Body Fluid Volume and Angiotensin II in Maintenance of One-Kidney, One Clip Hypertension

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To investigate the possible role of body fluid volume or the renin-angiotensin system in the maintenance of high blood pressure in chronic one-kidney, one clip (1K1C) hypertension, we studied whether blood pressure remained high after removal of the clip while the body fluid volume was kept constant or when angiotensin II (Ang II) was infused in conscious 1K1C rats. Blood pressure fell 58 ± 13 mm Hg in 1K1C rats after removal of the clip. When body fluid volume was kept at the same level as before “unclipping,” blood pressure fell only 9 ± 2 mm Hg after removal of the clip; if body fluid volume was then allowed to decrease, blood pressure fell an additional 55 ± 8 mm Hg. When Ang II was infused after removal of the clip, blood pressure fell 26 ± 7 mm Hg despite the fact that plasma Ang II increased to nonphysiological concentrations (1,161 ± 353 pg/ml). After Ang II infusion was stopped, blood pressure fell an additional 44 ± 13 mm Hg. When Ang II was infused and body fluid volume kept constant, blood pressure still did not change after removal of the clip, although plasma Ang II concentrations increased to nonphysiological levels (618 ± 98 pg/ml). After the Ang II infusion was discontinued and the body fluid volume was no longer kept constant, blood pressure fell 78 ± 9 mm Hg. These data further support the hypothesis that a volume factor, not the renin-angiotensin system, is important in the maintenance of high blood pressure in 1K1C hypertension. (Hypertension 1989; 14:269-273)

Partial constriction of one renal artery combined with contralateral nephrectomy produces one-kidney, one clip (1K1C) renal hypertension. Although it has been suggested that increased body fluid volume (BFV) contributes to the development of 1K1C hypertension,1-2 the extent to which it contributes to maintenance of hypertension in this model remains controversial. Similarly, while there is general agreement that the renin-angiotensin system contributes to the development of 1K1C hypertension, it does not appear to be a necessary component in the maintenance of high blood pressure in this model.1-5

It is well known that, after removal of the clip in animals with 1K1C hypertension, blood pressure falls rapidly and is associated with a decrease in plasma volume and an increase in urinary volume.2,6 Such experiments suggest that BFV plays an important role in the chronic phase of 1K1C hypertension. However, Neubig and Hoobler7 found that replacement of voided urine by saline infusion every 15-30 minutes failed to prevent a reduction in blood pressure after removal of the clip. Replacement of spontaneously voided urine causes volume replacement to lag behind, since some urine is retained in the bladder. Moreover, even when urine is returned to the vascular space by ureterocaval anastomoses, blood pressure decreases after “unclipping.”8,9 However, in those experiments the effects of the urinary kallikrein-kinin system and prostaglandins were not assessed. Also, voided urine does not represent net fluid volume loss, since the rat normally loses significant amounts of water from the lungs due to their high respiratory frequency.

Although plasma renin activity is normal in chronic 1K1C hypertension and is therefore not considered to be a major factor in its maintenance, Gavras et al10 reported that acute inhibition of angiotensin II (Ang II) reduced blood pressure in this model. They suggested that seemingly normal renin levels were inappropriately high for the state of volume expansion in rats with 1K1C renal hypertension; however, they observed the effect of Ang II inhibition only when the rats were previously sodium depleted and not when the sodium was replaced.
The present study was undertaken to investigate the role of BFV and the renin-angiotensin system in the maintenance of hypertension in chronic 1K1C hypertensive rats. We also investigated the possible interaction between these two factors in the maintenance of blood pressure after removal of the clip. Maintenance of high blood pressure after unclipping when BFV is kept constant would indicate that a volume factor plays a role in this phenomenon. Since it is well known that plasma renin activity and plasma Ang II concentrations decrease after unclipping, a role for Ang II in the maintenance of 1K1C hypertension would be indicated if Ang II infusion to replace endogenous Ang II prevented blood pressure from decreasing, provided that the infusion of Ang II needed to maintain blood pressure at hypertensive levels caused Ang II plasma concentrations similar to those found in the clipped rats. A possible interaction between Ang II and BFV would be indicated only if both BFV replacement and maintenance of a constant plasma Ang II concentration were effective in preventing a decrease in blood pressure.

Materials and Methods

All surgical procedures were performed with the rats under ether anesthesia. Hypertension was induced in male Sprague-Dawley rats (200–250 g) by removal of the right kidney and placement of a silver clip (0.23 mm gap) around the left renal artery. All rats were fed normal rat chow (0.42% sodium content) and tap water ad libitum and housed in separate cages until the day of the experiment. Once each week, systolic blood pressure was measured in conscious rats by means of the tail-cuff method, and body weight was recorded. Three weeks after induction of hypertension, two catheters were implanted into the bladder (groups 2, 3, and 5). One was inserted via a ventral incision for urine collection; the other, used to flush out the bladder, was passed underneath the skin and brought out at the scapular region. Four weeks after hypertension was induced, rats with a systolic blood pressure of 180 mm Hg or higher were selected; then, 2 days before the experiment, catheters were implanted in the abdominal aorta and inferior vena cava via the right femoral artery and vein. Both were passed underneath the skin and brought out at the scapular region.

On the day of the experiment, the rats were placed in plastic restrainers and direct mean blood pressure (BP) recorded from the arterial catheter with a Micron MP15 transducer (Micron Instruments, Inc., Los Angeles, California) and a Brush 440 recorder (Gould Inc., Cleveland, Ohio). Only rats with a direct BP of 160 mm Hg or higher were used for the experiments. BP was recorded continuously for 1 hour before unclipping (or sham unclipping in the case of the control rats, in which the clip was touched but not removed). The total surgical time was 15–20 minutes. After the rats had recovered from the anesthetic, BP was measured for an additional 2 hours; the first hour was the experimental period and the second was the postinfusion period.

Five groups of rats were studied.

Group 1 (unclipped+Ang II; n=5). After the control period, rats were unclipped and Ang II (100 μg/ml in 5% dextrose) was infused by means of a Harvard pump connected to a servo-controlled 990 amplifier (Harvard Apparatus Co., Millis, Massachusetts) that was activated by a fall in BP of about 5 mm Hg in an effort to maintain BP at or near control values. Ang II infusion was then stopped and BP recorded during the postinfusion period. The maximum infusion rate was set at 309 ng/min because when a higher rate of infusion was used in preliminary studies, many rats died due to hemoconcentration.

Group 2 (unclipped+saline; n=6). Rats with catheters implanted in the bladder were placed on a balance and BP recorded continuously. At the beginning of the experiment, the bladder was flushed out with 4 ml distilled water and then completely emptied by flushing with 3 ml air, after which it was kept empty by flushing with air every 2 minutes throughout the study. One milliliter saline (0.9% NaCl) was injected through the venous catheter. Body weight and BFV were kept constant during the control and experimental periods by infusion of saline to replace urine and water lost through respiration. The experimental period lasted for only 1 hour after unclipping, since preliminary studies had revealed considerable retroperitoneal edema when fluids were replaced for longer periods. Saline infusion was stopped and BP recorded for an additional 1 hour (postinfusion period).

Group 3 (unclipped+saline+Ang II; n=6). BFV was kept constant during the control and experimental periods as in group 2. After the control period, rats were unclipped and Ang II (10 μg/ml in 0.9% saline) was infused during the experimental period as in group 1. After the experimental period, saline and Ang II infusions were discontinued and BP recorded for 1 hour.

Group 4 (unclipped; n=5). After the control period, the rats were unclipped and BP recorded for 2 hours.

Group 5 (sham-unclipped+saline; n=6). The protocol was the same as in group 2, except that the rats were sham unclipped.

In groups 1 and 3, blood (1 ml) was sampled at the end of the control, experimental, and postinfusion periods for measurement of Ang II. Samples were replaced with blood from rats nephrectomized 24 hours earlier. Ang II was measured by radioimmunoassay. In groups 2, 3, and 5, urine was collected hourly throughout the experiment, and volume was measured gravimetrically and normalized to the body weight of the rats. Hematuria was assessed by Multistix (Ames Division, Miles Laboratories, Inc., Elkhart, Indiana); rats with hematuria were eliminated from the study. Urinary sodium concentration was measured with an autoanalyzer. In all
groups, the hematocrit was measured at the end of the control, experimental, and postinfusion periods. After the experiments were completed, an autopsy was performed on the rats. Rats with retroperitoneal edema were rejected.

Results were expressed as mean±SEM. Analysis of variance (ANOVA) and Scheffe’s contrasts were used to evaluate significance between groups and paired t tests for significance within groups. p<0.05 was considered significant.

Results

Average BP during control periods for groups 1–5 was 191.4±6.2, 209.5±5.0, 215.2±3.0, 202.6±4.9, and 203.2±5.6 mm Hg, respectively. Figure 1 shows the change in BP (ΔBP) after unclipping (or sham unclipping) in each group. BP did not change after sham unclipping (group 5). In group 4, unclipped but infused with neither Ang II nor saline, BP decreased rapidly after unclipping; ΔBP at 30 minutes (-39.4±10.4 mm Hg) was larger than the sham-unclipped group (p<0.001) and remained so at 60, 90, and 120 minutes (-50.2±9.4 mm Hg, p<0.01; -48.6±9.6 mm Hg, p<0.01; and -58.2±12.5 mm Hg, p<0.001, respectively). In group 1, BP decreased during the experimental period despite Ang II infusion; the decrease seen at 60 minutes (-26.0±6.5 mm Hg, p<0.01) was larger than in the sham-unclipped group (p<0.001) and remained so at 60, 90, and 120 minutes (-50.2±9.4 mm Hg, p<0.01; -48.6±9.6 mm Hg, p<0.01; and -58.2±12.5 mm Hg, p<0.001, respectively). In group 5, ΔBP decreased during the experimental period despite Ang II infusion; the decrease seen at 60 minutes (-26.0±6.5 mm Hg, p<0.01) was larger than in the sham-unclipped group (p<0.001). In group 3, urinary volume and U\text{Na:\text{V}} increased significantly during the experimental period. During the postinfusion period, urinary volume and U\text{Na:\text{V}} decreased from experimental values but remained higher than control values.

When BFV was not kept constant (groups 1 and 4), the hematocrit increased significantly (group 1) or tended to increase (group 4) during both experimental and postinfusion periods (Figure 3). In groups 2, 3, and 5, in which BFV was kept constant during the control and experimental periods, hematocrit did not change from the control to the experimental period. During the postinfusion period, when BFV was no longer kept constant, hematocrit tended to increase in group 2 and increased significantly in group 3 but did not change in group 5.

Discussion

While activation of the renin-angiotensin system and increases in BFV both appear to play a role in the initiation of 1K1C renal hypertension,\textsuperscript{1,2} the relative importance of these factors for mainte-
nance of hypertension in this model remains controversial.5,7-9 Our findings suggest that BFV plays an important role in the maintenance of 1K1C hypertension, but that Ang II either alone or in combination with BFV is not important.

In the present study, blood pressure remained high after removal of the clip when BFV was kept constant (group 2), but fell rapidly when BFV was allowed to decrease (group 4, experimental period; group 2, postinfusion period). These data suggest that a volume factor plays an important role in the maintenance of high blood pressure in chronic 1K1C hypertension. These results are inconsistent with earlier reports in which blood pressure did not remain high after unclipping despite maintenance of a positive fluid balance.7-9 Although Neubig and Hoobler7 infused saline every 15-30 minutes to replace urine voided during that period, it is doubtful that BFV was kept constant, since the saline was not sufficient to compensate for urine in the bladder and water lost through respiration. Muirhead and Brooks8 found that blood pressure decreased after unclipping even though body weight was kept constant by saline injection and infusion; however, the absence of retroperitoneal edema was not studied. In preliminary studies, we observed considerable retroperitoneal edema in two thirds of our rats when BFV was kept constant; this would contribute to body weight but not effective plasma volume. In the present study, BFV was maintained at control levels after unclipping by keeping body weight constant. The accuracy of our method was confirmed by the hematocrit data (see below). In addition, an autopsy was performed on all rats at the end of the experiments, and those with retroperitoneal edema were eliminated. In the experiments of Muirhead and Brooks8 and Floyer,9 although urine was returned by ureterocaval anastomoses, neither respiratory water loss nor possible hemodynamic effects of the urinary kallikrein-kinin system and urinary prostaglandins were assessed even though these factors could have contributed to the decrease in BP seen in those studies.

The importance of holding BFV constant to keep BP at hypertensive levels in 1K1C hypertension appears to be related to maintenance of plasma volume. This is consistent with the experiments of Liard and colleagues2-6 in which decreases in blood pressure were accompanied by decreases in hematocrit. The present data confirm this relationship, as shown in Figure 3. A decrease in hematocrit is accompanied by a decrease in blood viscosity, which in turn reduces cardiac output and ultimately lowers BP.

**Figure 2.** Line graphs of urinary volume (left panel) and urinary sodium excretion (right panel) in groups where body fluid volume was kept constant during experimental period. Crosses denote significance with respect to control values (*p<0.01; **p<0.001). Asterisks denote significance compared with group 5 at corresponding times (**p<0.01; ***p<0.001).

**Figure 3.** Hematocrit (left panel) and changes in hematocrit (right panel) before and after unclipping or sham unclipping. Crosses denote significance compared with control values (*p<0.05). Asterisks denote significance compared with group 5 at corresponding times (**p<0.05; ***p<0.001).
pressure after unclipping correlated significantly with decreases in plasma volume. They suggested that high blood pressure in chronic 1K1C hypertension is causally related to retention of sodium and water. Although we did not measure plasma volume in the present study, changes in plasma volume can be estimated from changes in hematocrit. In group 4, where BFV was not controlled, BP decreased in the experimental and postinfusion periods whereas hematocrit increased. On the other hand, when BFV was kept constant during the experimental period (group 2), both hematocrit and BP remained unchanged; only after BFV was allowed to decrease did BP decrease and hematocrit increase. This point is also demonstrated by the data from group 3 in which hematocrit remained constant until BFV was allowed to decrease. Thus, the fact that decreases in BP after unclipping were always associated with increases in hematocrit suggests a volume factor, presumably plasma volume, is causally related to maintenance of high blood pressure in chronic 1K1C hypertensive rats.

Although our data are consistent with involvement of a volume factor in the maintenance of 1K1C hypertension, they do not support either a direct or indirect role for Ang II. When only Ang II was infused after removal of the clip (group 1), high blood pressure was not maintained despite the fact that [Ang II] increased to nonphysiological levels during the infusion. This demonstrates that Ang II alone is insufficient to maintain a constant blood pressure after unclipping. Gavras et al reported that a competitive inhibitor of Ang II decreased blood pressure in sodium-deprived 1K1C hypertensive rats. They suggested that a "normal" renin level is actually inappropriately high for the state of volume expansion in such animals and further proposed an interaction between a volume factor and the renin-angiotensin system in the maintenance of high blood pressure in this model. To test this hypothesis, we infused Ang II in the presence of a constant BFV after unclipping. Although high blood pressure was maintained under these circumstances, the [Ang II] achieved by the infusion was nonphysiological. Thus, our results are inconsistent with an interaction between the renin-angiotensin system and a volume factor in the maintenance of blood pressure after removal of the clip in 1K1C hypertension; however, they do not rule out the possibility of such an interaction in rats on a low sodium diet.

Although our data are consistent with involvement of BFV in the maintenance of high blood pressure after unclipping, they do not rule out a role for vasoactive factors in the decrease in BP brought about by unclipping. Thus, it has been suggested that after unclipping, the kidney releases vasodilator substances that may contribute to the reduction in BP under these circumstances. Such substances may play a role in the insignificant decrease in BP seen in group 2. In that group, BP decreased 9.2 mm Hg by the end of the experimental period, although BFV was held constant. This decrease could have been related to the release of vasodepressor substances, which could also decrease BP by causing natriuresis and diuresis, thereby explaining the extremely high volume of saline needed to maintain BFV after unclipping (Figure 2), although the high diuresis and natriuresis could also be pressure related. Nevertheless, the larger decreases in BP observed after BFV was allowed to fall were probably due to fluid loss from the intravascular compartment.

References


Key Words blood pressure • fluid volume • Goldblatt hypertension • rat studies
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