Reversibility of Arterial and Venous Changes in Renal Hypertensive Rats

GEZA SIMON, M.D., PH.D.

SUMMARY The effect of reversal of hypertension on vascular function and composition was investigated in renal-hypertensive rats. The study comprised three groups of rats: 1) 21 male Sprague-Dawley rats with one-kidney, one clip Goldblatt hypertension of 9 weeks' duration; 2) 20 rats with one-kidney, one clip hypertension that underwent removal of renal artery clip (were "unclipped") at 6 weeks of hypertension, 3 weeks prior to the study; and 3) sham-operated normotensive control rats. Venous pressure-volume and arterial pressure-flow relationships were measured at maximal vasodilatation (sodium nitroprusside and papaverine) in the denervated, pump-perfused vascular beds of the hindquarters of rats. Anatomically defined segments of the aorta and of the vena cava were removed from rats for water, sodium, and potassium analysis. Hypertension was completely reversed in the "unclipped" rats. Compared to values obtained in normotensive control rats, the water concentration of the aorta and of the vena cava, the potassium concentration of the aorta, and the sodium concentration of the vena cava were increased \((p < 0.05)\) in rats with one-kidney, one clip hypertension. These changes were reversed in the "unclipped" rats. In contrast, the shift of the venous pressure-volume and of the arterial pressure-flow curves toward the pressure axis at maximal vasodilatation in hypertensive rats \((p < 0.02)\) persisted following reversal of hypertension in the "unclipped" rats \((p < 0.05)\). In chronic, one-kidney renovascular hypertension, the contribution of vascular wall "water-logging" to increased structural arterial resistance and decreased structural venous capacity appears to be minor.

(Key Words: experimental renal hypertension • reversal of hypertension • venous capacity • structural arterial resistance • vascular wall water and electrolyte composition)

INCREASED structural arterial resistance, decreased structural venous capacity, and increased water and sodium concentration of arteries and veins are common features of several experimental models of renovascular hypertension.\(^1\)\(^-\)\(^8\) The pathophysiologic significance of these changes and the role of the Goldblatt kidney in the maintenance of these abnormalities are unclear. Therefore, the effect of reversal of hypertension on structural arterial resistance and venous capacity and on arterial and venous wall water and electrolyte composition was investigated in rats with chronic one-kidney, one clip hypertension. It was postulated that the reversal of hypertension may have a different effect on the abnormalities of vascular function and composition so that their interrelationship and pathogenesis may be better understood.

Methods

Animal Preparation

Goldblatt hypertension was produced in 49 male Sprague-Dawley rats (5-6 week old) by applying a silver clip with an inner diameter of 0.2 mm to the left renal artery. Sham surgery was performed on 32 male Sprague-Dawley rats. The contralateral intact kidney was removed from all rats 1 week later. The systolic blood pressure of rats was measured twice, on two different days, 6 weeks later, by the microphonic manometer technique. In 28 hypertensive rats, the renal artery clip was then removed (unclipped). The remaining hypertensive rats \((n = 21)\) and the sham-operated normotensive control rats \((n = 32)\) underwent sham surgery. The systolic blood pressure of all rats was again measured twice, 2 to 3 weeks later. Eight unclipped but still hypertensive (average systolic...
blood pressure > 140 mm Hg) rats were rejected from further study. During the entire study period, all rats were kept under the same conditions, fed regular rat chow and given water ad libitum. The rats were weighed to the nearest 1 gm on the final day of the experiment.

**Venous Pressure-Volume and Arterial Pressure-Flow Relationships in the Hindquarters of Rats**

Three weeks after the unclipping or sham-uncclipping procedure, eight hypertensive, nine unclipped hypertensive, and 20 normotensive control rats were anesthetized with pentobarbital (30 mg/kg i.p.) and allowed to breathe spontaneously through a tracheal cannula. The left femoral vein of each rat was cannulated (PE 50), and the femoral vein pressure was recorded with a Statham P23V pressure transducer. Heparin (500 units i.v.) was given to all rats for anticoagulation. A midabdominal incision was made, and the intestines, from the duodenum to the sigmoid colon, were removed. The aorta distal to the left renal artery and the adjacent inferior vena cava (IVC) were dissected free. Mass ligatures were placed and tightened around the vertebral column, including the lumbar musculature and skin, and bilaterally to include the remaining lumbar musculature, the abdominal muscles and skin. The aorta and the IVC were cannulated with a blunt 16-gauge needle and a PE240 polyethylene tubing respectively, with the cannulae pointing in the direction of the hindquarters. During cannulation of the aorta, there was no evidence for backflow of blood indicating that the arterial collateral circulation to the lower body of rats was effectively tied off by the mass ligatures.

Venous pressure-volume measurements were performed as described in a previous publication. The lower body vascular beds were perfused (Sigma motor pump) with oxygenated (95% O₂, 5% CO₂) Krebs-Ringer solution containing dextran, 7 g/100 ml, at 37°C. The perfusate drained from the cannulated IVC into a reservoir and was recirculated. Perfusion pressure, monitored through a T-tube arrangement between the pump and the aorta, was detected with a Statham P23Db pressure transducer and recorded on an oscillographic recorder. Perfusion pressure was kept below 50 mm Hg. Perfusion of the vascular beds of the hindquarters was interrupted every 9 minutes. The perfusate drained from the IVC for 50 seconds. Venous pressure-volume measurements were repeated twice at 10-minute intervals.

After the completion of these venous pressure-volume measurements, the rats were killed by asphyxia. During asphyxia, there was no rise in the perfusion pressure, indicating that the earlier placement of mass ligatures around the vertebral column and the lumbar musculature resulted in effective acute vasomotor denervation of the vascular beds of the hindquarters. Sodium nitroprusside (50 μg/ml) was then added to the perfusate, followed 5–10 minutes later by papaverine (150 μg/ml). The dose of both drugs was sufficient to produce maximal vasodilatation. Perfusion pressure fell after the addition of sodium nitroprusside in all rats. There was no further reduction of perfusion pressure with papaverine, which suggests that maximal vasodilatation was reached. Booster doses of sodium nitroprusside and papaverine also did not lower perfusion pressure further. After killing the rats and adding vasodilators to the perfusate, we obtained two additional venous pressure-volume curves at 10-minute intervals.

In six hypertensive, eight unclipped hypertensive, and eight normotensive control rats, arterial pressure-flow measurements were performed in vivo and at maximal vasodilatation in addition to the venous pressure-volume measurements. After the completion of the in vivo venous pressure-volume measurements (see above), pump flow was increased in 2.0 ml/min increments from 2.0 ml/min to 10.0 ml/min. At each level of flow, several minutes were allowed until a steady perfusion pressure was obtained. The flow of perfusate to the hindquarters was measured by duplicate, 1-minute collections of the effluent from the cannulated IVC. Arterial pressure-flow measurements were repeated in the same manner after killing the rats and adding vasodilators to the perfusate. At the conclusion of the hemodynamic measurements, the pressure drop across the arterial cannula, over the same flow range that was used for the perfusion experiments, was recorded and subtracted from the perfusion pressures. The lower body of rats was transected at the point of cannulation of the aorta and of the IVC and was weighed to the nearest 1 gram.

Venous volumes and arterial flows were expressed in terms of 100 g rat hindquarters weight. Venous pressure-volume and arterial pressure-flow curves obtained in vivo and after maximal vasodilatation were averaged for each rat. Entire venous pressure-volume curves, in the pressure range of 0–30 mm Hg, and arterial pressure-flow curves, in the flow range of 1.0–5.0 ml/100 g-min⁻¹, of hypertensive and unclipped hypertensive rats were compared with those of normotensive control rats by profile analysis. For comparison of the arterial pressure-flow curves, the pressure readings were converted to the natural logarithmic scale to achieve homogeneity of variance in the three groups. The null hypotheses of parallelism and of lack of strata differences were tested. Body weights, systolic blood pressures, femoral vein pressures, and lower body weights of the three groups of rats were compared by Student’s t test for independent variables. Null hypotheses were rejected at p < 0.05.
Arterial and Venous Wall Water and Electrolyte Analysis

Three weeks after the unclipping and the sham-unclipping procedure, the inferior vena cava from the iliac veins to the left renal vein, the superior vena cava from the diaphragm to the right clavicle excluding the right atrial appendage, and the thoracic aorta from the aortic valve to the diaphragm were removed from 13 hypertensive, 11 unclipped hypertensive, and 11 normotensive control rats in a cold room (4°C). For the procedure, the rats were anesthetized with methoxyflurane. The inferior and superior vena cava specimens were combined. All specimens were cleaned of perivascular tissue, opened longitudinally, blotted once with filter paper to remove blood, cut into 3-4 mm segments and then weighed to the nearest 0.1 mg. The specimens were oven-dried at 105°C for 24 hours to evaporate the nitric acid. Tissue contents of sodium and potassium were measured by flame photometry. Ion concentrations were expressed as milliequivalents per kilogram dry, defatted weight.

Results

Body weights, systolic blood pressures, femoral vein pressures, and lower body weights of rats are shown in table 1. The body weight of normotensive control rats was greater than that of hypertensive or of unclipped hypertensive rats. The greater weight of normotensive control rats appeared to be due to excess body fat. Hypertension in the unclipped hypertensive rats was completely reversed 2 to 3 weeks after the unclipping procedure. Venous pressure of hypertensive, unclipped hypertensive, and normotensive control rats, submitted to the hemodynamic measurements, was the same. There were no statistically significant differences in the lower body weights of the three groups of rats. The ratio of lower body weight to total body weight in normotensive controls, hypertensive, and unclipped hypertensive rats, submitted to the hemodynamic measurements, was 0.41 (mean, n = 21), 0.41 (n = 8), and 0.42 (n = 9) respectively. Since lower body weight was obtained after the completion of the hemodynamic measurements, the findings suggest that the edema formation that may have occurred during perfusion of the hindquarters with an artificial solution was of the same degree of severity in the three groups of rats.

Figure 1 illustrates the arterial pressure-flow relationships in vivo and at maximal vasodilatation in the three groups of rats. The shift of the arterial pressure-flow curves toward the flow axis after maximal vasodilatation was statistically significant in the control (p < 0.05) but not in the hypertensive or the unclipped hypertensive group of rats. Compared to values obtained in normotensive control rats, the pressure-flow curves of hypertensive rats both in vivo and at maximal vasodilatation were shifted in the direction of the pressure axis (p < 0.02 and p < 0.01). In contrast, in comparison to values obtained in normotensive control rats, the pressure-flow curves of unclipped rats were shifted toward the pressure axis only at maximal vasodilatation (p < 0.05) but not in vivo. Parallelism of arterial pressure-flow curves existed in vivo among the three groups (p > 0.05), but it was no longer present after maximal vasodilatation, the pressure-flow curves being steeper in hypertensive (p < 0.05), and in unclipped hypertensive rats (p < 0.05) than in control rats.

There was no significant shift of the venous pressure-volume curves after the administration of vasodilators in any of the three groups of rats. Venous pressure-volume curves at maximal vasodilatation are illustrated in figure 2. Compared to values obtained in normotensive control rats, there is a shift of the

---

**Table 1. General Observations in Normotensive Control, One-Kidney, One Clip Hypertensive (1-k, 1 clip HT) and Unclipped Hypertensive Rats**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>1-k, 1 clip HT</th>
<th>Unclipped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>419 ± 9.0 (32)</td>
<td>374 ± 14.8 (21)*</td>
<td>375 ± 8.6 (20)*</td>
</tr>
<tr>
<td>Systolic BP (6 weeks) (mm Hg)</td>
<td>124 ± 3.4 (32)</td>
<td>180 ± 6.9 (21)f</td>
<td>206 ± 6.1 (20)f</td>
</tr>
<tr>
<td>Systolic BP (9 weeks) (mm Hg)</td>
<td>124 ± 4.2 (32)</td>
<td>194 ± 8.3 (21)f</td>
<td>115 ± 4.4 (20)</td>
</tr>
<tr>
<td>Venous pressure (mm Hg)</td>
<td>2.2 ± 0.13 (21)</td>
<td>2.6 ± 0.40 (8)</td>
<td>2.4 ± 0.27 (9)</td>
</tr>
<tr>
<td>Lower body weight (g)</td>
<td>170 ± 4.9 (21)</td>
<td>156 ± 11.2 (8)</td>
<td>156 ± 5.7 (9)</td>
</tr>
</tbody>
</table>

All values are means ± se.

The numbers in parentheses represent the number of observations.

*p < 0.01, for comparison of body weights of hypertensive and unclipped hypertensive rats with those of normotensive control rats.

†p < 0.001, for comparison of systolic blood pressures of hypertensive and unclipped hypertensive rats with those of normotensive control rats.
venous pressure-volume curves toward the pressure axis in hypertensive rats \((p < 0.02)\). The shift persisted in the hypertensive rats that underwent reversal of hypertension by unclipping \((p < 0.05)\). In addition, venous pressure-volume curves in hypertensive and unclipped hypertensive rats were steeper, as demonstrated by a lack of parallelism \((p < 0.05)\) between these curves and those of normotensive control rats.

The water and electrolyte composition of the thoracic aorta and of the vena cava in the three groups of rats is shown in table 2. Compared to values obtained in normotensive control rats, the water concentration of the aorta and of the vena cava, the dry, defatted weight and the potassium concentration of the aorta, and the sodium concentration of the vena cava were increased in hypertensive rats. The sodium concentration of the aorta in hypertensive rats was similar to that of normotensive control rats. Changes in the composition of the aorta and of the vena cava that were found in hypertensive rats were reversed in the unclipped hypertensive rats.

**Discussion**

In this study, hemodynamic measurements were performed in the pump-perfused vascular beds of the hindquarters of rats. Collateral arterial circulation to the hindquarters, with the exception of the vertebral column, was interrupted by the removal of the intestines and the placement of mass ligatures around the lumbar and abdominal tissues. The latter procedure resulted in effective acute vasomotor denervation of the hindquarters. Killing the rats by asphyxia had no further effect on the hemodynamic

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Arterial pressure-flow relationships in vivo (solid lines) and at maximal vasodilatation (interrupted lines) in the vascular beds of the hindquarters of eight normotensive control, six one-kidney, one clip hypertensive (1-K, 1 clip HT), and eight unclipped hypertensive rats. Circles and bars indicate mean values ± SE.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Venous pressure-volume relationships (mean values) at maximal vasodilatation in the vascular beds of the hindquarters of 21 normotensive control (solid line), 8 one-kidney, one clip hypertensive (interrupted line), and 9 unclipped hypertensive (dash-dot line) rats. Vertical and horizontal bars represent SE of mean pressures and volumes. \(p < 0.02\) and \(< 0.05\) for lack of strata differences between pressure-volume curves in normotensive control and hypertensive and unclipped hypertensive rats respectively.
measurements. The contribution of circulating humoral stimuli to vasoconstrictor tone was eliminated by the use of an artificial perfusion medium. Maximum vasodilatation was achieved by the inhibition of the residual myogenic tone with potent vasodilators.

The study confirmed previous findings of increased arterial resistance at maximal vasodilatation, referred to as structural arterial resistance, and decreased venous capacity in rats with chronic one-kidney, one clip hypertension. Decreased venous capacity, like increased arterial resistance, was not abolished by maximal vasodilatation. Like other investigators, we found evidence for “waterlogging” of the aorta and of the vena cava in hypertensive rats. Smooth muscle hypertrophy or hyperplasia of the thoracic aorta in hypertensive rats was suggested by its increased water and potassium concentration and by its increased dry, defatted weight. Evidence for hypertrophy of the vena cava in hypertensive rats was not found despite the fact that, as in the case of the aorta, the same anatomically defined segments of the inferior and superior vena cava were removed from all rats. Sodium concentration was increased in the large veins but, in contrast to the findings of previous investigators, not in the aorta of hypertensive rats. An increase in the weight of aortic wall elements rich in potassium but relatively deficient in sodium, such as vascular smooth muscle, might have accounted for the unchanged sodium concentration of the aorta in hypertensive rats.

Reversal of hypertension resulted in the reversal of the abnormal water and electrolyte concentration of the aorta and of the vena cava of hypertensive rats. Changes in the composition of the aorta and of the vena cava appear to reflect similar changes in the intervening segments of the circulation. Tobian et al. found that “waterlogging” and increased electrolyte concentration of the mesenteric arterioles also were reversible with reversal of hypertension in rats with chronic one-kidney, one clip hypertension. In contrast to the reversibility of vascular wall water and electrolyte changes in hypertension, increased structural arterial resistance and decreased structural venous capacity persisted in rats that underwent reversals of hypertension. Therefore, waterlogging and increased electrolyte concentration of blood vessels cannot completely account for the observed changes in structural vascular function in this model of hypertension. Additional abnormalities in vascular wall composition must be invoked.

In the course of development of chronic experimental renovascular hypertension, several investigators reported accumulation of aortic wall collagen, elastin, and alkali-soluble, noncollagenous proteins, in addition to waterlogging. The accumulation of noncollagenous proteins appears to represent hypertension-related smooth-muscle hypertrophy or increased interstitial acidic glycosaminoglycans or both. Some aortic wall abnormalities, such as waterlogging, are completely reversible; some, such as hypertrophy, are partially reversible; and some, such as the accumulation of collagen, appear to be irreversible with reversal of hypertension. Histopathologic studies have long ago demonstrated that connective tissue changes also occur in the arterioles of patients and experimental animals with long-standing hypertension, although biochemical confirmation of this finding is lacking. Connective tissue changes also may occur in the veins of experimental animals with hypertension. Greenberg et al. found an increase in the density of mucopolysaccharide and glycoprotein staining of veins in spontaneously hypertensive rats. In the same rats, there was a greater

### Table 2. Water Concentration, Dry, Defatted Weight (DDW), and Sodium and Potassium Concentration of the Thoracic Aorta and of the Inferior and Superior Vena Cava in Normotensive Controls, One-Kidney, One Clip Hypertension (1-k, 1 clip HT), and Unclipped Hypertensive Rats

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control n = 11</th>
<th>1-k, 1 clip HT n = 13</th>
<th>Unclipped n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aorta</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (ml/kg wet wt)</td>
<td>682 ± 5.4</td>
<td>706 ± 4.1*</td>
<td>677 ± 5.4</td>
</tr>
<tr>
<td>DDW (mg)</td>
<td>12.9 ± 0.52</td>
<td>17.6 ± 1.14*</td>
<td>13.8 ± 0.80</td>
</tr>
<tr>
<td>Sodium (mEq/kg DDW)</td>
<td>321 ± 7.0</td>
<td>326 ± 6.7</td>
<td>332 ± 6.2</td>
</tr>
<tr>
<td>Potassium (mEq/kg DDW)</td>
<td>120 ± 3.1</td>
<td>147 ± 4.3†</td>
<td>123 ± 2.7</td>
</tr>
<tr>
<td><strong>Vena cava</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (ml/kg wet wt)</td>
<td>665 ± 8.2</td>
<td>689 ± 7.1†</td>
<td>647 ± 8.8</td>
</tr>
<tr>
<td>DDW (mg)</td>
<td>9.7 ± 0.47</td>
<td>10.2 ± 1.07</td>
<td>9.3 ± 0.24</td>
</tr>
<tr>
<td>Sodium (mEq/kg DDW)</td>
<td>385 ± 8.2</td>
<td>426 ± 8.8*</td>
<td>392 ± 7.4</td>
</tr>
<tr>
<td>Potassium (mEq/kg DDW)</td>
<td>156 ± 5.3</td>
<td>165 ± 3.2</td>
<td>166 ± 5.8</td>
</tr>
</tbody>
</table>

All values are means ± SE.

* p < 0.01, for comparison of values in 1-k, 1 clip HT and normotensive control rats.
† p < 0.001, for comparison of values in 1-k, 1 clip HT and normotensive control rats.
‡ p < 0.05, for comparison of values in 1-k, 1 clip HT and normotensive control rats.
uptake of [14C] glucosamine into veins than in Wistar-Kyoto control rats. Preliminary findings in our laboratory suggest that the vena cava concentration of acidic glycosaminoglycans may be increased in both spontaneously hypertensive rats and in rats with chronic one-kidney, one clip hypertension. These vascular wall connective tissue changes, some of which have been shown to be irreversible, may explain our findings of increased structural arterial resistance and decreased venous capacity in hypertensive rats that underwent reversal of hypertension by unclipping.

Lundgren studied the reversibility of increased structural arterial resistance in rats with two-kidney, one clip hypertension, from 3 to 4 weeks’ duration. In contrast to our findings in rats with one-kidney, one clip hypertension, he found that the increased structural arterial resistance of the vascular beds of rat hindquarters was completely reversed by 3 weeks following the unclipping procedure. His findings supported the hypothesis that reversible medial hypertrophy (and possibly waterlogging) accounted for increased structural arterial resistance. Lundgren’s and our findings suggest that the pathophysiology of structural vascular changes in hypertension may depend on the stage and, perhaps, on the model of hypertension. In the early stages of hypertension, reversible waterlogging and medial hypertrophy may account for the limitations of maximal vasodilatation. In the chronic stages of hypertension, irreversible connective tissue changes may be primarily responsible for increased structural arterial resistance and decreased structural venous capacity.

Finally, despite increased arterial resistance at maximal vasodilatation, the in vivo arterial resistance of unclipped hypertensive rats was not significantly different from that of normotensive control rats. In this regard, the unclipped rats differed from the hypertensive rats. In our preparation, using an artificial solution to pump-perfuse an acutely denervated vascular bed, the difference between vascular resistance before and after maximal vasodilatation and after maximal vasodilatation represents vascular myogenic tone. The findings suggest that vascular myogenic tone might have been reduced in the unclipped rats following the reversal of hypertension and of vascular wall water and electrolyte abnormalities.

Acknowledgments

The author thanks Jan Weigenand and Stephen Altman for their technical assistance.

References

Reversibility of arterial and venous changes in renal hypertensive rats.

G Simon

Hypertension. 1980;2:192-197
doi: 10.1161/01.HYP.2.2.192

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/2/2/192