Angiotensin and Sodium Balance: Their Role in Chronic Two-Kidney Goldblatt Hypertension

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SUMMARY The purpose of this study was twofold: 1) to determine whether the failure of rats with chronic renovascular hypertension to respond to the angiotensin II antagonist (AHA) with a decrease in mean blood pressure (BP) was due to the agonistic effect of the antagonist; and, 2) if this was not the case, to examine whether a positive sodium balance impaired the reversal of the hypertension, after unclamping, in the rats that did not respond to angiotensin inhibitors. For this purpose, rats with chronic, two-kidney Goldblatt hypertension (one renal artery clamped and contralateral untouched) were tested for their BP response to the AHA (1-Sar-8-Ala-angiotensin II) and to the converting enzyme inhibitor (CEI) SQ20,881, which is devoid of agonistic effect. Approximately 50% of the rats responded to both inhibitors either with no change or with a decrease in BP of less than 20 mm Hg (nonresponders). The other 50% had a decrease in BP of 20 mm Hg or greater (responders). The decrease in BP produced by the AHA and the CEI correlated significantly ($r = 0.76$). Nonresponders to both inhibitors were unclamped or sham unclamped. A positive sodium balance was produced before surgery by injecting either 400 or 1000 µEq of sodium and was maintained for 12 hours. Direct BP significantly decreased 12 hours after surgery in the unclamped rats despite a continuous positive sodium balance. In the sham unclamped rats, BP did not change. These data indicate that the failure to respond to the AHA is not due to the agonistic effect of this peptide. Furthermore, these data suggest that a positive sodium balance is not a major pathogenetic factor in maintaining the high BP in the nonresponder rats, since a positive sodium balance failed to maintain the hypertension after unclamping. (Hypertension 1: 389–396, 1979)

KEY WORDS • renovascular hypertension • converting enzyme inhibitor • sodium balance • angiotensin antagonist • reversal of renovascular hypertension

PARTIAL constriction of one renal artery, while leaving the contralateral kidney untouched, produced hypertension (two-kidney renal hypertension) in rats. Evidence suggests that the renin-angiotensin system participates in the pathogenesis of the development of this type of hypertension. However, the factors that contribute to the maintenance of high blood pressure in the chronic phase of the hypertension are not well determined. The role of the renin-angiotensin system in the pathogenesis of this phase of renovascular hypertension has been extensively studied by inhibiting this system with antibodies against renin or angiotensin II or with angiotensin antagonists and converting enzyme inhibitor (CEI). Although these studies have yielded contradictory results, it is clear that some rats or even patients with chronic moderate hypertension, do not respond to the inhibition of the renin-angiotensin system, but do respond to renal revascularization with a decrease in blood pressure.

The role of sodium and water retention has also been studied in the two-kidney model. Tobian et al. found that exchangeable sodium was normal. Swales et al. noted a negative or normal cumulative sodium balance and also found that removal of sodium by peritoneal dialysis had no effect on the blood pressure. However, in these sodium metabolism studies the severity of hypertension was not considered. In a recent study, Möhring et al. showed a significant correlation between increased blood pressure and sodium retention in rats with moderate two-kidney hypertension. Thus, it is possible that a positive sodium balance plays a role in the pathogenesis of this phase of chronic renovascular hypertension. The purpose of this study was to determine if the failure to respond to the angiotensin antagonist was due to the agonistic effect of this peptide and to examine whether a positive sodium balance impaired the reversal of renovascular hypertension, after unclamping, in the rats that did not respond to the angiotensin inhibitors.
Methods

Male Sprague-Dawley rats weighing between 150–200 g were fed Purina rat chow (0.42% sodium content) and tap water ad libitum. All surgical procedures were done under ether anesthesia. Hypertension was induced by placing a U-shaped silver clip with an internal gap of 0.23 mm or 0.25 mm around the left renal artery. One end of the clip was bent outward to facilitate unclamping. The contralateral kidney was left untouched. Systolic blood pressure was measured by the tail-cuff method. Ten to 14 weeks after clamping, those rats having a systolic blood pressure of 160 mm Hg or higher at two consecutive measurements were placed in metabolic cages. Three days later, a catheter was implanted into the urinary bladder, and after an additional 7 days, catheters were also chronically implanted into the abdominal aorta and inferior vena cava through the femoral artery and vein, as previously described. Catheters were threaded subcutaneously to the scapular region of the rats.

Two days after vessel catheterization (12–16 weeks after clamping: chronic phase), direct mean blood pressure (BP) was recorded by connecting the arterial catheter to a pressure transducer (Micron, Model MP 15) and a four-channel recorder (Brush, Model 440). Only those rats having a BP of 140 mm Hg or above were used. During the experiments, the rats were unanesthetized and free to move about in their metabolic cages, only partially restrained by a harness attached to springs on their backs. After the blood pressure had stabilized, 100 ng/rat of angiotensin I (1-Asp-5-Ile-Ang-I, Schwarz/Mann), angiotensin II (1-Asp-5-Val-Ang II, Hypertensin-CIBA), and 1-norepinephrine (Levophed, Winthrop) were administered separately as bolus injections through the venous catheter. All injections were given in a volume of 0.1 ml followed by 0.2 ml of 5% dextrose. Subsequently, 1-Sar-8-Ala-Ang II (AlI) was infused through the venous catheter at 4 μg/min/rat (a solution of 588 μg/ml in 5% dextrose using a Harvard pump at 6.8 μl/min) for 1 to 2 hours. The BP was continuously recorded during the infusion of the AIIA, and the response to pressor substances was obtained at the end of the infusion. One to 4 hours later, during which time the BP returned to control levels, the response to pressor substances was repeated. CEI (SQ20.881) was then injected through the venous catheter as a bolus at the dose of 4 mg/kg. The BP was continuously recorded for the next 60 minutes.

Response to pressor substances was again obtained within 30 minutes of the injection of CEI. Blood (0.5 ml) was drawn from each rat for plasma renin activity (PRA) determinations before (initial PRA) and within 30 minutes of the injection of CEI. Blood (0.5 ml) was drawn from each rat for plasma renin activity (PRA) determinations before (initial PRA) and after administration of the AIIA and the CEI. Rats showing a decrease in BP of 20 mm Hg or more either to the AIIA or to the CEI were classified as responders; those with an increase, no change, or a decrease of less than 20 mm Hg were classified as non-responders. Throughout the text, ΔBP indicates the difference between the lowest BP either during AIIA infusion or after CEI injection and the BP before each inhibitor (initial BP). Thus, when the BP increases, the Δ is positive; and when the BP decreases, it is negative.

Twenty-four hours after the infusion study, direct BP was recorded for 30 to 60 minutes in 15 nonresponder rats. Thereafter, the bladder was completely emptied by flushing twice with 2 ml of distilled water, and then 3 ml of air through the chronically implanted bladder catheter. These rats were then anesthetized for unclamping (nine rats) or sham unclamping (six rats). Immediately before surgery either 400 (2.3 ml of 1% NaCl) or 1000 (5.9 ml of 1% NaCl) μEq of sodium was injected intravenously to produce a positive sodium balance; then the left renal artery was unclamped or sham unclamped. The rats were then returned to their metabolic cages, and no further anesthesia was given. The BP was recorded continuously, and the positive sodium balance was maintained for the next 12 hours as follows: every 30 to 60 minutes the bladder was flushed and emptied. Sodium and urinary volume were measured immediately, and both replaced with NaCl (1 mEq/ml) and 5% dextrose. Food was withheld during the first 12 hours after unclamping or sham unclamping, but water was available ad libitum.

Twenty-four hours after unclamping or sham unclamping, the blood pressure was recorded once more for 30 to 60 minutes. In addition, a third group of seven nonresponder rats was unclamped without replacement of sodium and served as a further control. Body weight was measured at the beginning and at the end of the experiment. Before, at 12 hours and at 24 hours after unclamping or sham unclamping, blood samples were drawn for PRA, hematocrit and serum sodium and potassium determinations. All blood samples were replaced immediately by injecting the same amount of blood obtained from donor rats which had been nephrectomized at least 2 hours earlier. Rats with a hematocrit of 40% or less at any time during the study were eliminated since this could indicate internal bleeding which, by itself, could alter the PRA and the response to the AIIA and the CEI.

We determined PRA by using a modification of the radiolmmunoassay method of Haber et al. Plasma renin activity was expressed as ng of generated angiotensin I/ml plasma/hr incubation. Serum and urinary sodium and potassium were measured using a flame photometer with lithium as an internal standard (Instrumentation Laboratory, Inc., Model 143). The hematocrit was determined by centrifuging the blood samples at 2000 rpm at 4°C for 20 minutes. All results were expressed as mean ± SEM. Statistical significance was determined by using the Student's t test.

Results

The pressor response to 100 ng of angiotensin I, angiotensin II and 1-norepinephrine before and at the end of infusion of AIIA and before and 30 minutes after CEI was tested in each rat used in these experi-
ments. The dose of AIIA used completely blocked the pressor effect of angiotensin I and II, but it did not affect the response to 1-norepinephrine. The dose of CEI used blocked the pressor response of angiotensin I but did not change the response to angiotensin II or 1-norepinephrine. Since no differences were found between responder and nonresponder rats, the results were combined (fig. 1).

Response to Angiotensin Antagonist

Changes in BP (ΔBP) and changes in PRA (ΔPRA) in each rat, and mean PRA before and at the end of the AIIA infusion are shown in figure 2. Thirty-five rats (56.5%) were classified as nonresponders (ΔBP < 20 mm Hg), and 27 (43.5%) as responders (ΔBP ≥ 20 mm Hg). The average BP in the responders before infusion was 179.3 ± 3.5 mm Hg and 158.9 ± 2.4 in the nonresponders (p < 0.0005). The average ΔBP during infusion in the responders was 43.1 ± 4.4 mm Hg but only 5.7 ± 1.1 in the nonresponders. The ΔBP during infusion correlated with the initial BP (r = −0.65, p < 0.01). The average initial PRA (PRA before infusion) was 6.3 ± 0.8 in the nonresponders and 23.7 ± 3.9 in the responders (p < 0.0005) (fig. 2, bottom). The ΔPRA during infusion correlated with the initial PRA levels (r = −0.49, p < 0.01). Infusion of the AIIA resulted in a significant increase in PRA (44.4 ± 7.4) in all of the nonresponders. In the responders, the mean PRA was significantly increased (47.1 ± 8.9); however, the PRA in some rats either did not change or decreased (fig. 2, middle).

Response to Converting Enzyme Inhibitor

In 39 rats the response to CEI was studied 1–4 hours after the AIIA infusion. Twenty-one rats

FIGURE 1. Blood pressure response (+ΔBP) to 100 ng of angiotensin I (Ag I), angiotensin II (Ag II) and norepinephrine (NE), before (B) and at the end (E) of angiotensin antagonist (AIIA) infusion (left) and before (B) and after (A) converting enzyme inhibitor (CEI) injection (right).

FIGURE 2. Changes in mean blood pressure (ΔBP, top) and PRA (ΔPRA, middle) during 1-Sar-8-Ala-angiotensin II (AIIA) infusion, and mean PRA (bottom) before (B, white column) and at the end (E, striped column) of the antagonist infusion in chronic renal hypertensive rats. Nonresponders are presented on the left and responders on the right.
(53.8%) were nonresponders and 18 (46.2%) were responders to CEI. Figure 3 shows ΔBP and ΔPRA, and mean PRA before and after CEI injection. The average initial BP was 170.8 ± 5.1 mm Hg in the responders and 156.8 ± 2.6 mm Hg in the nonresponders (p < 0.01). The average ΔBP after injection of CEI in the responders was 41.1 ± 2.8 mm Hg, and 7.5 ± 1.2 in the nonresponders. The ΔBP after CEI injection correlated significantly with the initial BP (r = −0.56, p < 0.01). The average ΔPRA was 10.0 ± 2.5 in the nonresponders and 19.3 ± 3.2 in the responders (p < 0.0005). Injection of the CEI caused the PRA to increase to 62.3 ± 10.2 and 79.9 ± 12.3 in the nonresponders and responders, respectively (fig. 3, bottom). The ΔBP after CEI correlated with the initial PRA (r = −0.44, p < 0.01).

The ΔBP during infusion of the AIIA significantly correlated with the −ΔBP after injection of the CEI (fig. 4). Only three (12.5%) of the 24 nonresponders to the AIIA showed a −ΔBP > 20 mm Hg in response to the CEI (open circles, fig. 4). In these rats, however, PRA level before injection of CEI (14.6, 21.0 and 15.7 ng/ml/hr, respectively) was higher than that before AIIA infusion (6.7, 10.1 and 5.2 ng/ml/hr, respectively).

Influence of Sodium Replacement in the Reversal of Hypertension

Nine nonresponder rats were unclamped and six sham unclamped. A positive sodium balance was produced, before unclamping or sham unclamping, and maintained for 12 hours as described in the Methods section. The average BP in the unclamped group decreased significantly (p < 0.0005) 12 hours after surgery. On the other hand, in the sham-unclamped group, the BP did not change. The postoperative course of average BP and cumulative sodium balance is shown in figure 5. The ranges of cumulative positive sodium balance during this 12-hour period in the unclamped and sham-unclamped groups were 122 to 473 and 93 to 488 μEq, respectively. In two unclamped rats, the positive sodium balance was maintained from 857 to 1063 μEq (fig. 6). The decrease in their BP was similar to that found in the group in which the positive sodium balance was
maintained at approximately 400 μEq. In the seven rats that were unclamped without sodium replacement, the average BP before, and at 1, 2, 4, 8 and 12 hours after unclamping was 157.1 ± 3.7, 143.1 ± 4.9, 140.9 ± 4.0, 132 ± 3.4, 128.9 ± 4.7, and 109.2 ± 1.5 mm Hg, respectively. When compared with the sodium-replaced group, the decrease in BP in this unclamped group without sodium replacement was not statistically significant (p > 0.05) at any time. Table 1 shows body weight, hematocrit, PRA, and serum electrolytes before and at 12 hours after surgery in the unclamped and sham-unclamped groups. The changes in

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<th>Table 1. Body Weight, Hematocrit, Plasma Renin Activity (PRA) and Serum Electrolytes Before and 12 Hours After Unclamping or Sham Unclamping*</th>
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<td><strong>Unclamped Group</strong></td>
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<td><strong>Body weight (g)</strong></td>
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*All values are means ± SEM, and numbers in parentheses indicate number of rats.
†Significant at p < 0.001.
the two groups were similar; however, with the exception of serum potassium, they were not significant (table I). At 24 hours after surgery, PRA decreased to $1.9 \pm 0.6 \text{ng/ml/hr}$ ($p < 0.025$) in the unclamped group, but it did not change ($3.2 \pm 0.9$) in the sham-uncalmed group.

### Discussion

The mechanism that contributes to the maintenance of high BP in chronic two-kidney renovascular hypertension is still unclear. Various agents which either antagonize the effect of angiotensin II or prevent its formation have provided a means of assessing the importance of the renin-angiotensin system in the pathogenesis of experimental and human renovascular hypertension.\textsuperscript{27-29} Recent studies using the AIIA have shown that some animals or even patients with chronic renovascular hypertension do not respond to the AIIA with a decrease in blood pressure. However, their blood pressure decreased after excision of the ischemic kidney or renal revascularization,\textsuperscript{14, 18, 17-18} thus indicating that the failure to respond to the AIIA is not due to permanent arteriolar changes. It has been postulated that sodium depletion unmasks the role of the renin-angiotensin system in the pathogenesis of renovascular hypertension.\textsuperscript{30} It could be that the renin-angiotensin system is an important factor in maintaining the elevated BP in renovascular hypertension when the rats are sodium depleted. However, it could also be that the renin-angiotensin system plays a homeostatic role during the sodium-depleted state, a role that tends to maintain BP at the level before sodium depletion, independent of the presence or absence of hypertension.\textsuperscript{31} Furthermore, it is also important to determine if angiotensin, through its direct vasoconstrictor effect, is the main pathogenetic factor that maintains the high blood pressure in renovascular hypertension when the rats or patients are on a “normal” sodium diet, since this diet is the more common situation. For this reason, the BP response to both AIIA and CEI was tested during the normal sodium state in this experiment. It has also been postulated that the failure of AIIA to decrease BP may be due to the agonistic effect of this peptide.\textsuperscript{32, 33} To examine this possibility, both AIIA and CEI (which is devoid of agonistic activity) were used. We found that more than 50% of the rats were nonresponders to either AIIA or CEI. The remaining rats were responders to both inhibitors. Moreover, the observed decrease in blood pressure produced by AIIA was similar to that produced by CEI. These data clearly indicate that the failure to respond to AIIA is not due to the agonistic effect of this peptide. In addition, these results do not lend support to the possibility that kinin accumulation may be involved in mediating the hypotensive response to CEI. However, this possibility cannot be completely eliminated since circulating levels of kinin were not measured in this study.\textsuperscript{34}

All rats that were nonresponders to AIIA were also nonresponders to CEI with the exception of three rats that showed a decrease in BP greater than 20 mm Hg after the CEI injection. It is not clear in which way these three rats differ from the other 18 that did not respond to either inhibitor. These three rats were not included in the unclamping experiment.

All the nonresponders showed an increase in PRA after the two inhibitors had been administered, an increase which may be explained by the inhibition of the angiotensin negative feedback\textsuperscript{35, 36} produced by these two agents. Some responders, however, showed a decrease in PRA at the end of infusion of AIIA. It may be that, in these rats, the agonistic effect of AIIA at the level of the juxtaglomerular apparatus was more pronounced than at the peripheral vessels, thus inhibiting renin release.

The PRA level after administration of AIIA and CEI in nonresponders was similar to the PRA level in responders (figs. 2 and 3). Since the two agents were effective in blocking the angiotensin generated by plasma renin in responders, they should also be effective in the nonresponders. When nonresponders were unclamped, a highly significant decrease in BP ($p < 0.0005$) was observed; thus, the lack of response in these rats was not the result of permanent vascular changes. Therefore, our study clearly indicates that there are two subgroups classified as responders and nonresponders in chronic renovascular hypertensive rats and that factors other than angiotensin play an important role in the pathogenesis of chronic hypertension in nonresponders.

There was a negative correlation between the BP before administration of AIIA or CEI and the ΔBP produced by these two peptides. This suggests that the renin-angiotensin system participates to a greater extent in maintaining severe rather than moderate renovascular hypertension. As observed by other investigators,\textsuperscript{14} we also found a negative correlation between the ΔBP and initial PRA.

Many investigators\textsuperscript{35, 37-39} have suggested that sodium and water retention may play a role in the pathogenesis of one-kidney renovascular hypertension, but not of two-kidney hypertension. Möhring et al.,\textsuperscript{33} however, recently showed that rats with two-kidney hypertension had a slight positive cumulative sodium balance. This was only the case for rats with moderate hypertension (systolic pressure < 180 mm Hg). Rats with more severe hypertension (systolic pressure ≥ 180 mm Hg) showed sodium and water loss and were in a negative sodium balance. Thus, it is possible that there are two populations in the two-kidney hypertensive group — one with moderate hypertension and a positive sodium balance, and one with more severe hypertension and a negative sodium balance. In the present study, the moderate hypertensive nonresponder rats, in which angiotensin II was eliminated as the main cause of the hypertension using AIIA and CEI, were unclamped and kept in a continuous positive sodium balance. A highly significant decrease in BP was observed despite a positive sodium balance (fig. 5). This decrease was significantly greater than the decrease produced by the administration of...
AIIA and CEI (fig. 7). It could be argued that the decrease in BP observed after unclamping plus positive sodium balance could have been greater if the rats had not been kept in positive sodium balance. However, the decrease in BP after unclamping with sodium replacement was similar to that found in the unclamped rats without sodium replacement. When six rats were sham unclamped, no change in BP was observed. These sham-unclamped rats were included only to prove that the stress of the surgery and the manipulation done to maintain the positive sodium balance did not affect the blood pressure. These data indicate that neither a positive sodium balance nor angiotensin are the main factors in maintaining the hypertension of nonresponders.

Our results in two-kidney renovascular hypertensive rats are in accordance with Neubig and Hoobler's study,\(^*\) in which reversal of one-kidney renovascular hypertension in rats was observed despite maintenance of positive sodium balance. In this experiment, although the greatest decrease in BP occurred in the first hour after unclamping, sodium balance was not measured until 6 hours later. In addition, since the bladders of these rats were not emptied at the end of the 6-hour period, it is possible that, at the end of this period, they retained urine which could contain enough sodium to make the difference between a positive and negative sodium balance. In the present study, the bladder was flushed through the chronically implanted catheter with water, followed by an injection of air to ensure complete emptying at the beginning and the end of each collection period.

Since, due to their high respiratory rate, rats lose considerable amounts of water which is very difficult to measure, a water-balance study was not done in this experiment. However, water was available, \textit{ad libitum} in the metabolic cages to avoid dehydration. Loss of body weight was observed in the unclamped and sham-unclamped groups, because food was withheld during the experiment. Similar potassium loss (approximately 60 mEq/g body weight loss), due mainly to the loss of body weight,\(^*\) was observed in both groups.

Skeggs et al.\(^{36,38}\) recently showed that an unknown substance other than renin may be the cause of the high BP in rabbits with chronic one-kidney hypertension. This substance, which they name renopressin, could be involved in maintaining elevated BP in nonresponders.\(^{40}\)

Although responder rats were not unclamped in the present study, it has been reported that there is less of a decrease in their BP after AIIA than after the exci-
sion of the ischemic kidney. This suggests that renin accounts for only part of the increase in BP and that this hypothetical factor may contribute to maintaining the hypertension in the responder rats.

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