Role of the Renin-Angiotensin System in the Pathogenesis of Severe Hypertension in Rats

By O. A. Carretero, P. Kuk, S. Piwonska, J. A. Houle, and M. Marin-Grez

ABSTRACT

Plasma renin levels are elevated in accelerated or malignant hypertension. To see if this increase of renin is a concurrent phenomenon or a pathogenetic factor in the increase of blood pressure, severe hypertension was produced in rats by occluding the aorta between the origins of the renal arteries. Eight days later, these animals had developed severe hypertension (mean blood pressure = 201 ± 3 mm Hg) and markedly elevated plasma renin levels (151 ± 35 ng angiotensin II/ml hr⁻¹; normal range = 11 ± 0.6). When the kidney distal to the ligature was excised at the time of the coarctation, the animals developed only a moderate increase in blood pressure (mean = 120 ± 13 mm Hg) and their plasma renin levels remained in the normal range. When the nonnephrectomized animals with severe hypertension were injected with antibodies against angiotensin II, blood pressure decreased, reaching its lowest point (126 ± 12 mm Hg) 2 days later. This work demonstrates that the severe increase in blood pressure is not due to the mechanical increase in resistance caused by complete coarctation of the aorta; rather, it is due to a humoral factor produced by the kidney, and this factor is renin.

KEY WORDS

angiotensin II antibody malignant hypertension aortic coarctation blood pressure nephrectomy

Increased angiotensin formation caused by an increase in renin activity can be postulated to be a contributory factor in the development and maintenance of renal hypertension. However, measurements of renin and angiotensin levels in plasma have been inconclusive (1-12).

Immunological approaches, namely the injection or endogenous production of antibodies against renin, have supported the hypothesis that the renin-angiotensin system is directly responsible for the increase of blood pressure in renal hypertension (13-20), although some negative results have been reported (21). The main criticism of these experiments is that kidney extracts which contained other proteins besides renin were used for the antibody preparation. The antibodies therefore lack specificity, since they are not only against renin, but also against other kidney proteins (22).

With the recent development of specific antibodies against angiotensin II, this problem of specificity has been circumvented. Still, the results obtained using these new antibodies have been contradictory (23-28) and have failed to clarify the role of the renin-angiotensin system in the pathogenesis of renal hypertension.

In accelerated or malignant hypertension, irrespective of its etiology, the renin and angiotensin levels in plasma are consistently elevated, suggesting that the renin-angiotensin system mediates the accelerated increase in blood pressure (29-32). Since other clinical states occur in which high plasma levels of renin exist even though blood pressure remains normal (4, 12, 33-36), the renin-angio-
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TENSIN SYSTEM CANNOT BE UNIVERSELY CONSIDERED THE PATHOGENETIC FACTOR UNDERLYING AN INCREASE IN BLOOD PRESSURE WHEN HIGH PLASMA LEVELS OF RENIN AND HIGH BLOOD PRESSURE EXIST CONCOMITANTLY.

The object of this study is to see if the increase in plasma renin in severe hypertension is a concurrent phenomenon or a causative factor in the pathogenesis of hypertension. The study of this problem required an experimental model in which the animal would develop both accelerated hypertension and high plasma renin levels. A modification of Selye's method which consists of tying the aorta between the renal arteries was used (37, 38) in rats.

We attempted to determine how much of the blood pressure increase in the experimental rats was produced by the mechanical increase in resistance caused by the complete coarctation of the aorta and how much by a humoral factor produced by the kidney distal to the aortic ligature. With this aim, blood pressure and plasma renin were measured in normal rats, in rats with aorta coarctation, and in rats with aorta coarctation and excision of the kidney distal to the ligature. To identify the humoral factor as renin, we employed an immunological approach. Antibodies against angiotensin II were injected into rats with severe hypertension caused by complete ligation of the aorta and into normal rats. Blood pressure, urine volume, urine sodium and potassium, and water intake were measured before and after antibody injection.

Materials and Methods

Studies were performed in conscious Sprague Dawley male rats weighing 200-260 g. Rats were fed laboratory chow and given free access to tap water. All surgical procedures were performed under ether anesthesia. Hypertension was induced by complete ligation of the aorta between the origin of renal arteries (38).

MEASUREMENTS

Blood Pressure Measurements.—Blood pressure was measured through a chronically implanted catheter inserted via the carotid artery into the aorta. Intravenous injections were made through a chronically implanted cannula inserted via the jugular vein into the superior vena cava (39). Intra-arterial pressures were recorded by connecting the indwelling catheter to a Sanborn pressure transducer, which was in turn connected to a Sanborn 350-1100C carrier preamplifier. The meter was electronically damped to register the mean pressure. The caged animals wore a harness, yet they were relatively unrestrained while their blood pressures were being measured. All animals were connected to the transducer by a cannular feedthrough swivel (LVE Model 1603).

Blood Samples.—Blood (0.5 ml) was drawn from the artery in a syringe wet with 10 μliters of 11% EDTA in saline.

Generation of Antibodies.—Antibodies against angiotensin II were generated in rabbits by repeated subcutaneous or intraperitoneal injections of angiotensin II conjugated with rabbit serum albumin and complete Freund's adjuvant as described by Goodfriend et al. (41).

Plasma Renin.—Renin concentration was estimated by the micromethod of Nasjletti et al. (40) and expressed as ng angiotensin II/ml plasma hr⁻¹.

Sodium and Potassium.—Sodium and potassium were measured by the standard flame photometer technique.

EXPERIMENTAL GROUPS

Control Group.—An intra-arterial catheter was inserted in 18 animals. In 6 of the animals no sham operation was performed, but a catheter was inserted via the carotid artery into the aorta. In 6 other animals, a sham aortic constriction was performed on the same day as the catheter implantation. The final 6 animals had a sham aortic constriction 2 days after the catheter insertion. Since no differences were found in blood pressure or in renin concentration, these animals were combined into a single control group.

Aorta Coarctation with Both Kidneys Present.—In 11 rats, hypertension was induced by complete ligation of the aorta between the renal arteries. In 6 of the rats, a catheter was implanted into the carotid at the same time as ligation. In the remaining 5 rats, the catheter was implanted 4 days later.

Aorta Coarctation with Left Kidney Excised.—In 12 rats, the aorta was tied, the aortic catheter was implanted, and the kidney distal to the ligature was excised. In 6 of the rats, the excised
kidney was ground in 1 ml of saline and injected subcutaneously. As no differences were found in blood pressure or renin concentration of these animals, they were combined into a single group.

In all of the animals in the above three groups, blood pressure, plasma renin, and hematocrits were measured daily for as long as the intra-arterial catheter remained open.

Aorta Coarctation and Antibody Injection.—The aorta was completely tied between the renal arteries in 13 rats. Four days later, arterial and venous catheters were implanted. These animals were then divided into two subgroups. In the first subgroup of 8 rats, antibodies against angiotensin II were injected (0.3 ml, iv and 0.7 ml, ip) 6 days after aortic constriction. Blood pressure was recorded immediately before and after antibody injection and on each day following until the animal died or the catheter was occluded. Response to angiotensin, renin, and norepinephrine was tested in 4 of the animals before and 30 minutes, 1 day, and 2 days after the antibody injections. Blood was drawn immediately before and 1 day subsequent to antibody injection, and the antibody titers were assessed. In the second subgroup of 5 rats, 1 ml of antibody was injected 8 days after aortic constriction. Daily urine sodium and potassium, urine volume, and water intake were measured during the 2 days prior to antibody injection and for 2 days after. In addition, blood pressure was measured 2 days before and again 2 days after antibody injection. Blood was drawn immediately before and 1 day subsequent to antibody injection, and the antibody titers were assessed.

Aorta Coarctation and Plasma Injection.—The protocol for rats receiving antibody injections was followed except that the rats were injected with normal rabbit plasma. The first subgroup consisted of 13 animals and the second of 6.

Normal Rats with Antibody Injection.—These animals were divided into two subgroups. In the first subgroup of 5 rats, arterial and venous catheters were implanted. Three days later, response to angiotensin II, renin, and norepinephrine was tested. Following this, the animals were injected with antibodies against angiotensin II (0.3 ml, iv and 0.7 ml, ip). The response to angiotensin, renin, and norepinephrine was tested again the same day, and 1–3 days after antibody injections. Blood pressure was recorded immediately before and after and 1 and 2 days after antibody injections. In vitro antibody titers were assessed by bioassay. In a second subgroup, 6 rats were placed in metabolic cages, and 24-hour urine specimens collected. Water and food were given ad libitum. Daily urine volume, urine sodium and potassium, and water intake were measured during the 2 days before and the 2 days after the injection of 1 ml of antibody (ip). Blood was drawn from 3 of the animals by heart puncture at the beginning of the experiment and

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

**FIGURE 1**

Top: Systolic and diastolic blood pressures, separated by shaded areas, in normal rats (closed circles) and in hypertensive rats (open circles). For the normal rat, the pressures are the average of 6–18 animals; for the hypertensive rats, the pressures are the average of 6–9 animals. Bottom: Plasma renin in normal rats (solid shading) and in hypertensive rats (stippled shading and broken column). Vertical bars represent the se.
1 day after antibody injection. The antibody titers were measured in vitro by the bioassay method.

Results

Control Group.—Both the blood pressure and the plasma renin concentration showed very little variation from day to day (Fig. 1). In these 18 rats, 122 consecutive renin determinations gave a mean of 11.0 ± 0.6 ng/ml hr⁻¹ (mean ± se). Hematocrit decreased during the course of the experiment from 49 ± 6.0 to 42 ± 0.9.

Aorta Coarctation with Both Kidneys Present.—The blood pressure in these animals was moderately increased 1 day after ligature. Four days later however, they had developed severe hypertension, and the blood pressure remained elevated until the animals died. Plasma renin levels were at least ten times above the normal range (Fig. 1). The hematocrit decreased during the course of the experiment from 50 ± 1.3 to 46 ± 1.5. All the animals in this group were dead 11 days after ligature.

Aorta Coarctation and Left Kidney Excised.—On the first and second day after ligature, there was an increase in blood pressure similar to that in the rats having both kidneys intact and complete ligature. However, no further increase in blood pressure was observed on the third and fourth day. By the seventh day there was, if anything, a tendency for the blood pressure to decrease. In the 12 rats of this group, 95 consecutive renin determinations gave a mean of 14.0 ± 1.0 ng/ml hr⁻¹, thus showing that plasma renin was in the normal range. Again, a decrease in the hematocrit was observed in these animals, going from 49 ± 0.6 to 42 ± 1.2.

Aorta Coarctation and Antibody Injection.—All of the animals in the first subgroup had developed severe hypertension 6 days after the tightening of the aorta. The average systolic and diastolic pressures were 228/174 ± 10/9 mm Hg. Immediately after the injection of antibody, the blood pressure started to decrease, reaching its lowest point 2 days later when the average systolic and diastolic pressures were 164/98 ± 11/12 mm Hg. After 2 days, the blood pressure started to increase (Fig. 2). In the 4 animals in which the response to angiotensin and renin was tested, a significant decrease appeared immediately after the injection of antibody and remained for at least 2 days. No significant changes were observed in the response to norepinephrine (Fig. 2). The angiotensin II-binding capacity of the plasma as tested by bioassay ranged from 0 to 40 ng/ml before antibody injection as compared to 230 to 416 ng/ml 1 day after antibody injection.

In the second subgroup, daily urinary sodium and potassium, urinary volume, and water intake were measured 2 days before and 2 days after the injection of antibody. No significant differences were found. When comparing the blood pressure before antibody injection and 2 days after, a significant decrease was observed (Fig. 3). The binding capacity of the plasma was 0–26 ng/ml before the antibody injection and 310–330 ng/ml 1 day after antibody injection.
In the second subgroup, no significant changes were observed in blood pressure, urinary sodium and potassium, urinary volume, or water intake after the injection of normal rabbit plasma (Fig. 3).

Normal Rats with Antibody Injections.—In the first subgroup, no decrease in blood pressure was observed after the injection of antibody. However, the response to angiotensin and renin decreased significantly immediately after the antibody injection and remained low for at least 2 days. No significant changes were observed in the response to norepinephrine (Fig. 5). One day after injection of antibody, 1 ml of plasma from these animals was able to bind 300–400 ng of angiotensin when tested in vitro by the bioassay.

In the second subgroup, urinary sodium and potassium, urinary volume, and water intake were measured daily for the 2 days before and 2 days after the antibody injection. No significant differences were observed (Fig. 6).

Aorta Coarctation and Plasma Injection.—In the first subgroup, the animals had severe hypertension 6 days after the tightening of the aorta. The systolic and diastolic pressures were 231/177 ± 3/5 mm Hg. Immediately following the injection of normal rabbit plasma, no changes were observed in blood pressure. Two days after, however, an increase in the blood pressure was observed (257/201 ± 6/5 mm Hg) (Fig. 4). In 5 of these animals, response to angiotensin, renin, and norepinephrine was tested. No significant changes were observed either immediately or 1 day after the injection of plasma. Two days later though, the response to a given dose tended to decrease (Fig. 4).
The capacity of plasma to bind angiotensin was tested in 3 of these animals and was 260, 380, 430 ng/ml.

Discussion

This experimental model fulfills the criteria for severe accelerated hypertension, since all of the animals developed a diastolic pressure over 150 mm Hg and a plasma renin level at least ten times higher than that of normal animals. Furthermore, these animals died during the first 12 days after ligature of the aorta.

On the other hand, animals with their aorta constricted and their left kidney excised developed only moderate hypertension, and no further increase in blood pressure occurred after the first day of coarctation. If anything, it had a tendency to decrease by day 7 after ligature of the aorta (Fig. 7). All of these animals were alive 30 days after the coarctation. Furthermore, they also had normal plasma renin levels. These results tend to confirm the hypothesis that the severe increase in blood pressure found in the animals with coarctation was not produced by a mechanical increase in resistance after ligation of the aorta but by the endocrine function of the ischemic kidney. Apparently the humoral factor produced by the kidney is renin, since the plasma levels of this enzyme were at least ten times greater in the animals with severe hypertension than in the control group or in the group with coarctation and left nephrectomy.
Another possibility is that the increase in blood pressure and plasma renin, observed in the animals with coarctation, was due to the necrosis of the kidney under the ligature, since it is feasible that the renin stored in the kidney was reabsorbed. However, in half of the animals with coarctation and nephrectomy of the left kidney, the kidney was ground and injected subcutaneously; no differences were observed when these animals were compared with those in which the excised kidney was not injected. Therefore, this latter hypothesis was discarded.

When plasma with antibodies against angiotensin II was injected in the hypertensive animals, an immediate decrease in blood pressure was observed. This decrease reached its lowest point 2 days after the antibody injection. It is not clear why the maximum decrease in blood pressure occurred 2 days after the antibody treatment, since inhibition of the pressor effect of angiotensin or renin was very similar immediately after and 1 or 2 days after the antibody injection. One possible explanation is that the increase in blood pressure, which also took 2 or 3 days to reach its highest point, is produced not only by active constriction of the arterioles but also by structural changes like the ones proposed by Tobian (i.e., water and sodium logging) (43). These changes would take time to be reversed. In the animals treated with normal rabbit plasma, the blood pressure did not change and, if anything, a further increase was
observed until the animal died. These experiments were done to establish that the decrease in blood pressure observed in the animals injected with plasma containing the antibodies was not produced by the heterologous plasma.

No significant changes were observed in urinary sodium and potassium, urinary volume, or water intake when the hypertensive group was treated with antibodies. Yet, it is difficult to interpret these findings since widely dispersed results were observed. This particular experiment was done to see if the decreased blood pressure observed after antibody injection was mediated by an inhibition of the secretion of aldosterone and, as a consequence, an increase in sodium excretion in the urine. Laragh has reported an increase in aldosterone in severe hypertension (44). Our results support the hypothesis that the renin-angiotensin system is the pathogenic factor responsible for the severe blood pressure increase in these animals. Furthermore, it would seem that angiotensin acts directly on the arterial wall and not through aldosterone.

No changes were observed in the blood pressure, urinary sodium and potassium, urinary volume, or water intake after the injection of antibody in the normal rats. This antibody was taken from the same pool as the one used in the hypertensive group. Thus any contamination with pyrogens or other substances that could nonspecifically decrease the blood pressure appears unlikely since these substances should also decrease the blood pressure in the normotensive animals. These results agree with those previously reported by Hedwall (23). They also concur with the findings of Bing and Poulsen (28), who were unable to decrease the blood pressure in unanesthetized normal rats. These results do not, however, coincide with the work of Worcel et al. (45). One possible reason could be that when the renin-angiotensin system is blocked, other mechanisms of regulating blood pressure (i.e., the baroreceptors) compensate for its deficiency. Yet, the same antibody was effective in reducing the blood pressure in animals with severe hypertension (Fig. 7). On the basis of our experiments, the direct role of the renin-angiotensin system in the regulation of blood pressure in normal rats cannot be completely ruled out. Yet our work fails to support this possibility.

It is pertinent to add here that these experiments were not designed to study the role of the renin-angiotensin system in the benign phase of renal vascular hypertension which, in general, is accompanied by a normal or moderate increase in plasma renin. Further, the pathogenesis of this benign phase of hypertension is probably different from the pathogenesis of the malignant phase. For this reason, we do not believe our results contradict those of Macdonald et al. (26) who were probably working in the benign rather than the malignant phase of hypertension. These investigators failed to produce a blood pressure decrease in rabbits with renal hypertension after active immunization against angiotensin II. Even though they considered those animals having a systolic blood pressure increase of more than 25 mm Hg to be severely hypertensive, they were probably working in the benign phase of renal hypertension since the plasma levels of angiotensin in their animals increased only 60% above normal levels. In our experimental model, however, the mean blood pressure increased approximately 100 mm Hg and the plasma renin increased at least ten times above normal, thus indicating that we are working in the malignant phase.

Another difference between our study and that of Macdonald et al. is that we used animals in which the nonischemic kidney was intact, while in Macdonald's experiments, this kidney was excised. This could represent a significant difference, since it has been reported that renin and aldosterone increase in the first case, but remain normal in the second
(46-48). Still another major difference is that we are producing passive immunization, which implies a sudden block of the angiotensin. Macdonald's group, on the other hand, used active immunization. This is important, since active immunization requires a prolonged period of time; this would give the kidney more time to adjust to the blockage of angiotensin by producing more renin. Consequently, it may be that Macdonald's rabbits were less responsive to renin and angiotensin, not only because they had high titers of circulating antibody, but also because they had higher plasma renin levels (49).

Finally, assuming that our experimental model is equivalent to the accelerated or malignant phase of hypertension, we conclude that accelerated hypertension could be a special case of renal hypertension and that, in this particular case, renin appears to mediate the accelerated rise in blood pressure. This work confirms Volhard's old hypothesis that pale hypertension (malignant hypertension) is due to active constriction of the small arteries and arterioles by a chemical substance which arises from the kidney as a result of the ischemia (50).

References
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