Sodium restriction and inhibition of the renin-angiotensin system in renovascular hypertension in the rat

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Summary

1. In the early phase of hypertension produced by renal artery constriction with the opposite kidney intact, infusion of the angiotensin antagonist Sar¹-Ala⁸-angiotensin II or bilateral nephrectomy lowered blood pressure. However, the extent of the fall was variable and some animals remained hypertensive after each procedure.

2. To assess whether sodium retention was the additional factor which maintained blood pressure when the renin-angiotensin system was suppressed, rats were maintained on a low-salt diet before and during the development of hypertension. The blood pressure-lowering effect of bilateral nephrectomy or antagonist infusion was not enhanced.

3. Infusion of antagonist or converting-enzyme inhibitor 6 h after bilateral nephrectomy had only a minor blood pressure-lowering action, indicating that, at this late stage after nephrectomy, the renin-angiotensin system makes only a very small contribution to blood pressure maintenance.

Key words: angiotensin II, bilateral nephrectomy, blood pressure, hypertension, sodium retention, vascular renin activity.

Introduction

In the first few weeks of hypertension produced in the rat by unilateral renal artery constriction, plasma renin and angiotensin II levels are frequently but by no means invariably elevated when the opposite kidney is left intact (Goldblatt two-kidney hypertension: Miksche, Miksche & Gross, 1970; MacDonald, Boyd & Peart, 1975). Infusion of the angiotensin II antagonist 1-sarcosine, 8-alanine-angiotensin II lowers the blood pressure but not always to normal pressures (MacDonald et al., 1975; Brunner, Kirshman, Sealey & Laragh, 1971). It has been postulated that mechanisms other than the renin-angiotensin system are involved (MacDonald et al., 1975). It has also been suggested that sodium retention is responsible for maintaining blood pressure in the presence of inhibition of the renin-angiotensin system (Leenen & de Jong, 1975). The present experiments were designed to assess the role of sodium retention by blocking the renin-angiotensin system in salt-depleted rats with Goldblatt two-kidney hypertension. Two methods of inhibition were used: antagonist infusion and bilateral nephrectomy. In addition we have combined the two methods by infusing antagonist into bilaterally nephrectomized animals to determine how far the renin-angiotensin system plays a role in blood pressure maintenance 6 h after bilateral nephrectomy, in view of evidence that persistent vascular renin activity plays a role in blood pressure control for at least 3 h after circulating renin has fallen to a low value (Swales, Tange & Thurston, 1975).

Methods

Operative procedures

Under ether anaesthesia, a silver clip (internal diameter 0.2 mm) was applied to the left renal
artery of female white Wistar rats weighing 150–250 g. Blood pressure was measured twice weekly by an indirect photoelectric method (Swales & Tange, 1970). Rats which had moderate or severe hypertension of less than 2 weeks duration and which were within 4 weeks of renal artery constriction were selected on the basis of a reading of 150 mmHg or more. For the acute studies, a carotid and jugular vessel were cannulated under sodium pentobarbitone anaesthesia (5 mg/100 g body weight). In some animals (groups 3 and 4), bilateral nephrectomy was first carried out through bilateral loin incisions. Access to water or food was not allowed after nephrectomy. Carotid arterial blood pressure was monitored by a Statham transducer and recorded by a Grass Polygraph recorder. Blood pressure was recorded for 5–10 min and, when stable, 10 pmol of Sar'-Ala8-angiotensin I1 dissolved in 0.01 ml of glucose solution (5 g/100 ml) was infused each minute by constant-infusion pump intravenously until a stable pressure had been obtained and maintained for at least 5 min. So long as the cannulae were first filled with the antagonist solution, the blood pressure response was complete within 3–4 min and blood pressure was therefore monitored for 9–15 min. At this point the blood pressure response to an intravenous injection of 50 pmol of Val5-angiotensin II was recorded.

Experimental groups

Group 1. (Normal diet, antagonist infusion: n = 8.) Animals were allowed free access to water and laboratory chow containing 0.166 mmol of sodium/g and 0.22 mmol of potassium/g until cannulation.

Group 2. (Low-salt diet, antagonist infusion: n = 8.) Animals were allowed free access to deionized water and a low-salt diet containing 0.004 mmol of sodium/g and 0.21 mmol of potassium/g for 3 days before renal artery constriction, and then throughout the post-operative period until cannulation.

Group 3. (Normal diet, bilateral nephrectomy: n = 9.) Animals were treated as in group 1 except that bilateral nephrectomy was performed 6 h before cannulation and Sar'-Ala8-angiotensin II was not infused in this group. However, 250 nmol of the nonapeptide converting-enzyme inhibitor SQ20881 was administered as a bolus dose and the blood pressure monitored for 20 min.

Group 4. (Low-salt diet, bilateral nephrectomy: n = 8.) Rats were treated as in group 2 except that bilateral nephrectomy was performed 6 h before cannulation. Antagonist infusion was then carried out.

Statistical significance was evaluated by Student's unpaired t-test. Results are expressed as mean values ± SD unless otherwise stated.

Results

During antagonist infusion at a rate of 10 pmol/min, the response to 50 pmol of angiotensin II ranged from 0 to 3 mmHg in all experiments. Pilot studies in nephrectomized animals given Sar'-Ala8-angiotensin II at a rate of 1 pmol/min yielded pressor responses of about 10 mmHg to the same dose of angiotensin II during antagonist infusion.

Group 1. Antagonist infusion produced a substantial fall (47.5 ± 31 mmHg) in mean blood pressure (Table 1). Individual response varied widely; two rats showing a fall of 2 and 7 mmHg.
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TABLE 1. Body weights of rats at renal artery clipping and at cannulation, and mean arterial pressures at cannulation and after administration of angiotensin antagonist and converting-enzyme inhibitor

Mean results ± SEM are shown. Nephrectomy in groups 3 and 4 was performed 6 h before cannulation.

<table>
<thead>
<tr>
<th>Group no. and treatment</th>
<th>Body wt. (g)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>1. Normal diet: antagonist</td>
<td>166.4 ± 5.9</td>
<td>178.6 ± 5.0</td>
</tr>
<tr>
<td>2. Low-salt diet: antagonist</td>
<td>175.5 ± 8.4</td>
<td>176.8 ± 7.7</td>
</tr>
<tr>
<td>3. Normal diet; nephrectomy</td>
<td>185.1 ± 10.1</td>
<td>199.1 ± 10.9</td>
</tr>
<tr>
<td>4. Low-salt diet; nephrectomy</td>
<td>207.4 ± 6.7</td>
<td>191.6 ± 8.0</td>
</tr>
</tbody>
</table>

* Sar¹-Ala⁵-Angiotensin II was administered to groups 1, 2 and 4, converting-enzyme inhibitor to group 3.

mmHg remained hypertensive, i.e. showed a mean arterial pressure of >120 mmHg (Table 1), whereas others showed a fall in blood pressure of 58 and 67 mmHg which reduced the final value to the low normal range (Fig. 1).

Group 2. Antagonist infusion produced a smaller fall in blood pressure (37.5 ± 31.0 mmHg). The differences in response between groups 1 and 2 was not significant (P > 0.1). Blood pressure falls were again variable and ranged from 6 to 53 mmHg, but initial and final blood pressures were closely similar in groups 1 and 2 (P > 0.1).

Groups 3 and 4. The blood pressure after nephrectomy was not significantly different in animals maintained on normal or low-salt diet (Table 1), and again some rats remained markedly hypertensive.

Inspection of data on individual rats showed the same range of normal and elevated blood pressures whether the renin–angiotensin system was blocked by bilateral nephrectomy or by antagonist infusion; previous salt depletion had no additional effect upon blood pressure (Fig. 1). There were no significant differences between mean blood pressures of any of the four groups whether rats had been treated by antagonist infusion or nephrectomy (P > 0.1).

The administration of converting-enzyme inhibitor or Sar¹-Ala⁵-angiotensin II produced only a very small blood pressure fall in groups 3 and 4 respectively. The fall in blood pressure of group 3 (6.3 ± 8.1 mmHg) just failed to reach statistical significance (0.05 < P < 0.1), whereas the fall in blood pressure of group 4 (6.9 ± 5.4 mmHg) is just significant (P < 0.05), although five rats in group 3 and four rats in group 4 still had blood pressures above 140 mmHg after inhibitor or antagonist infusion.

Discussion

We have previously shown that in rats, a low-salt diet produces a sustained sodium deficit, equal to approximately 10% of the rat’s exchangeable sodium (Thurston & Swales, 1976). In the present experiments, a diet containing rather less sodium was employed, so that it is reasonable to assume that a similar or slightly greater degree of sodium depletion was obtained. The small fall in weight shown by the animals on low-salt diet compared with the rise shown by rats on normal diet would support such a view, although interpretation of these values is complicated by increase in weight associated with growth. The present results indicate that pretreatment of animals with this diet does not reduce the final blood pressure achieved when the renin–angiotensin system is blocked pharmacologically or by nephrectomy. After both procedures, a wide range of blood pressures was observed with some rats remaining markedly hypertensive whether sodium-depleted or not. The heterogeneity of response to angiotensin II antagonist has previously been noted (MacDonald et al., 1975). It seems probable therefore that one or more additional mechanisms are important in maintaining blood pressure in Goldblatt two-kidney hypertension in the rat. The efficacy of the antagonist declines with increasing duration of hypertension (Thurston & Swales, 1974), suggesting that vascular changes induced by hypertension (Folkow, Hallback, Lundgren, Sivertsson & Weiss, 1973) may play a role. However, in the present experiments and those of MacDonald et al. (1975) antagonist was frequently ineffective when such vascular changes have probably not had time to develop.

It is theoretically possible for sodium reten-
tion to act as an additional factor in short-term Goldblatt two-kidney hypertension; this has been postulated in Goldblatt one-kidney hypertension (Gavras, Brunner, Vaughan & Laragh, 1973). Tight clipping of the renal artery is associated with a reduction in urea clearance and a positive post-operative sodium balance (Leenen & de Jong, 1975), and two groups have shown that the sodium retention so produced is still present during the development of hypertension (Leenen & de Jong, 1975; Möhring, Möhring, Naumann, Philippi, Homsy, Orth, Dauda, Kazda & Gross, 1975). In another strain of rat with a rather longer latent phase before the onset of hypertension, we have found no evidence of such sodium retention; as blood pressure rises, hypertension is associated with a negative sodium balance (Swales, Thurston, Queiroz & Medina, 1972). Other groups have been unable to detect elevation of exchangeable sodium when hypertension is established (Tobian, Coffee & McCrea, 1969). Gradual constriction of the renal artery of conscious sheep (Blair-West, Coghlan, Denton, Orchard, Scoggins & Wright, 1968) and of the dog (Bianchi, Baldoli, Lucca & Barbin, 1972) likewise produced hypertension without sodium retention. It has been argued that fluid and sodium shifts from the intracellular space would produce extracellular fluid expansion in the absence of a positive sodium balance (Möhring et al., 1975) and thereby help to sustain hypertension. The present experiments throw no light upon this hypothesis. Such sodium shifts, however, would require to be very great for expansion of the extracellular fluid space to be sustained in spite of a negative sodium balance and changes in diffusibility of extracellular fluid markers have to be excluded as an alternative explanation (Swales, 1975). The present work, taken in conjunction with evidence from other species and the resistance of this model to either dialytic (Swales & Tange, 1971) or dietetic (Thurston & Swales, 1976) sodium depletion, indicates that in this strain of rat at least sodium retention is not a causal factor. Where it occurs after renal artery constriction, it may be incidental to the reduction in renal blood flow (Selkurt, 1951) and not causally related to the elevation of blood pressure. In another strain of rat, on the other hand, hypertension was prevented by dietetic salt depletion (Miksche et al., 1970), suggesting that the role of sodium may be strain-specific and indicating perhaps an analogy with sodium-dependent and sodium-resistant forms of genetic hypertension (Dahl, Heine & Tassinari, 1962). Water (rather than sodium) retention might also be responsible for the maintenance of blood pressure. Our results do not exclude such a possibility, although previously published data suggest increased water loss in this model rather than the reverse (e.g. Swales et al., 1972; Möhring et al., 1975).

Sar-Ala*-angiotensin II has agonist as well as antagonist properties (Pals, Masucci, Denning, Sipos & Fessler, 1971). Such activity, however, cannot explain the failure of the antagonist to reduce the blood pressure of all animals to normal, since bilateral nephrectomy was equally ineffective in this respect. Further converting-enzyme inhibitor, which of course has no agonist action, was also ineffective.

The present experiments suggest that blood pressure in Goldblatt two-kidney hypertension in the rat is maintained partially by factors other than the renin–angiotensin system and sodium retention. Such factors appear to vary in importance in individual animals.

Acknowledgment

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References


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