GENERATION OF ANGiotENSIN II AT PERIPHERAL VASCULAR LEVEL: STUDIES USING ANGiotENSIN II ANTISERA

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SUMMARY

1. When the renin–angiotensin system of rats had been suppressed by a high salt diet or by bilateral nephrectomy, large doses of angiotensin II antiserum were required to block the pressor action of exogenous angiotensin II. Infusion of renin profoundly lowered the blocking requirement of such animals.

2. It is postulated that renin bound to blood vessels generates angiotensin locally which is taken up by vascular receptors. Where such receptors are left unoccupied and free to bind exogenous angiotensin, high doses of blocking antisera are required.

3. Animals with hypertension produced by renal artery constriction with contralateral nephrectomy were shown to be in positive sodium balance. Nevertheless their blocking requirement was low.

4. It is suggested that the local generation of angiotensin may play a role in the production of renal hypertension and that this accounts for the development of hypertension even in animals immunized against angiotensin.

Key words: angiotensin II antiserum, renin, sodium depletion and sodium loading, pressor response, clip-nephrectomy hypertension, bilateral nephrectomy.

Most studies of the role of the renin–angiotensin system in hypertension depend upon circulating renin and angiotensin II levels, which do not necessarily represent the concentration of these substances at the vascular receptor site. Brunner, Chang, Wallach, Sealey & Laragh (1972) measured the amount of angiotensin II antibody required to block the blood pressure response to exogenous angiotensin II in the rat. Where the endogenous formation of renin and angiotensin II had been stimulated by a low salt diet, the pressor response to 50 ng doses of exogenous angiotensin II was blocked by very small doses of antiserum. Conversely, where the renin–angiotensin system had been suppressed by a high salt intake, large amounts of antiserum were required. To explain this anomaly, they suggest that vascular affinity for
angiotensin II is increased by salt loading, and decreased by salt deprivation. This explanation is open to the objection that it requires affinity to be decreased in those situations where the peripheral vasoconstrictor action of angiotensin II comes into play. Such a process would, therefore, tend to nullify a homeostatic mechanism for blood pressure preservation.

The purpose of the present paper is to provide evidence for an alternative theory that renin is taken up by the peripheral arterioles and generates angiotensin II locally and to suggest that renin levels which are inappropriately high for the state of sodium balance may play a role in renal hypertension.

METHODS

Production of antiserum

Antisera to angiotensin II ('Hypertensin', Ciba) were prepared in a rabbit by the method of Goodfriend, Fasman, Kemp & Levine (1966). The in vivo titre of this antiserum was tested by the method of Eide & Aars (1970). Volumes (0.1 ml) of serial dilutions of antiserum were mixed with 50 ng of angiotensin II in 0.9 ml of NaCl and the pressor action of the resultant mixture assessed by intravenous infusion of 0.1 ml quantities into a ganglion-blocked bilaterally nephrectomized rat. A dose–response curve showed that 1 ml of antiserum would block the pressor response to approximately 16000 ng of angiotensin II.

Infusion experiments

White Wistar rats (males or females), weighing 150–200 g, were used throughout. The jugular vein and carotid artery were cannulated under ether anaesthesia and the pressor response to 50 ng of intravenously injected angiotensin II was recorded. After the blood pressure had returned to the baseline, 0.1 ml of antiserum was injected over a 5–10 s period. At 3–5 min later, the pressor response to angiotensin II was again recorded. If necessary, further doses of antiserum and angiotensin II were infused until the response to angiotensin II was blocked (i.e. the pressor effect was 5 mmHg or less). Inert serum from a non-immunized rabbit was found to have no effect upon the pressor response of a normal rat to angiotensin II.

Pretreatment of rats

Experimental groups 1–4 and 6 comprised six animals each.

Group 1 (normal) was given tap water to drink and free access to laboratory chow.

Group 2 (salt-loaded) was given 1% saline to drink and free access to laboratory chow for 8–14 days.

Group 3 (salt-depleted) was given deionized water to drink and low salt food ('Edosol', Trufood Limited) for 8–10 days.

Group 4 (salt-loaded, renin preinfused) was pretreated as group 2. After cannulation, 0.1–0.2 unit of purified hog renin (Miles–Seravac Limited and M.R.C. standard porcine renin) in 0.1 ml of NaCl (9 g/l) was infused over 5–10 s. Antiserum and angiotensin II were then infused according to the above protocol. The time-interval between renin infusion and the first dose of antiserum was 15–20 min and the experiment completed within a further 10–20 min.

Group 5 (bilateral nephrectomy). Eighteen animals were cannulated 6–18 h after bilateral nephrectomy, having been deprived of food and water in the post-operative period. Pilot
TABLE 1. Blood pressure response to angiotensin II antiserum and hog renin and amount of antibody required to block the pressor effect of 50 ng of angiotensin II in the six experimental groups (means ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Normal</th>
<th>Group 2 Salt-loaded</th>
<th>Group 3 Salt-depleted</th>
<th>Group 4 Salt-loaded, renin-infused</th>
<th>Group 5 Bilateral nephrectomy</th>
<th>Group 6 Clip-nephrectomy</th>
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<tbody>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td></td>
<td></td>
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<tr>
<td>(a) Before antiserum</td>
<td>104.9 ± 6.1</td>
<td>109.7 ± 5.0</td>
<td>105.3 ± 10.1</td>
<td>114.0 ± 7.2</td>
<td>90.8 ± 6.5</td>
<td>139.1 ± 5.2</td>
</tr>
<tr>
<td>(b) After blocking</td>
<td>100.4 ± 8.2</td>
<td>99.7 ± 4.0</td>
<td>97.4 ± 10.1</td>
<td>102.6 ± 7.5</td>
<td>89.0 ± 5.3</td>
<td>134.7 ± 5.2</td>
</tr>
<tr>
<td>Blocking dose (ml)</td>
<td>0.32 ± 0.03</td>
<td>0.65 ± 0.03</td>
<td>0.22 ± 0.04</td>
<td>0.18 ± 0.05</td>
<td>0.62 ± 0.03</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Response to 1.6 units of renin (mmHg)</td>
<td>23.8 ± 3.5</td>
<td>34.5 ± 1.7</td>
<td>8.5 ± 1.9</td>
<td>—</td>
<td>43.3 ± 5.1</td>
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experiments indicated that the post-operative interval, if 3 h or longer, did not influence the results. In twelve animals, before the blocking requirement was estimated, doses of renin (0.025-1.0 unit) were infused according to the protocol used with group 4 rats. The remaining six animals received no renin infusion until after blocking with angiotensin II antiserum.

Group 6 (clip-nephrectomy) underwent right nephrectomy 1–2 months before study. Each was maintained in a metabolic cage for 5–6 days, and then a 0.2 mm silver clip applied to the left renal artery. The blocking dose of antiserum was measured 7–17 days after clipping. Post-operative cumulative sodium balance was measured by our previously described technique (Swales, Thurston, Queiroz & Medina, 1972).

Renin (1.6 units) was infused into animals in groups 1–3 and the six rats in group 5 not pretreated with renin immediately after the last test dose of angiotensin (i.e. when the animal was blocked).

RESULTS

Groups 1–4

The mean blocking dose of salt-loaded animals was significantly higher ($P<0.05$) and that of salt-depleted animals significantly lower ($P<0.05$) than that of normals (Table 1). Renin infusion lowered the blocking requirement of salt-loaded animals to values close to those of the salt-depleted rats. Comparison of dose pressor response curves to angiotensin II showed near identity between salt-depleted animals and salt-loaded rats infused with renin (Fig. 1). There was no relationship between the initial blood pressure of the animals and the blocking requirement (Table 1).

Group 5

The blocking dose of the bilaterally nephrectomized rats was almost identical to that of the salt-loaded animals (group 2; Table 1). This was confirmed when the blocking dose–response curves were compared (Figs. 1 and 2). With the infusion of small quantities of renin, the decrease in blocking requirement was proportional to the amount infused (Fig. 3). Larger doses of renin produced a marked rise in blood pressure (60–70 mmHg) and the pressor effect of angiotensin II was blocked even without the infusion of antiserum (Fig. 3).

Group 6

All the clip-nephrectomy hypertensive rats were in positive sodium balance at the time of study (mean sodium balance = $+1.54\pm0.91$ mmol of sodium). The blocking dose of antiserum, nevertheless, did not differ significantly from that observed with salt-depleted animals ($P>0.05$; Table 1). Blocking dose–response curves of salt-depleted normal animals and clip-nephrectomy hypertensives were also similar (Figs. 1 and 4).

Despite blocking a marked pressor response to renin occurred in all animals tested. This was significantly greater than normal in high salt-pretreated and bilaterally nephrectomized rats ($P<0.05$).

DISCUSSION

The importance of both renin and salt in some forms of hypertension was recognized at the
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Vascular generation of angiotensin II of the turn of the century (Tigerstedt & Bergman, 1898; Ambard & Beaujard, 1904). The inter-relationship of the two has remained obscure, however, despite great advances in the understanding of the renin–angiotensin system and of the physiological role of the sodium ion. It has been suggested that an abnormal relationship of renin to body sodium may be responsible

![Graph](image)

**Fig. 1.** Pressor response (means ± SEM) to 50 ng of angiotensin II after successive 0.1 ml doses of angiotensin II antiserum. Dose–response curves for normal (○), salt-depleted (▲), salt-loaded (●) and salt-loaded renin-infused rats (■).

for the hypertension found in some patients with end-stage renal disease (Brown, Düsterdieck, Fraser, Lever, Robertson, Tree & Weir, 1971; Ledingham, 1971) and in patients with malignant hypertension (Davies, Schalekamp, Beevers, Brown, Briggs, Lever, Medina, Morton, Robertson & Tree, 1973). Most studies upon the renin–angiotensin system in hypertension have been concerned with circulating levels of these hormones. There is, however, evidence that changes in
vascular responsiveness to and avidity for angiotensin II may be important and that the sodium ion may be involved in such changes (Brunner et al., 1972).

Sodium depletion produces a decrease in pressor response to angiotensin II (Kaplan & Silah, 1964; Reid & Laragh, 1965). This effect is preserved in fresh in vitro preparations of aortic strips from sodium-depleted rabbits. The explanation cannot, therefore, lie in a decrease in plasma volume, or saturation of vascular receptors by high circulating angiotensin II levels (Strewler, Hinrichs, Guiod & Hollenberg, 1972). This is supported by our experiments where differences in vascular responsiveness manifested in differences in blocking dose still occurred despite the decrease in free angiotensin II levels to very low values by antiserum.

![Pressor response of bilaterally nephrectomized rats to 50 ng of angiotensin II after successive 0.1 ml doses of angiotensin II antiserum.](image)

**Fig. 2.** Pressor response of bilaterally nephrectomized rats to 50 ng of angiotensin II after successive 0.1 ml doses of angiotensin II antiserum.
Our results indicate that both exogenous renin infused into high salt-pretreated rats (group 4) and endogenous renin stimulated by the low salt diet (group 3) facilitated blocking of the pressor response to infused angiotensin II by a specific antiserum. Since renin elevates circulating angiotensin II levels, by increasing production of the peptide, it would be expected that the antibody requirement would be increased. The present results clearly show the converse. This phenomenon could be explained by a local generation of angiotensin at the arteriolar...
level. If local renin receptors are left unoccupied in animals in which renin secretion has been suppressed (i.e. groups 2 and 5), little or no angiotensin would be locally generated. Almost all angiotensin receptors would, therefore, be free to react with any unbound circulating angiotensin and a large dose of antiserum would be needed to decrease free angiotensin below the critical level at which a pressor response occurs. In the presence of high vascular renin levels produced by renin infusion or low salt diet, large amounts of angiotensin II would be generated locally, and would occupy most of the vascular angiotensin II receptors, maintaining peripheral resistance. Few angiotensin receptors would be unoccupied to take up circulating angiotensin and the level of unbound angiotensin required to produce a pressor response is therefore high, and the blocking dose correspondingly low.

The increase in blocking requirement after bilateral nephrectomy (group 5) is readily explained by a fall in vascular renin levels. Recent studies (J. D. Swales, H. Thurston & J. D. Tange, unpublished observations) have shown that blocking requirement increases rapidly over the first 1–2 h after bilateral nephrectomy and that, at 6 h, the blocking requirement is identical in salt-depleted and non-salt-depleted nephrectomized rats. An increase in both the pressor response to renin and the duration of this response is, of course, well known to occur after bilateral nephrectomy although the mechanism of this effect is disputed (Collins & Harakal, 1952; Blaquier, 1965). An increase in the velocity of angiotensin formation by the plasma of nephrectomized animals can be demonstrated in vitro (Collins & Harakal, 1952; Montague, 1968; Romero & Hoobler, 1972). Increased pressor sensitivity to renin cannot, therefore, be attributed wholly to free renin vascular receptor sites in this model. The protracted pressor response to angiotensin II, however (Gabelman & Randell, 1966), may be related to the increase in free receptor sites postulated by our hypothesis. This is also consistent with the hypothesis that renin rather than sodium status determines the blocking dose of antibody.

This hypothesis demands that renin in the arteriolar wall generates angiotensin II which is not accessible at least to the circulating antibody protein molecules. This view is supported by the pressor response to renin infusions which was much greater in high salt-pretreated animals (Table 1). Where renin receptor sites are free, renin could generate sufficient angiotensin II locally to cause a substantial elevation of blood pressure and the angiotensin II so generated would be active without release into the circulation. It is difficult to see how such differences in pressor responsiveness to renin could be mediated by circulating angiotensin II since the pressor action of infused angiotensin II was blocked by antiserum, and remained blocked, indicating that saturation of antibody had not occurred.

There is support for our hypothesis from several observations by other workers. (i) Renin-like material has been demonstrated in hog arterial wall extracts (Gould, Skeggs & Kahn, 1964). (ii) The pressor action of renin persists even when circulating pressor material can no longer be demonstrated by cross-circulation studies (Schaechtelin, Regoli & Gross, 1964), (iii) Blocking doses of angiotensin II antiserum are relatively ineffective in impairing the pressor response of bilaterally nephrectomized rats to renin (Bing & Poulsen, 1970). (iv) In rabbits immunized against angiotensin II, the pressor action of angiotensin I was 5–10 times greater than the pressor action of angiotensin II (Stokes, Storey & Oates, 1972).

An inappropriately high vascular level of renin might play a role in maintaining peripheral resistance in hypertension. We have shown that hypertension in rats with a single ischaemic kidney is generally associated with marked sodium retention (Swales, Thurston, Queiroz, Medina & Holland, 1971; Swales et al., 1972); this is confirmed by measurement of exchange-
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able sodium levels in this experimental model (Tobian, Coffee & McCrea, 1969). This is well shown in the six clip-nephrectomy animals studied here (group 6). In spite of this salt loading, however, this group showed an ease of blocking which corresponded with the salt-depleted animals (Table 1, Fig. 4). If the present hypothesis is correct this suggests that these animals have vascular renin levels which are inappropriately high for their sodium status. Since circulating renin levels are not elevated in this model (Miksche, Miksche & Gross, 1970), this would suggest a disturbance in the relationship between vascular and blood renin concentration. As far as we know, no reliable direct observations have been made upon vascular renin content in this situation.

We have not as yet studied the two kidney Goldblatt model by the present technique. Since the majority of rats with this form of hypertension already show a negative sodium balance (Swales et al., 1972), which would act as a stimulus for renin secretion, an 'inappropriate' blocking dose of antiserum would be difficult to establish.

The resistance of hypertension to immunization against angiotensin II has been adduced as powerful evidence against participation of circulating angiotensin in the underlying haemodynamic mechanisms (Eide & Aars, 1969; Louis, Macdonald, Renzini, Boyd & Peart, 1970). The present observations suggest a possible alternative role for angiotensin which is independent of circulatory levels.

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REFERENCES


