Indomethacin or aprotinin infusion: effect on reversal of chronic two-kidney, one-clip hypertension in the conscious rat

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Summary
1. Blood pressure was monitored continuously in conscious rats with chronic Goldblatt two-kidney, one-clip hypertension.
2. Hypertension was rapidly reversed by removal of the constricting clip and the reversal was complete by 12 h, although structural vascular changes persisted for much longer.
3. Blood pressure in this model of hypertension was not affected by a 15 h infusion of either indomethacin or aprotinin.
4. The pattern of fall in blood pressure after removal of the clip was similar when animals were infused with either indomethacin or aprotinin. Blood pressure at 24 h was normal and did not differ significantly from that of unclipped rats given a glucose infusion.
5. The fall in blood pressure produced by unclipping therefore did not appear to be mediated via the prostaglandin or kallikrein systems.

Key words: aprotinin, Goldblatt hypertension, indomethacin, kallikrein, prostaglandin.

Abbreviation: PRC, plasma renin concentration.

Introduction
The maintenance of established hypertension in the chronic phase (> 16 weeks after clipping) of the Goldblatt two-kidney, one-clip model in the rat and its rapid reversal by unclipping in some studies [1–3] although not all [4] remains unexplained. Plasma renin concentrations are initially raised in this model but then fall and may become normal with increasing duration of hypertension [5–8] and renin–angiotensin blockade results in only partial correction of hypertension at this stage [8–10]. Negative sodium balance occurs during the development of hypertension, probably as a consequence of pressure natriuresis and diuresis through the untouched kidney [11]. This sodium deficit probably persists, as unclipping and correction of hypertension results in sodium retention which is maintained over several days [2]. In our hands unclipping produces a blood pressure fall to normal at 24 h [2, 12] before structural changes in the resistance vasculature could have regressed [13]. It would seem therefore that surgical correction of established hypertension in this model is not dependent on reversal of renin hypersecretion, vascular hypertrophy or sodium retention.

A possible alternative mechanism is that a vasodepressor system is activated by relief of ischaemia. Two such candidates within the kidney are the prostaglandin and kallikrein systems. To test the hypothesis that one of these systems may be involved in the lowering of blood pressure seen after unclipping, we have removed the clip from rats with two-kidney, one-clip hypertension of greater than 16 weeks' duration whilst they were infused with either indomethacin, a prostaglandin synthase inhibitor, or aprotinin, a potent kallikrein inhibitor.

Methods
Female white Wistar rats (160–250 g weight) were used throughout and all surgical procedures
were performed under ether anaesthesia. Two-kidney, one-clip hypertension was produced by placing a silver clip (0.2 mm internal diameter) on the left renal artery through a loin incision; the right kidney was not disturbed. Indirect blood pressures were measured by a light-plethysmographic method [14] and animals with blood pressures in excess of 150 mmHg more than 16 weeks after clipping were used for the study.

Polythene catheters were placed in the carotid artery (P50) and jugular vein (P30, P10) and then exteriorized between the scapulae and protected by a light flexible metal tube attached to the animals by a linen jacket. This tube was maintained under minimal tension by means of a lightly counter-balanced arm. On recovery from the anaesthetic animals were placed in a plastic container (30 cm × 30 cm) with free access to food and water. Blood pressure was monitored continuously with a Statham P23 gb transducer connected to a Grass polygraph recorder. Patency of the arterial catheter was maintained throughout this procedure and for the subsequent 24 h.

Plasma renin concentration (PRC) was measured on samples of tail vein blood obtained under light ether anaesthesia before the study began. The technique and effect of anaesthesia have been described previously [2].

Normal values for blood pressure and PRC were derived from female Wistar rats that were kept for the same period of time as those that were clipped, i.e. greater than 16 weeks.

The mean direct arterial blood pressure was calculated from the diastolic plus one-third of the pulse pressure. All results were expressed as mean values ± SEM and paired and unpaired Student's t-tests were used for statistical comparisons. PRC was transformed into logarithms before such comparisons were made, since PRC is not normally distributed.

Results

Initial plasma renin concentrations (PRC) in all four groups were similar and not significantly different (Table 1). No group demonstrated a significantly raised PRC when compared with PRC values obtained in normal rats by the same technique (n = 8, 65 ± 15-5 pmol of ANG I h⁻¹ ml⁻¹). All groups had a significantly raised blood pressure before infusion (Table 1) when compared with normal rats from the same colony (n = 8, 123 ± 34 mmHg, P < 0.01).

There was no significant change in blood pressure during the pre-operative infusion period whether animals were infused with glucose, aprotinin or indomethacin.

Table 1. Initial plasma renin concentration (PRC) and mean arterial blood pressure pre-infusion, pre-operation and 24 h postoperation in rats with chronic Goldblatt two-kidney, one-clip hypertension.

<table>
<thead>
<tr>
<th>Group (n = 8)</th>
<th>PRC (pmol of ANG I h⁻¹ ml⁻¹)</th>
<th>Direct mean blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-infusion</td>
<td>Pre-operation</td>
</tr>
<tr>
<td>Sham operation</td>
<td>96.6 ± 17</td>
<td>171 ± 5-6</td>
</tr>
<tr>
<td>Unclipped, 5% glucose</td>
<td>74.2 ± 7-1</td>
<td>173 ± 5-8</td>
</tr>
<tr>
<td>Unclipped, aprotinin</td>
<td>54.7 ± 19</td>
<td>192 ± 7-0</td>
</tr>
<tr>
<td>Unclipped, indomethacin</td>
<td>86.9 ± 20</td>
<td>179 ± 8-1</td>
</tr>
</tbody>
</table>

* P < 0.01 (comparison of blood pressure with pre-infusion blood pressure).
Reversal of renovascular hypertension

FIG. 1. Blood pressure response in chronic two-kidney, one-clip hypertension after sham operation (○) or unclipping during infusion of glucose (■), indomethacin (O) or aprotinin (▲). Operative procedures are indicated by the arrow.

Glucose infusion (unclipped and sham-operated rats)

Both groups demonstrated similar falls in blood pressure during ether anaesthesia. The sham-operated group rapidly regained its previously hypertensive level and remained hypertensive for the following 24 h. At the end of this period blood pressure was not significantly different from the pre-operative value. Animals that were unclipped behaved differently. There was a small rise in blood pressure post-operatively but not to hypertensive levels, and then followed a gradual decline in blood pressure to normal levels by 12 h, after which blood pressure remained normal (Fig. 1).

Indomethacin infusion

This group demonstrated a blood pressure response similar to that of unclipped glucose-infused animals. There was a small recovery of blood pressure after the operative fall and then a further fall to normal levels by 24 h. The vasodepressor response seen after an intravenous bolus injection of 0·5 mg of arachidonic acid before indomethacin infusion was 11·9 ± 4·2 mmHg and 15 h after commencement of indomethacin infusion it was 1·1 ± 1·1 mmHg.

Aprotinin infusion

The rise in blood pressure after unclipping reached hypertensive levels but did not reach pre-operative levels. The pattern of response was similar to that of the two groups which were unclipped, although the decline in blood pressure was slower. At 12 h the aprotinin group had a blood pressure that was significantly higher than that of the glucose-infused unclipped group (136 ± 9·3, 112 ± 5·4 mmHg respectively, \( P < 0·05 \)). However, at 24 h there were no significant differences between any of the unclipped groups (Fig. 1).

Discussion

We have previously demonstrated that complete reversal of two-kidney, one-clip hypertension in the rat cannot be achieved by renin–angiotensin system inhibition either in the early phase (< 6 weeks from clipping) when plasma renin concentrations are raised or in the chronic phase (> 16 weeks) where renin concentrations are lower
or normal. In the chronic phase the fall in blood pressure after a 12 h infusion of saralasin was small, whereas unclipping even after several months of sustained hypertension was effective in lowering blood pressure to normal [3]. In a recently reported study [12] we removed the longstanding clip from hypertensive rats during continuous infusions of either captopril or saralasin, infused in doses sufficient to block the renin-angiotensin system, and demonstrated an additional and far greater fall in blood pressure with unclipping than that achieved by pharmacological inhibition. That study also demonstrated that the pattern of response to unclipping was not altered by blockade of the renin-angiotensin system and that correction of blood pressure took place within 24 h of operation. Thus the fall in blood pressure seen after unclipping is neither dependent on reversal of renin hypersecretion, nor is it associated with a diuresis and natriuresis [2], and it occurs before vascular changes secondary to hypertension could have regressed [13]. It is therefore necessary to consider what alternative mechanisms may contribute to this correction of hypertension.

It has been reported that the fall in blood pressure after unclipping is mediated via a fall in total peripheral resistance [15] and this in the presence of vascular hypertrophy implies that smooth muscle tone is subnormal. This could be due to the removal of a vasopressor agent other than renin and although such renal vasopressor agents have been postulated, no physiological role has been defined [16–18]. An alternative explanation is that a vasodopressor substance is released. Three intrarenal vasodoppressor systems have been proposed: prostaglandins, kallikrein and the renomedullary lipids [19].

Although different effects of prostaglandins have been demonstrated in the isolated kidney and mesenteric preparations in the rat from those of other species [20, 21] no such differences have been seen on the systemic vasculature with respect to arachidonic acid, PGE₂, PGI₂, PGF₂α or indomethacin [22]. Indomethacin is a potent prostaglandin synthase inhibitor [23] and its acute administration in the dog [24] and rabbit [25] caused a rise in blood pressure. Further work on Goldblatt one-kidney, one-clip hypertension demonstrated that those treated with indomethacin developed higher pressures than did controls and this was associated with a significant suppression of prostaglandin E synthesis [28]. In the present experiments, with the same dose of indomethacin although for a shorter period of time, there was a small but non-significant rise in direct conscious blood pressure in hypertensive rats (P > 0.1). This period of infusion was sufficient in our view as prostaglandins are not stored in the kidney and inhibition of production occurs within a short time of administration of indomethacin [29]. There was no alteration in the blood pressure response to unclipping, with a similar pattern of response seen to that of glucose-infused unclipped rats; blood pressure was normal by 24 h. If prostaglandins were involved in the fall in blood pressure after unclipping, administration of indomethacin should result in either the blood pressure remaining elevated or at least some delay in the blood pressure fall.

The kallikrein system has potent vasodilator properties which are mediated by the generation of kinins. Aprotinin inhibits conversion of inactive prekallikrein and thereby suppresses kinin generation [30]. The dose regimen used in the present study was similar to that used by Kramer et al. in conscious rats [31], based on previous work [32, 33]. This regimen was reported to have no effect on systemic arterial blood pressure in normal rats but did reduce urinary immuno-reactive PGE₂ by 60% [31]. Other workers have shown that 50 000 k-i.u. of aprotinin subcutaneously twice daily over 4 days suppressed urinary kallikrein activity in normal rats by 60% with a similar fall in urinary prostaglandin in E₂ [34]. However, measurement of urinary kallikrein activity in vitro may not reflect accurately the effectiveness of aprotinin as an inhibitor of kallikrein activity in vivo [34]. It has been demonstrated that although high doses of aprotinin produce immediate and complete inhibition of kininogenase activity, lower concentrations produce a gradually increasing inhibition with time [32]. Therefore, although it could be expected that kinin generation would be significantly suppressed by the dose of aprotinin used in the present study, no assessment of this was made and complete inhibition cannot be assumed.

In our study no effect on blood pressure was seen when hypertensive rats were infused with aprotinin for 15 h, and the response of blood pressure fall after unclipping was similar to that in glucose-infused rats, although the decline to
normal blood pressure was slightly delayed but complete at 24 h. Furthermore, a previous study has demonstrated that urinary kallikrein levels fall in the 8 h after unclipping in this model of hypertension [35]. It is possible that kallikrein acts as a local hormone, perhaps acting through prostaglandin [36], but the present experiments suggest that neither is necessary for the surgical correction of hypertension in this model.

Other studies have shown that the presence of an intact renal medulla is essential to the restoration of normal blood pressure by surgical correction in this model [37]. It would seem that other vasodepressor substances, possibly renomedullary lipid [19], will have to be investigated further if one contends that the fall in blood pressure seen after unclipping is due to release of a vasodilator.

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References


