Effect of Theophylline and Adrenergic Blocking Drugs on the Renin Response to Norepinephrine In Vitro

By Hector L. Nolly, Ian A. Reid, and William F. Ganong

ABSTRACT

The effects of norepinephrine, theophylline, and adrenergic blocking drugs on renin release from rat kidney slices were studied in vitro. 3-Norepinephrine increased renin release into the incubation medium; this increase was accompanied by an increase in the renin content of the slices. Statistically significant increases in renin release were produced by $10^{-7}$ M and $2 \times 10^{-8}$ M 3-norepinephrine. d-Norepinephrine in the same doses was ineffective. Theophylline ($10^{-5}$ M) had no effect by itself, but it potentiated the effect of 3-norepinephrine on renin release. The response to 3-norepinephrine was markedly suppressed by 3-propranolol ($10^{-8}$ M) but not by d-propranolol ($10^{-8}$ M). The a-receptor blocking agents phentolamine ($10^{-4}$ M) and phenoxybenzamine ($10^{-4}$ M) increased rather than decreased the effect of 3-norepinephrine. These results are consistent with a direct intrarenal effect of norepinephrine on renin release; this effect appears to be mediated by a $\beta$-adrenergic mechanism.

KEY WORDS

alpha receptors  
phentolamine  
renin release in vitro  
beta receptors  
propranolol  
phenoxybenzamine  
rat kidney slices

Substantial evidence now indicates that the sympathetic nervous system plays an important role in the regulation of renin secretion via both the renal sympathetic nerves and circulating catecholamines (1-5). There is also reason to believe that sympathetic stimuli increase renin secretion through a $\beta$-adrenergic mechanism, although some studies have also suggested the involvement of $\alpha$ receptors (6-8). In view of the closeness of adrenergic nerve endings to the juxtaglomerular cells (9, 10), a direct effect of catecholamines on renin secretion seems likely; Johnson et al. (11) have recently presented evidence that both renal nerve stimulation and norepinephrine increase renin secretion by a direct action on the juxtaglomerular cells. On the basis of these observations, it has been suggested that the effect of catecholamines on renin secretion is mediated by $\beta$ receptors on the juxtaglomerular cells (12-14). However, for several reasons it has been difficult to test this hypothesis in the intact animal. For example, administration of catecholamines frequently produces systemic effects which are thought to influence renin secretion by indirect actions.

To avoid such actions, we studied the effect of norepinephrine and adrenergic blocking drugs on the release of renin from rat kidney slices. Since the effects of $\beta$-adrenergic stimulation are mediated via the activation of adenyl cyclase and the formation of cyclic adenosine monophosphate (cyclic AMP), the effect of the phosphodiesterase inhibitor theophylline on the renin response to norepinephrine was also determined.

Methods

INCUBATION OF SLICES

Male and female Wistar rats (200-250 g) maintained on a regular diet were killed by a blow on the head, and both kidneys were rapidly removed. Slices of renal cortex approximately 0.3 mm thick were cut with a razor blade and washed with a modified Krebs-Ringer's solution. The slices were paired so that both control and experimental incubation vessels contained approximately 100 mg of tissue from the same kidney. The incubation technique was a modification of a method described previously (15). The slices were incubated in 2.5 ml of the Krebs-Ringer's solution for 60 minutes in a shaking incubator at 37°C in an atmosphere saturated with 95% O$_2$-5% CO$_2$. In the oxygen consumption and the initial 3-norepinephrine experiments, no preincubation was carried out. In all other experiments, the slices were preincubated for 15 minutes, and then the incubation medium was discarded. The millimolar composition of the modified Krebs-Ringer's solution was: NaCl 140, KCl 4, MgSO$_4$ 0.6, NaH$_2$PO$_4$ 1.2, CaCl$_2$ 2.5, dextran 0.1, and glucose 8. The pH of the solution was 7.45, and the
Osmolarity was 350 mosmoles/liter. In several control experiments, oxygen consumption during the 60-minute incubation period or for longer periods was measured using a Warburg apparatus.

Kidney slices were incubated in the presence of \(l\)-norepinephrine (\(l\)-arterenol bitartrate, Sigma) in concentrations ranging from \(7.5 \times 10^{-7} \text{M}\) to \(2 \times 10^{-5} \text{M}\). \(d\)-Norepinephrine in concentrations of \(10^{-8}\text{M}\) and \(2 \times 10^{-6}\text{M}\) was also tested. The effect of \(l\)-norepinephrine (\(10^{-4}\text{M}\)) on renin release was tested in the presence of \(d\)- and \(l\)-propranolol (\(10^{-4}\text{M}\)) (Ayerst), phentolamine (\(10^{-4}\text{M}\)) (Ciba), phenoxybenzamine (\(10^{-4}\text{M}\)) (Smith, Kline and French), and theophylline (\(10^{-4}\text{M}\)) (Searle). The effects of these doses of blocking drugs and theophylline without the addition of norepinephrine were also studied. All blocking drugs and theophylline were added in the preincubation period as well as at the start of the 60-minute incubation period. To determine whether the increase in osmolarity produced by the addition of the drugs affected renin release, choline chloride was added to the incubation medium of control slices in amounts that duplicated the largest increase in molar concentration produced by the addition of \(l\)-norepinephrine alone or together with propranolol, phenoxybenzamine, or phentolamine.

**RENIN ASSAY**

At the end of each experiment, a small volume of the incubation medium or of a saline extract of homogenized renal tissue was acidified to \(pH 2.7-2.9\) by the addition of 25% phosphoric acid. The solution was then incubated for 30 minutes at 37°C, adjusted to \(pH 5.5\) by the addition of 5n NaOH, and centrifuged at 32,000 g for 20 minutes. Renin concentration was measured by a modification of the method of Reid et al. (16). The incubation mixture consisted of 0.02 ml of the sample, 0.93 ml of nephrectomized dog plasma, and 0.05 ml of a solution containing the following inhibitors of converting enzyme and angiotensinas: disodium ethylenediaminetetraacetic acid \(15 \text{mM}\), dimercaprol \(5 \text{mm}\), and \(8\)-hydroxyquinoline \(3.5 \text{mm}\). The mixture was incubated at \(pH 5.5\) for 2 hours at 37°C; the angiotensin I thus generated was measured by radioimmunoassay (17). Recovery of angiotensin I was 85-90%. Renin concentration was expressed as ng angiotensin I/mg kidney tissue hour\(^{-1}\) under these conditions. The rate of renin release was expressed as the amount of renin released into the medium/mg fresh tissue hour\(^{-1}\). All results are expressed as means ±SE.

Statistical significance was evaluated using the paired \(t\)-test.

**Results**

**RENIN RELEASE AND OXYGEN CONSUMPTION**

Renin release and oxygen consumption were linear during the 60-minute incubation period, indicating that the tissue was viable during this period. The results of a typical experiment are shown in Figure 1. In this group of seven kidneys in which renin was released at a rate of \(6.28 \pm 0.48\) ng angiotensin I/mg kidney tissue hour\(^{-1}\), oxygen consumption was \(0.98 \pm 0.16\) μliters O\(_2\)/mg fresh tissue hour\(^{-1}\).

Raising the osmolarity by adding choline chloride in amounts that duplicated the increase in molar concentration produced by drugs did not alter renin release.

**EFFECT OF NOREPINEPHRINE**

Addition of \(l\)-norepinephrine to the incubation medium produced a dose-related increase in the rate of renin release; the increases produced by norepinephrine concentrations of \(10^{-7}\text{M}\) and \(2 \times 10^{-5}\text{M}\) were statistically significant (Figs. 2 and 3).

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 calorie restriction and dietary fiber are protective factors for reducing the risk of cardiovascular disease (21). Circulating levels of angiotensin II, as well as sympathetic nerve activity, are increased in calorie-restricted subjects (19, 23). An increase in angiotensin II plasma levels can be a consequence of increased sympathetic nervous system activity that increases heart rate and renal vascular resistance (21). These findings support the hypothesis that sympathetic nervous system activity is a major determinant of angiotensin II production.

The results of the present study suggest that dietary fiber may be beneficial for reducing the risk of cardiovascular disease. In animal models, high dietary fiber intake reduces blood pressure and can prevent atherosclerosis (6, 28). Dietary fiber may have beneficial effects on other cardiovascular risk factors such as hyperlipidemia and insulin resistance (28). Further research is needed to determine the mechanisms by which dietary fiber reduces cardiovascular risk.

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### References


TABLE 1

Effect of l-Norepinephrine on Kidney Renin Concentration In Vitro

<table>
<thead>
<tr>
<th>Concentration of l-norepinephrine (M)</th>
<th>Kidney renin concentration (ng angiotensin I/mg kidney tissue hour⁻¹)</th>
<th>Control</th>
<th>Norepinephrine</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 10⁻⁴</td>
<td>71.50 ± 12</td>
<td>92.40 ± 15</td>
<td>10</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>1 x 10⁻⁵</td>
<td>65.50 ± 9</td>
<td>85.00 ± 12</td>
<td>8</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>2 x 10⁻⁵</td>
<td>74.25 ± 7</td>
<td>94.62 ± 8</td>
<td>8</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Kidney slices were incubated for 60 minutes. Values are means ± SE. N = number of kidneys tested.

Norepinephrine also increased the concentration of renin in the incubated kidney tissue, but the magnitude of the increase was not related to the dose of norepinephrine (Table 1). Renin release was unaffected by d-norepinephrine in concentrations of 10⁻⁵M and 2 x 10⁻⁶M.

EFFECT OF THEOPHYLLINE

Addition of theophylline to the incubation medium had no effect on renin release, but it did potentiate the renin response to l-norepinephrine at several different concentrations of l-norepinephrine (Figs. 3 and 4).

EFFECT OF ADRENERGIC BLOCKING DRUGS

The effects of d- and l-propranolol on the renin response to l-norepinephrine are shown in Figure 5. The increase in renin release produced by l-norepinephrine was unaffected by d-propranolol but was markedly suppressed by l-propranolol. In these concentrations, neither d- nor l-propranolol alone had any significant effect on renin release. Phentolamine (10⁻⁴M) by itself had no effect on renin release (Fig. 6) but produced significant potentiation of the response to l-norepinephrine. Similar results were obtained when the concentration of phentolamine was increased to 10⁻³M. Phenoxybenzamine also had no effect by itself (Fig. 7) but produced significant potentiation of the renin response to l-norepinephrine.

Discussion

The present study was designed to investigate the effects of adrenergic agonists and antagonists on the release of renin from kidney slices in vitro. In such a preparation, the variables of blood pressure and urine formation are eliminated, and, although
Effect of norepinephrine (NE) on renin secretion in vivo (21). The α-receptor blocking agent a-propranolol, which has all of the properties of d-propranolol, was ineffective. Further evidence that norepinephrine stimulates renin release via a β-adrenergic mechanism and cyclic AMP was provided by the experiments with theophylline. This phosphodiesterase inhibitor by itself had no effect on renin release in vitro, but it potentiated the effect of norepinephrine at all dose levels tested. Theophylline also appears to potentiate the effect of endogenous catecholamines on renin secretion in vivo (21). The lack of response to theophylline in vitro is probably explained by the lack of stimulating amounts of catecholamines in the incubation medium.

The α-receptor blocking agents not only failed to block but actually potentiated the renin response to l-norepinephrine in vitro. This effect is consistent with a previous finding (12) that phenoxybenzamine potentiates the renin response to hypoglycemia in vivo. Winer and his associates (7) have reported that α-receptor blocking agents block the renin response to catecholamines, but this finding has not been confirmed. One possible explanation for the potentiating effect of α-receptor blocking agents in vitro is that they prevent the interaction of norepinephrine with α receptors in the tissue slices and, therefore, leave more norepinephrine available to interact with β receptors. Addition of phenoxybenzamine or phentolamine without norepinephrine did not significantly affect renin release, presumably because the amounts of endogenous catecholamines in the incubation medium were insufficient to produce stimulation.

Acknowledgment

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References

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