Isoprenaline-induced secretion of active and inactive renin in anaesthetized rabbits and by kidney cortex slices

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(Received 4 November 1980/6 April 1981: accepted 26 May 1981)

Summary

1. The effect of the $\beta$-adrenoceptor agonist isoprenaline on the secretion of active and inactive renin was investigated in two preparations.

2. In ten urethane-anaesthetized rabbits isoprenaline, given as a renal artery infusion, had relatively minor effects on renal sodium excretion (increased) and systemic arterial blood pressure (decreased). Urine volume, potassium excretion, creatinine clearance and serum electrolytes were all unchanged. Plasma active and inactive renin both increased immediately and returned to basal values after ceasing the isoprenaline infusion.

3. No significant changes in either plasma renin activity or renal function were observed in a group of ten control animals.

4. The magnitude of the isoprenaline-induced changes in plasma active renin was similar to that in a previous study of frusemide diuresis, but the time course was quite different. Inactive renin disappeared from plasma during frusemide diuresis.

5. Renin release by rabbit kidney cortex slices was also studied. Isoprenaline, added to the incubation medium, caused a dose-related increase in active renin secretion, but inactive renin release remained unchanged. This is in marked contrast to a previous study where reducing $[\text{Na}^+]$ increased active renin and inhibited inactive renin output.

6. These data support our previous suggestion that activation of inactive renin is regulated by a sodium-sensitive intrarenal mechanism.

Key words: active renin, inactive renin, isoprenaline, kidney slices.

Introduction

The $\beta$-adrenoceptor agonist isoprenaline stimulates the release of `renin' by the kidney when given as an intrarenal artery infusion to intact animals [1, 2] or isolated perfused kidney preparations [3, 4]. A component of the renin released may result from intrarenal vasodilatation. However, isoprenaline also stimulates renin secretion by kidney cortex slices [5] and isolated kidney cortex cells [6]. This supports the hypothesis that a $\beta$-adrenoceptor located on the juxtaglomerular cells is involved in renin release.

It is now known that multiple forms exist of both plasma and kidney renin [7]. Acidification of plasma samples from several species results in an increase in renin activity. This, it is thought, represents activation of an inactive form of renin. We have shown in previous studies, using the rabbit as an experimental animal, that the relative amounts of active and inactive renin secreted in response to a given physiological stimulus depends on both the nature and duration of the stimulus. Frusemide diuresis [8] and the phase of dietary sodium depletion when the animals are in negative sodium balance [9] are both associated with raised plasma active renin and the disappearance of inactive renin. A similar response was found when the sodium concentration was reduced in buffers bathing kidney...
cortex slices [10]. In contrast, other experimental manipulations such as haemorrhage [11] and urethane anaesthesia [9] result in parallel increases in both forms of renin. These stimuli might be expected to promote renin secretion via a combination of the intrarenal baroreceptor and renal nerve (β-adrenergic) mechanisms. All these studies taken together led us to propose tentatively that activation of inactive renin, which we consider to be primarily an intrarenal event, is regulated by some form of sodium-sensitive mechanism. However, other experimental conditions must be studied to confirm or refute this hypothesis.

We have therefore studied the effect of isoprenaline on the release of active and inactive renin. Two different preparations were used. Close renal artery infusion of the drug was studied in anaesthetized rabbits. To complement these studies, and to help evaluate the contribution of intrarenal vasodilatation, we have also investigated the response of rabbit kidney cortex slices to isoprenaline. A preliminary report of these studies has been published as an abstract [12].

**Methods**

**Experiments in vivo**

Male New Zealand White rabbits (3.0–4.5 kg body weight) were anaesthetized with intraperitoneal urethane (1-25 g/kg). A tracheostomy was performed and a carotid artery cannulated to allow continuous monitoring of blood pressure and collection of blood samples.

In ten rabbits a radio-opaque cannula was inserted via a femoral artery and positioned by X-ray fluoroscopy so that the tip was located in the dorsal aorta just above the two renal arteries. This cannula was subsequently used to infuse isoprenaline into the animals. A further group of ten rabbits was used as a control group and did not receive isoprenaline.

Each animal received a continuous infusion (60 ml/h) of sodium chloride solution (150 mmol/l: saline) containing creatinine (100 mg/l) as a clearance marker. This was given via a jugular vein cannula. A priming dose of 50 ml of the infusate was given on completion of surgical preparation. Urine was collected with a balloon catheter (Warne Surgical Products, Andover, U.K.; size 10FG) passed into the bladder.

Seven successive 30 min clearance periods were monitored, commencing 30 min after the priming dose of infusate. Whenever urine flow rate exceeded 30 ml in any 30 min period the additional volume was replaced intravenously with saline. Body fluid depletion was therefore avoided. A blood sample (3 ml) was collected at the mid-point of each clearance period. The following measurements were made in each of the seven clearance periods: urine volume, urinary sodium and potassium excretion rates, creatinine clearance and serum sodium and potassium. Electrolytes were estimated with an integrating flame photometer (EEL) and creatinine samples were processed on an autoanalyser (Technicon). Plasma samples were retained for renin assay as described below.

In the group of animals receiving isoprenaline this was given as a continuous infusion for 45 min, starting on completion of the first 30 min clearance period. A dose of 0.5 mg of isoprenaline min⁻¹ kg⁻¹ was used.

**Experiments in vitro**

Animals were killed by neck fracture or with a lethal dose of pentobarbitone sodium. Both kidneys were excised, placed on ice and the poles and medulla removed. Eleven transverse slices of renal cortex were hand-cut from each kidney with a razor blade. Slices were approximately 200 μm thick and weighed between 50 and 100 mg. After accurate weighing, and retention of one slice as a non-incubated control, each slice was incubated in a stoppered flask with 5 ml of oxygenated (O₂ + CO₂ 95:5, v/v) Krebs-Ringer bicarbonate buffer (pH 7.4) containing glucose (10 mmol/l). Flasks were incubated for 90 min at 37°C and the medium was gassed every 10 min during this time. After incubation active and inactive renins were assayed in the slice supernatant as described below. The non-incubated control slice was immediately frozen at −20°C and subsequently ground in a Potter homogenizer in 2 ml of the buffer. The renins extracted into the buffer were estimated.

**Estimation of active and inactive renin**

Plasma samples and slice supernatants were divided into two portions and subjected to one of the following dialysis protocols. One group of samples was dialysed to pH 2-8 against glycine/HCl buffer (170 mmol/l) for 24 h and then redialysed for a further 24 h to pH 7-5 against a phosphate buffer (175 mmol/l). Acidification is considered to activate inactive renin and so subsequent renin assay yielded a value for active and inactive renin. The remaining group of samples was dialysed for 48 h against the pH 7-5 buffer and later analysis provided a value for active renin. Inactive renin was therefore deter-
mimed as the difference between these two sets of measurements.

Active renin was estimated by radioimmuno-assay of generated angiotensin I [13] after incubation of dialysed samples with excess sheep renin substrate [14]. Details of the evaluation of assay procedures described for active and inactive renin have been published previously [8].

Results for renin estimations on plasma samples are expressed as renin activity per millilitre of plasma (pmol of ANG I h⁻¹ ml⁻¹). Data for renin measurements for experiments in vitro are expressed as renin activity released per milligram of tissue wet weight (pmol of ANG I h⁻¹ mg⁻¹ of tissue).

Statistical analysis of results

The experimental design for the experiments in vivo permits two comparisons to be made for evaluation of results. Unpaired statistical tests were used to compare isoprenaline-infused animals with the control group. For correlations between different clearance periods in each animal paired tests were used. As isoprenaline infusion only commenced after the first clearance period, the initial period provided an additional set of controls.

For renin estimations, non-parametric statistical tests were used: Wilcoxon's signed-rank test for paired data and the Mann–Whitney U-statistic for unpaired analysis. Paired tests were used for comparisons between acidified and non-acidified samples. Paired and unpaired Student's t-tests were used for the analysis of results for the percentage increase in renin activity after acidification and for all data concerned with aspects of renal function and arterial blood pressure.

Renin measurements for the experiments in vitro were analysed by Wilcoxon's test and linear regression analysis was used to relate renin released to isoprenaline concentration in the buffer.

Results

Isoprenaline infusion in anaesthetized rabbits

Infusion of isoprenaline failed to produce any significant changes in urine volume, potassium excretion or creatinine clearance (Table 1). Sodium excretion did not alter significantly. However, it did appear that there was a modest increase in excretion rate when the isoprenaline-infused animals were compared with the control group. Sodium excretion attained peak values in clearance period 6, some 1 ½ h after the end of the isoprenaline infusion. Serum [Na⁺] did not change significantly from the initial 157 ± 2 mmol/l in the group which received isoprenaline. Similarly, there were no changes in serum [K⁺] from initial values of 3-0 ± 0-2 mmol/l (control) and 2-8 ± 0-1 mmol/l (isoprenaline group).

Isoprenaline was given by close renal artery infusion to minimize changes in systemic arterial blood pressure. Fig. 1 shows that there was a fall in pressure during the infusion, but this was limited to a mean of only 5 mmHg.

Results for active and inactive plasma renin are shown in Table 1. Infusion of isoprenaline led to an increase in plasma active renin from 20.2 ± 3-8 to 47.7 ± 8-9 pmol of ANG I h⁻¹ ml⁻¹ (P < 0-05 for both paired and unpaired comparison). At the end of the infusion, plasma active renin fell progressively towards baseline levels over a period of approximately 1 ½ h. Inactive plasma renin followed a similar pattern to active renin. It increased significantly (paired comparison) by 74% during the isoprenaline infusion and subsequently returned to control levels. Calculation of the percentage increase in renin activity after acidification shows that this remained between 10-5 and 16-2% throughout the experiment (Table 1). The corresponding range for the control group was 8-4–14-8%.

Therefore isoprenaline infusion caused an immediate increase in both active and inactive renin in plasma. Both forms returned to basal values relatively quickly after the infusion ceased and there was no evidence of a difference in time course for changes in the two forms of renin.

The isoprenaline-induced changes in sodium excretion rate and arterial blood pressure were relatively small, especially compared with experimental manipulations such as haemorrhage [11] or frusemide diuresis [8]. It was nevertheless important to obtain other evidence to confirm that the changes in renin secretion were specifically mediated by a β-adrenoceptor mechanism. This was provided by the data in the next section, which reports the effects of isoprenaline on the secretion of renin by kidney cortex slices.

Isoprenaline in vitro: kidney cortex slices

Isoprenaline was added to the buffers bathing kidney cortex slices in concentrations up to 40 µg/ml (112 mmol/l). Results for measurements of the renin released into the slice supernatant during the 90 min incubation period are shown in Table 2. At the highest dose of isoprenaline used, active renin release from the slices was 122% above the control value. There was a linear
Isoprenaline (0-5 μg min⁻¹ kg⁻¹) was given for 45 min starting after clearance period 1. Total renin (active and inactive) was measured after dialysis to pH 2.8 and back to pH 7.5 to activate inactive renin. Samples for active renin estimation were dialysed at pH 7.5. Results are means ± SEM. Significance of difference: *P < 0.05 for paired analysis (Wilcoxon's signed-rank test); †P < 0.05 for unpaired analysis (Mann-Whitney U-statistic). For data other than renin estimations no significant changes occurred. Student's paired and unpaired t-tests were used for these results.

<table>
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<tr>
<th>Clearance period</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>Time (min)</td>
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<td>90</td>
<td>120</td>
<td>150</td>
<td>180</td>
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<td>Isoprenaline infusion...</td>
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<tr>
<td>Active renin (pmol of ANG I h⁻¹ ml⁻¹)</td>
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<tr>
<td>Control</td>
<td>21.3 ± 2.5</td>
<td>20.5 ± 3.0</td>
<td>20.1 ± 3.4</td>
<td>22.9 ± 3.9</td>
<td>26.3 ± 5.8</td>
<td>25.7 ± 5.3</td>
<td>22.5 ± 4.4</td>
</tr>
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<td>Isoprenaline</td>
<td>20.2 ± 3.8</td>
<td>37.2 ± 8.4†</td>
<td>47.7 ± 8.9†</td>
<td>31.2 ± 6.3</td>
<td>25.2 ± 4.6</td>
<td>22.2 ± 3.9</td>
<td>18.5 ± 3.3</td>
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<td>Inactive renin (pmol of ANG I h⁻¹ ml⁻¹)</td>
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<tr>
<td>Control</td>
<td>2.9 ± 0.5</td>
<td>2.6 ± 0.9</td>
<td>2.6 ± 1.35</td>
<td>2.5 ± 0.7</td>
<td>3.7 ± 1.6</td>
<td>4.4 ± 1.9</td>
<td>2.25 ± 0.6</td>
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<td>Isoprenaline</td>
<td>3.1 ± 0.6</td>
<td>5.3 ± 1.1*</td>
<td>5.4 ± 1.1*</td>
<td>4.3 ± 1.2</td>
<td>4.1 ± 0.8</td>
<td>2.8 ± 0.6</td>
<td>3.1 ± 0.8</td>
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<tr>
<td>Increase in renin activity after acidification (%)</td>
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<tr>
<td>Control</td>
<td>11.7 ± 1.9</td>
<td>10.7 ± 2.5</td>
<td>10.9 ± 4.1</td>
<td>9.2 ± 2.7</td>
<td>12.2 ± 2.9</td>
<td>14.8 ± 2.5</td>
<td>8.4 ± 2.7</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>14.9 ± 3.7</td>
<td>12.7 ± 1.6</td>
<td>10.5 ± 1.9</td>
<td>12.8 ± 1.4</td>
<td>15.5 ± 2.0</td>
<td>11.3 ± 2.2</td>
<td>16.2 ± 4.5</td>
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<td>Urine volume (ml/30 min)</td>
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<tr>
<td>Control</td>
<td>2.9 ± 0.4</td>
<td>2.4 ± 0.4</td>
<td>2.2 ± 0.3</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>2.1 ± 0.4</td>
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<tr>
<td>Isoprenaline</td>
<td>1.8 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>1.9 ± 0.4</td>
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<td>Sodium excretion (mmol/30 min)</td>
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<tr>
<td>Control</td>
<td>0.96 ± 0.16</td>
<td>0.94 ± 0.22</td>
<td>0.78 ± 0.24</td>
<td>0.82 ± 0.24</td>
<td>0.82 ± 0.24</td>
<td>0.66 ± 0.14</td>
<td>0.68 ± 0.10</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>1.24 ± 0.14</td>
<td>1.11 ± 0.16</td>
<td>1.17 ± 0.13</td>
<td>1.22 ± 0.23</td>
<td>1.34 ± 0.41</td>
<td>1.68 ± 0.48</td>
<td>1.19 ± 0.29</td>
</tr>
<tr>
<td>Potassium excretion (mmol/30 min)</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
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<tr>
<td>Creatinine clearance (ml/min)</td>
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</tr>
<tr>
<td>Control</td>
<td>5.6 ± 1.4</td>
<td>5.3 ± 1.2</td>
<td>5.6 ± 1.4</td>
<td>5.4 ± 1.2</td>
<td>5.7 ± 1.5</td>
<td>6.5 ± 1.2</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>5.0 ± 0.7</td>
<td>4.9 ± 0.8</td>
<td>5.7 ± 0.9</td>
<td>4.1 ± 0.6</td>
<td>5.2 ± 0.7</td>
<td>5.7 ± 0.8</td>
<td>6.3 ± 1.0</td>
</tr>
</tbody>
</table>

**Fig. 1.** Carotid artery blood pressure in ten rabbits receiving isoprenaline by close renal artery infusion (a) and ten control rabbits (b). Results are means ± SEM. *P < 0.05 compared with initial control period (Student's paired t-tests).

A relationship ($r^2 = 0.93$; slope = +0.23) between active renin released and isoprenaline. The amount of inactive renin released into the slice incubation media remained constant. There was, therefore, a fall in the proportion of total renin released that was inactive. This is shown by the inverse linear relationship ($r^2 = 0.67$; slope = −0.19) between the percentage increase in renin activity after acidification and isoprenaline (Table 2).
Active renin extracted from the non-incubated slices was 268.5 ± 58.2 pmol of ANG I h⁻¹ mg⁻¹ of tissue. The maximum isoprenaline-induced release of active renin therefore represented 4.5% of the total extractable from the slices. Acidification of the extract resulted in a 35.4 ± 1.6% increase in renin activity.

Discussion

On the basis of previous studies using the rabbit as an experimental animal we have proposed that the relative amounts of active and inactive renin in plasma are controlled by a sodium-sensitive intrarenal mechanism. Some of our earlier studies utilized similar experimental protocols to the two series of investigations of isoprenaline-induced renin secretion contained in the present paper. Direct comparison of the results is therefore facilitated.

Furosemide diuresis in intact animals was associated with a maximum increase in active renin of 160% [8]. The magnitude of the peak isoprenaline-induced changes was similar, a 136% increase, but the time course was quite different. For furosemide the maximum was reached in clearance period 5, some 1½–2 h after the single injection of furosemide and 1–1½ h after the greatest alterations in urine flow and other aspects of renal function. Isoprenaline generated an immediate increase in plasma active renin. The highest plasma renin activity measured was during the isoprenaline infusion and it declined quickly on completion of the infusion (Table 1). Clearly isoprenaline and furosemide are stimulating renin secretion through quite different pathways.

The two experimental manipulations discussed produced opposing changes in plasma inactive renin. Furosemide diuresis was accompanied by disappearance of inactive renin, whereas isoprenaline produced parallel increases in both forms of renin. The latter situation was also found to occur in the response of rabbit plasma renins to two intensities of haemorrhage [11]. In this case the changes were slower in onset than for isoprenaline.

There are some difficulties in interpreting data from whole-animal experiments in relation to the classically described renin secretion-control receptor mechanisms; the macula densa, the intrarenal baroreceptor and β-adrenoceptor systems associated with the renal nerves [15, 16]. Although the isoprenaline-induced alterations in sodium excretion rate and systemic arterial blood pressure were quantitatively small, they nevertheless could contribute to the renin secretion responses observed. Similarly, haemorrhage, a stimulus originally considered to affect primarily the intrarenal baroreceptor, is now known to have a major component of its effect via the renal nerves and elevated catecholamine secretion [17, 18]. Isolated preparations provide a way of minimizing, if not entirely circumventing, these problems.

Reducing the sodium concentration of media bathing rabbit kidney cortex slices caused an increase in the release of active renin and suppression of inactive renin release [10]. Isoprenaline, however, in the same preparation, was accompanied by elevated secretion of active renin, but no change in inactive renin release. Again, the magnitude of the changes induced by these two experimental manipulations were similar.

The studies reported here have shown that isoprenaline in the rabbit increases active renin release with either no change (kidney cortex slices) or increased secretion (close renal artery infusion) of inactive renin. A similar pattern of response has been reported for studies of isoprenaline administered to human subjects [19]. In addition, the β-adrenoceptor antagonist propranolol suppresses active renin, but has no effect.

<table>
<thead>
<tr>
<th>Conc. of isoprenaline (µg/ml)…</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active renin (pmol of ANG I h⁻¹ mg⁻¹ of tissue)†</td>
<td>7.6 ± 1.1</td>
<td>9.2 ± 1.5</td>
<td>11.6 ± 1.6*</td>
<td>14.3 ± 2.7*</td>
<td>16.9 ± 3.5*</td>
</tr>
<tr>
<td>Inactive renin (pmol of ANG I h⁻¹ mg⁻¹ of tissue)</td>
<td>1.4 ± 0.3</td>
<td>1.45 ± 0.35</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Increase in renin activity after acidification (%)‡</td>
<td>17.2 ± 2.1</td>
<td>15.0 ± 1.7</td>
<td>12.6 ± 1.3</td>
<td>8.3 ± 1.4</td>
<td>9.5 ± 1.9</td>
</tr>
</tbody>
</table>

† Active renin: correlation coefficient (r²) vs isoprenaline = 0.93; slope = +0.23.
‡ Increase in renin activity (%) after acidification: correlation coefficient (r²) vs isoprenaline = 0.67; slope = —0.19.
on inactive renin [19–23]. In a study of active and inactive renin release by the pig kidney Bailie et al. [24] found that isoprenaline resulted in parallel increases in both active and inactive renin and that propranolol suppressed both forms equally. The reasons for this apparent anomaly may rest with the preparation used. Studies with the isolated perfused rat kidney, while demonstrating that isoprenaline increases the release of active renin, failed to provide evidence of the existence of an inactive form of renin at all in this species [25].

In conclusion, β-adrenoceptor stimulation with isoprenaline, either given as a close renal artery infusion to intact rabbits or added to the medium bathing kidney cortex slices, caused an increase in active renin secretion. Inactive renin release was either increased or unchanged. This is similar to the changes we have reported previously for the changes we have reported previously for the sodium concentration bathing kidney cortex slices in vivo. The present study provides further evidence that secretion control mechanisms for active and inactive renin can respond independently to differing physiological stimuli.

References


