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Effect of Dopamine and Dopamine-Receptor Blockade on *in vitro* Renin Release, Tissue Renin Content and Tissue Cyclic AMP Content in the Rat

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This study evaluated the *in vitro* renin release, tissue cyclic AMP content (TcAMPc), and tissue renin content (TRC) changes with time, in response to administration of dopamine (DOP) and of the dopamine-receptor blocking agent pimozide (PIM) to renal cortical slices from sodium deficient (SD) rats. Addition of 10^{-3}M DOP to the slice preparation resulted in a gradual stimulation of RR with time, which was significantly different from that seen in control samples after 60 min of incubation. In contrast, TcAMPc of the DOP-treated samples was significantly greater than that of controls after 5 min of incubation. At 60 min, mean TRC of DOP-treated samples was greater than that of controls but not significantly. Two PIM doses (10^{-5}M and 10^{-6}M), whether added alone or together with 10^{-3}M DOP to the cortical slice system, significantly increased RR in each instance while simultaneously depressing TcAMP content markedly below that of unstimulated controls at all incubation times examined. Mean TRC of pimozide-treated samples was also lower than that of controls by 60 min. These *in vitro* data in the SD rat suggest that: 1) stimulation of renin release by DOP is time-dependent and is mediated by a TcAMP-generating mechanism, and 2) the increase in renin release by PIM administration appears to involve pharmacological inactivation of TcAMP-generating pathways and disruption of membrane permeability, leading to uncontrolled RR.

Key words: Dopamine, Renin release, Renin content, Tissue cyclic AMP content, Rat.

Data from studies which have utilized a rat renal cortical slice system (9) or the isolated, perfused rat kidney (22), suggest that dopamine can directly stimulate renin release and that this effect is partially mediated by a β-adrenergic receptor mechanism, probably operating at the level of the juxtaglomerular cell membrane. We have further supported this concept (16, 17) by demonstrating that both the stimulation of renin release by dopamine and the blockade of this effect by the beta-receptor antagonist propranolol, are coupled with corresponding changes in the cAMP content

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of rat renal cortical tissue incubated for one hour in the presence of these agents.

We have also preliminarily investigated the possibility that renal cortical dopamine-specific receptors may participate in the direct regulation of renin release by dopamine in the rat. In these studies (16, 17), several doses of the dopamine-receptor blocker pimozide, added either alone or together with dopamine to renal cortical slices, markedly increased rather than blocked renin release, while simultaneously inhibiting cAMP generation in the cortical tissue during a one-hour incubation period. These results may reflect either a direct effect of pimozide itself on renin secretion via inactivation of membrane-bound cAMP generating mechanisms, or a release of dopamine receptor-mediated inhibition of renin release as shown by QUESADA *et al.* (22) in the case of the dopamine-receptor blocker, haloperidol, in the isolated rat kidney. Pimozide has been extensively used as a central dopamine-receptor blocker in various species (7, 8, 24, 25), but its use as a peripheral dopamine-receptor antagonist is limited (11, 18, 28) and has not included the kidney. Therefore, this study further examines the nature of the mechanisms by which dopamine and the dopamine-receptor antagonist pimozide influence renin release in the rat, by simultaneously evaluating the changes in renin secretion and tissue cAMP content ($TcAMP_c$) in relation to the length of time elapsed after administration of these agents. Also, changes in tissue renin concentration are concurrently measured in order to determine if changes in renin release produced by treatment with these agents, involve a modification in the available tissue pool of renin. We have utilized a renal cortical slice preparation from sodium deficient rats, in view of reports (5, 14, 15, 21) which suggest that dietary sodium deficiency significantly potentiates the renin secretory responses to a variety of stimuli. A portion of these data has been reported in preliminary form (13).

Materials and Methods

The *in vitro* kidney slice system used in this study has been previously described (17). Briefly, renal cortical tissue was obtained from male, Sprague-Dawley rats, which had been maintained for 2-3 weeks on a sodium deficient diet (Teklad Test Diets) providing less than 0.02 mEq/Na^+ per day. The tissue was sliced using a Stadie-Riggs microtome (A. Thomas Co.), preincubated for 15 min in Robinson's buffer (3) at 37 °C, and subsequently incubated for 5, 20 or 60 min under continuous gassing with 95 % O_2 — 5 % CO_2. Pimozide (Janssen Pharmaceutical) solutions were prepared in 1.2 % tartaric acid and were added to the tissue preparation before both the preincubation and incubation periods. In turn, dopamine (Sigma) was prepared in 0.1 % ascorbic acid to prevent oxidation, and was added to the kidney slice system prior to the incubation periods only. The dopamine-β-hydroxylase enzyme inhibitor, FLA-63 (Regis Chemical Co.), was prepared in 0.1 % ascorbic acid and added to all dopamine-treated samples to prevent conversion of dopamine to norepinephrine in the cortical slice tissue (2). At the end of the respective incubation periods the supernatant medium was collected and assayed for renin concentration by a modified radioimmunoassay of angiotensin I (23), and the values were expressed as ng of angiotensin I/mg wet tissue/h. Similarly, tissue used for the determination of cAMP content was collected at the end of all three incubation periods, and the nucleotide was extracted (17) and measured by a modified competitive protein-binding assay (6) and the values expressed as pMmol of cAMP/mg wet tissue. Tissue samples used for the evaluation of intrarenal renin

content changes were collected at the end of the 60-min incubation period only, homogenized in 1 ml of 0.9 % sodium chloride at 4 °C, and centrifuged for 15 min in the cold at 5,000 rpm. The supernatant was then dialyzed to pH 3.3 over 24 h at 3 °C against a disodium EDTA-containing buffer, followed by heating to 32 °C for 60 min to denature endogenous renin substrate and to inactivate angiotensinases, and subsequently dialyzed again to pH 7.5 over 24 h at 3 °C against a sodium phosphate EDTA-containing buffer, as described by SKINNER (26). The purified material was then measured for renin concentration by radioimmunoassay of angiotensin I (23). The results were evaluated for statistical significance by modified paired and unpaired t-test (27).

Results

Addition of 10^{-3} dopamine to the renal cortical tissue preparation resulted in a gradual stimulation of renin release with time in relation to control samples, which was statistically significant after 60 min of incubation (fig. 1). In contrast, the $TcAMP_c$ of dopamine-treated samples was significantly greater than that of controls by 5 min of incubation (fig. 1). Mean tissue renin content levels in samples treated with dopamine were higher by about 80 ng than those seen in control samples but not significantly.

In another study (fig. 2), two doses (10^{-8}M and 10^{-6}M) of the dopaminereceptor blocker pimozide, added separately or together with 10^{-3}M dopamine during a 60-minute incubation period, markedly increased renin release and simultaneously depressed $TcAMP_c$ as compared to the changes previously caused by 10^{-3}M dopamine alone during a corresponding incubation period (fig. 1). Tissue renin content in response to these treatments also appeared depressed but not significantly (fig. 2).

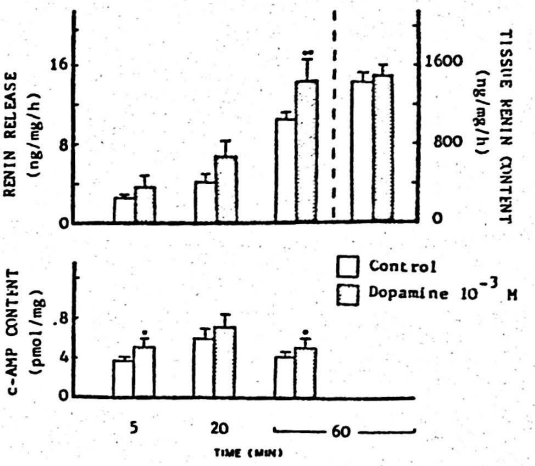

Fig. 1. *Effect of 10^{-3} dopamine on renin release, tissue cyclic AMP content, and tissue renin content in renal cortical slices from sodium deficient rats.*

The data represent the mean ± S.E. of 14-32 observations for renin release in control and dopamine-treated samples, and of 12-14 observations for tissue cyclic AMP content in the same samples, at three incubation periods. Mean tissue renin content values ± S.E. represent 23 observations in control and dopamine-treated samples at 60 min incubation. Mean control renin release at 5, 20, and 60 min was 2.6 ± 0.3, 3.9 ± 1.0, and 10.3 ± 0.9 ng/mg/h, respectively. Corresponding control values for tissue cyclic AMP content were 0.4 ± 0.03, 0.6 ± 0.08, and 0.4 ± 0.05 pMol/mg, respectively. Mean control tissue renin content at 60 min was 1419 ± 103 ng/mg/h. * Significantly different from control ($p < 0.05$). ** Significantly different from control ($p < 0.01$).

To further evaluate this clear dissociation of the renin release and $TcAMP_c$ responses to pimozide administration, the effect of the same two doses of this agent on these two parameters and on tissue renin content was examined in relation to the length of incubation time (fig. 3). The results of this study show that, as opposed to the gradual stimulation of renin release with time seen upon treatment with 10^{-3}M dopamine alone (fig. 1), both 10^{-8}M and 10^{-6}M pimozide doses added alone significantly increased renin

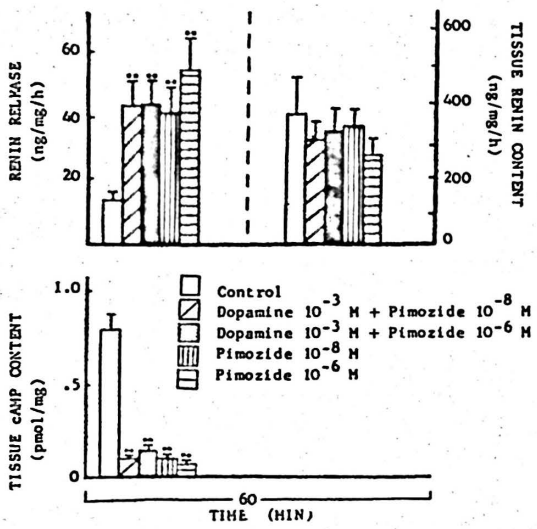

Fig. 2. *Renin release, tissue cyclic AMP content, and tissue renin content responses to 10^{-6}M and 10^{-8}M pimozide added alone or together with 10^{-3}M dopamine to renal cortical slices from sodium deficient rats.*
The data represent the mean value ± S.E. of 11 observations for renin release, 9 observations for tissue cyclic AMP content, and 11 observations for tissue renin content, in response to each treatment during a 60 min incubation period. Mean values were 14.2 ± 3.6 ng/mg/h for renin release, 0.8 ± 0.1 pMol/mg for tissue cyclic AMP content, and 3833 ± 866 ng/mg/h for tissue renin content, respectively, in untreated control samples. * Significantly different from control ($p < 0.05$). ** Significantly different from control ($p < 0.01$).

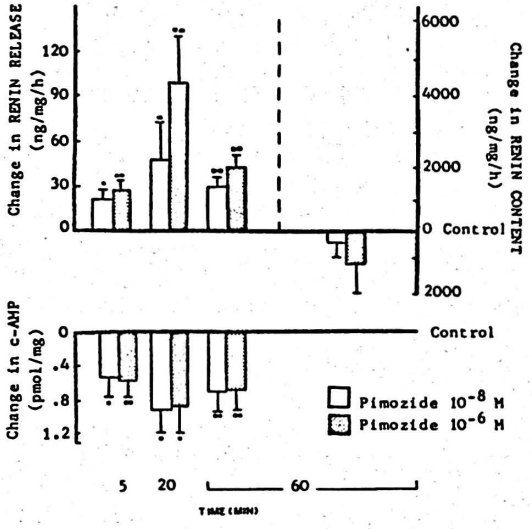

Fig. 3. *Changes in renin release, tissue cyclic AMP content, and tissue renin content, in response to two doses of pimozide added to a renal cortical slice system from sodium deficient rats.*
The data represent the mean change ± S.E. of 11 observations for renin release, 6-9 observations for tissue cyclic AMP content, and 11 observations for tissue renin content, for each treatment used at three incubation periods. Control values at 5, 20, and 60 min were 3.4 ± 0.9, 8.8 ± 1.9 and 14.2 ± 3.6 ng/mg/h for renin release. The corresponding control values for tissue cyclic AMP content were 0.77 ± 0.18, 1.1 ± 0.33 and 0.79 ± 0.19 pMol/mg, respectively. The mean control level for tissue renin content at 60 min was 3833 ± 866 ng/mg/h. * Significantly different from control ($p < 0.05$). ** Significantly different from control ($p < 0.01$).

release over that of controls at each incubation period evaluated. In turn, $TcAMP_c$ of the pimozide-treated samples appeared significantly depressed at each period studied, and tissue renin content levels in response to pimozide administration were also lower by 60 min than those seen in control samples, although not significantly (fig. 3).

Discussion

The results of this study support previous *in vitro* findings suggesting that dopamine can directly stimulate renin release in the rat (9, 16, 17, 22), and that this effect may be mediated via a cAMP-dependent membrane receptor mechanism which is probably β-adrenergic in nature since it can be blocked by propranolol (16, 17). Furthermore, these data also demonstrate that stimulation of renin secretion by dopamine is gradual and may involve an interaction between this amine and a membrane-bound cAMP-generat-

ing receptor complex, perhaps similar to that proposed for dopamine in the frontal cortex of the primate brain (1) and for norepinephrine in rat renal cortical tissue (5, 14-15). Our observation that renin release in dopamine-treated samples was significantly greater than that of controls only after 60 min of incubation, and which was in turn preceded by a significant increase in $TcAMP_c$ at the earliest incubation time examined, is consistent with this view. Alternatively, the report by DeVito *et al.* (4) that resting renin content of rat kidney slices exhibits wide variations between animals as oppossed to renin release which is stable, may partially explain the lack of a significant effect of dopamine on this parameter in this study, since the standard errors were large.

The present data confirm previous reports from our laboratory (16, 17) regarding the marked dissociation in renin release and $TcAMP_c$ caused by various doses of administered pimozide after 60 min of incubation. At shorter incubation periods in this study, two additional doses of this agent produced the same marked changes as those observed at 60 min, suggesting that this effect is pharmacological rather than physiological in nature. The immediate and significant increase in renin release over that of controls caused by both doses of added pimozide is clearly in contrast to the gradual stimulation of renin release with time seen in this study upon dopamine administration, and it probably reflects an acute, generalized alteration in juxtaglomerular cell permeability leading to uncontrolled renin release as well as pharmacological inactivation of cAMP-generating mechanisms, as judged by the significant depression in cAMP content of the pimozidetreated tissue. A generalized, pharmacological alteration of membrane permeability resulting in uncontrolled renin release has been reported to mediate the stimulatory effect of the beta-adrenergic receptor agonist, isoproterenol, on renin secretion (12). Whether or not $TcAMP_c$ was also affected by this agent cannot be determined, since only changes in renin secretion were evaluated in that study.

Utilization of pimozide as a central and peripheral dopamine-receptor blocker in various species (11, 19, 20, 28), has resulted in an enhanced secretion of target tissue hormonal products presumably dew to psysiological blockade of the inhibitory effect of dopamine at those sites. Our results, however, suggest that at doses ranging from 10^{-8}M to 10^{-5}M the effect of pimozide in potentiating *in vitro* renin release in response to dopamine administration, is actually pharmacological in nature since it can be exerted by this agent itself. In view of reports (10) questioning the specificity of pimozide as a dopamine-receptor blocker in the dog, it is clear that further evaluation of the possibility that specific dopamine receptors may participate in the overall direct regulation of renin release in the rat is in order.

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Resumen

Se investigan los efectos cronológicos producidos por la administración de la dopamina y del pimozide, bloqueador de receptores dopaminérgicos, sobre la secreción de la renina (SR), al contenido intrarrenal de la adenosina monofosfática cíclica (CAMC), y al contenido intracelular de la renina (CIR), en secciones de corteza renal obtenidas de ratas deficientes en sodio. La dopamina 10^{-5}M, estimula SR en forma gradual y la diferencia entre las seccio-

nes tratadas y las de control es estadísticamente significativa a los 60 min de incubación. En contraste, el CAMC muestra un aumento significativo desde los 5 min de incubación y el CIR también muestra un aumento a los 60 min, aunque no significativo. La administración de 2 dosis de pimozide (10^{-5}M y 10^{-6}M), sea en forma independiente o en combinación con la dopamina (10^{-5}M), muestra en todos los casos un aumento agudo y significativo en SR y una disminución simultánea, e igualmente significativa, en CAMC desde los 5 min de incubación. El CIR también se muestra disminuido a los 60 min, aunque no significativamente. Los resultados de este estudio indican que, la estimulación de SR producida por la dopamina es un proceso fisiológico gradual y posiblemente mediado por cambios inmediatos en el CAMC, y que el aumento en SR, producido por pimozide no es, a diferencia del causado por la dopamina, el resultado de un proceso fisiológico normal, sino de un efecto agudo farmacológico que inactiva la producción de la AMC y que provoca una secreción incontrolable de la renina, posiblemente debido a alteraciones en permeabilidad de la membrana de las células yuxtaglomerulosas.

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