The influence of sodium intake on the pressor response to angiotensin II in the unanaesthetized rat

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Summary
1. Dose–response curves for the pressor activity of angiotensin II have been determined in unanaesthetized rats receiving diets containing 2.5% (w/w) or 0.007% (w/w) sodium; the different diets were administered in various sequences.

2. In comparison with those from rats receiving a low sodium diet, the dose–response curves were displaced to the left on the high sodium diet, indicating a greater response to angiotensin, and this displacement persisted for a period of approximately 7 days after the diet was changed from high to low sodium. The dose–response curve subsequently shifted to the right when the low sodium diet was maintained for longer.

3. There was a negative correlation between the slope of the dose–response curve and the basal blood pressure in all groups; the correlation was significant in three out of the five different treatment groups.

4. Basal blood pressures were significantly raised in rats on the high sodium diet for 7 days.

5. A number of possible mechanisms have been considered to explain both the parallel shift of the dose–response curve and alteration in its slope. It is concluded that the observed findings are compatible with an action of sodium-loading on the sensitivity of the smooth muscle cell to angiotensin, on the resting of the renin–angiotensin system, on the rate of inactivation of angiotensin and on a change in initial length of the muscle fibre.

Key words: angiotensin II, blood pressure, dietary sodium, hypertension.

Introduction
It has been recognized for some years that the pressor activity of angiotensin II is dependent on the sodium status of the individual (Ames, Borkowski, Sicinski & Laragh, 1965; Bianchi, Brown, Lever, Robertson & Roth, 1968). This becomes of particular importance in clinical situations where renin secretion is inappropriate to the prevailing sodium status, as in chronic renal failure (Brown, Fraser, Lever & Robertson, 1971; Ledingham, 1971; Dathan, Johnson & Goodwin, 1973; Davies, Beevers, Briggs, Medina, Robertson, Schalekamp, Brown, Lever, Morton & Tree, 1973). This dependence of the pressor effect of angiotensin II on sodium status has been demonstrated by a shift in the dose–response curve to the right in sodium-deprived dogs (Bianchi et al., 1968).

The present study was undertaken to investigate this phenomenon in both the sodium-depleted and sodium-loaded, conscious rat and to determine the factors responsible.

Methods

Animals
Female albino Wistar rats from an inbred strain were randomly selected in a weight range 200–250 g, at which time they were approaching the horizontal portion of their growth curve. This was of importance in ensuring that the cannulae used for blood pressure measurement and angiotensin infusion continued to function throughout the course of the experiment.
Cannulae and measuring techniques

Angiotensin II was injected into a polyethylene and silicone elastic (Silastic: Dow Corning) right atrial cannula implanted via the right external jugular vein and exteriorized behind the right ear. This cannula was adapted from one first designed by Weeks & Davis (1964). Mean arterial pressure was measured with a polyethylene cannula implanted in the abdominal aorta distal to the renal arteries (Browning, Ledingham & Pelling, 1970). The aortic cannula was connected to a Statham P23G transducer with an output to both a direct-reading scale and a recorder.

Animal conditions for experiment

Angiotensin injections and blood pressure measurements were carried out in the unanaesthetized state. The rats were trained to remain at rest in a Perspex restraining cage for periods of approximately 1 h. Environmental temperature in the restraining cage was maintained between 30 and 34°C, within the thermoneutral range for the rat. Angiotensin injections and blood pressure monitoring were commenced when the rats had attained a resting state, usually after about 10–15 min in the restraining cage.

Operative techniques

Cannulae were implanted at least 7 days before the start of the experiment. A period of approximately 3 days was allowed between the implantation of the atrial cannula and the aortic cannula. Operations were carried out under light ether anaesthesia and antibiotic was applied topically after operation. The cannulae were subsequently filled with heparin (1000 units/ml) and plugged with a short length of stainless steel.

Diet

The standard laboratory diet contained 0·5% (w/w) sodium. The low sodium diet (0·007%, w/w, sodium) was modified from one devised by Cuthbertson (1957) and fulfilled all the dietary requirements with the exception of sodium. The high sodium diet was made by adding sodium to the low sodium diet to a concentration of 2·5% (w/w) sodium.

The low sodium diet, supplemented with lesser amounts of sodium, had previously been shown to produce the same growth rate of weanling rats as standard laboratory rat cake.

A subsequent study to monitor the sodium intake of the rats on each of the experimental diets showed that the rats on the diet containing 2·5% (w/w) sodium ingested 148·0 mmol of sodium day⁻¹ kg⁻¹. Those on a low sodium diet ingested 0·34 mmol of sodium day⁻¹ kg⁻¹. The rats in all groups consumed approximately 20 g of diet in 24 h.

Experimental procedure (Table 1)

Before implantation of cannulae, rats were maintained on the standard laboratory diet and afterwards were placed on the high or low sodium diets and given free access to distilled water. After 6 days on the diet, a dose–response curve for angiotensin II was constructed. This was repeated on day 7. The observations on days 6 and 7 were referred to as H, high sodium, and L, low sodium. Thereafter, the rats were changed to the opposite diet. Dose–response experiments were carried out on days 13 and 14 [groups HL(7), LH(7) respectively]. A proportion of the group on low sodium was maintained on this diet for a further week and dose–response experiments were carried out on days 20 and 21 [group HL(14)]. The fewer number of animals in the later phases of the procedure was partly due to cannula failure.

Mean blood pressure was continuously monitored for a 5 min period after the rats had attained the resting state and the basal blood pressure was derived from the mean of five observations at 1 min intervals.

Dose–response experiments

Preliminary experiments in which angiotensin infusions were given continuously led to rather poor dose–response curves and a liability to tachyphylaxis at the high doses. It was therefore decided to administer single bolus injections at each dose. Asp¹,Val⁵-angiotensin amide (Hypertensin, Ciba) was made up in sodium chloride solution (150 mmol/l; saline) to a concentration of 5000 ng/ml and was frozen in aliquots. Just before each experiment it was diluted to a concentration of 400 ng/ml and injected into the right atrial cannula in 4, 8, 12, 16 ng doses from a micrometer syringe. The order in which the doses were given was in accordance with one of two 4 x 4 Latin squares:
Table 1. Experimental protocol

Group H standard diet: high sodium diet for 6 and 7 days. Group L standard diet: low sodium diet for 6 and 7 days. Group HL(7) high sodium diet for 7 days: low sodium diet for 6 and 7 days. Group LH(7) low sodium diet for 7 days: high sodium diet for 6 and 7 days. Group HL(14) high sodium diet for 7 days: low sodium diet for 13 and 14 days.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Standard laboratory rat cake</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>High sodium</td>
</tr>
<tr>
<td>6</td>
<td>Dose-response</td>
</tr>
<tr>
<td>7</td>
<td>Dose-response {Group H}</td>
</tr>
<tr>
<td></td>
<td>Changed to low sodium</td>
</tr>
<tr>
<td>13</td>
<td>Dose-response</td>
</tr>
<tr>
<td>14</td>
<td>Dose-response {Group HL(7)}</td>
</tr>
<tr>
<td></td>
<td>Continued on low sodium</td>
</tr>
<tr>
<td>20</td>
<td>Dose-response</td>
</tr>
<tr>
<td>21</td>
<td>Dose-response {Group HL(14)}</td>
</tr>
</tbody>
</table>

Doses were given at 3 min intervals, since this was the maximum time for recovery after a 16 ng dose of angiotensin. The total volume of the sixteen injections was 0.4 ml. Blood pressure change was estimated as the percentage increase in pressure from the pre-injection level. A mean value for the percentage blood pressure change was calculated for each dose, the final value thus being the mean of the four values. The dose–response curve was constructed from the mean of the two curves determined on consecutive days (e.g. days 6 and 7). The mean percentage blood pressure increases at each dose on two consecutive days were correlated with the dose of angiotensin. The resultant regression line was considered to be the dose–response curve. All individual animal dose–response curves had correlation coefficients \(r\) \(\geq 0.9\).

Statistical treatment

Differences between means of basal blood pressures were assessed by Student's t-test. Possible differences between position and slopes of dose–response curves were evaluated by using a comparison of regression line technique (Davies & Goldsmith, 1972).

Results

Effects of dietary sodium intake on basal (initial, resting) blood pressure (Table 2)

The basal mean arterial blood pressure of group H was significantly greater than that of group L \(P < 0.001\) and that of group HL(7) \(P < 0.005\).

The basal pressure of group LH(7) was significantly higher than that of group L \(P < 0.005\) and group HL(7) \(P < 0.005\). The basal blood pressure of group HL(14) was not significantly different from that of any other group.

Angiotensin dose–response

Satisfactory linear dose–response curves over the
Table 2. Basal blood pressures
Pressure values are shown ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean arterial pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>18</td>
<td>121.8±2.2</td>
</tr>
<tr>
<td>L</td>
<td>17</td>
<td>107.6±2.7</td>
</tr>
<tr>
<td>HL(7)</td>
<td>12</td>
<td>107.9±3.7</td>
</tr>
<tr>
<td>LH(7)</td>
<td>5</td>
<td>123.7±5.3</td>
</tr>
<tr>
<td>HL(14)</td>
<td>4</td>
<td>111.9±6.5</td>
</tr>
</tbody>
</table>

Selected dose range were obtained in all groups. The percentage increases in blood pressure in response to the four doses of angiotensin in each group are shown in Table 3, and the significance of differences in slope and position between regression lines in the various groups in Table 4.

The mean dose–response curves for groups H and L are shown in Fig. 1, from which it will be seen that the two curves lie parallel ($P = 0.78$) and that the curve for HL(14) is also parallel ($P = 0.51$ and 0.80) but shifted further to the right. There is a significant difference in position between groups H and L ($P = 0.00013$) and a difference of borderline significance ($P = 0.058$) between groups L and HL(14).

Group HL(7) did not give the diminished responses which would be expected from rats on a low sodium intake but gave responses which were significantly greater than those of group L and of group HL(14) (Fig. 2). This group, HL(7), which had for the preceding 7 days been on a high sodium intake, continued to give the enhanced response characteristic of sodium-loaded animals. In comparison with group H, the slope of the curve was steeper ($P = 0.015$) and the mean values were significantly different ($P = 0.00065$).

The response in group LH(7) lay intermediate between that of group H and L (Fig. 3). The slope of

Table 3. Percentage increases in mean arterial blood pressure at each dose of angiotensin
Results are shown ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>4 ng</th>
<th>8 ng</th>
<th>12 ng</th>
<th>16 ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>18</td>
<td>18.9±0.8</td>
<td>29.0±1.1</td>
<td>34.4±2.3</td>
<td>37.6±1.5</td>
</tr>
<tr>
<td>L</td>
<td>17</td>
<td>14.3±1.2</td>
<td>23.4±2.1</td>
<td>29.7±2.5</td>
<td>33.3±2.3</td>
</tr>
<tr>
<td>HL(7)</td>
<td>12</td>
<td>21.4±1.8</td>
<td>29.1±2.4</td>
<td>39.6±2.7</td>
<td>47.2±3.0</td>
</tr>
<tr>
<td>LH(7)</td>
<td>5</td>
<td>15.5±3.4</td>
<td>25.8±2.7</td>
<td>34.1±2.3</td>
<td>38.5±1.6</td>
</tr>
<tr>
<td>HL(14)</td>
<td>4</td>
<td>10.4±1.6</td>
<td>17.7±1.3</td>
<td>25.0±2.1</td>
<td>31.5±2.8</td>
</tr>
</tbody>
</table>

Fig. 1. Mean dose–response curves for groups H (○), L (●) and HL(14) (△). MAP = mean arterial pressure.

Fig. 2. Mean dose–response curves for groups H (○), L (●) and HL(7) (△). MAP = mean arterial pressure.
the line appeared greater, but statistically this was only of borderline significance [$P = 0.052$, H versus LH(7)]. The mean values were not significantly different.

Analysis of the slopes of the individual curves in the separate groups revealed a negative correlation between the slope and the basal blood pressure. This correlation for group L is shown in Fig. 4 ($r = -0.56, n = 17, P < 0.01$): corresponding values in the other groups were: H, $r = -0.47, n = 18, P < 0.05$; HL(7), $r = -0.65, n = 12, P < 0.05$; LH(7), $r = 0.67, n = 5, P < 0.01$; HL(14), $r = -0.43, n = 4, P < 0.3$.

**Discussion**

The results show a significantly increased pressor response to angiotensin in rats on a high compared with a low sodium intake. This confirms previous work in rats (Reid & Laragh, 1965; Samwer, Schreiber, Molzahn & Oelkers, 1974; Thurston & Laragh, 1975) and dogs (Bianchi et al., 1968). The effect is seen within 6 days of changing from a normal to a high or low sodium diet. When a change is made from a high to a low sodium diet, the response remains characteristic of the high sodium diet for 7 days and then falls to that characteristic of the low sodium diet after a further 7 days. When a change is made from a low to a high sodium diet, the response changes towards that characteristic of the high sodium within 7 days. The change in the dose-response curve appeared to be mainly attributable to a parallel shift in the position of the curve rather than to a change in slope, although one group showed a slope greater than the others [group HL(7)]. An approximately parallel shift of this kind has been observed by others studying the effect of sodium-loading on the pressor response to angiotensin (Bianchi et al., 1968) and also on the vasoconstrictor response after direct injection of angiotensin into the femoral artery (Strewler, Hinricks, Guiod & Hollenberg, 1972). Such a shift implies that the high relative to the low sodium diet either brought about an
increase in sensitivity to angiotensin or reduced the rate of destruction of angiotensin. The observation that arterial strips, derived from the aortae of rabbits, were significantly more sensitive to angiotensin when the rabbits had previously been maintained on a high than on a low sodium diet is strong evidence in support of some local change induced in the state of the vessel wall, making the muscle cells more sensitive to angiotensin (Strewler et al., 1972). The second possibility, that of an alteration in the destruction rate of angiotensin, is being explored and preliminary results suggest that angiotensin is being inactivated at a greater rate in animals having a low sodium intake. This finding is in accord with the observation that the kidney from sodium-depleted rats inactivates angiotensin at an enhanced rate (Leary & Ledingham, 1970) and that angiotensinase activity changes in man in states of hypertension with oedema (Hickler, Lanter & Thorn, 1963).

The dose–response curve for group HL(7) did not shift to the right as in group L, but became steeper than group H. This could be due to a delay in the fall in sensitivity to the contractile stimulus; data from the literature are not available to confirm this possibility. Another possibility is that the inactivation rate of injected angiotensin in group HL(7) is closer to that in group H than in group L. This possibility is being investigated.

In all groups there was a negative correlation between slope and basal blood pressure (Fig. 4). The observation can most readily be explained if it is postulated that the minimal length of the muscle fibre which can be induced by maximal stimulation with angiotensin is unaffected by alterations in the basal length of the fibre. In other words, at higher basal pressures (resistance), the muscle fibre is already partly constricted and the proportional shortening at maximal stimulation will be less.

On the assumption that a higher basal blood pressure is reflected in a higher resistance, it would be expected that the slope of the curve on the high sodium diet would be less steep than that on the low sodium diet. This was not found to be the case. One explanation of this could be that the basal (endogenous) concentration of angiotensin in the group on the low sodium diet is higher and this should have the effect of lessening the slope of the curve (Deheneffe & Bernard, 1974). These two effects clearly act in opposite directions and it may be fortuitous that they cancel out, leaving the slopes the same for the two diets.

A significantly steeper slope was observed in group HL(7) than in group H. This could be accounted for by the difference in basal blood pressure in the two groups.

It is evident that the interpretation of the dose–response curve for the pressor effect of angiotensin is most complex in view of the many variables involved. We consider that the most important variables are likely to be the action of the sodium ion on smooth muscle sensitivity to angiotensin, the initial activity of the renin–angiotensin system and the rate of inactivation of angiotensin in different states of sodium-loading. However, alteration in the geometry of the vessels at different initial blood pressures, perhaps determined by autoregulation, could modify the extent of contraction of the smooth muscle cell and so affect the dose–response curve.

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References


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