Exchangeable sodium in Goldblatt one-kidney one-clip hypertension in the rat

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(Received 12 April/12 September 1983; accepted 31 October 1983)

Summary

1. Exchangeable sodium (NaE), plasma active renin concentration and blood pressure were measured in rats with a sole remaining kidney before and after the development of hypertension induced by clipping of the single renal artery and again after unclipping.

2. Control observations were made in sham-clipped and sham-unclipped uninephrectomized rats.

3. Renal artery clipping caused hypertension and expansion of NaE, the latter being sustained throughout the 6 weeks during which the renal artery was constricted.

4. Hypertension in the clipped rats was progressive over 6 weeks, whereas the expansion of NaE was not; thus the two measurements were not significantly correlated.

5. Two rats which remained normotensive after clipping did not show expansion of NaE.

6. Plasma active renin was elevated in comparison with the sham-clipped controls on the day after clipping, but not thereafter.

7. Unclipping in hypertensive rats was followed by a return of NaE and blood pressure to control values.

8. Both the sustained expansion of NaE and the transient rise in active renin probably contribute to the development of hypertension in this model, but neither alone nor together do they provide a full satisfactory explanation.

Key words: experimental hypertension, Goldblatt hypertension, fluid balance, renin, sodium.

Introduction

There is controversy concerning whether or not body sodium content is expanded in rats with hypertension after removal of one kidney and application of a constricting clip to the artery to the remaining kidney (Goldblatt one-kidney one-clip hypertension). Swales et al. [1] reported sodium retention concurrently with the development of hypertension in this model; however, external balance studies of this kind are prone to cumulative errors in long-term experiments [2]. By contrast, Doyle et al. [3], estimating exchangeable sodium (NaE), did not observe significant differences between hypertensive and control rats in measurements made serially from 7-28 days after the application of the renal artery clip. Tobian et al. [4] found that, 3 months after operation, rats with one-kidney one-clip hypertension had higher exchangeable sodium than normotensive sham-operated rats with two kidneys remaining; this was, however, not an appropriately controlled comparison. Albertini et al. [5], also making single measurements of NaE 8-14 weeks after operation, found significantly higher values in rats with one-kidney one-clip hypertension than in uninephrectomized, sham-clipped controls.

The present study was designed to make serial measurements of NaE in rats before, during and after the development of one-kidney one-clip hypertension and in one-kidney sham-clipped controls, in an attempt to resolve these issues. A
second objective, should expansion of NaE be found, was to examine whether such changes correlated with the development and resolution of hypertension. A preliminary account of this work has been given [6].

Methods
Thirty-six male Sprague-Dawley rats (initially weighing 160-200 g) were housed in individual cages, fed with a sodium-free diet and given sodium chloride solution (85 mmol/l) containing $^{22}$Na [37 kBq/l (1 μCi/l)] to drink. After 14 days, equilibration with radioisotope was complete. Serial measurements of NaE were made in each rat twice weekly thereafter; details of the method have been described earlier [7, 8]. Under ether anaesthesia, each animal had a right nephrectomy performed. Twelve days later, again under ether anaesthesia, 21 animals had a left renal artery clip applied and 15 had a sham operation. Ten of the clipped animals died in the first postoperative week and a further two remained normotensive; these were excluded from subsequent statistical analyses. Three hypertensive rats died 3 weeks after clipping; thus analyses in this group were made on nine animals up to 3 weeks post-clipping and on six thereafter. Six weeks after clipping, each of the surviving hypertensive rats and the controls had an unclipping or sham-unclipping operation performed. Measurements were continued for a further 2 weeks, when the animals were killed with intravenous pentobarbitone. In addition to the assessments of NaE, blood pressure and plasma active renin concentration were measured twice weekly throughout.

Systolic blood pressure was measured in lightly restrained unanaesthetized animals with a tail occlusion cuff and a piezo-electric crystal detector connected to a blood pressure recorder (W & W Electronics, Basel, Switzerland). Plasma active renin concentration was measured by a radioimmunoassay technique in venous blood samples (50 μl) drawn from the tail of unanaesthetized rats [9]; the only modification to the method as previously described was that incubation was with rat renin substrate made as described by Nasjletti et al. [10].

Statistical methods comprised repeated measures analyses of variance and paired t-test where appropriate.

Results

Weight

There was a transient loss of weight in each group after renal artery clipping or sham clipping. On days 3 and 5 after operation, weight was marginally lower in the clipped group ($P < 0.05$) than in the sham-clipped group (Fig. 1c), but subsequently there were no significant differences in weight between the two groups. From 1 week before nephrectomy to the end of the entire experiment, mean weight increased from 209.6 ± SEM 10.5 g to 402.8 ± 16.7 g in the clipped group and from 215 ± 7.4 g to 413.1 ± 7.9 g in the sham-clipped group.

Fluid and sodium intake

There was no significant difference in fluid intake between the clipped rats and controls at
any time. Fluid intake tended to rise during the experiment as the weight of the animals increased. In the clipped group, after nephrectomy, mean daily fluid intake was 24.6 ± SEM 1.8 ml/day before renal artery clipping, 25.1 ± 2.0 ml/day in the first week after application of the clip, 31.3 ± 2.8 ml/day in the week before removal of the clip and 30.8 ± 4.1 ml/day after unclipping.

Similarly, in the sham-clipped animals fluid intake (after unilateral nephrectomy) was 26.4 ± 1.1 ml/day before the sham-clip, 24.9 ± 0.9 ml/day in the first week after sham-clipping, 28.6 ± 1.1 ml/day after sham-uncipping and 28.5 ± 1.0 ml/day after sham-uncipping. Sodium intake depended on fluid intake, and there was no systematic difference in sodium intake between the two groups throughout the experiment.

**Blood pressure**

There was no significant change in blood pressure in the sham-clipped group throughout the entire experiment (Fig. 1a).

Blood pressure in the clipped group rose progressively from 139.4 ± SEM 3.2 mmHg to 220.1 ± 6.0 mmHg (P < 0.001) in the 6 weeks after clipping (Fig. 1a). After unclipping, blood pressure fell to 136.3 ± 12.5 mmHg, which was not significantly different from the pressure in the contemporary sham-operated group (144.6 ± 2.3 mmHg).

In the two clipped rats which did not become hypertensive, respective blood pressures were 135 and 140 mmHg immediately before clipping, and 140 and 150 mmHg 27 days later.

**Exchangeable sodium (NaE)**

Immediately before clipping, NaE was similar in the clipped (39.43 ± SEM 0.45 mmol/kg) and sham-clipped (39.61 ± 0.25 mmol/kg) groups. After the application of a renal artery clip the clipped animals retained significantly more sodium than the sham-clipped rats (48.03 ± 0.11 mmol/kg vs 43.52 ± 0.42 mmol/kg by the third day, P < 0.01; 44.87 ± 1.17 mmol/kg vs 41.75 ± 0.33 mmol/kg by the fifth postoperative day, P < 0.05). Exchangeable sodium in the clipped group then remained consistently and significantly higher than that in the sham-clipped group during the next 6½ weeks (Fig. 1b). Sodium retention was not progressive in the clipped rats, remaining some 3-5 mmol/kg above control values, and was not correlated with the steady rise in blood pressure over the same period.

After unclipping or sham-uncipping, there was a transient rise in NaE in both groups. Thereafter NaE was no longer expanded in the previously clipped group, and remained similar to values in the contemporary control rats (Fig. 1b).

In the two clipped rats which remained normotensive, NaE was respectively 38.06 and 41.07 mmol/kg immediately before clipping, and 41.59 and 39.97 mmol/kg 27 days later; all these values are close to the means for the sham-clipped control group at the same times.

**Plasma renin concentration**

At the beginning of the experiment there was no significant difference in plasma active renin concentration between the two groups (0.45 ± 0.07 μunit/ml; 0.46 ± 0.07 μunit/ml in the clipped and sham-clipped animals respectively). Plasma renin concentration fell gradually in both groups and immediately before clipping was 0.18 ± 0.04 μunit/ml in the clipped group and 0.32 ± 0.06 μunit/ml in the sham-clipped group (difference between clipped and sham-clipped groups not significant).

On the first day after clipping, plasma renin concentration in the clipped group (0.45 ± 0.09 μunit/ml) was significantly higher than in the control group (0.23 ± 0.03 μunit/ml; P < 0.01). Thereafter plasma renin concentration fell and remained low (0.28 ± 0.09 μunit/ml in the clipped group; 0.19 ± 0.03 μunit/ml in the sham-clipped group immediately before unclipping; the difference between groups was not significant. Two weeks after unclipping, plasma renin concentration remained insignificantly different in the two groups (0.17 ± 0.07 μunit/ml; 0.33 ± 0.03 μunit/ml respectively).

**Renal histology**

There were no histological abnormalities in the kidneys of the sham-clipped animals. In rats with a previously clipped kidney, there were occasional calcified infarcts but no other evidence of ischaemia.

**Discussion**

The present study has shown consistent sustained expansion of NaE in one-kidney one-clip Goldblatt hypertensive rats throughout a 6-week period after application of a renal artery clip. After removal of the clip and alleviation of the hypertension, NaE returned to control values. Exchangeable sodium in the hypertensive rats was, after a sharp rise in the first week followed by a decline, fairly steady thereafter, remaining some 3-5 mmol/kg above values in the control group. Thus NaE was
not correlated with the hypertension, which progressed markedly throughout the same period (Fig. 1).

Two clipped rats which did not develop hypertension did not show expansion of NaE.

Our findings of expanded NaE in rats with one-kidney one-clip hypertension are broadly consistent with the external balance data of Swales et al. [1], and with the more limited NaE measurements of Albertini et al. [5]. Swales et al. [1] saw evidence of cumulative sodium retention, particularly over the first 8 days after clipping, less markedly so thereafter.

Our results are, however, in contrast to the findings of Doyle et al. [3], who used a closely similar method of serial NaE estimation to us but did not observe expansion of body sodium. A possibly important difference between the two studies was that in the experiments of Doyle et al. [3] the rats were given sodium chloride solution at 130 mmol/l to drink, whereas in the present experiments we gave only 85 mmol/l. However, it is difficult to see how the more dilute saline offered to our rats could have led to sodium retention, whereas the more concentrated saline drunk by the rats of Doyle et al. [3] did not cause this.

The present results are to be contrasted with our findings using the same methodology in two-kidney one-clip hypertension in the rat, in which NaE was throughout no different from control values [11].

In the present studies plasma active renin concentration was markedly elevated on the first day after clipping but not subsequently; again there was no correlation between plasma renin and the severity of the progressive hypertension (Fig. 1b). Our findings in this respect are in agreement with the more limited data of Doyle et al. [3], who found no difference between control and experimental values of plasma renin activity 28 days after clipping. Our results are also consistent with reports of normal or diminished renal renin content and decrease of juxtaglomerular granulation in this model [12, 13].

The present renin results should be contrasted with the pattern of changes seen in two-kidney one-clip hypertension in the rat reported from this department [14]. In the latter model, plasma active renin concentration is raised for the first week after clipping, subsides transiently during the second and third weeks, and then rises again, variably, but often markedly, for up to 20 weeks from clipping.

Biochemical changes after the application of a clip to the artery of a sole remaining kidney were examined in detail in conscious dogs by Bianchi et al. [15]. They found that plasma renin concentration was elevated markedly immediately after clipping; although values declined subsequently, they remained significantly above pre-clipping levels 7 days later; at 14 days after clipping renin was no longer significantly raised. External balance data showed expansion of body sodium content immediately after clipping and 2 weeks later. Bianchi et al. [15] concluded that the pathogenesis of this form of hypertension could not be explained simply in terms of the changes in renin and sodium balance.

Our present studies in the rat with one-kidney one-clip hypertension likewise indicate that although both the sustained rise in NaE and the more brief elevation of active renin may contribute importantly to the hypertension, additional factors require to be invoked to provide a full explanation of its pathogenesis.

References

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