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 secretagogue, 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.

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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). Phentolamine 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-Ringer bicarbonate 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 phentolamine (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+phentolamine 145±10%, or DA+propranolol 195±13%); whereas propranolol...
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 (10⁻⁵ M)</td>
<td>159±10</td>
</tr>
<tr>
<td>DA+phenolamine (10⁻⁴ M)</td>
<td>145±10</td>
</tr>
<tr>
<td>DA+propranolol (2×10⁻⁴ M)</td>
<td>195±13</td>
</tr>
<tr>
<td>Isoproterenol (10⁻⁶ M)</td>
<td>145±5</td>
</tr>
<tr>
<td>Isoproterenol+propranolol (2×10⁻⁴ 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.

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, perfusion 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 2×10⁻⁴ M did not significantly affect the DA-stimulated renin secretion (Figure 2A). Whereas, in the same model propranolol (2×10⁻³ M) blocked the isoproterenol (10⁻⁶ 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 DA₁ receptor agonist fenoldopam and the DA₂ 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 DA₂ 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 DA₁ receptor agonist, may also have similar actions was investigated. In static incubations, both phenolamine (10⁻⁴ M) and propranolol (2×10⁻⁴ M) did not block fenoldopam-induced renin release (Table 2). Similarly, in perfused slices the presence of pro-
TABLE 2. Effects of FenoMopain Alone and With Adrenergic 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>FenoMopam (10⁻⁵ M)</td>
<td>148±14*</td>
</tr>
<tr>
<td>FenoMopam+phenolamine (10⁻⁴ M)</td>
<td>124±7</td>
</tr>
<tr>
<td>FenoMopam+propranolol (2x10⁻⁴ 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.

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. 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 phenolamine or the β-adrenergic blocker propranolol. This was seen not only in static incubations, but also in the perfusion 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 perfusion 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, phenolamine did not alter DA-induced renin release as indicated by us, but con-
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Dopaminergic fibers end in proximity to the juxtaglomerular apparatus, and DA receptors have been identified in the rat adrenal gland and renal glomeruli. Furthermore, low doses of DA have a vasodilatory effect, and this effect is more pronounced in the kidney than in other organs. The mechanisms by which DA receptors modulate renal hemodynamic changes are not fully understood. 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 α- and β-adrenergic as well as at DA1 and DA2 receptors), the potential renin-stimulating activity of DA appears to be restricted to specific DA1 receptor activation.

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References


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