Determinants of high blood pressure in salt-deprived renal hypertensive rats: role of changes in plasma volume, extracellular fluid volume and plasma angiotensin II

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Summary

1. Rats with indwelling aortic and right atrial cannulae were maintained on a sodium-free diet before and after renal arterial constriction combined with contralateral nephrectomy. Control animals underwent the same protocol except that non-constricting clips were used.

2. Plasma volumes in the salt-deprived animals were lower than previously determined values in animals with free access to sodium. After clipping plasma volume increased in the hypertensive animals. Extracellular fluid volume was increased equally in both normotensive and hypertensive animals on the second postoperative day only.

3. Before clipping and contralateral nephrectomy plasma angiotensin II values were higher than normal. After the operation angiotensin II concentrations fell to normal over a period of 14 days without significant differences between experimental and control groups.

4. It is concluded that high blood pressure after clipping may be in part maintained by increases in plasma volume. However, the results strongly suggest that other renal mechanisms are likely to be of major pathogenic importance.

Key words: angiotensin II, extracellular space, plasma volume, renal hypertension, salt-free diet.

Introduction

Experimental evidence suggests that interlocking mechanisms are involved in the production and maintenance of one-kidney renal hypertension. The initial rise in blood pressure after clipping is associated with increased activity of the renin-angiotensin system (Brown, Davis, Olichney & Johnston, 1966; Bianchi, Tilde Tenconi & Lucca, 1970; Liard, Cowley, McCaa, McCaa & Guyton, 1974), but over the next 72 h sodium retention occurs (Conway, 1968; Bianchi et al., 1970; Swales, Thurston, Queiroz & Medina, 1972) and activity of the renin-angiotensin system returns to normal (Bianchi et al., 1970; Miksche, Miksche & Gross, 1970; Liard et al., 1974). Early increases in plasma volume and extracellular fluid volume take place (Ledingham & Cohen, 1964; Bianchi et al., 1970), and in established one-kidney renal hypertension both plasma volume and total exchangeable sodium are higher than in control animals (Lucas & Floyer, 1974; Tobian, Coffee & McRea, 1969). It has been concluded that these changes in body fluid volumes lead to a sustained increase in cardiac output (Ledingham, 1971; Guyton, Coleman, Cowley, Scheel, Manning & Norman, 1972), and that the high peripheral arterial resistance characteristic of established one-kidney renal hypertension is maintained by tissue autoregulation in the face of these small increases in blood flow.

Sodium deprivation does not prevent the development of one-kidney renal hypertension.
The role of the renin–angiotensin system in these sodium-deprived animals has not been clearly defined; although plasma renin activity is not increased (Miksche et al., 1970), the administration of an angiotensin blocking agent causes a substantial fall in blood pressure, which is abolished by sodium repletion (Gavras et al., 1973).

The present experiments were undertaken in order to investigate further the significance of the renin–angiotensin system and body fluid volumes in the pathogenesis of experimental one-kidney renal hypertension in sodium-deprived rats.

Materials and methods

All experiments were carried out in female Wistar rats of an inbred strain weighing 180–200 g. Two weeks before renal arterial clipping a modified Weeks' cannula (Weeks & Jones, 1960) was inserted under ether anaesthesia into the abdominal aorta below the level of the renal arteries. After this operation the animals were fed on a laboratory diet based on that of Hartroft & Eisenstein (1957), containing 0.003 mmol of sodium/g of diet. This diet was continued for the remainder of the experiment, and the animals were allowed free access to demineralized water. One week later a further similar cannula was implanted into the right atrium through the right jugular vein (Weeks & Davis, 1964). Patency of the cannulae was maintained by filling with heparin, 1000 i.u./ml (Weddel Pharmaceuticals Ltd, London), which was withdrawn before the cannula was used for blood sampling, isotope injection or direct pressure measurement. Before clipping animals were divided in a random manner into two equal groups. In the first (experimental) group, under ether anaesthesia, through a dorsal incision in the abdominal wall a tight silver clip of internal diameter 0.23 mm (0.009 inch) was applied to the left renal artery and right nephrectomy was performed. The second (control) group underwent the same operation, including right nephrectomy, but a non-constricting clip 0.5 mm (0.02 inch) in diameter was used. Blood loss during the operation was negligible.

Experiment 1

Mean arterial pressure was measured in the conscious animal 8 and 5 days before renal arterial clipping, on the morning of surgery and 1, 3, 6, 10, 13 and 16 days after clipping, Packed cell volume, plasma volume and extracellular fluid volume were measured on the day before clipping and 2 and 15 days after the operation.

Experiment 2

Mean arterial pressure and plasma angiotensin II were measured 1 day before and 1, 2, 7, 13, 17 and 23 days after clipping; plasma volume, extracellular fluid volume and packed cell volume were measured 15 days after the operation (as in Experiment 1).

Mean arterial pressure was measured in conscious animals conditioned to sit quietly in a small restraining cage pre-warmed to 34–36°C, with a Statham P23G transducer linked to an ultraviolet recording system [SE 2005, S.E. Laboratories (Engineering) Ltd, London]. Arterial pressure was recorded for approximately 20 min, the reported pressure being the average in the steady state during the last 10 min.

Packed cell volume, plasma volume and extracellular fluid volume were measured in conscious animals. A portion (0.4 ml) of blood was withdrawn through the aortic cannula into a test tube containing approximately 10 i.u. of dried heparin, and replaced with fresh normal donor blood. After centrifuging and measurement of packed cell volume, two 50 μl plasma samples were taken for duplicate measurement of packed cell volume, 10 μl plasma samples were then injected through the right atrial cannula, and 1, 2 and 3 h after isotope injection blood samples (0.3 ml) were withdrawn through the aortic cannula, each being replaced immediately with an equal volume of donor blood. Duplicate portions (50 μl) of plasma were taken from each sample. 131I and 36Cl standards were made in triplicate with 5 μCi of each isotope and 50 μl volumes taken.

All samples were counted directly for 131I radioactivity, with a gamma counter. The 131I count at zero time was extrapolated from a logarithmic plot of the mean of duplicate 1, 2 and 3 h sample counts less the basal count. Plasma volume was then calculated as 131I-labelled human serum albumin space at zero time. 25% Trichloroacetic acid (BDH Chemicals, Poole, Dorset, U.K.), 1 ml, was then added to all samples in order to precipitate 131I-labelled albumin. After centrifugation, 0.5 ml of the supernatant fluid was withdrawn and added to 10 ml of scintillation fluid
Renal hypertension with salt deprivation

Salt-free diet (Unisolve, Koch–Light Laboratories, Maidenhead, Berks., U.K.) which was then counted for β radioactivity. Because $^{36}\text{Cl}$ radioactivity did not decrease over the 3 h period of measurement, the $^{36}\text{Cl}$ count at zero time was taken to be the mean value obtained from duplicate 1, 2 and 3 h sample counts, less the basal count. Extracellular fluid volume was then derived from the chloride space (McMurrey, Boling, Davis, Parker, Magnus, Ball & Moore, 1958).

Plasma angiotensin II was measured by taking 1 ml of arterial blood in less than 60 s, and mixing immediately at 4°C with 50 μl of angiotensinase inhibitor solution containing disodium EDTA (disodium ethylenediamine tetra-acetate; BDH Chemicals) and o-phenanthroline (1,10-phenanthroline monohydrate; Sigma Chemicals, St Louis, Mo., U.S.A.) (Düsterdieck and McElwee, 1971). The samples was centrifuged at 4°C, after which plasma was removed and stored at -20°C. Blood samples were replaced with 1 ml of whole normal donor blood. At the end of the experiments plasma angiotensin II concentration was measured in all samples concurrently by radioimmunoassay in the laboratories of the Medical Research Council Blood Pressure Unit, Glasgow, Scotland, using the method of Düsterdieck & McElwee (1971), with an internal standard to correct for extraction losses.

Statistical comparisons were made by unpaired t-test analysis, with two-tailed t-test significance values given for all data except for changes in blood pressure after renal artery clipping in the hypertensive animals, when the one-tailed t-test was used. Mean values of plasma angiotensin II were calculated after logarithmic transformation to normalize the data. All values are given as mean ± SEM.

Results

Experiment 1

Of 28 animals starting the experiment, four from each group (control and experimental) were withdrawn before the renal artery clip operation because of technical problems associated with the aortic cannulae. The weights of the 10 remaining animals in each group during the course of the experiment were identical (Fig. 1), periods of weight loss being associated with operative procedures. No animals of the experimental group developed malignant hypertension.

Blood pressure in animals with a tight renal artery clip rose to 115 ± 3 mmHg after clipping, compared with 100 ± 2.5 mmHg in control animals ($P < 0.005$, Fig. 2). This difference was maintained throughout the experiment, during which blood pressure of the hypertensive animals rose steadily.

Packed cell volume, plasma volume and extracellular fluid volume measurements were made in 10 animals in each group before, and 2 days after, surgery and in eight normotensive and nine hypertensive animals 15 days after surgery. The remaining three animals were not included because of late aortic cannula failure. The results of measurements made 15 days after surgery in animals from experi-

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Fig. 1. Rat weight before and after renal artery clipping and contralateral nephrectomy in experiment 1. O, Normotensive rats; ●, hypertensive rats. Day 0 refers to date of clip operation.
ment 2 are included with those of the remaining 17 animals from experiment 1 (Table 1). On the second postoperative day, the packed cell volume had fallen in hypertensive animals from 0.46 ± 0.014 to 0.39 ± 0.008 (P < 0.001) and in normotensive animals from 0.46 ± 0.006 to 0.41 ± 0.006 (P < 0.001). The difference between hypertensive and normotensive groups was also statistically significant (P < 0.05). At 15 days after operation the packed cell volume was not significantly different from preoperative levels in either group of animals.

Plasma volume increased in hypertensive animals from 2.97 ± 0.12 ml/100 g pre-operatively to 3.55 ± 0.08 ml/100 g (p < 0.001) on the second postoperative day. Small increases in plasma volume, from 3.11 ± 0.14 ml/100 g to 3.33 ± 0.08 ml/100 g, were found in control animals; these changes were not statistically significant. Differences in plasma volume between hypertensive and normotensive animals did not attain statistical significance. At 15 days after surgery plasma volume continued to be significantly raised in hypertensive animals (3.40 ± 0.10 ml/100 g, P < 0.02) when compared with pre-operative values, whereas in control animals the volume had remained stable. Differences in slope of 131I decay curves were apparent on only the second postoperative day, when that of the hypertensive animals was significantly lower than that of the control group (16.0 ± 1.8 units vs 22.0 ± 1.8 units, P < 0.05). A slope of 20 arbitrary units implied that the differences between 1 and 2 h, and 2 and 3 h 131I radioactivity was of the order of 20%.

Extracellular fluid volume increased in both hypertensive animals, from 27.27 ± 0.62 ml/100 g to 29.11 ± 0.59 ml/100 g (P < 0.05), and control animals, from 26.12 ± 0.29 ml/100 g to 28.50 ± 0.78 ml/100 g (P < 0.02) on the second post-operative day. At 15 days after surgery this volume had returned to pre-operative values in all animals. At no time was the difference in extracellular fluid volumes between hypertensive and control animals significant.

**Experiment 2**

Two animals from each group (control and experimental) were withdrawn before renal artery clipping because of aortic cannula failure, leaving 12 rats in each group. Blood pressure in animals with a tight renal artery clip rose to 110 ± 3.5 mmHg when measured 24 h after surgery, com-

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**Table 1. Changes in packed cell volume, plasma volume, extracellular fluid volume and slope of 131I decay curve**

Renal artery clipping and contralateral nephrectomy were carried out in all animals on day 0. NT, Normotensive control group of animals with wide renal artery clips; HT, experimental group with constricting renal artery clips. Mean values ± SEM are shown with the numbers of animals in each group in parentheses. P values refer to statistical significance coefficients within groups of animals between results for 2 days and 15 days after surgery when compared with pre-operative data. N.S., Not significant. Any differences between control and experimental groups were not statistically significant apart from packed cell volume and 131I decay slope on day 2 (see the text).

<table>
<thead>
<tr>
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<th>Day –1</th>
<th>Day 2</th>
<th>Day 15</th>
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<tbody>
<tr>
<td><strong>Packed cell volume</strong></td>
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<tr>
<td>NT</td>
<td>0.46 ± 0.006 (10)</td>
<td>0.41 ± 0.006 (10)</td>
<td>0.44 ± 0.009 (20)</td>
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<tr>
<td>HT</td>
<td>0.46 ± 0.014 (10)</td>
<td>0.39 ± 0.008 (10)</td>
<td>0.42 ± 0.013 (21)</td>
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<tr>
<td><strong>Plasma volume (ml/100 g body weight)</strong></td>
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<tr>
<td>NT</td>
<td>3.11 ± 0.14 (10)</td>
<td>3.33 ± 0.08 (10)</td>
<td>3.33 ± 0.08 (20)</td>
</tr>
<tr>
<td>HT</td>
<td>2.97 ± 0.12 (10)</td>
<td>3.55 ± 0.08 (10)</td>
<td>3.40 ± 0.10 (21)</td>
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<tr>
<td><strong>Slope of 131I decay curve (arbitrary units)</strong></td>
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<tr>
<td>NT</td>
<td>20.0 ± 2.2 (10)</td>
<td>22.0 ± 1.8 (10)</td>
<td>23.0 ± 1.1 (20)</td>
</tr>
<tr>
<td>HT</td>
<td>19.0 ± 1.7 (10)</td>
<td>16.0 ± 1.8 (10)</td>
<td>21.0 ± 1.3 (21)</td>
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<tr>
<td><strong>Extracellular fluid volume (ml/100 g body weight)</strong></td>
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<tr>
<td>NT</td>
<td>26.12 ± 0.29 (10)</td>
<td>28.50 ± 0.78 (10)</td>
<td>26.39 ± 0.27 (20)</td>
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<tr>
<td>HT</td>
<td>27.27 ± 0.62 (10)</td>
<td>29.11 ± 0.59 (10)</td>
<td>26.62 ± 0.37 (21)</td>
</tr>
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pared with 98 ± 1.6 mmHg in control animals \((P < 0.005)\). Differences in blood pressure between the two groups gradually increased over the following 3 weeks (Fig. 3). At no time were differences in plasma angiotensin II between hypertensive and normotensive animals statistically significant (Fig. 0.005). Within the hypertensive group, mean plasma angiotensin II was significantly lower than pre-operative levels (190 pmol/l) when measured 13 days (93 pmol/l, \(P < 0.01\)), 17 days (49 pmol/l, \(P < 0.001\)) and 23 days (70 pmol/l, \(P < 0.001\)) after clipping.

**Discussion**

Increases in plasma volume in these salt-deprived animals after clipping were similar to those observed by other workers in salt-replete animals (Ledingham & Cohen, 1964; Conway, 1968; Bianchi et al., 1970; Liard et al., 1974) in the first 2 weeks of one-kidney renal hypertension. However, because the initial pre-clipping values of plasma volume in the present experiment were below normal, the final value attained after operation, although increased, was no higher than that of normotensive intact salt-replete animals, where plasma volume was \(3.65 \pm 0.10\) ml/100 g body weight \((n = 16)\).

The rate of \(^{131}\)I decay when measuring plasma value in hypertensive animals was significantly lower than in normotensive control animals on the second day after clipping. This difference may have been due to decreased capillary permeability to albumin in developing hypertension; however, because urinary losses of \(^{131}\)I-labelled albumin were not measured in these experiments, the possibility that the observed changes in \(^{131}\)I decay slopes arose from reduction in renal blood flow accompanied by a parallel decrease in urinary protein loss after tight clipping cannot be excluded.

Extracellular fluid volume was also increased in both normotensive and hypertensive animals, but only on the second postoperative day. When this volume was expressed in absolute terms rather than in relation to body weight, no such rise occurred, indicating that postoperative weight loss did not alter the absolute value of extracellular fluid volume.

Changes in plasma volume and packed cell volume in the salt-deprived hypertensive animals could not have been due to sodium retention, which is likely to be responsible for increases in plasma volume and extracellular fluid volume in salt-replete animals (Conway, 1968; Bianchi et al., 1970; Swales et al., 1972; Liard et al., 1974). The salt-deprived hypertensive animals may therefore have undergone redistribution of fluid within the extracellular compartment; changes of this nature have been described in salt-replete animals (Lucas & Floyer, 1974). Alternatively, the increases in plasma volume may have resulted simply from water retention consequent on reduction of renal perfusion pressure after tight clipping.

Plasma angiotensin II concentrations fell within 2 weeks of surgery to values approximately 50% of those found pre-operatively in both normotensive and hypertensive animals, as found by Miksche et al. (1970), who measured plasma and renal renin activity in intact, unilaterally nephrectomized, and one-kidney renal hypertensive rats, all of which had been deprived of dietary sodium for 2 weeks before the measurements were made. Measurements in our animals, made on six different occasions after renal arterial clipping, showed that plasma angiotensin II did not fall until 13 days after surgery, when compared with pre-operative levels. In the
control animals this fall in plasma angiotensin II concentration 13 days after operation is likely to have been a direct result of the reduction in renin-producing tissue after unilateral nephrectomy; the delay in this fall may have been a non-specific effect of surgery, or perhaps a consequence of handling the remaining kidney during the application of a non-constricting renal artery clip.

These experiments have confirmed that sodium retention is not essential for either the development or the maintenance of one-kidney renal hypertension. A small increase in plasma volume has been observed, and the findings are therefore compatible with theoretical models of renal hypertension in which an increase in plasma volume is thought to lead to a rise in cardiac output with consequent autoregulation of blood flow (Ledingham, 1971; Guyton et al., 1972). However, because the changes in plasma volume in the present experiment were small, an increase in venous return sufficient to increase cardiac output seems unlikely, unless the haemodynamic characteristics of capacitance vessels were altered, and in fact the mean circulatory filling pressures of one-kidney renal hypertensive dogs (Richardson, Fermo & Guyton, 1964), perinephritic hypertensive dogs (Ferrario, Page & McCubbin, 1970) and one-kidney renal hypertensive rats (Gotzen, Herberg, Schultzze & Lohmann, 1971) are known to be increased.

The finding that plasma angiotensin II concentrations in hypertensive animals were similar to those of normotensive control animals may seem to be at variance with the experiments of Gavras et al. (1973) in rats deprived of dietary sodium for 4 weeks. In those experiments, substantial falls in blood pressure occurred after infusion of the angiotensin II antagonist saralasin in one-kidney renal hypertensive animals, whereas blood pressure fell to a lesser extent in one- and two-kidney normotensive control animals. However, percentage decreases in blood pressure in both the intact and the hypertensive rats were very similar. As our experiments indicate that the renin–angiotensin system played no part in the development of one-kidney renal hypertension under conditions of sodium deprivation, the experiments of Gavras and his co-workers are interpreted to suggest that angiotensin II contributes fractionally to the blood pressure of both normotensive and hypertensive salt-deprived animals in proportion to the level of blood pressure resulting from other pressor mechanisms. Our experiments suggest that such mechanisms, which may include increases in plasma volume brought about by processes other than sodium retention, are likely to be of major importance in the development of one-kidney renal hypertension.

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References


