Renin Release by Rat Kidney Slices Incubated in Vitro

Role of Sodium and of α- and β-Adrenergic Receptors, and Effect of Vincristine

Alessandro M. Capponi and Michel B. Vallotton, M.D.

SUMMARY The role of sodium concentration, of α- and β-adrenergic receptors, and of a microtubular inhibitor (vincristine) on renin release was studied in rat kidney slices in vitro. Renin release was an active, linear, and temperature-dependent process. Kidneys from young rats released much more renin than those from adults. Lowering sodium concentration inhibited renin release by one-half, even when osmolality was kept constant. Isoproterenol (10⁻⁴ to 10⁻⁶ M) stimulated renin release significantly in a partially dose-related manner. dl-propranolol inhibited this stimulation. Significant (P < 0.05) inhibition of renin release was induced by l-epinephrine or l-norepinephrine (10⁻⁴ M). In the presence of an α-receptor blocking drug, phenoxybenzamine (10⁻⁴ M), inhibition no longer occurred with epinephrine and stimulation was observed with l-norepinephrine. Vincristine (10⁻⁶ M) did not affect renin release when slices from the kidneys of normal rats or adrenalectomized, sodium-depleted rats were incubated, but significantly inhibited (P < 0.01) release that had been stimulated in vitro by isoproterenol. These results suggest to us that there may be (1) a direct or indirect (mediated through the macula densa) effect of sodium on juxtaglomerular cells, (2) an inhibitory role for α-adrenergic receptors on renin release, in addition to the stimulatory role of β-receptors, (3) possible participation of microtubules in isoproterenol-stimulated renin release, and (4) an alternative mode of secretion of renin under stimulation by adrenalectomy and salt depletion.

INCUBATION of kidney slices in vitro is a practical approach for studying the variables which affect renin secretion. However, even in this simple system, some points remain unsettled. For example, it is not clear whether sodium concentration of the incubation medium plays an important role, or whether changes in renin release observed after modifying sodium concentration actually depend only on changes in osmolality. Furthermore, although studies in vivo generally point to a stimulatory role of β-adrenergic receptors on renin release, there has been some controversy concerning the effect of catecholamines on renin release in vitro. Also, the role, if any, of α-adrenergic receptors still is to be elucidated. Finally, the mechanisms by which renin is released remain unknown. Since secretory granules containing renin have been shown in the epithelial cells of the juxtaglomerular apparatus, it is possible that secretion is mediated, as in other polypeptide-secreting cells, by the microtubular-microfilamentous system.

Our present study was undertaken to clarify the effect of sodium in the incubation medium on renin release by rat kidney slices, as well as the role of α- and β-adrenergic agonists and inhibitors. Furthermore, we tested the hypothesis that renin release is dependent on the microtubular system by studying the effect of a microtubular inhibitor, vincristine, on renin secretion.

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To stimulate the juxtaglomerular apparatus, some rats were adrenalectomized and placed first on a normal diet for 2 days, then fed a sodium-free diet for 3 days and given furosemide (Lasix, Hoechst, Germany), 30 mg/kg of body weight, during the 2nd day of the sodium-free diet. They then were nephrectomized and the kidney slices were processed as described above.

**RENNIN ASSAY**

Samples (50-μl) of the incubation media, taken at different times, were diluted 10-40 times in an ice-cold Krebs-Ringer solution at pH 6.5. Samples of 50 μl of these diluted media then were incubated for 3 hours at 37°C with 50 μl of a rat renin substrate solution prepared according to Skeggs et al.14,17 [pH 6.5, containing ethylenediamine-tetraacetic acid (EDTA)] and 10 μl of anti-angiotensin I antiserum. The angiotensin I thus generated was related directly to the renin content and was measured by the radioimmunoassay technique of Vallotton,18 modified according to Poulsen et al.19 The recovery of angiotensin I added in various amounts to the incubation medium was 90.9 ± 8.1% (SD). Renin release was expressed as nanograms of angiotensin I generated per milligram of fresh kidney tissue.

Statistical significance was evaluated with the Wilcoxon rank test and the unpaired Student's t-test when justified. All results are expressed as mean ± standard deviation.

**RESULTS**

**ASSESSMENT OF THE SYSTEM**

Renin release was active and linear during the 90-minute incubation period. Moreover, it was temperature-dependent. The results of a typical experiment are shown in Figure 1. Lowering the temperature from 37°C to 22°C and 4°C resulted in a decrease in renin release after 90 minutes of incubation, from 115.6 ± 7.2 ng of angiotensin I per mg of fresh tissue to 70.1 ± 14.0 ng/mg and 42.8 ± 3.4 ng/mg, respectively. These temperature-induced changes were reversible (Table 1).

Table 2 shows the effect of lowering the sodium concentration in the incubation medium from 140 mM to 30 mM. The low sodium concentration diminished renin release by about one-half. When constant osmolality was maintained by adding choline chloride, renin release remained significantly lower than in controls. All differences were significant after 45 minutes of incubation. Choline chloride in itself, when added at the same concentration to the medium containing 140 mM sodium, had no effect on renin release.

Table 2: Effect of Temperature on Renin Release by Rat Kidney Slices in Vitro

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>First period</th>
<th>Temperature (°C)</th>
<th>Second period</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>55.6 ± 7.9</td>
<td>37</td>
<td>44.5 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>27.2 ± 3.4</td>
<td>4</td>
<td>21.9 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>28.0 ± 5.9</td>
<td>37</td>
<td>73.7 ± 26.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>37</td>
<td>59.3 ± 12.6</td>
<td>4</td>
<td>31.5 ± 7.3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.

After 45 minutes of incubation (first period), incubation medium was replaced by fresh medium at the temperature indicated for 45 minutes (second period).

P = levels of significance between first and second period (n = 4). NS = not significant.

**EFFECT OF CATECHOLAMINES AND ADRENERGIC BLOCKING DRUGS**

Isoproterenol (10^-4 to 10^-5 M) stimulated renin release by kidney slices. This stimulation was in part concentration-related (Table 3). dl-Propranolol (10^-4 M) incompletely inhibited the effect of isoproterenol (10^-5 M) (Table 4). At higher concentrations, dl-propranolol had a significant agonistic effect. Figure 2 shows the results of experiments with epinephrine (10^-4 M) and /-norepinephrine (10^-4 M). These catecholamines significantly inhibited renin release from kidney slices (P < 0.05).

In the presence of an /-adrenergic blocking agent, phenoxybenzamine (10^-4 M), inhibition no longer occurred.

**TABLE 2 Effect of Osmolality and Sodium Concentration of the Medium on Renin Release in Vitro by Rat Kidney Slices.**

<table>
<thead>
<tr>
<th>Na⁺ (mM)</th>
<th>Osmolality (mOsmol/kg)</th>
<th>Renin release (ng angiotensin I per mg fresh tissue)</th>
<th>After 45 min</th>
<th>After 90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>290</td>
<td>41.0 ± 7.6</td>
<td>64.0 ± 8.9</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>85</td>
<td>23.5 ± 2.9</td>
<td>33.6 ± 6.7</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>30</td>
<td>290†</td>
<td>31.7 ± 4.2</td>
<td>41.8 ± 4.5</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.

* Levels of significance vs. controls (n = 8).
† Osmolality was adjusted with choline chloride.
TABLE 3  Effect of Isoproterenol on Renin Release in Vitro after 90 Minutes of Incubation

<table>
<thead>
<tr>
<th>Isoproterenol concentration (M)</th>
<th>Controls (n = 4)</th>
<th>10^{-4} (n = 8)</th>
<th>10^{-3} (n = 10)</th>
<th>10^{-2} (n = 10)</th>
<th>10^{-1} (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin release (ng per mg fresh tissue)</td>
<td>84.0 ± 6.1</td>
<td>128.0* ± 17.5</td>
<td>153.6* ± 27.6</td>
<td>152.3* ± 29.9</td>
<td>152.4* ± 34.7</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
* P < 0.01 vs. controls.

with epinephrine, and there was a significant (P < 0.05) stimulation of renin release with l-norepinephrine. Phenoxycyanine by itself had no effect.

EFFECT OF ADRENALECTOMY AND VINCristINE ADMINISTRATION

The microtubular inhibitor vincristine (10^{-4} M) had no effect on renin release by control rat kidney slices (Fig. 3). Renin release after 90 minutes of incubation was 82.6 ± 13.4 ng of angiotensin I per mg of tissue in control rats and 212.9 ± 37.7 ng/mg in adrenalectomized, salt-depleted rats (P < 0.001). Despite this marked stimulation, vincristine had no effect on renin release (Fig. 3). On the contrary, vincristine significantly inhibited (P < 0.01) renin release when stimulated by isoproterenol (10^{-4} M) (Fig. 3).

It should be noted that kidneys from young adrenalectomi-zed and salt-depleted rats showed no greater release of renin (269.5 ± 122.9 ng of angiotensin I per mg of tissue) than kidneys from control young rats (356.2 ± 64.3 ng/mg (n = 4).

Discussion

Renin release in vitro has been shown to be an active process. Our results support this finding since (1) the secretion depends upon temperature, as already noted by Hammersen et al., (2) this effect of temperature is reversible, and (3) more important, the system can be stimulated. It is noteworthy, however, that some renin is still released at 4°C. This could be due to the lysis of some cells which release their content into the medium. Although Weinberger and Rosner reported a greater stability of release at 25°C than at 37°C we did not note such difference.

TABLE 4  Effect of Propranolol on Renin Release in Vitro Stimulated by Isoproterenol

<table>
<thead>
<tr>
<th>n</th>
<th>Renin release (ng angiotensin I per mg fresh tissue)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>9</td>
<td>63.6 ± 8.1</td>
</tr>
<tr>
<td>10^{-4} M isoproterenol</td>
<td>5</td>
<td>110.3 ± 11.6</td>
</tr>
<tr>
<td>10^{-3} M isoproterenol</td>
<td>5</td>
<td>77.7 ± 15.5*</td>
</tr>
<tr>
<td>+ 10^{-4} M dl-propranolol</td>
<td>4</td>
<td>102.8 ± 14.0*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
* P < 0.05 vs. controls.

FIGURE 2  Effect of phenoxybenzamine (DIB), l-norepinephrine (NE), and epinephrine (E) on renin release after 90 minutes of incubation (mean ± SD). Asterisk denotes P < 0.05 vs. controls; NS = not significant; n = 6 in each group.

Renal renin content decreases with age in the rat. In accord with this finding, we observed a greater release of renin with slices taken from kidneys of young rats than with slices prepared from adult rats. This high capacity of young rats to release renin could not be further enhanced when they were subjected to adrenalectomy and salt depletion.

The role of sodium concentration in the incubation medium has been the subject of contradictory reports. Michalakis found an inverse relationship between sodium concentration and renin release. Aoi et al. could not demonstrate any effect of changing sodium concentration in the medium when osmolality was maintained constant. In contrast, Hammersen et al. and Oelkers et al. reported a direct dependence of renin release on sodium content, although less marked when osmolality was corrected with choline chloride. In our experiments, diminution of sodium concentration strongly inhibited renin release, even with constant osmolality. Sodium may exert a direct effect on the epithelial cells, thus modifying their secretion. It is also possible that some functional relations are maintained between the macula densa and the epithelial cells within the

FIGURE 3  Effect of vincristine (VCR) on renin release by kidney slices after stimulation in vivo by adrenalectomy (ADRENEX) or in vitro by isoproterenol (mean ± SD). Asterisk denotes P < 0.001 vs. controls; NS = not significant; numbers in parentheses are the numbers of observations.
slices. In this case, sodium could affect renin secretion through the macula densa.

It is generally assumed that renin release can be increased in vivo and in perfusion studies through β-adrenergic stimulation. Although some authors were unable to demonstrate any effect of catecholamines, other reports demonstrated a stimulatory effect of norepinephrine and epinephrine on renin release in vitro. In contrast to these data, our results show a significant inhibition when either epinephrine or norepinephrine is added to the medium. Moreover, the addition of an α-receptor blocking agent, phenoxybenzamine, suppresses this inhibition and leads to a significant stimulation in presence of norepinephrine. These data suggest that, in vitro, the α-adrenergic effect of catecholamines is predominant and inhibits renin release as long as it is not blocked. In the presence of phenoxybenzamine, the β-adrenergic effect can manifest its stimulatory action. Further support of this assumption is given by the fact that isoproterenol, an essentially β-stimulating drug, increases renin release in a partially dose-related manner, and that dl-propranolol, a β-receptor blocking agent, abolishes this stimulation. The inhibitory action of α-adrenergic receptors was described in the perfused rat kidney by Vandongen and Peart and offers an explanation for the potentiating effect of phenoxybenzamine reported in vitro by Nolly et al. Weinberger et al. proposed that the potentiating effect of α-receptor blockers is due to the uptake blockade induced by the α-receptor antagonists. Yet this explanation does not account for the fact that agents with α-adrenergic action inhibit renin release. As already pointed out by Aoi et al., we did not find a dose-response relationship with catecholamines. Degradation of these substances might have occurred in the incubation medium, thus modifying their concentration at the receptor sites. The discrepancy between our results and some previous ones most likely result from differences in techniques: in the bioassay for renin, incomplete removal of the catecholamines from the medium will give increase of blood pressure which can be misinterpreted as increased release of renin.

The results obtained with vincristine are difficult to interpret. Although vincristine inhibition of isoproterenol-stimulated renin release may indicate the involvement of microtubules in the process of renin secretion, the failure of vincristine to inhibit renin release stimulated by adrenoreceptors and salt depletion seems to contradict this hypothesis. However, the present experiments cannot exclude the possibility that isoproterenol and adrenoreceptors stimulate the juxtaglomerular cells at different levels, for example, by releasing renin not associated with storage granules. Indeed, adrenoreceptors have been shown to maintain a sustained level of renin release despite a depletion of juxtaglomerular cells in secretory granules.

In conclusion, this work has shown an important role for the sodium concentration in the incubation medium. Moreover, the data point to an inhibitory effect of α-adrenergic receptors on renin release. Finally, the experiments with vincristine suggest alternative means for the secretion of renin, one of these possibly involving the microtubules.

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References


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