Renal Glomerular Atrial Natriuretic Factor Receptors in One-Kidney, One Clip Rats

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SUMMARY One-kidney, one clip (1K1C) hypertension is often associated with an expanded plasma volume and (once the arterial clip is removed) with natriuresis. Blood pressure (BP), atrial and plasma concentrations of atrial natriuretic factor (ANF), hematocrit, and renal glomerular ANF receptors were therefore studied in 1K1C rats and in their normotensive uninephrectomized controls before and after unclipping. Six hours after removal of the clip, BP was normal in the 1K1C group and plasma ANF presented a sharp decline but was still significantly higher than in the normotensive controls, with a slight difference being evident 24 hours after unclipping. Hematocrit was lower in the 1K1C rats than in their control counterparts, but this difference tended to disappear once the clip was removed, indicating a contraction of plasma volume in these unclipped 1K1C animals. The renal glomerular ANF receptor population was markedly smaller in 1K1C rats than in the uninephrectomized controls but showed a twofold increase in number and affinity 24 hours after unclipping. It is concluded that the up-regulation and enhanced affinity of glomerular ANF receptors (probably secondary to the decrease in plasma levels of ANF) may contribute to the natriuresis reported in hypertensive 1K1C animals on removal of the arterial clip. (Hypertension 11: 185-190, 1988)

KEY WORDS • atrial natriuretic factor • one-kidney, one clip hypertension • renal glomerular atrial natriuretic factor receptors • natriuresis

THE one-kidney, one clip (1K1C) paradigm is believed to be a volume-expanded model of experimental hypertension,1 with early sodium retention2,3 and increased exchangeable sodium levels.4 Unclipping of the remaining kidney is usually followed by marked natriuresis5 and permanent, almost immediate reversal of hypertension.1,5

We have recently reported6 that the high plasma concentrations of atrial natriuretic factor (ANF) in 1K1C animals with 4 weeks of hypertension declined rapidly 6 hours after the clip was removed but re-mained significantly higher than in the controls for 5 days after unclipping. In these experiments, removal of the clip was also accompanied by a rapid normalization of blood pressure (BP) and by weight loss, which quickly returned to control levels. This acute transient decrease in weight was suggestive of increased natriuresis and depletion of body fluid. Since it has been well documented that specific ANF receptors present in renal glomeruli7,8 can be manipulated by physiological interventions, which result in changes in the plasma concentration of ANF,9,10 and since the glomerulus is a major target of ANF, we have hypothesized6 that the natriuresis observed in 1K1C rats after unclipping could be partially due to their higher plasma ANF levels and to modifications of specific glomerular ANF binding sites. Indeed, it has recently been shown that vascular ANF receptors, both in cultured smooth muscle11 and in blood vessels of 1K1C12 and deoxycorticosterone acetate–salt hypertensive rats,13 vary inversely with the ambient concentration of ANF. To test this hypothesis, we investigated whether the number and affinity of glomerular ANF receptors are altered after removal of the arterial clip in 1K1C rats. Plasma and atrial concentrations of ANF were also measured in this study.
Materials and Methods

Animal Experiments

1K1C hypertension was produced in male Sprague-Dawley rats (weight, 180–200 g; Charles River Breeding Laboratories, St.-Constant, Quebec, Canada) under sodium pentobarbital anesthesia (60 mg/kg i.p.) by partial constriction of the left renal artery with a silver clamp (internal gap, 0.20 mm) and by removal of the contralateral kidney 1 week later. Nortemisotiv controls underwent a sham operation and were subjected to a right nephrectomy after 1 week. All the animals were maintained on regular rat chow and tap water ad libitum. Systolic BP was monitored once a week by means of a tail cuff with the rats under light ether anesthesia. When a reading of 150 mmHg or more was obtained for 6 consecutive weeks in the 1K1C rats, nortemisotiv and hypertensive groups were killed before or 6 or 24 hours after unclipping. Systolic BP was measured and blood samples were taken before the animals were killed. Blood collection and unclipping were both performed with the rats under pentobarbital anesthesia. The uninephrectomized, normotensive controls were subjected to sham unclipping, where the abdominal cavity was exposed and the left renal artery manipulated. Systolic BP was measured in these conscious, preheated animals (37°C for 10 minutes) by the tail-cuff method (Narco Biosystems, TX, USA).

Biochemical Methods

Blood was collected in glass tubes containing the following protease inhibitors at final concentrations of 1.0 x 10^-5 M EDTA, 5 x 10^-6 M pepstatin, 1000 U/ml aprotinin, and 3 x 10^-3 M phenylmethylsulfonyl fluoride (PMSF). The samples were immediately centrifuged at 2000 g for 10 minutes at 6°C. ANF was extracted from plasma with Vycor glass beads (Corning Glass Works, Corning, NY, USA) and measured by radioimmunoassay, as described elsewhere.14 The detection limit of the assay is 0.75 pg/tube. The interassay and intra-assay coefficients of variance are below 16%, and the recovery of added 123I-ANF is 75%.

Hearts and kidneys were rapidly removed after death. The right and left atria were dissected separately, and atrial ANF concentrations were measured by radioimmunoassay.15 Briefly, atria were homogenized for 1 minute with a Polytron (setting 8; Brinkmann Instruments, Westbury, NY, USA) in 2 ml of 0.1 M acetic acid containing same protease inhibitors as already described. The detection limit of the assay is 3 pg/tube. The interassay and intra-assay coefficients of variance are below 16%, and the recovery of added 123I-ANF is 85%. Atrial protein content was assessed by a modification of Bradford’s method.16

Preparation of Glomeruli

The kidneys were excised from the renal capsules, placed in ice-cold 0.9% NaCl, dissected longitudinally, and the medulla and papilla eliminated. Renal tissue was then homogenized by passing the cortical tissue through a 0.5-mm grid. The mesh was diluted with 0.9% NaCl and filtered through 200-μm, 50-μm, 100-μm and 150-μm mesh nylon sieves. Glomeruli retained in the sieve were collected (4°C, 2000 g). After the last centrifugation, they were suspended in 0.05 M Tris HCl (pH 7.2) and kept at ~70°C. This glomerular suspension was homogenized for 1 minute with a Polytron (Setting 6), centrifuged at 30,000 g for 30 minutes, and resuspended in 1 ml of 0.05 M Tris HCl (pH 7.2). An aliquot was taken for protein determination by a modification of Bradford’s method.16 The glomerular suspension was then added to the final assay buffer containing 50 mM Tris HCl (pH 7.2) 1 μM aprotinin, 0.1% bacitracin, 0.5 mM bovine serum albumin, 5 mM MgCl2, and 0.4% PMSF.

ANF Binding Assay

The binding assay was performed as follows: Aliquots (35 μg) of glomerular membrane protein were incubated in duplicate for 60 minutes at 22°C, as reported by Carrier et al.,17 in the presence of increasing concentrations of unlabeled ANF (10^-13 to 10^-7 M) and 20 pM 125I-ANF in a final volume of 1 ml. The reaction was stopped by dilution with 3.5 ml of assay buffer and rapid filtration through polyethyleneimine-treated Whatman GF/C filters (Clifton, NJ, USA), which were then rinsed three times with 3 ml of Tris HCl (pH 7.2), allowed to dry, and counted in a gamma counter (LKB, Turku, Finland) with 65% efficiency. 125I-ANF was prepared by the lactoperoxidase method.18 It had a specific activity of approximately 1000 Ci/mmol. Rat ANF (Ser 99-Tyr126) was purchased from BioMega (Laval, Quebec, Canada).

Analysis of Data

Results are expressed as means ± SEM. The data were evaluated when applicable by two-way analysis of variance with repeated measures to globally test the time effect, the group effect, and the group interaction by time (F test). An a posteriori contrast test, according to Bonferroni’s method, was applied to globally test the time effect whenever a level of significance was found (p < 0.05). The binding data were analyzed using the computer-based LIGAND program,19 after preliminary treatment of the data using the program EBDA,20 to determine the density and affinity of binding sites in the competition experiments. These results were analyzed by the unpaired Student’s t test and were considered significant at a p level below 0.05.

Results

Figure 1 depicts the BP, relative heart weight, and hematocrit values before and 6 and 24 hours after removal of the renal artery clip. Before unclipping, BP was 98 ± 2 mmHg in the normotensive rats and 187 ± 6 mmHg in the 1K1C group (p < 0.01). There were no differences in BP between the hypertensive and normotensive animals 6 hours after the clip was removed as well as thereafter. The heart weight of 1K1C rats, normally higher than that in normotensive controls at all times, tended to be lower 24 hours after unclipping, but this difference was not significant. He-
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FIGURE 1. Effect of removal of renal artery clip on systolic BP, heart weight, and hematocrit in controls (C; n = 15) at time periods 1, 2, and 3 and in 1K1C rats (n = 9) at time periods 1, 2, and 3 (n = 6). (bw = body weight.)

matocrit, before unclipping, was significantly lower in 1K1C animals than in normotensive rats (33.3 ± 2.1 vs 41.7 ± 0.5%). This difference tended to diminish 24 hours after the renal artery clip was removed, but it remained significant (p<0.05), with values of 35.6 ± 1.3% in unclipped 1K1C rats and 40.4 ± 0.6% in the normotensive group. Body weight was higher in normotensive than in hypertensive animals (429 ± 8 vs 332 ± 19 g; p<0.01) before unclipping.

Figure 2 illustrates the plasma ANF levels, atrial ANF concentrations, and number of specific ANF binding sites in renal glomeruli (B^) before and at the two periods after unclipping or sham unclipping. Before removal of the renal artery clip, plasma ANF was significantly higher in hypertensive 1K1C rats than in their normotensive controls (133.5 ± 21.8 vs 40.0 ± 4.5 pg/ml). Six hours after unclipping, plasma ANF presented a sharp decline, but it was still significantly higher in the now normotensive unclipped 1K1C rats (p<0.05). Twenty-four hours after the arterial clamp was removed, plasma ANF still tended to be higher in the 1K1C rats, but because of intra-group variations, no statistically significant differences were found. A significant positive correlation was obtained between plasma ANF and systolic BP in both hypertensive and normotensive rats before unclipping (r = 0.79, p<0.01) and when all periods were considered (r = 0.62, p<0.001).

The atrial ANF concentration was lower in the right atrium of 1K1C hypertensive animals than in their normotensive counterparts (see Figure 2). Six hours after removal of the arterial clip, this difference was also observed in the left atrium. In the 24-hour period after unclipping, the ANF concentration in the right atrium was similar in both groups but in the left atrium it was higher in 1K1C animals.

The number of ANF binding sites (B_m) in the renal glomeruli was significantly lower (p<0.01) before unclamping in 1K1C hypertensive rats than in uninephrectomized normotensive controls (see Figure 2). This difference was still observed 6 hours after the clip was removed, but after 24 hours an approximately twofold rise in glomerular ANF binding sites was seen in 1K1C animals, which increased from 348 ± 50 fmol/mg protein before unclipping to 610 ± 12 fmol/mg protein 24 hours after clip removal. At the same time, the glomerular receptor affinity (K_d) of 1K1C rats was increased from 76 ± 12 pM before to 35 ± 3 pM after unclipping. Figure 3 shows a representative binding curve for control and 1K1C animals before unclipping and 6 and 24 hours after removal of the renal artery clamp.

**Discussion**

In both hypertensive 1K1C rats and dogs, unclamping of the constricted renal artery is followed by
ANF is a potent natriuretic and vasorelaxant peptide that may play a role in body fluid and BP regulation. However, the mechanism of ANF-induced natriuresis has not been clearly identified, although its effect on glomerular filtration appears to be predominant.\textsuperscript{19, 20} Recently, it has been reported that the number and affinity of ANF-specific receptors in renal glomeruli\textsuperscript{7–9} can be modified by chronic sodium intake\textsuperscript{6} and that these changes are inversely correlated with plasma ANF levels.\textsuperscript{10} A short-term down-regulation of glomerular ANF receptors has also been noted during mineralocorticoid escape in the rat,\textsuperscript{21} similar to that observed in cultured vascular smooth muscle cells\textsuperscript{11} and in blood vessels during sodium loading and in volume-expanded models of hypertension.\textsuperscript{12, 13}

We have now confirmed our previous results\textsuperscript{6} by killing groups of rats at different time intervals. The substantially higher levels of plasma ANF in animals with chronic 1K1C hypertension in comparison to normotensive controls could be secondary to the dual mechanism of volume expansion and elevated BP. The latter, by increasing left ventricular end-diastolic pressure, could raise left atrial pressure, thus stimulating ANF release. On the other hand, volume expansion, by enhancing venous return, could elevate right atrial pressure. 1K1C rats have lower hematocrit values than normotensive controls have, which may reflect an expanded plasma volume. Once the clip was removed, however, these values approached control levels, suggesting a reduction of plasma volume, due either to a redistribution of extracellular fluid or to urinary loss, as reported by other investigators.\textsuperscript{1, 3, 5} Hypertensive 1K1C dogs present a negative sodium and fluid balance during the 3 days following removal of the clip, and these changes are accompanied by a plasma volume decreased to preconstriction levels, with a gradual hematocrit rise,\textsuperscript{1} indicating that the normalization of BP is probably associated with the diminution of extracellular fluid. Our results are in accordance with those in the dog in the sense that the unclipping of 1K1C rats produced a slight rise in hematocrit in the present experiments, with rapid weight loss,\textsuperscript{6} suggestive of fluid depletion. It has been reported that urinary sodium excretion follows variations in mean arterial BP,\textsuperscript{22} and thus decreases or increases in the latter induce parallel changes in the former. It has been recently reported\textsuperscript{23} that the acute natriuresis induced by exogenously administered ANF may be blunted or abolished when renal arterial pressure is lowered. That mechanism could partially explain why 1K1C animals, with the only kidney having the renal artery clipped and therefore with a diminished renal arterial pressure, may have a positive sodium balance even in the presence of high plasma ANF. In the chronic situation, another factor promoting sodium retention could be the lower glomerular ANF receptor density.

An elevation of renal artery pressure has been invoked as the mechanism responsible for the natriuresis observed once the arterial clip is removed.\textsuperscript{3} However, this mechanism could not be the only factor involved in acute\textsuperscript{22} or chronic experiments, since the clipped
kidney in two-kidney, one clip hypertension manifested greatly depressed sodium excretion in the presence of normal perfusion pressure. Moreover, following the release of the clip in 1K1C hypertensive dogs, the aortorenal pressure gradient falls immediately to pre-constriction values, but a negative sodium balance may last for several days. These experiments indicate that however important pressure may be, it is not the only element present to explain natriuresis and plasma volume reduction once the arterial clip is removed. As illustrated in Figure 3, the hematocrit was still lower in unclipped and normotensive 1K1C animals than in controls 24 hours after removal of the clip, suggesting that the circulatory volume was still expanded. Only 4 days after the unclipping, the hematocrit returned to the values observed in controls. At this time the density of glomerular ANF receptors is identical in both groups (R. Garcia et al., unpublished results, 1987).

These data suggest that pressure by itself could be an important natriuretic mechanism immediately after the clip is removed, but another mechanism may be involved in provoking a more prolonged negative sodium balance and consequently reestablishing a normal plasma volume.

We have now obtained evidence that the reversal of hypertension is accompanied by a rapid decline in plasma ANF, which, however, still tends to be higher than in control animals 24 hours after the arterial clip is removed. Previously, we reported that these higher levels of ANF in plasma were maintained for as many as 5 days after the renal artery was unclamped. Our present experiments also demonstrate that, 24 hours after unclipping, there is an increment in both glomerular ANF receptor density ($B_{max}$) and affinity ($K_d$) that, as previously suggested, may be involved, together with the high plasma ANF levels, in the fluid loss occurring in this model of hypertension once the arterial clip is removed. This glomerular ANF receptor up-regulation does not, however, reach the receptor density observed in uninephrectomized controls. This is not unexpected, since the appearance of new receptors requires protein synthesis and it may take a longer time (yet to be determined) for the receptors in the unclipped kidney to attain the same number as in the remaining kidney of uninephrectomized rats. A 12-hour down-regulation of glomerular ANF receptors has been reported during mineralocorticoid escape in the rat. Together with the results of the present experiments, this finding suggests that the regulation of glomerular ANF receptors, by changing the renal responsiveness to ANF, may play as important a role as plasma ANF levels in maintaining sodium and water homeostasis.

In summary, removal of the renal artery clip in hypertensive 1K1C rats results in an increase in glomerular ANF receptor density and affinity with a rapid decline in plasma ANF, which, however, remains slightly higher than in uninephrectomized controls 24 hours after unclipping. The data indicate that a dual mechanism could be responsible for the natriuresis induced by unclipping 1K1C rats. Early after clip removal, pressure natriuresis may play a predominant role. Later, plasma ANF and the up-regulation of glomerular ANF receptors may take over as the main mechanism producing natriuresis during hypertension reversal after unclipping 1K1C Goldblatt hypertensive rats.

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