficient rats. The data represent the mean renin release change ± SE and the mean tissue cyclic AMP content change ± SE, respectively, of 12 observations for each dopamine concentration. The mean control (nontreated) rate of renin release was 5.43 ± 0.19 ng/mg/hr and the mean control cyclic AMP content was 0.42 ± 0.02 pmole/mg wet tissue. Incubation time was 60 min. * Significantly different from control ($P < 0.05$).

that which we had observed in experiments in which FLA-63 was not used (Fig. 3). FLA-63 added alone did not affect resting renin release or tissue cyclic AMP content in this study or in any other in which it was used.

To further evaluate the possibility of a dopamine-specific renal receptor mediating the stimulatory effect of dopamine on renin secretion, a previously stimulatory dopamine dose (10^{-3} *M*) was added to the slice preparation either alone or together with pimozide (10^{-6} *M*), with FLA-63 being present at all times. As opposed to the results obtained in previous experiments in which FLA-63 was not utilized (Fig. 2), the stimulatory effect of dopamine on renin release, still significant in the presence of the enzyme inhibitor, was now dramatically potentiated rather than inhibited by the addition of pimozide (Fig. 4). The significantly negative changes in tissue cyclic AMP content in response to pimozide added either alone or together with dopamine remained unchanged, however, whether FLA-63 was present or not (Figs. 2 and 4). A similar dissociation of the renin secretory responses from those seen in tissue cyclic AMP content was observed in another study in which various pimozide concentrations were added to the slice tissue preparation either alone or in conjunction with 10^{-3} *M* dopamine in the presence of FLA-63 (Fig. 5).

To determine if an alternative type of re-

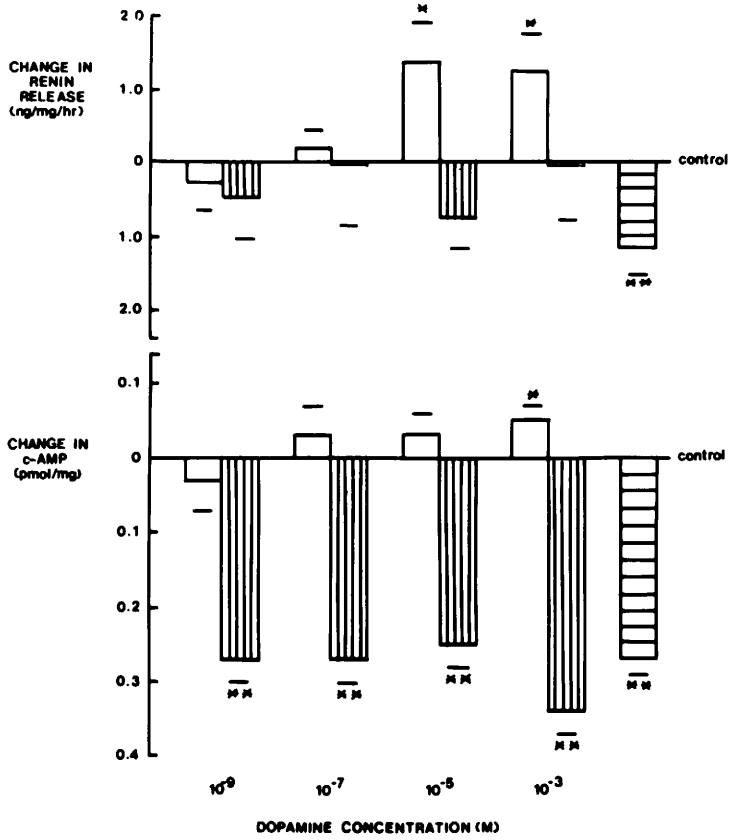

FIG. 2. Changes in renin release and tissue cyclic AMP content in response to various concentrations of dopamine, added either alone or together with 10^{-6} *M* pimozide to renal cortical slices from sodium-deficient rats. The data represent the mean renin release change ± SE and the mean tissue cyclic AMP content change ± SE, respectively, of 12 observations for each treatment. The mean control (nontreated) rate of renin release was 5.43 ± 0.19 ng/mg/hr and the mean control cyclic AMP content was 0.42 ± 0.02 pmole/mg wet tissue. Incubation time was 60 min. ▤, 10^{-6} *M* pimozide alone; □, dopamine alone; ▥, dopamine + 10^{-6} *M* pimozide. * Significantly different from control ($P < 0.05$). ** Significantly different from control ($P < 0.01$).

ceptor mechanism may be involved in mediating the stimulatory effect of dopamine on renin release and tissue cyclic AMP content seen in these studies, an additional study was undertaken. In this study, dopamine (10^{-3} *M*) was added to the slice system either alone or together with the β-adrenergic receptor blocker propranolol (10^{-4} *M*), in the presence of FLA-63 (Fig. 6). At the concentration used, propranolol effectively prevented the significant stimulatory effect of dopamine on renin release and tissue cyclic AMP content. Propranolol added alone did not affect renin release but it decreased tissue cyclic AMP content.

Discussion. Studies utilizing *in vitro* kidney preparations (5–9, 12–16) have provided evidence supporting the concept of a direct stimulatory effect of catecholamines on renin secretion from the juxtaglomerular cells of the kidney. In these preparations, the influence of blood pressure and macula densa flux changes which are known to influence renin release in the live animal is effectively eliminated, and although a number of cell types are still present the effect of sympathetic agonist and antagonist agents added to the tissue system is most likely a direct one on the renin-secreting cells.

The data reported in this study clearly indicate that dopamine is capable of directly stimulating renin release, which supports previous *in vitro* observations (17). Furthermore, these results show that the stimulation of renin secretion by dopamine is caused by this catecholamine itself rather than by its con-

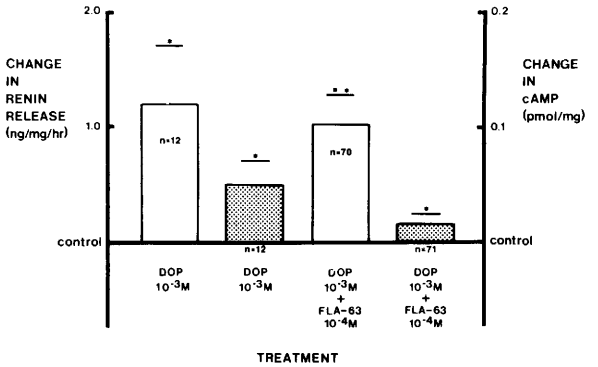

FIG. 3. Effect of dopamine (DOP) (10^{-3} *M*), added either alone or in conjunction with FLA-63, on renin release and cyclic AMP content in renal cortical slices from sodium-deficient rats. The data represent the mean release change ± SE and the mean tissue cyclic AMP content change ± SE, respectively, of 12 observations in experiments without FLA-63 and of 70 and 71 observations, respectively, in experiments in which FLA-63 was used. The mean control (nontreated) rate of renin release was 5.43 ± 0.19 ng/mg/hr in experiments without FLA-63 and 7.04 ± 0.32 in experiments with FLA-63. The mean control tissue cyclic AMP content was 0.42 ± 0.02 pmole/mg wet tissue in experiments without FLA-63 and 0.32 ± 0.01 in experiments with FLA-63. Incubation time was 60 min. □, change in renin release; ▨, change in cAMP; *n*, number of observations. * Significantly different from control ($P < 0.05$). ** Significantly different from control ($P < 0.01$).

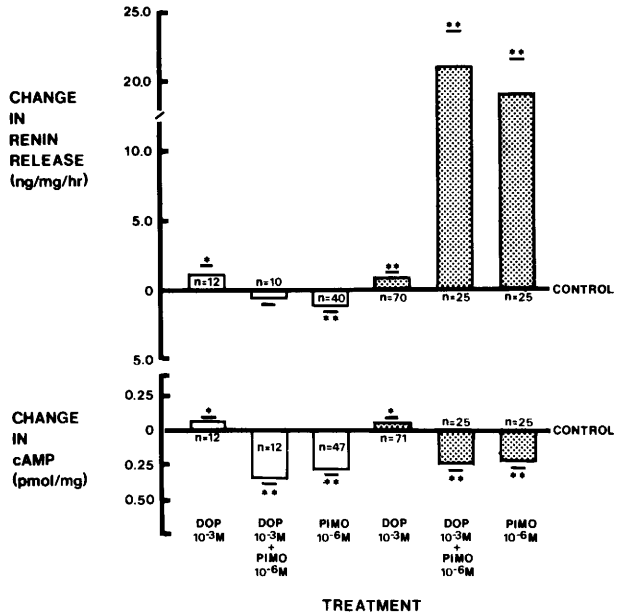

FIG. 4. Changes in renin release and cyclic AMP content in response to 10^{-3} *M* dopamine (DOP) added alone or together with 10^{-6} *M* pimozide (PIMO) to renal cortical slices from sodium-deficient rats, in the presence or absence of FLA-63 in the incubating medium. The data represent the mean renin release change ± SE and the mean tissue cyclic AMP content change ± SE, respectively. The number of observations (*n*) per treatment is indicated. The mean control (nontreated) rate of renin release was 5.43 ± 0.19 ng/mg/hr for DOP alone and DOP + PIMO treatments and 5.23 ± 0.18 for PIMO added alone, in experiments without FLA-63; alternatively, it was 7.04 ± 0.32 ng/mg/hr for DOP added alone and 7.7 ± 0.55 for DOP + PIMO and PIMO added alone, in experiments with FLA-63. Similarly, the mean control tissue cyclic AMP content values were, respectively, 0.42 ± 0.02 and 0.40 ± 0.02 pmole/mg wet tissue in experiments without FLA-63, and 0.32 ± 0.01 and 0.31 ± 0.02 pmole/mg wet tissue in experiments with FLA-63, for the same treatment distribution as for renin. Incubation time was 60 min. □, no FLA-63; ▨, with FLA-63 (10^{-4} *M*). * Significantly different from control ($P < 0.05$). ** Significantly different from control ($P < 0.02$).

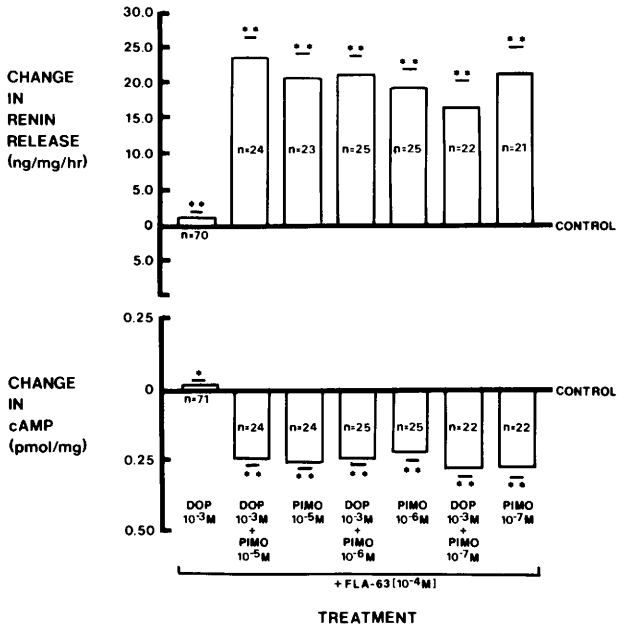

FIG. 5. Changes in renin release and tissue cyclic AMP content in response to 10^{-3} M dopamine (DOP) added alone or together with several pimozide (PIMO) concentrations to renal cortical slices from sodium-deficient rats. FLA-63 (10^{-4} *M*) was present in all instances. The data represent the mean renin release change ± SE and the mean tissue cyclic AMP content change ± SE, respectively. The number of observations (*n*) per treatment is indicated. The mean control (nontreated) rate of renin release was 7.04 ± 0.32 ng/mg/hr for DOP alone, 6.4 ± 0.53 for DOP + 10^{-5} *M* PIMO and 10^{-5} *M* PIMO alone, 7.7 ± 0.55 for DOP + 10^{-6} *M* PIMO and 10^{-6} *M* PIMO alone, and 4.4 ± 1.22 for DOP + 10^{-7} *M* PIMO and 10^{-7} *M* PIMO alone, respectively; similarly, the mean control tissue cyclic AMP content values were, respectively, 0.32 ± 0.01, 0.30 ± 0.02, 0.31 ± 0.02, and 0.34 ± 0.02 pmole/mg wet tissue, for the same treatment distribution as for renin. Incubation time was 60 min. * Significantly different from control ($P < 0.05$). ** Significantly different from control ($P < 0.01$).

version to norepinephrine in the slice tissue, since the significant stimulatory effect exerted by 10^{-3} *M* dopamine on renin release persisted in the presence of FLA-63, an effective dopamine-β-hydroxylase enzyme inhibitor (31).

The concept of a specific renal dopaminergic receptor mediating the effect of dopamine on renin secretion has been previously proposed (35, 36), although these data are inconclusive. Our results do not presently support the view that the stimulation of renin release and tissue cyclic AMP content seen in the presence of dopamine is mediated by a dopamine-specific receptor mechanism, since the meaning of our findings using the postulated dopamine-receptor blocker pimozide is unclear to us at this time. In our studies, three different pimozide concentrations added either alone or in conjunction with 10^{-3} *M* dopamine produced quite similar effects on either renin release or tissue cyclic AMP content. The tissue content of this nucleotide appeared markedly inhibited by pimozide in the presence or absence of FLA-63, but the presence of the enzyme inhibitor caused a dramatic stimulation of renin release by pimozide as opposed to a previously significant inhibition caused by this agent when FLA-63 was not added to the tissue preparation. We do not have a reasonable explanation for the significant dissociation of the renin secretory and tissue cyclic AMP content responses to added pimozide when FLA-63 was present in the incubate, but some studies (20) have questioned the specificity of pimozide as a dopamine-receptor blocker, by demonstrating its inability to prevent the stimulation of renin release by the dopamine-receptor agonist apomorphine in the dog. Thus, the observed effects may be due to a generalized action of pimozide independent of its association with a dopamine-specific membrane receptor. Alternatively, they may reflect a blockade by

FIG. 6. Changes in renin release and tissue cyclic AMP content in response to 10^{-3} *M* dopamine (DOP) added alone or together with 10^{-4} *M* propranolol (PROP) to renal cortical slices from sodium-deficient rats. FLA-63 (10^{-4} *M*) was present in all instances. The data represent the mean renin release change ± SE and the mean tissue cyclic AMP content change ± SE, respectively. The number of observations (*n*) per treatment is indicated. The mean control (nontreated) rate of renin release was 7.04 ± 0.32 ng/mg/hr and the mean control tissue cyclic AMP content was 0.32 ± 0.01 pmole/mg wet tissue. Incubation time was 60 min. * Significantly different from control ($P < 0.05$). ** Significantly different from control ($P < 0.01$).

pimozide of dopamine-induced intracellular cyclic AMP content changes in cortical cells other than the juxtaglomerular cells, while dopamine may be simultaneously modulating both renin release and cyclic AMP content by a propranolol-blockable mechanism. Regardless, it is obvious that further *in vitro* studies utilizing additional dopamine-receptor agonist and antagonist agents, phosphodiesterase inhibitors, additional pimozide concentrations to find a dose which by itself does not affect renin release and cAMP content, and the measurement of intracellular renin changes are needed before the possibility of the existence of a dopamine-specific renal receptor modulating renin release can be properly evaluated. These studies are currently being conducted in our laboratory.

It has been proposed (22, 37) that structurally, dopamine has the capability of binding to various types of membrane receptors. Our data support this view by clearly showing that the simultaneous stimulation of renin release and tissue cyclic AMP content by 10^{-3} *M* dopamine was effectively prevented by the β-adrenergic receptor blocker propranolol. These results agree with previous observations (17), and they additionally suggest that the stimulatory effect of dopamine on renin secretion is at least partially mediated by a β-adrenergic receptor mechanism involving intracellular cyclic AMP changes.

Summary. The effect of dopamine (DOP), dopamine-receptor antagonist, and β-adrenergic receptor antagonist agents on simultaneously measured renin release (RR) and

cyclic adenosine 3′:5′-monophosphate (cAMP) content of renal cortical slices from sodium-deficient rats was studied *in vitro*. A DOP dose of 10^{-5} *M* significantly increased RR while a higher (10^{-3} *M*) dose significantly stimulated both RR and cAMP content in the slice preparation. Addition of the DOP-receptor blocker pimozide (PIMO) at 10^{-6} *M* effectively prevented stimulation of RR and tissue cAMP content by DOP. However, PIMO added alone also significantly decreased resting RR and tissue cAMP content levels. In subsequent experiments, the DOP-β-hydroxylase (DBH) enzyme inhibitor FLA-63 (10^{-4} *M*) was used in conjunction with the other agents to prevent conversion of DOP to norepinephrine (NE) in the tissue preparation. In the presence of FLA-63, 10^{-3} *M* DOP again significantly stimulated both RR and cAMP content as previously seen without FLA-63. Conversely, a previously inhibitory effect on RR was converted to significant stimulation by several PIMO doses in the presence of the DBH enzyme inhibitor. Tissue cAMP content appeared significantly inhibited by all PIMO doses added alone or together with 10^{-3} *M* DOP whether FLA-63 was present or not. FLA-63 was ineffective by itself. A 10^{-4} *M* dose of the β-adrenergic receptor blocker DL-propranolol (PROP), added together with 10^{-3} *M* DOP and FLA-63, significantly prevented the stimulatory effect of DOP on RR and tissue cAMP content. These data indicate that (i) DOP itself can directly stimulate RR from the renal juxtaglomerular cells, (ii) a direct stimulatory effect of DOP on RR is at least partially mediated by a β-adrenergic receptor mechanism involving intracellular cAMP changes, and (iii) further studies are needed before the possibility of the existence of dopamine-specific renal receptors mediating the action of DOP on RR can be properly evaluated.

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