Mechanism of Enhanced Blood Pressure Rise After Reclipping Following Removal of a Renal Artery Clip in Rats

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SUMMARY The effect of removing a renal artery clip 14 to 18 days after its application was studied in two-kidney, one clip hypertensive rats. Blood pressure decreased to a normotensive level within 24 hours after the removal of the clip. The cardiovascular effects of reapplication of the clip and infusion of renin, angiotensin II, and norepinephrine were assessed at that time. Reapplication of the clip to the unclipped renal artery was followed in 1 hour by an increase in blood pressure to the level of sham-operated renal hypertensive rats. After reapplication of the clip, plasma renin activity increased to the same level as after the initial application. Application of the clip to the contralateral renal artery did not affect blood pressure and failed to increase plasma renin activity. It appears that renin release is a prerequisite for the rapid rise of blood pressure after reapplication of the clip. The infusion of renin, angiotensin II, and norepinephrine for 90 minutes caused an enhanced blood pressure response similar to that observed following reapplication of the clip. The increase in blood pressure in control rats was associated with bradycardia, which was absent in unclipped rats. The enhanced response of unclipped rats to an infusion of renin was abolished by pithing. It is concluded that removal of a renal artery clip unmasks a hyperreactivity of the cardiovascular system to reapplication of the clip. (Hypertension 2: 4-13, 1980)

KEY WORDS • two-kidney, one clip hypertension • renin • angiotensin II • norepinephrine • plasma renin activity

In rats with an intact contralateral kidney, a small rise of blood pressure (10-20 mm Hg) occurs within 1 to 2 hours following the application of a solid silver clip to the left renal artery.1,2 The blood pressure increases gradually to a stable level within 1 to 3 weeks, depending on the size of the clip used.3 One day after the removal of the clip in rats whose systolic blood pressure had reached 200 mm Hg or more, the blood pressure had decreased to a normotensive level.1,3,4,5 Reapplication of the clip to the unclipped renal artery was followed by a rise in blood pressure with hypertensive levels being reached within 2 hours.1,5 In two-kidney, one clip hypertensive rats an augmented secretion of renin and a retention of water and sodium appear to be important factors for the development and maintenance of hypertension.6,8 On the other hand, the enhanced blood pressure response of unclipped rats to reapplication of the clip points to an increased reactivity of the cardiovascular system. Such a change in the reactivity of the cardiovascular system could equally well contribute to the maintenance of the hypertension.9 The present study in renal hypertensive rats, unclipped rats, and normotensive control rats explored the blood pressure response and the response of the renin-secreting system to application of the clip to the unclipped or to the contralateral renal artery. The blood pressure response to renin, angiotensin II, and norepinephrine given intravenously was assessed as well.

Methods

Animals

Male rats of a Wistar strain (outbred stock, WU/Cpb, Zeist, the Netherlands) weighing 140-160 g were used. Rats had free access to food (Trouw, Amsterdam, the Netherlands; 111 μEq sodium/g) and tap water. They were housed in a temperature-controlled room (22-24°C) illuminated between 5 a.m. and 7 p.m.

General Procedures

Under ether anesthesia, all rats underwent two operations. During the first operation a solid silver clip with an internal diameter of 0.20 mm was applied to the left renal artery, leaving the contralateral kidney undisturbed.3 Control rats were operated on in the same way, but the clip was not applied (sham procedure). After 14 to 18 days the systolic blood pressure was checked, under light ether anesthesia, by a tail sphygmographic method. The systolic blood pressure and heart rate of the sham-operated rats were 127 ± 1 mm Hg and 390 ± 3 beats/min (n = 106) and of the hypertensive rats 196 ± 2 mm Hg and
Contralateral (right) renal artery (n = 8); NS (Normal-pressure was thus recorded for 2 hours after the operation. Angiotensin II (A) was started 30 minutes after the operation, as in Experiment 1. This procedure enables us to compare blood pressure increases, induced by reclip-

Experiment 1

The caudal artery of the tail was cannulated 24 hours after the removal of the clip or after sham operation. A U-shaped piece of a cannula (PP 25, Portex; 1.5 cm length) was inserted 1 cm into the caudal artery at the base of the tail. The PP 25 cannula was connected to a piece of PP 60 cannula (15 cm length) which passed subcutaneously to the neck. Blood pressure was measured with a Statham transducer (model P 23 AC) and monitored on a Grass polygraph. Heart rate was calculated from the blood pressure recordings. Thereafter a clip was applied to the left or right renal artery, or the kidney was exposed. The duration of the operation (cannulation followed by application of the clip or by sham operation) was 10–15 minutes. Blood pressure was recorded for 2 hours. In previous experiments (unpublished data), we found that blood pressure of normotensive or renal hypertensive rats was restored to the preoperative level within 30 minutes after the operation. At the end of the 2-hour observation period, the rats were decapitated, and trunk blood was collected for the estimation of plasma renin activity. There were seven groups tested: HS (Hypertensive-Sham) renal hypertensive rats that were sham-operated (n = 8); US (Unclip-Sham) unclipped rats that were sham-operated (n = 7); UL (Unclip-Left recclip) unclipped rats that received a clip on the left renal artery (n = 10); UR (Unclip-Right recclip) unclipped rats that received a clip on the right renal artery (n = 8); NS (Normal-Sham) normotensive control rats that were sham-operated (n = 7); NL (Normal-Left clip) normotensive control rats that received a clip on the left renal artery (n = 8); NR (Normal-Right clip) normotensive control rats that received a clip on the right renal artery (n = 8).

Experiment 2

The caudal artery was cannulated in the same way as in Experiment 1. A cannula (PP 25, Portex) was inserted into the jugular vein and passed subcutaneously to the neck. An infusion with saline (V), renin (Re), or angiotensin II (A) was started 30 minutes after the operation and continued for 90 minutes. Blood pressure was thus recorded for 2 hours after the operation, as in Experiment 1. This procedure enables us to compare blood pressure increases, induced by reclip-

Estimation of the Plasma Renin Activity

Blood was collected from the neck for 30 seconds immediately after decapitation; plastic tubes were used that contained 10 mg EDTA dissolved in 0.2 ml 0.9% NaCl. The blood samples were immediately placed on ice, and the plasma was stored at —20°C.
Plasma renin activity was measured according to the method of Haber et al., using a radioimmunoassay for angiotensin I. Plasma renin activity was calculated as the amount of angiotensin I generated from endogenous substrate per ml plasma during incubation of the sample at pH 6.5 for 1 hour at 37°C.

Statistical Analyses

The results were expressed as mean ± SEM. The significance of differences between two groups was determined with Student's t test. Regression analysis was applied to the data from the dose-response curves. A probability level of 0.05 was the criterion of significance.

Results

Experiment 1

The mean blood pressure of renal hypertensive rats had decreased to a normotensive level within 24 hours after removal of the clip (106 ± 5 mm Hg, compared with 107 ± 4 mm Hg for sham-operated control rats US-NS, fig. 1). Reapplication of the clip to the unclipped renal artery was followed by a rise of blood pressure to hypertensive levels within 1 hour (165 ± 5 mm Hg, compared with 169 ± 9 mm Hg for sham-operated renal hypertensive rats (UL-HS). Application of the clip to the contralateral renal artery in unclipped rats did not induce a significant blood pressure rise (113 ± 3 mm Hg, compared to 106 ± 5 mm Hg for sham-operated unclipped rats, UR-US). Application of the clip to the left or right renal artery in control rats induced a moderate blood pressure rise (left: 134 ± 3 mm Hg; right: 129 ± 3 mm Hg, compared with 107 ± 4 mm Hg for sham-operated control rats NL-NR-NS). The mean blood pressure of the different groups did not change significantly during the second hour of the observation period (fig. 1, dotted lines). From previous experiments in which the blood pressure was measured for 24 hours, we knew that the blood pressure reached maximal levels within 2 hours after reapplication of the clip (ten Berg and de Jong, unpublished data).

Plasma renin activity showed a pattern similar to that of the blood pressure levels in the various groups (fig. 1). Following removal of the clip, plasma renin activity decreased to the level seen in sham-operated normotensive control rats, but it returned to the level in sham-operated hypertensive rats when the clip was reapplied. Application of a clip to the contralateral (right) renal artery of unclipped rats resulted in a level similar to that in sham-operated normotensive control rats or sham-operated normotensive unclipped rats. In rats clipped for the first time, plasma renin activity increased to the same level as in replipped rats; the latter showed a much larger rise in blood pressure.

Experiment 2

As shown in figure 2, the blood pressure of normotensive control rats and of normotensive unclipped rats (renal hypertensive rats in which the renal artery clip had been removed 24 hours before) did not differ during an infusion with saline (at the end of the 90 min infusion, the levels were 112 ± 3 and 116 ± 4 mm Hg respectively) (NV-UV). There was a significant difference in blood pressure between normotensive control rats, renal hypertensive rats, and unclipped rats (rats in which the clip had been removed 24 hr previously). The bars express the mean blood pressure determined 1 hour after the operation. The dotted lines express the mean blood pressure determined 2 hours after the operation. HS = Hypertensive-Sham (renal hypertensive rats that were sham-operated, n = 8); US = Unclip-Sham (uncropped rats that were sham-operated, n = 7); UL = Unclip-Left (uncropped rats that received a clip on the left renal artery, n = 10); US = Unclip-Right (uncropped rats that received a clip on the right renal artery, n = 8); NS = Normal-Sham (normotensive rats that were sham-operated, n = 7); NL = Normal-Left clip (normotensive rats that received a clip on the left renal artery, n = 8); NR = Normal-Right clip (normotensive rats that received a clip on the right renal artery, n = 8).

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Experiment 1

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respectively (NRe-URe). The difference in mean blood pressure between control and unclipped rats was nearly the same (Δ 28 and Δ 29 mm Hg respectively) 2 hours after the application of a clip to the left renal artery or at the end of the infusion with renin (2 hours after the operation). There was no difference in heart rate between control and unclipped rats in the case of sham application of a clip or infusion with saline. There was, however, a marked difference in all groups of rats in which a rise of blood pressure had been induced (fig. 2 upper panels); only the control rats showed pronounced bradycardia.

Infusion with angiotensin produced differences in blood pressure and heart rate similar to those caused by renin (NA-UA). The difference in mean blood pressure at the end of the 90-minute infusion of saline and of renin was enhanced in unclipped rats (Δ 33 and Δ 58 mm Hg respectively for control and unclipped rats) (fig. 2 lower panels). Blood pressure responses to the infusion of saline and renin were also assessed in renal hypertensive rats, which were sham-unclipped. The difference at the end of the infusions was attenuated in renal hypertensive rats (Δ 18 mm Hg).

**Experiment 3**

The relationship between the change in blood pressure and the bolus dose of angiotensin II (i.v.) in normotensive control rats and normotensive unclipped rats is shown in figure 3. The regression line for control rats was \( y = 28 \log x + 7 \) and that for unclipped rats, \( y = 39 \log x - 3 \). The difference between the slopes was small but significant \( p < 0.005 \), indicating an increase in responsiveness of the unclipped rats.

The pressor response to the 10-minute infusions with angiotensin II did not differ between control and unclipped rats, but the decrease of blood pressure after infusion termination did differ significantly \( p < 0.01 \) for doses of 90 and 270 ng/kg/min (fig. 4). Infusions with angiotensin II were followed by infusions with norepinephrine. There was a similar difference \( p < 0.01 \) in the blood pressure decrease of control and unclipped rats after the termination of the infusions with norepinephrine, for the highest dose (fig. 4). Infusions with angiotensin II were given in the same way to the same animals 24 hours later (i.e., 48 hr after the removal of the clip), and a similar difference in blood pressure decrease \( p < 0.05 \) was observed between control and unclipped rats (−44 ± 5 and −30 ± 4 mm Hg decrease respectively) at 2.5 min after a dose of 270 ng/kg/min.

The blood pressure increase and heart rate change during the 90-minute infusions with various doses of renin were significantly different for the two groups (fig. 5). The lowest dose of renin resulted in the greatest difference in blood pressure increase between unclipped and control rats (166 – 138 = Δ 28 mm Hg, at 45 min). This difference was similar to that observed after application of the clip (168 – 140 = Δ 28 mm Hg). The plasma renin activity in control and unclipped rats did not differ significantly at the end of the renin infusions (table 1). The lowest dose of renin

**Figure 2.** Heart rate (upper panels) and mean blood pressure (lower panels) of control rats (N) and unclipped rats (U). On the left side is shown the mean blood pressure and heart rate of four groups in Experiment 1: NS (Normal-Sham); US (Unclip-Sham); NL (Normal-left clip); UL (Unclip-Left recap). On the right side is shown the mean blood pressure and heart rate determined at the end of an infusion with vehicle: NV (Normal-Vehicle) and UV (Unclip-Vehicle); renin (6.25 × 10^4 GU/kg/min); NRe (Normal-Renin) and URe (Unclip-Renin); or angiotensin II (270 ng/kg/min): NA (Normal-Angiotensin) and UA (Unclip-Angiotensin). Infusions were started 30 minutes after sham operation and continued for 90 minutes. Thus, the blood pressure and heart rate values presented in fig. 2 are all determined 2 hours after an operation. Data are mean values ± SEM of 9 to 13 rats.
Figure 3. Increase in mean blood pressure after intravenous injections with angiotensin II. Closed circles = normotensive unclipped rats (renal hypertensive rats, in which the clip was removed 24 hours previously). Open triangles = normotensive control rats. Data are mean values ± SEM (vertical lines) for 11 rats in each group. The lines express the dose-response curves: y = 39 log x - 3, and y = 28 log x + 7 respectively for unclipped and control rats. Difference in slope of both curves was significant (p < 0.005).

Figure 4. Change in mean blood pressure during the intravenous administration of angiotensin II (upper panels) and norepinephrine (bottom panels) for 10 minutes. The stippled horizontal bars indicate the time of the infusions with angiotensin or norepinephrine. These infusions were followed by saline infusions. Closed circles = normotensive unclipped rats (renal hypertensive rats, in which the clip had been removed 24 hours previously). Open triangles = normotensive control rats. Data are mean values ± SEM (vertical lines) for 11 rats in each group.
FIGURE 5. Mean blood pressure (left side) and heart rate (right side) during infusions with renin for 90 minutes. Renin was given in three different doses to control rats and to unclipped rats (renal hypertensive rats in which the clip was removed 24 hr previously). Each dose was given to different rats. Closed circles = unclipped rats; open circles = control rats. Data are mean values from six to nine rats. Level of significance between control and unclipped rats: x = p < 0.05; xx = p < 0.01; xxx = p < 0.001.

TABLE 1. Plasma Renin Activity (Al ng/ml/hr) in Peripheral Blood

<table>
<thead>
<tr>
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<th>Control rats</th>
<th>Unclipped rats</th>
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<tr>
<td></td>
<td>Plasma renin activity (Al ng/ml/hr)</td>
<td>Mean blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Application of clip to left renal artery</td>
<td>68 ± 8</td>
<td>140 ± 4</td>
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<tr>
<td>Sham application of clip</td>
<td>14 ± 2</td>
<td>104 ± 2</td>
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<tr>
<td>Infusions with renin (GU/kg/min):</td>
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<tr>
<td>5 10^4</td>
<td>50 ± 4</td>
<td>133 ± 4</td>
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<tr>
<td>12.5 10^4</td>
<td>107 ± 24</td>
<td>144 ± 7</td>
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<tr>
<td>31.3 10^4</td>
<td>220 ± 13</td>
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Plasma renin activity in peripheral blood of normotensive control rats and normotensive unclipped rats (renal hypertensive rats, in which the clip was removed 24 hours before). The blood was collected at the end of the infusions with renin (see fig. 5), or 2 hours after the application of the clip or sham operation (see fig. 1). Mean blood pressure just prior to the moment of blood collection are mentioned. Data are mean values ± SEM.
increased plasma renin activity to a level similar to that measured 2 hours after application of the clip. During the infusion with norepinephrine (fig. 6), the differences in blood pressure and heart rate were similar to those observed during the infusions with renin.

**Experiment 4**

The enhanced response of unclipped rats to renin was abolished by pithing (fig. 7). The pithing procedure lowered the mean blood pressure to a similar level in unclipped as in control rats (47 ± 4 and 53 ± 4 mm Hg respectively). To obtain a blood pressure increase in pithed control rats (PN) similar to that obtained in intact control rats (IN) (induced with a dose of $5 \times 10^{-8}$ GU/kg/min renin), a dose six times higher was required. This higher dose of renin caused a blood pressure rise in pithed unclipped rats (PU) which was not significantly different from the increase observed in pithed control rats (PN) (fig. 7). In the pithed control and unclipped rats, no bradycardia was observed during the infusion with renin.

**Discussion**

The blood pressure of renal hypertensive rats decreased to normotensive levels within 24 hours after removal of the renal artery clip; this is consistent with previous reports. The blood pressure regulating systems of these normotensive unclipped rats, however, may still operate at a different level at that time. The enhanced blood pressure rise following the application of a clip to the unclipped renal artery points to such an altered reactivity. The difference in blood pressure rise between unclipped and control rats over a 2-hour period following the application of a clip was very similar to that reported by Skulan et al. The enhanced response of unclipped rats to the application of a renal artery clip could be caused by an exaggerated secretion of pressor factors by the kidney and/or altered reactivity of the cardiovascular system. Plasma renin activity reached a similar level in unclipped and control rats after application of a clip, as well as after infusions with renin. The difference in the increase in blood pressure induced by infusing renin into unclipped and into control rats was similar to the
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The mean blood pressure increase (mm Hg) is shown in the figure below for intact, conscious (solid lines) unclipped rats (closed circles) and control rats (open circles). The mean blood pressure of these two groups are shown in fig. 5. A six-times-higher dose of renin was given to pithed (dotted lines) unclipped rats (closed circles) and control rats (open circles). Basal blood pressure levels at the beginning of the infusions were for: IU (Intact-Undipped) 99 ±4 mm Hg, n = 6; IN (Intact-Normal) 97 ±3 mm Hg, n = 9; PU (Pithed-Unclipped) 47 ±4 mm Hg, n = 7; and PN (Pithed-Normal) 53 ± 4 mm Hg, n = 7.

Figure 7. Change in mean blood pressure during a 90-minute infusion with renin (5 10^-4 GU/kg/min) to intact, conscious (solid lines) unclipped rats (closed circles) and control rats (open circles). The mean blood pressure of these two groups are shown in fig. 5. A six-times-higher dose of renin was given to pithed (dotted lines) unclipped rats (closed circles) and control rats (open circles). Basal blood pressure levels at the beginning of the infusions were for: IU (Intact-Undipped) 99 ± 4 mm Hg, n = 6; IN (Intact-Normal) 97 ± 3 mm Hg, n = 9; PU (Pithed-Unclipped) 47 ± 4 mm Hg, n = 7; and PN (Pithed-Normal) 53 ± 4 mm Hg, n = 7.

A slight but significant difference in the slope of the angiotensin II dose-response curves of unclipped and control rats was observed in the present study, indicating an enhanced reactivity of the cardiovascular system in unclipped rats. Infusing angiotensin II and norepinephrine for 10 minutes did not lead to a significant difference in pressor response between unclipped and control rats. It is likely that the highest dose was still too low to show a difference in reactivity, as was indicated by the occurrence of such a difference for the highest injected doses of angiotensin. After termination of the infusions, the return of blood pressure to baseline was delayed in the unclipped rats. The similarity of the delayed return after administration of angiotensin and norepinephrine does not point to an important role of a decreased metabolism of angiotensin in such a delay.

It is supposed that the enhanced reactivity of the cardiovascular system of hypertensive rats is caused by an increased reactivity of the peripheral vessels to constrictor stimuli. This could be an enhanced contractility or enhanced sensitivity of the smooth muscles. Difference in threshold between unclipped and normal rats was not observed in the present study, which would point to an enhanced contractility.

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the enhanced pressor response of unclipped rats to renin, it is unlikely that only an enhanced reactivity of the peripheral vessels was involved. The absence of reflex bradycardia during the blood pressure increase in unclipped rats and the retarded normalization of blood pressure after termination of the 10-minute infusions in unclipped rats point to altered autonomic reflexes. It is generally accepted that the baroreceptor reflex is reset within 1 to 2 days after the onset of hypertension.21 Liard et al.22 reported that elimination of the baroreceptor reflex in dogs caused an enhanced blood pressure rise after narrowing the renal artery. The difference in blood pressure rise between denervated and control dogs, after narrowing the renal artery, was of the same order as that found between unclipped and control rats in our study. It is possible that the baroreceptor reflex is still reset 24 hours after the removal of the clip, notwithstanding the normalization of the blood pressure. Salgado and Krieger,23 however, showed that baroreceptor function was normalized within 6 hours after the removal of the clip in chronic renal hypertensive rats.

It is suggested that an increase in the reactivity of the cardiovascular system, as is observed in unclipped rats in our study, gradually develops during the course of renal hypertension. This course can be divided into several stages.24 During the initial phase, hypertension is renin-dependent, but in the later stages a discrepancy has been reported between the level of the blood pressure and the activity of the renin angiotensin system. The discrepancy perhaps can be explained by an altered reactivity of the cardiovascular system to angiotensin.5,25-27 It is tempting to speculate that such an enhanced reactivity to angiotensin could be induced by angiotensin itself during the early phase of the development of renal hypertension; the term "autopotentiation" has been suggested to describe this effect.28 Several investigators showed that systemic arterial pressure rose progressively over a period of 1 day to 3 weeks to reach a plateau during the chronic i.v. administration of a subpressor or mild pressor dose of angiotensin.29-31 Recently, this was confirmed by Slack et al.,31 who reported that the blood pressure of dogs increased gradually, reaching a plateau within 2 weeks, during the chronic infusion of angiotensin II given in a subpressor dose. An enhanced blood pressure response to this peptide developed in the course of the administration of angiotensin II.29

In conclusion, our study shows that the removal of the renal artery clip in two-kidney, one clip hypertensive rats, which is followed by rapid normalization of the blood pressure, unmaska a hyperreactivity of the cardiovascular system. The data point to a role of renin secretion and altered autonomic reflexes in the enhanced blood pressure response.

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