Evidence That Specific Dopamine-1 Receptor Activation Is Involved in Dopamine-Induced Renin Release

I. Antonipillai, M.I. Broers, and D. Lang

Direct effects of dopamine on renin release were examined using static incubations and perfusions of rat renal cortical slices. Dopamine (10^-5 M) significantly stimulated renin release compared with control. To determine which receptors are involved in dopamine-elicited renin release, studies were performed with specific dopamine-1 and dopamine-2 receptor agonists and antagonists, as well as with α- and β-adrenergic antagonists. Fenoldopam, a dopamine-1 receptor agonist, dose dependently stimulated renin secretion both in static incubations and perfusions; whereas quinpirole (10^-7-10^-5 M), a dopamine-2 receptor agonist, was ineffective. Phentolamine (10^-4 M), an α-adrenergic antagonist, did not alter dopamine- or fenoldopam-induced renin release. Similarly, propranolol, a β-blocker, did not interfere with the renin stimulation of dopamine (10^-5 M) or fenoldopam (10^-4 M) in incubations or perfusion experiments; whereas propranolol significantly blocked isoproterenol action. SCH 23390 (10^-5 M), a specific dopamine-1 antagonist, blocked dopamine- and fenoldopam-induced renin. In contrast, pimozide, a dopamine-2 receptor antagonist, was ineffective. These studies indicate that dopamine is a direct renin secretogogue, and its effects seem to be mediated by specific dopamine-1 receptor activation, as neither α- nor β-adrenergic blockers nor dopamine-2 receptor antagonists altered dopamine actions. The results suggest that dopamine produced locally in the kidney may stimulate renin secretion directly by dopamine-1 receptor activation.

(Hypertension 1989;13:463-468)

In recent years, it has become apparent that dopamine (DA), an endogenous catecholamine, plays an important role in physiological regulation outside the central nervous system. For example, endogenous DA may inhibit aldosterone release and increase renal sodium excretion, and variations in hypophysial portal blood DA levels may influence leutinizing hormone, follicle-stimulating hormone, growth hormone, and thyroid-stimulating hormone secretion and play a key role in pituitary prolactin secretion. Since the kidney can produce DA and dopaminergic endings terminate directly on the kidney juxtaglomerular cells, it has been reported that DA may directly stimulate renin release. However, since DA activates not only two distinct peripheral dopamine-1 (DA1) and dopamine-2 (DA2) receptors, but also α- and β-adrenergic receptors, the mechanisms of DA stimulation of renin release are not clear. When DA was injected into canine renal arteries, there was release of renin by specific dopaminergic receptor activation. Other studies of direct effects of DA on the kidney juxtaglomerular cells were interpreted to indicate that DA stimulates renin via β-adrenergic receptor activation.

The recent availability of selective DA1 and DA2 receptor agonists and antagonists prompted us to use these compounds to study the presence and subtype of DA receptors involved in juxtaglomerular cell renin secretion. Fenoldopam mesylate is a specific DA1 receptor agonist. Compared with DA, this compound is four times more potent as a renal vasodilator in dogs. Furthermore it has minimal α2- but no α1- or β-adrenergic receptor activity. Similarly, quinpirole (LY171555) is reported to be a selective DA2 receptor agonist in peripheral tissues. In the present studies, we examined the direct actions of fenoldopam and quinpirole (DA1 and DA2 receptor agonists) on renin secretion. In addition, we have evaluated the role of α- and
Materials and Methods

Methods

Fenoldopam mesylate was a gift from Smith Kline and French Laboratories (Philadelphia, Pennsylvania); quinpirole hydrochloride (LY171555) was a gift from Eli Lilly and Company (Indianapolis, Indiana), and SCH 23390 maleate was a gift from Schering Corporation (Bloomfield, New Jersey). Phenolamine was obtained from Ciba Pharmaceutical Company (Summit, New Jersey). Dopamine (3-hydroxytyramine hydrochloride), isoproterenol, and dl-propranolol hydrochloride were from Sigma Chemical Company (St. Louis, Missouri), and pimozide (R-6238) was purchased from Janssen Pharmaceutica (Piscataway, New Jersey).

Male Sprague-Dawley rats (150–250 g) were decapitated, and superficial slices from the dorsal and ventral side of the kidney (0.5-mm thick) were used for static incubation and perifusion experiments (Endotronics Acusyst S Perifusion System, Marietta, Ohio), as previously described.22,23 For static incubations, slices (15–30 mg) were washed with Krebs bacterinate with glucose (KRBG) medium that contained 0.01% bovine serum albumin. Slices were preincubated in a metabolic shaker, saturated with 95% O2-5% CO2 at 37° C for 15 minutes and then incubated for five consecutive 15-minute incubation periods. Each slice was incubated for two 15-minute baseline periods, after which various agents were added; the response to an agent was observed for the next three 15-minute periods, thus enabling each slice to serve as its own control. The standard KRBG medium contained (mM): NaCl 120, KCl 4.7, MgSO4 1.2, CaCl2 2.5, KH2PO4 1.2, NaHCO3 26.8, and glucose 10, pH 7.4. For perifusions, slices were placed in culture chambers and perifused with KRBG buffer, as described previously.24 After an initial 60-minute stabilization period, 10-minute fractions were collected. After a 30-minute baseline sampling, the agents were dissolved in 20 ml KRBG buffer and perifused over a 20-minute period. This was followed by a control KRBG buffer for a period of 30 minutes.

In experiments where DA or isoproterenol effects were studied, ascorbic acid (6×10^-4 M) was added to the KRBG medium as an antioxidant.25 Fenoldopam, quinpirole, and SCH 23390 were dissolved in KRBG medium immediately before use. Pimozide was dissolved in a minimum volume of acetone and diluted to the required concentration in ethanol. The final concentration of ethanol in KRBG medium was 0.05%. This concentration of ethanol was also added to control incubations not exposed to test compounds and did not influence renin activity. To investigate the effects of α, β, or DA antagonists on renin release induced by DA or fenoldopam, slices were incubated or perifused with KRBG that contained these antagonists for 15 minutes before administration of the agents. Renin release in the supernatant of the incubations or perifusion medium was determined by radioimmunoassay to measure the generation of angiotensin I by the method of Haber as used by one of us before.22,23

The results are expressed as the mean±SEM percent control renin release. Statistical analysis was performed using the CL INFO System. Analysis of variance with both unpaired t tests and Duncan's multiple range tests was used to assess the significance of renin release, as previously described.23 In the studies of perifusion of kidney slices, the area under the curve was subjected to one-way analysis of variance, followed by Duncan's multiple comparison tests.

Results

Effects of Dopamine on Renin Release

In static incubations, DA at 10^-7 or 10^-6 M increased renin slightly at 30 minutes (Figure 1), but 10^-5 M concentration significantly increased renin release compared with control slices (control 91±6%, DA 143±9%, p<0.001).

Effects of α- and β-Adrenergic Blockade on Dopamine-Induced Renin Release

Since DA, at certain doses, activates both α- and β-adrenergic receptors, we first examined the effects of adrenergic blockade on DA-induced renin release. As shown in Table 1, in static incubations addition of the α-adrenergic blocker phenolamine (10^-4 M) or the β-adrenergic blocker propranolol (2×10^-5 M) did not alter DA (10^-5 M)-induced renin release (DA 159±10%, DA+phenolamine 145±10%, or DA+propranolol 195±13%); whereas propranolol
significantly blocked isoproterenol (a β-adrenergic agonist)-induced renin release, p<0.001 (Table 1).

To fully evaluate the more definite role of DA and its mechanisms, perifusion studies were performed. Renin release in the control slices was relatively stable over the 80-minute time period (Figure 2A). DA (10^{-5} M) increased renin secretion over the control slices. However, propranolol at concentrations as much as 2x10^{-4} M did not significantly affect the DA-stimulated renin secretion (Figure 2A). Whereas, in the same model propranolol (2x10^{-5} M) blocked the isoproterenol (10^{-4} M)-induced renin release (Figure 2B).

### Effects of Specific Dopamine Agonists on Renin Secretion

To determine if the action of DA to increase renin release involves a specific dopaminergic mechanism, the effects of the specific DA_{1} receptor agonist fenoldopam and the DA_{2} agonist quinpirole were studied. In static incubations fenoldopam increased renin in a dose-related manner (fenoldopam, 10^{-6} M 117±10, p<0.02, 10^{-5} M 148±14, p<0.001) and was 10 times more potent than DA in its action (Figure 3). In contrast, quinpirole, a DA_{2} agonist (10^{-7}-10^{-5} M), did not alter basal renin release.

### Effects of α- and β-Adrenergic Blockade on Fenoldopam-Induced Renin

Since DA is capable of activating adrenergic receptors, the possibility that fenoldopam, a DA_{1} receptor agonist, may also have similar actions was investigated. In static incubations, both phentolamine (10^{-4} M) and propranolol (2x10^{-4} M) did not block fenoldopam-induced renin release (Table 2). Similarly, in perifused slices the presence of pro-

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**TABLE 1. Lack of Inhibitory Effect of α- or β-Blocker on Dopamine-Induced Renin Release**

<table>
<thead>
<tr>
<th>Agents or vehicle added</th>
<th>% control renin release at 30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99±5</td>
</tr>
<tr>
<td>DA (10^{-4} M)</td>
<td>159±10</td>
</tr>
<tr>
<td>DA+phentolamine (10^{-4} M)</td>
<td>145±10</td>
</tr>
<tr>
<td>DA+propranolol (2x10^{-5} M)</td>
<td>195±13</td>
</tr>
<tr>
<td>Isoproterenol (10^{-4} M)</td>
<td>145±5</td>
</tr>
<tr>
<td>Isoproterenol+propranolol (2x10^{-5} M)</td>
<td>108±4*</td>
</tr>
</tbody>
</table>

Static incubations were carried out as indicated in Materials and Methods. Values (mean±SEM) represent five to eight experiments in comparison with controls at 30 minutes.

*p<0.001 versus isoproterenol.

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**FIGURE 3. Effects of fenoldopam (a dopamine-1 (DA_{1}) agonist) and quinpirole (a dopamine-2 (DA_{2}) agonist) on renin release by rat renal cortical slices at 30 minutes. Each value represents the mean±SEM of five to six experiments. Fenoldopam 10^{-6} M significantly increased basal renin release. A higher dose (10^{-5} M) further stimulated renin secretion. *p<0.02; **p<0.001.**
TABLE 2. Effects of FenoMopain Alone and With Adrenergk Blocking Agents on Renin Release

<table>
<thead>
<tr>
<th>Agents or vehicle added</th>
<th>% control renin release at 30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90±3</td>
</tr>
<tr>
<td>FenoMopain (10⁻⁵ M)</td>
<td>148±14*</td>
</tr>
<tr>
<td>FenoMopain+phenotolamine (10⁻⁴ M)</td>
<td>124±7</td>
</tr>
<tr>
<td>FenoMopain+propranolol (2×10⁻⁴ M)</td>
<td>161±18</td>
</tr>
</tbody>
</table>

Static incubations were done as described in Materials and Methods. Values (mean±SEM) represent five to seven experiments.

*p<0.001 versus control.

pranolol did not alter renin activity stimulated by fenoMopam (Figure 4). In fact, both in static incubations and perifusion studies with fenoMopam, the renin stimulatory activity of slices was slightly higher in the presence of propranolol, as was seen in studies with DA (Table 1, Figure 2A).

Effects of Specific Dopamine-1 and -2 Receptor Antagonists on Dopamine and FenoMopam

To further characterize the specificity of dopaminergic mechanism, studies were performed with specific DA1 and DA2 receptor antagonists, SCH 23390 and pimozide, respectively.

In static incubations, SCH 23390 significantly blocked both DA and fenoMopam-induced renin secretion (DA 149±9%, DA+SCH 23390 93±5%, p<0.001; fenoMopam 142±9%, fenoMopam+SCH 23390 89±4%, p<0.001) and was specific in its actions as it did not alter isoproterenol-induced renin release (isoproterenol 145±5%, isoproterenol+SCH 23390 165±9%) (Table 3). Pimozide had no effect on either DA or fenoMopam-induced renin secretion (Table 3).

Discussion

The findings of the present study confirm earlier reports that DA is a direct renin secretagogue, although its actions in inducing renin release are seen at relatively high concentrations (10⁻⁵ M, see Figure 1), an observation consistent with the other reports. Peripheral DA receptors have been classified into two subtypes. The DA1 receptor is located on several blood vessels and when activated leads to vasodilation. DA2 receptors are located presynaptically, and stimulation of presynaptic DA2 receptors inhibits the release of norepinephrine from the sympathetic ganglia and nerve terminals. Since juxtaglomerular renin-secreting cells are of vascular origin and free DA is generated in the kidney, we evaluated the direct role of DA1 and DA2 receptors on renin release. FenoMopam, a selective DA1 receptor agonist, mimicked the effects of DA on renin secretion. This phenomenon of increased renin activity with fenoMopam has been noted by others in in vivo studies. In contrast, quinpirole, a selective DA1 receptor agonist, did not alter renin secretion. Since DA also possesses α- and β-adrenergic agonistic properties, we determined if adrenergic activation or a specific dopaminergic mechanism was involved in a DA-induced rise in renin release. The stimulation of renin release by DA or fenoMopam in the present study was not blocked by the α-adrenergic antagonist phenotolamine or the β-adrenergic blocker propranolol. This was seen not only in static incubations, but also in the perifusion system of renal cortical slices, which is considered more physiological. In this system, products from slices and medium are continuously washed out, and the contents of the medium are kept constant. Moreover, the perifusion system is thought to be more sensitive in detecting the effects of agents on renin secretion. With this system, propranolol, at a dose that completely blocked the isoproterenol-induced increases in renin, had no significant effect on DA or fenoMopam-induced renin release. These results suggest that DA stimulates renin release independent of the α- or β-adrenergic system. These findings are in conflict with reports by Henry et al. In their studies, phenotolamine did not alter DA-induced renin release as indicated by us, but con-

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** Lack of effect of propranolol (Prop) on fenoMopam (F)-induced renin release in the perifusion system. Results are mean±SEM of five to seven separate sets of experiments.

TABLE 3. Effects of Dopaminergic Antagonists on Dopamine or FenoMopam-Induced Renin Release

<table>
<thead>
<tr>
<th>Agents or vehicle added</th>
<th>% control renin release at 30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>149±9</td>
</tr>
<tr>
<td>Dopamine+SCH 23390 (10⁻³ M)</td>
<td>93±5*</td>
</tr>
<tr>
<td>Dopamine+pimozide (10⁻³ M)</td>
<td>151±10</td>
</tr>
<tr>
<td>FenoMopam (10⁻⁵ M)</td>
<td>142±9</td>
</tr>
<tr>
<td>FenoMopam+SCH 23390</td>
<td>89±41</td>
</tr>
<tr>
<td>FenoMopam+pimozide</td>
<td>148±11</td>
</tr>
<tr>
<td>Isoproterenol (10⁻⁴ M)</td>
<td>144±6</td>
</tr>
<tr>
<td>Isoproterenol+SCH 23390</td>
<td>165±9</td>
</tr>
</tbody>
</table>

Static incubations were done as described in Materials and Methods. Values (mean±SEM) represent five to eight experiments.

*p<0.001 versus dopamine.

p<0.001 versus fenoMopam.
trary to our results, propranolol at \(2 \times 10^{-4}\) M concentrations significantly blocked DA-secreted renin release. We have no explanation for these results. We did notice, however, that in our system propranolol at \(2 \times 10^{-4}\) M levels showed a significant agonistic effect on renin release (unpublished observations). In addition, in DA- and fenoldopam-treated groups, the presence of propranolol caused a further slight rise in renin. Other in vitro reports have similarly indicated agonistic actions of propranolol on basal renin levels.\(^{25,26}\) It is unclear whether this effect of propranolol is due to a nonspecific action. Propranolol has been reported to have direct actions on cell membranes and can act as a membrane stabilizing agent.\(^{37}\) It is interesting to note, however, that angiotensin II-induced aldosterone inhibition by DA has also been shown not to be mediated by the activity of DA at \(\beta\)-adrenergic receptors.\(^{38}\)

Our data importantly indicate that this stimulatory action of DA on renin is due to selective action of DA\(_1\) receptors and does not involve DA\(_2\) receptors. This is based on the following observations: 1) the DA\(_1\) receptor agonist, fenoldopam, was active in inducing renin release; whereas, the DA\(_2\) receptor agonist quinpirole was inactive; 2) the most specific DA\(_1\) receptor antagonist SCH 23390\(^{39,40}\) completely antagonized the actions of both DA and fenoldopam; 3) the two different DA\(_2\) receptor antagonists, pimozide\(^{36}\) and metoclopramide\(^{16}\) (unpublished observations), did not alter either DA- or fenoldopam-promoted renin release. Other investigators have similarly shown that fenoldopam-induced renin is not altered by metoclopramide.\(^{35}\)

These data clearly show that receptors on renin-secreting cells that respond to DA involve specific DA\(_1\)-type receptors and support the findings made by earlier investigators that identified the presence of DA receptors using dopaminergic antagonists. Imbs et al\(^{13}\) showed in anesthetized dogs that haloperidol, a dopaminergic blocker, but not propranolol, was effective in antagonizing the renin release. Similarly, Mizoguchi et al\(^{12}\) infused DA intrarenally into conscious dogs and showed a significant increase in renin secretion that was not inhibited by propranolol, but was inhibited by two DA antagonists, sulpiride and haloperidol.

The cellular mechanisms by which DA induces its stimulatory effect on renin remain to be established. Dopaminergic fibers end in proximity to the juxtaglomerular apparatus, and DA receptors have been identified in the rat adrenal gland and renal glomeruli.\(^{4,5,46,47}\) Furthermore, low doses of DA have a vasodilatory effect, and this effect is more pronounced in the kidney than in other organs.\(^{14,15}\) The mechanisms by which DA\(_1\) receptors modulate renal hemodynamic changes are not fully understood.\(^{15,39,48}\) The possibility that local alterations in DA production in kidney may be responsible for physiological or pathophysiological modifications of renin-angiotensin II–aldosterone responses awaits future study.

In conclusion, our data demonstrate that DA may play a direct stimulatory role in the control of renin release by kidney juxtaglomerular cells. Although DA has effects at multiple receptor sites (i.e., at \(\alpha\)- and \(\beta\)-adrenergic as well as at DA\(_1\) and DA\(_2\) receptors), the potential renin-stimulating activity of DA appears to be restricted to specific DA\(_1\) receptor activation.

Acknowledgments

We thank Dr. Richard Horton for his advice and criticism on this study and Susan Bitolas for her excellent secretarial assistance.

References

29. Lokhandwala MF, Jandhyala BS: The role of sympathetic nervous system in the vascular actions of dopamine J Pharmacol Exp Ther 1976;210:120–126
36. Caero I, Massingham R, Lefeuvre-Borg F: Peripheral dopa-mine receptor antagonists KEY WORDS • renin • kidney • dopamine • fenoldopam • dopamine-receptor antagonists
Evidence that specific dopamine-1 receptor activation is involved in dopamine-induced renin release.
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Hypertension. 1989;13:463-468
doi: 10.1161/01.HYP.13.5.463

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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