Contrasting Regression of Blood Pressure and Cardiovascular Structure in Declipped Renovascular Hypertensive Rats

Stinne Kvist, Michael J. Mulvany

Abstract—We investigated the time relationship between changes in blood pressure and changes in the structure of the resistance vasculature. Blood pressure, heart/body weight ratio, and morphology and function of mesenteric resistance arteries from 1-kidney, 1-clip renovascular hypertensive rats were followed before and after declipping at age 14 weeks. The rats were divided into 5 groups, which were investigated 6 hours, 24 hours, 1 week, 4 weeks, and 8 weeks after declipping and compared with 2 normotensive and 2 renovascular hypertensive control groups at 14 weeks and 18 weeks. Systolic blood pressure was elevated 2 weeks after application of the clip and stabilized after 6 weeks. Declipping induced a prompt fall in blood pressure within 6 hours, and blood pressure was normalized within 1 week. Heart/body weight ratio was increased in renovascular hypertensive rats, and declipping induced a gradual decrease in the ratio, which was normalized within 4 weeks. Media/lumen ratio and media area of mesenteric resistance arteries were increased in renovascular hypertensive rats, and declipping did not affect media/lumen ratio and media area within 8 weeks, although there was a tendency for some regression of media/lumen ratio. There were no differences in response to high potassium, noradrenaline, or acetylcholine. Thus, these findings show definitively that declipping causes rapid reversal of renovascular hypertension in rats accompanied by gradual reduction of the heart/body weight ratio but lack of normalization in the mesenteric resistance vessels. This provides clear evidence that neither vascular nor cardiac structural changes are capable of keeping rats hypertensive. (Hypertension. 2003;41:540-545.)

Key Words: hypertension, renal rats hypertrophy arteries blood pressure heart kidney remodeling

Hypertension is complicated by hypertrophy of the heart and abnormal structure of peripheral arteries in renovascular hypertensive rats and spontaneously hypertensive rats (SHR), as well as in humans with essential or renovascular hypertension. Hypertension and the abnormal structure of the heart and vessels in SHR and essential hypertension can be reversed by pharmacological treatment, but the cause of the disease has not been treatable. In contrast, the cause of renovascular hypertension is known and can be removed and indeed appears to be the only way to normalize the blood pressure, as pharmacological treatment is difficult and seldom effective. Because the structural changes of heart and vessels occur in parallel with the high blood pressure in all forms of hypertension, it has been suggested that the structural changes may be one of the causes of hypertension, or at least contribute to the maintenance of hypertension. However, there are a number of reports indicating that after an intervention, vascular structure and pressure are not correlated. For example, when the renal artery stenosis is removed in 1-kidney, 1-clip (1K-1C) rats, blood pressure returns to normotensive levels almost immediately, whereas similar observations have been made concerning the angiotensin II infusion model of hypertension after cessation of infusion. In both cases, the rapidity of the blood pressure fall makes it likely that the fall occurs before any regression of resistance vessel structure, but there is no direct evidence on this point. Stacy and Prewitt found little change in cremaster arteriole structure either before or 4 weeks after declipping measured histologically, although in vivo measurements indicated that 1K-1C hypertension was associated with structural changes that partially regressed after declipping. Indirect evidence for slow regression of resistance vessel structure after declipping was obtained in perfusion experiments in the hindquarter preparation. Slow regression of heart weight after declipping has also been reported. We therefore decided to examine this question directly in the resistance vasculature and to test critically the hypothesis that blood pressure and resistance vessel structure are not necessarily correlated after an intervention that corrects hypertension. To address this hypothesis, the current study was performed in 1K-1C rats in which hypertension is pronounced and independent of renin. After development of a stable high blood pressure, we removed the clips and studied the time relationship of the regression of blood pressure, heart and kidney weight, and resistance artery morphology measured on a myograph from 6 hours to 8 weeks thereafter.
Methods

Materials
One hundred ninety-nine Male Wistar rats were received (5 weeks old) from Møllegaard Breeding Center, Lille Skensved, Denmark. An equilibration period for at least 4 days was allowed before the start of experimental procedures. The rats had free access to food and tap water except at the time of blood pressure measurements (see below). All rats were killed by exposure to CO₂ for final studies.

Health status was supervised by the local animal welfare officer, and all experiments were performed according to Danish legislation.

Protocol for Grouping
The rats were divided into a noninvasive, sham-operated group (sham) of 30 rats and a hypertensive 1K-1C group of 101 rats. All operations were performed in rats 6 to 7 weeks of age. The sham rats were subdivided in 2 groups with 15 rats in each, which were studied at 14 and 18 weeks of age, respectively. The 1K-1C rats were randomly subdivided into 7 groups: 2 groups with 15 rats in each, which served as hypertensive controls (control 1K-1C) at 14 and 18 weeks of age, respectively, and 5 groups that were declipped (declipped 1K-1C) at 14 weeks of age, and the rats were subsequently killed 6 hours (16 rats), 24 hours (14 rats), 1 week (16 rats), 4 weeks (15 rats), and 8 weeks (10 rats) after declipping.

Blood Pressure
Systolic blood pressure was measured by the tail-cuff method with a plethysmograph (LE5000, Lettica). Before measurements, rats were preheated for 20 to 30 minutes at 35°C in their cages. Rats were then moved to a small heated container, where the rats were trained to stay for periods up to 20 minutes. In each rat, 4 to 6 measurements were made and mean values were used. The equipment was calibrated every day by comparison with a mercury column.

Blood pressure measurements in 1K-1C rats were made before insertion of clips, 2 weeks after and every week hereafter until systolic blood pressure increased above 160 mm Hg. Afterward, blood pressure was measured in control 1K-1C rats at the time of killing, whereas in declipped 1K-1C it was measured just before declipping and at the time of killing. Blood pressure measurements in the sham rats took place just before sham operation and at the time of killing.

Preparation of Sham and 1K-1C Rats
Rats were anesthetized with 7 mg/kg IP methohexital, and, if necessary, supplemented with small doses of methohexital directly to the abdominal cavity during the operation. The fur was shaved off the abdomen and the operation was performed through a 4-cm longitudinal incision as described by Stacy and Prewitt. The left renal artery was dissected free of fat and connective tissue, and in animals chosen to be hypertensive, a U-shaped silver clip, 230- to 250-μm internal diameter, was placed around the renal artery as far as the clips could be inserted. Sham rats were prepared by the same procedure as the 1K-1C rats apart from the application of a clip. In declipped rats, the clips were gently removed from 1K-1C rats after cleaning for adhesive tissue in an operation through the initial incision.

In Vitro Protocols
Dissection
After killing with exposure to CO₂, the rat was weighed and heart, kidney, and a piece of the mesenteric vascular bed were cut out and placed in ice-cold physiological salt solution (PSS, see below for composition). The atria of the heart were cut away and the heart was wiped with paper and weighed. The kidney was cleaned from fat and connective tissue, wiped with paper, and weighed. From the mesenteric vascular bed, 2 segments of vessels from the third branch, 2 mm long and with an internal diameter of ~230 μm, were dissected free from fat and mounted in a wire myograph. In the myograph, vessels were bubbled with 5% CO₂ in air and allowed to equilibrate at 37°C for 30 minutes.

Morphometric Measurements
After equilibration, the myograph was placed on a microscope and wall structure was measured at 6 different positions by an ocular micrometer. Vessels were then set to normalized diameter, L₀, that is, 0.9 times the internal diameter the vessels would have if fully relaxed and exposed to a transmural pressure of 100 mm Hg, calculated on the basis of the Laplace relation. Mean of media thickness and internal diameter was expressed as media/lumen ratio.

Functional Measurements
After normalization, vessels were exposed to PSS with potassium (K-PSS, see below for composition) for 2 minutes as a measure of contractile capacity. A concentration response curve to acetylcholine (10⁻⁸–10⁻⁴ mol/L) was performed on top of a precontraction with 3 μmol/L noradrenaline by adding the drug in half-logarithmic concentrations to the bath every second minute.

Exclusion Criteria
Rats were excluded from the study if (1) systolic blood pressure in rats with clips was below 165 mm Hg after 8 weeks; (2) systolic blood pressure in sham rats was above 140 mm Hg; (3) internal diameter of the vessels in the myograph was below 150 μm or above 350 μm; (4) active pressure response of the vessels [active wall tension/(L₀/L₁)]² to K-PSS for 2 minutes was below 13 kPa; (5) if renal scars caused by renal infarction were present at the time of killing.

Solutions and Drugs
Physiological salt solution consisted of (mmol/L): 119 NaCl, 4.7 KCl, 1.17 MgSO₄·7H₂O, 25 NaHCO₃, 1.18 KH₂PO₄, 0.0026 EDTA, 5.5 glucose, and 1.6 CaCl₂. In K-PSS, NaCl was replaced with equimolar KCl.

Drugs used were methohexital (Brietal 7, Lilly), (−)-noradrenaline hydrochloride (Sigma Chemicals Co), and acetylcholine (Fluka AG).

Data Analysis
Analysis was based on the mean of the 2 vessels taken from each animal. All values are given as mean ± SEM. One-way ANOVA was used for comparisons between groups. If ANOVA showed a significant difference, data were analyzed by the Student 2-tailed, unpaired t test and linear regression for specified groups. A value of P<0.05 was considered significant.

Results
Basal Characteristics
After the application of the clips or sham operation at age 6 to 7 weeks old, 68 rats (34%) were lost as the result of uremia/malignant hypertension (n=46), rupture of the renal artery when declipping (n=8), anesthesia (n=6), infarction of the kidney (n=1), unknown reason (n=7), or exclusion according to the above exclusion criteria. This left 131 rats for investigation.

Before application of clips, the average weight of the rats was 163±3 g, and the average systolic blood pressure and heart rate were 116±1 mm Hg and 409±4 beats/min, respectively. There were no differences in the parameters between the groups.

Blood Pressure
The blood pressure of the 1K-1C rats was increased 2 weeks after application of clips and increased further until 6 weeks after application of clips. Afterward, the blood pressure remained stable. After declipping, there was an immediate
When rats were 14 weeks old, 8 weeks after the application of clips, the media/lumen ratio of mesenteric resistance arteries was increased in the 1K-1C rats compared with the age-matched sham rats. There was no further increment in media/lumen ratio between 14 and 18 weeks of age, either for sham rats or control 1K-1C rats. Declipping did not induce regression of media/lumen ratio of mesenteric resistance arteries (ANOVA for declipped 1K-1C groups; \( P=0.11 \)). Linear regression of media/lumen ratio of resistance arteries from declipped 1K-1C rats showed a tendency for regression (\( P=0.07 \)).

Media area and media thickness of the mesenteric resistance arteries were increased in the control 1K-1C rats compared with the sham rats at 14 and 18 weeks of age. There was no regression of media area and media thickness after declipping. There were no significant differences in internal diameter of arteries from sham rats, control 1K-1C rats, or declipped 1K-1C rats.

**Functional Measurements**

No functional differences were found (Table 3). Thus, there were no differences in maximal effect of acetylcholine between the sham, control 1K-1C, or declipped 1K-1C groups. The vessels relaxed by \( \approx 65\% \) of the noradrenaline preconstriction when exposed to acetylcholine in all groups. The acetylcholine concentration for half-maximal relaxation was similar in all groups (compared with 0.1 \( \mu \)mol/L, data

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**Table 1. Weight of Body, Left Kidney, and Heart in SHAM, Control 1K-1C, and Declipped 1K-1C Rats**

<table>
<thead>
<tr>
<th>Rat Type</th>
<th>BW, g</th>
<th>KW, g</th>
<th>KW/BW( \times 10^{-3} )</th>
<th>HW, g</th>
<th>HW/BW( \times 10^{-3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declipped 1K-1C rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 wk</td>
<td>304±11*</td>
<td>1.6±0.06**</td>
<td>5.2±0.2</td>
<td>1.2±0.05**</td>
<td>4.0±0.2**</td>
</tr>
<tr>
<td>18 wk</td>
<td>395±8</td>
<td>1.9±0.06</td>
<td>4.9±0.1</td>
<td>1.2±0.07</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>SHAM rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 wk</td>
<td>361±5</td>
<td>1.9±0.10</td>
<td>5.3±0.3</td>
<td>0.9±0.03</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>18 wk</td>
<td>405±8</td>
<td>1.9±0.08</td>
<td>4.8±0.2</td>
<td>1.0±0.07</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>Control 1K-1C rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 wk</td>
<td>315±12*</td>
<td>1.5±0.05**</td>
<td>4.9±0.2</td>
<td>1.3±0.05**</td>
<td>4.1±0.2**</td>
</tr>
<tr>
<td>18 wk</td>
<td>374±18</td>
<td>1.9±0.09</td>
<td>5.1±0.2</td>
<td>1.3±0.08*</td>
<td>3.6±0.2**</td>
</tr>
</tbody>
</table>

BW indicates body weight; KW, kidney weight; and HW, heart weight.

* \( P<0.05 \) compared with age-matched SHAM rats; ** \( P<0.01 \) compared with age-matched SHAM rats.
not shown). There were no differences in contractile responses to noradrenaline and K-PSS between the groups.

**Discussion**

The main finding of this study was that while declipping caused a rapid normalization of blood pressure, heart weight and resistance vessel morphology regressed either slowly or not at all.

Given the strong association between blood pressure and vascular structure, as discussed in the Introduction, it was surprising that the structure of the small arteries was not significantly regressed 8 weeks after declipping, despite normal pressure for almost the whole of this time. This is in apparent contrast to the relation seen in SHR. Here, for example, treatment of established hypertension with different vasodilator antihypertensive drugs for 8 to 12 weeks results in a gradual reduction of blood pressure and regression of cardiac hypertrophy and regression of abnormal structure of small resistance vessels. Similarly, in humans, successful treatment of essential hypertension with vasodilators causes regression of heart weight and resistance vessel structure, at least after 1 year. As described in the Introduction, the retarded regression of the hypertrophy of the heart despite fast normalization of blood pressure in renovascular hypertensive rats was also observed by Friberg and Nordborg and Lundgren and similar findings were observed in the arterioles of the rat cremaster muscle by Stacy and Prewitt and indirectly in the hindquarter perfusion preparation by Lundgren. These indications of fast normalization of blood pressure and the retarded or absent regression of heart weight and resistance vessel structure in declipped renovascular hypertensive rats are now strongly supported by the direct investigations of the structure of the vessels in our experiments. It appears therefore to be a general finding in these animals that hypertrophy of vessels and heart are not able to keep blood pressure high, and any hypertensive action of the hypertrophy is eliminated either by removal of a vasoconstrictor agent or by activation of a vasodilator agent. The existence of such an agent has also been proposed by Muirhead et al., who provided evidence for an antihypertensive neutral renomedullary lipid, medullipin, suggested to be released from the declipped kidney.

The divergence in the rates of normalization in blood pressure and cardiovascular structure seen in this study confirms other situations in which blood pressure and cardiovascular structure are not always tightly associated. Thus, although this tight association is normally seen in most forms of hypertension, as discussed above, specific interventions may interrupt this association. For example, treatment of SHR or of patients with essential hypertension patients with β-blockers causes blood pressure reduction without affecting small-artery structure. As another example, withdrawal of treatment, both in humans and in SHR, induces a rise in blood pressure that is not time-related to changes in resistance artery structure, and the data emphasize that abnormal vascular structure is not in itself sufficient to maintain a high blood pressure. As regards normalization of blood pressure, normalization of resistance vessel structure may not therefore be important. However, it cannot be

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**TABLE 2. Morphometric Measurements in Rat Mesenteric Resistance Vessels**

<table>
<thead>
<tr>
<th>Rat Type</th>
<th>Internal Diameter, μm</th>
<th>Media Thickness, μm</th>
<th>Media Area, μm²×10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declipped 1K-1C rats</td>
<td>233±10</td>
<td>17.37±0.81</td>
<td>12.96±0.96</td>
</tr>
<tr>
<td>6 h (14 wk)</td>
<td>207±10</td>
<td>17.21±1.15</td>
<td>11.51±1.13</td>
</tr>
<tr>
<td>24 h</td>
<td>227±8</td>
<td>15.80±1.16</td>
<td>11.39±1.02</td>
</tr>
<tr>
<td>1 wk</td>
<td>244±10</td>
<td>16.85±0.74</td>
<td>13.02±0.90</td>
</tr>
<tr>
<td>4 wk (18 wk)</td>
<td>223±6</td>
<td>14.62±0.70</td>
<td>10.11±0.45</td>
</tr>
<tr>
<td>8 wk</td>
<td>233±9</td>
<td>12.53±0.39</td>
<td>9.07±0.57</td>
</tr>
<tr>
<td>SHAM rats</td>
<td>244±9</td>
<td>13.01±0.60</td>
<td>9.76±0.60</td>
</tr>
<tr>
<td>Control 1K-1C rats</td>
<td>229±10</td>
<td>18.25±1.60**</td>
<td>13.92±2.09*</td>
</tr>
<tr>
<td>14 wk</td>
<td>228±8</td>
<td>17.60±1.13**</td>
<td>12.98±1.22*</td>
</tr>
</tbody>
</table>

*P<0.05 compared with age-matched SHAM rats; **P<0.01 compared with age-matched sham rats.

**TABLE 3. Maximal Responses of Mesenteric Resistance Arteries to Acetylcholine (Relaxation) and to K-PSS and Noradrenaline (Contraction)**

<table>
<thead>
<tr>
<th>Rat Type</th>
<th>Acetylcholine, % of Precontraction</th>
<th>K-PSS, mN/mm</th>
<th>Noradrenaline, mN/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declipped 1K-1C rats</td>
<td>74±4</td>
<td>2.53±0.19</td>
<td>2.14±0.22</td>
</tr>
<tr>
<td>6 h (14 wk)</td>
<td>59±9</td>
<td>2.74±0.25</td>
<td>2.26±0.22</td>
</tr>
<tr>
<td>24 h</td>
<td>41±7</td>
<td>3.05±0.28</td>
<td>3.73±0.35</td>
</tr>
<tr>
<td>1 wk</td>
<td>70±6</td>
<td>3.26±0.27</td>
<td>3.56±0.37</td>
</tr>
<tr>
<td>4 wk (18 wk)</td>
<td>67±7</td>
<td>2.83±0.11</td>
<td>3.17±0.17</td>
</tr>
<tr>
<td>8 wk</td>
<td>60±7</td>
<td>2.26±0.18</td>
<td>2.33±0.22</td>
</tr>
<tr>
<td>SHAM rats</td>
<td>75±6</td>
<td>2.79±0.21</td>
<td>2.69±0.23</td>
</tr>
<tr>
<td>Control 1K-1C rats</td>
<td>72±6</td>
<td>2.79±0.26</td>
<td>2.66±0.33</td>
</tr>
<tr>
<td>14 wk</td>
<td>62±7</td>
<td>2.55±0.22</td>
<td>2.82±0.24</td>
</tr>
</tbody>
</table>

There were no differences between any of the groups.

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**Figure 3.** Time relationship of media/lumen ratio after declipping. ○, Declipped 1K-1C rats; ●, sham rats; ■, control 1K-1C rats. Results are mean±SEM. ***P<0.001 vs age-matched control 1K-1C rats. **P<0.01 vs age-matched declipped 1K-1C rats.
excluded that abnormal structure may have other consequences, such as reduced vascular reserve; further studies are, however, needed to establish this.

The role of the endothelium in hypertension has been investigated intensively, and the results in normotensive rats and hypertensive rats of different kinds are conflicting. However, as regards renovascular hypertensive rats, there has been general agreement that the response to acetylcholine is reduced (in 1K-1C as well as in 2K-1C rats) compared with normotensive rats. This is in contrast to our results that showed no differences in response to acetylcholine. We found no obvious reason for the conflicting results because the conditions we have used are in general similar, such that some subtle difference in our protocol probably accounts for the discrepancy.

It was of some concern that approximately one third of the animals did not survive the consequences of the operation, the discrepancy.

Some subtle difference in our protocol probably accounts for the discrepancy.

It was of some concern that approximately one third of the animals did not survive the consequences of the operation, which we ascribed to the crucial importance of the clip aperture: Variations of ≈30 μm were found to span the difference between no hypertensive effects and induction of uremia. However, our finding that for the survivors after placement of the clip, blood pressure was elevated after 2 weeks and remained stable after 6 weeks is in agreement with the findings of Stacy and Prewitt. The hypertrophy of the heart and increased media/lumen ratio and media area of the resistance arteries is also in agreement with previous findings, as is the normalisation of the weight of the clipped kidney after an initial weight reduction. The rapid fall in blood pressure within a few hours after declipping is also similar to that previously reported, although it took more than 1 day to normalize. The model thus appears to have been satisfactory.

Perspectives

This study has provided clear evidence that rapid reduction of blood pressure, achieved in this study by declipping of 1K-1C rats, is not necessarily accompanied by a corresponding normalisation of resistance vessel structure over a period of 8 weeks, although heart weight was normalized after 4 weeks. This thus provides definitive evidence that an abnormal resistance vessel structure is not in itself sufficient to maintain a high blood pressure. Conversely, reduction of blood pressure does not in itself necessarily result in a rapid normalisation of resistance vessel structure. As indicated, treatment of genetic hypertension does in some cases result in normalisation of resistance vessel structure, but it is not known how quickly this occurs. Furthermore, some treatments of genetic hypertension do not normalise resistance vessel structure even after 1 year or more. Taken together with the results of the present study, this indicates a need for a better understanding of the mechanisms that allow normalisation of resistance vessel structure in the treatment of hypertension. The results of this study also underline the need for understanding the degree to which an abnormal resistance vessel structure should be a target for antihypertensive therapy.

Acknowledgments

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References

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