Response of chronic renovascular hypertension to surgical correction or prolonged blockade of the renin–angiotensin system by two inhibitors in the rat

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Summary
1. Removal of the renal artery constriction but not of the clipped kidney restored the blood pressure to normal levels in Goldblatt two-kidney rats with hypertension of more than 4 months' duration.

2. Despite the differences in blood pressure response, both surgical procedures lowered plasma renin concentration to normal or below normal values.

3. Administration of the oral converting enzyme inhibitor SQ 14 225 produced a marked fall in blood pressure in Goldblatt kidney rats with chronic hypertension. However, a prolonged infusion of the angiotensin II antagonist saralasin was quite ineffective. The difference in response to the two inhibitors may have been due to bradykinin potentiation by the converting enzyme inhibitor.

4. Although plasma renin is often elevated in Goldblatt two-kidney rats with hypertension of more than 4 months' duration, the renin–angiotensin system plays no role in the maintenance of blood pressure at this stage.

Key words: angiotensin II, converting enzyme inhibitor, Goldblatt two-kidney hypertension, removal of clip, saralasin.

Introduction
The mechanism of the blood pressure rise induced by renal ischaemia is only partly understood. Despite a plethora of experimental studies. In the early phases of hypertension produced by unilateral renal ischaemia without contralateral nephrectomy (Goldblatt two-kidney hypertension), plasma renin is elevated in the rat (Miksche, Miksche & Gross, 1970) and dog (Watkins, Davis, Hanson, Lohmeier & Freeman, 1976). Long-standing hypertension is associated with a fall in plasma renin to normal in the dog (Watkins et al., 1976) and, according to some groups, also in the rat (Koletsky & Rivera-Velez, 1970). After several months' hypertension pharmacological blockade of the renin–angiotensin system with the angiotensin antagonist saralasin no longer produces a blood pressure fall (Thurston & Swales, 1974; Gavras, Brunner, Thurston & Laragh, 1975), and it has been concluded that the renin–angiotensin system plays little role at this stage. One explanation is that vascular structural changes induced by hypertension maintain blood pressure (Folkow, Hallback, Lundgren, Sivertsson & Weiss, 1973). An alternative possibility is that the renin–angiotensin system maintains blood pressure by an action distinct from its acute pressor effect. This has been suggested by the demonstration that prolonged infusion of saralasin lowered blood pressure of rats with hypertension of 6 weeks' duration, slowly over 12 h (Riegger, Millar, Lever, Morton & Slack, 1977). Such a 'slow' pressor mechanism would be in keeping with studies in which hypertension gradually developed in response to the infusion of normally sub-pressor doses of angiotensin II (Dickinson & Yu, 1967).

The present studies were designed to assess the efficacy of such prolonged pharmacological block-
ade of the renin–angiotensin system with saralasin and the oral converting enzyme inhibitor SQ 14 225 in Goldblatt two-kidney hypertension of several months' duration. The results were compared with attempted surgical correction by removing either the renal artery clip or the clipped kidney, in order to evaluate the relative importance of the renin–angiotensin system and vascular hypertrophy at this stage.

Methods
Female white Wistar rats of the same strain weighing 170–250 g were used throughout. Hypertension was induced by the application of a silver clip (internal diameter 0.2 mm) to the left renal artery under ether anaesthesia; the opposite kidney was left untouched. Control non-hypertensive rats were prepared with a non-constricting 'loose' clip (internal diameter 0.5 mm). Systolic blood pressure was measured indirectly by light plethysmography (Swales & Tange, 1970) and each animal was weighed at approximately weekly intervals thereafter until the time of experimental study. Animals were studied after 4–12 months of hypertension (systolic blood pressure > 150 mmHg). On the day of, and 10 and 60 days after, the experimental procedure a blood sample was obtained from the tail under light ether anaesthesia for measurement of plasma renin concentration. A blood sample was obtained from the control rats under the same conditions on the day before and 1 and 8 days after surgery, whereas for experimental groups blood samples were taken at days 1 and 10 after surgery.

Plasma renin concentration assay

The blood sample was collected in a pre-cooled tube moistened with a drop of concentrated solution of potassium ethylenediamine tetra-acetate (EDTA). Plasma was separated after spinning in a refrigerated centrifuge and frozen at −20°C. A 100 μl sample of plasma was incubated with 400 μl of nephrectomized rat plasma as substrate at pH 6-5 and the generated angiotensin I measured by radioimmunoassay. The method used was similar to that of Sealey, Laragh, Gerten-Banes & Aceto (1974), except that phenylmethylsulphonyl fluoride was used as an angiotensinase inhibitor. A normal range for rat plasma renin concentration was obtained with blood samples taken from 12 normotensive rats.

Experimental groups
There were eight rats in each group.

Control rats. Group 1 (loose clip–declipping): the non-constricting clip was removed from the left renal artery.

Group 2 (nephrectomy): the left kidney was removed from normotensive rats.

Hypertensive rats. Group 3 (declipping): the constricting clip was removed from the left renal artery in the same manner as in the controls (group 1).

Group 4 (left nephrectomy): the clipped kidney was removed under anaesthesia.

Group 5 (converting enzyme inhibitor): the tripeptide oral converting enzyme inhibitor captopril (SQ 14 225) was added to the food paste and mixed. The total amount was calculated so that each animal took approximately 2.5 mg of inhibitor in the food intake for one 24 h period.

Group 6 (saralasin infusion). Polythene cannulae were inserted into the jugular and carotid vessels and exteriorized at the back of the neck. The animals were allowed to regain consciousness and maintained under loose constraint in a Perspex box. Glucose solution (5%) was infused for 1 h, followed by [sarcosine⁴, alanine⁸]angiotensin II (saralasin) in 5% glucose, the rate of infusion being adjusted to give 10 μg min⁻¹ kg⁻¹ for 10–12 h.

Throughout the study mean arterial blood pressure was measured directly through the carotid cannula, which was connected to a Statham P23gb strain-gauge transducer. A continuous recording was made by a Grass polygraph. Angiotensin II blockade was assessed by observing the blood pressure response to a large (80 pmol) test dose of angiotensin II dissolved in 0.01 ml of 5% glucose given intravenously during the saralasin infusion.

Expression of results
All results were expressed as mean ± SEM and the statistical analysis was performed either with the paired or unpaired Student t-test as appropriate. Since the measurements of plasma renin concentration were not normally distributed, logarithmic transformation was required before statistical analysis could be performed.

Results
The plasma renin concentration in 12 normal rats was 116.0 ± 16.96 pmol of angiotensin I·h⁻¹·ml⁻¹.
Control rats

Plasma renin concentration was within the normal range in six out of the eight loose-clipped rats with a non-significantly raised mean of $173 \pm 34.85$ pmol of angiotensin I h\(^{-1}\) ml\(^{-1}\) ($P > 0.05$), which fell after removing the clip to $125.2 \pm 18.58$ pmol h\(^{-1}\) ml\(^{-1}\) at 24 h ($P > 0.05$) and to $84.9 \pm 13.73$ pmol h\(^{-1}\) ml\(^{-1}\) at 7 days ($P < 0.01$).

Removal of the loose clip caused a non-significant fall in blood pressure from $91.8 \pm 3.4$ to $85.0 \pm 3.70$ mmHg at 7 days ($P > 0.05$). Unilateral nephrectomy had no effect on the blood pressure, which was $97.5 \pm 1.9$ mmHg before and $97.5 \pm 3.3$ mmHg 7 days after surgery.

Hypertensive rats

Surgical correction. The mean plasma renin concentration before operation was significantly raised, at $1057.9 \pm 438.2$ pmol of angiotensin I h\(^{-1}\) ml\(^{-1}\) when compared either with normal rats ($P < 0.001$) or sham-clipped rats ($P < 0.02$) and fell into or below the normal range after removal of the constricting renal artery clip or the clipped kidney (Fig. 1a, b). The difference between plasma renin at 10 and 60 days in the two groups was not significant ($P > 0.1$). When the renal artery clip was removed (group 3), a rapid fall in blood pressure had occurred at the end of 24 h and the blood pressure of all animals remained normal thereafter (Fig. 2a). However, unilateral nephrectomy (group 4) produced a different response in that although there was a comparable immediate fall, blood pressure then rose again to hypertensive levels (Fig. 2b).

Angiotensin blockade. Administration of the oral converting enzyme inhibitor SQ 14 225 for 24 h was associated with a significant fall in blood pressure ($P < 0.001$) (Fig. 3), although all but two animals remained hypertensive. By contrast, infusion of the angiotensin antagonist saralasin into conscious animals for 12 h produced virtually no change in the blood pressure (Fig. 4) despite complete pharmacological blockade of pressor response to the test dose of angiotensin II.

Discussion

In this study plasma renin concentrations remained elevated for more than 4 months after renal artery constriction in Goldblatt two-kidney hypertension.

![Fig. 1. Plasma renin concentration in Goldblatt two-kidney rats with chronic hypertension before and after removal of either the constricting clip (a) or the clipped left kidney (b). The range (lowest to highest values) for normal rat plasma renin is indicated by the broken lines.](image-url)
These results confirm and extend observations of others in which renin concentrations were still elevated at 5 weeks (Leenen, de Jong & de Wield, 1973) and 10 weeks (Miksche et al., 1970). On the other hand, one group has reported that hyperreninaemia is limited to 2–3 weeks in this model (Koletsky & Rivera-Velez, 1970). In addition it has been reported that juxtaglomerular granularity is initially increased but then returns to normal at 4 months (Latta, Osvaldo & Johnston, 1975). The discrepancies may be due to strain differences.

More consistent results have been obtained by measuring the blood pressure response to infusion of the angiotensin II antagonist, saralasin. Thus although this agent caused a partial fall in blood pressure of rats with Goldblatt two-kidney hypertension of a few weeks' duration (Thurston & Swales, 1974; MacDonald, Boyd & Peart, 1975), no depressor response occurred in animals at 3–4 months (Thurston & Swales, 1974; Gavras et al., 1975). However, each study involved a short-term infusion of saralasin and Riegger et al. (1977) have suggested that angiotensin II may raise blood pressure in different ways.

**FIG. 2.** Mean systolic blood pressure of Goldblatt two-kidney rats with chronic hypertension (a) before and after removal of the constricting clip (at the point indicated by the arrow) and (b) before and after removing the clipped kidney.

**FIG. 3.** Effect of oral converting enzyme blockade (given over 1 day as denoted by the arrows) on mean systolic blood pressure of Goldblatt two-kidney rats with chronic hypertension.

**FIG. 4.** Effect of prolonged infusion of saralasin on the mean arterial blood pressure of Goldblatt two-kidney rats with chronic (>4 months) hypertension.
pressure by means of a 'slow mechanism', which can only be reversed by prolonged angiotensin blockade. In their study the mean interval between renal artery constriction and infusion was 42 days. In the present study, with hypertension of much longer duration, the antagonist was infused for up to 12 h without any effect on the blood pressure: thus there seems to be no evidence for such a slow mechanism maintaining blood pressure in the chronic phase of Goldblatt two-kidney hypertension as defined by us. On the other hand, the oral converting enzyme inhibitor produced a significant fall in blood pressure at 12 and 24 h after administration. However, this agent has also been shown to lower the blood pressure of spontaneously hypertensive rats to near normal levels, although plasma renin activity is not elevated in these animals (Muirhead, Prewitt, Brooks & Brosius, 1978). The discrepancy between the response to converting enzyme inhibitor and saralasin could be explained by partial agonist properties of the latter, but recently studies with converting enzyme inhibitor indicate an additional vasodepressor effect independent of the renin-angiotensin system probably due to bradykinin potentiation (Thurston & Swales, 1978). It would seem unlikely therefore that the renin-angiotensin system plays a role in blood pressure maintenance in chronic phase Goldblatt two-kidney renovascular hypertension in the rat. There is also disagreement about the blood pressure response to surgery in chronic Goldblatt two-kidney hypertension in the rat. Thus, in keeping with the present results, others have shown that the blood pressure remains elevated after removing the clipped kidney (Wilson & Byrom, 1941; Koletsky & Rivera-Velez, 1970; Thurston & Swales, 1974), but one group found the blood pressure was restored to normal (Gross, 1971). On the other hand, removal of the constricting clip in this and a previous study (Gross, 1971) caused an immediate fall in blood pressure, which remained normal thereafter.

Although the blood pressure responses were quite different, plasma renin concentration fell and remained at low or normal values no matter which surgical procedure had been performed. This supports the view therefore that plasma renin, although often elevated, is not responsible for blood pressure maintenance. The difference in blood pressure responses to declipping and nephrectomy suggests that the declipped kidney plays a major role in restoring blood pressure to normal. The hypothesis is strengthened by studies where nephrectomy was performed in declipped Goldblatt two-kidney hypertension. Thus, although removal of the 'untouched' kidney tended to reduce blood pressure, when the previously clipped kidney was removed a rise in blood pressure occurred (Floyer, 1951). Whether the mechanism depends on a change in sodium balance or possibly the secretion of a vasodilator substance by the previously clipped kidney has not been determined by the present studies.

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References


