Renin Dependency of the Effect of Chronically Administered Atrial Natriuretic Factor in Two-Kidney, One Clip Rats

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SUMMARY Conscious two-kidney, one clip rats with 150 mm Hg or higher systolic blood pressure were infused with saralasin for 60 minutes. Those with a blood pressure decline of 30 mm Hg or more were classified as saralasin-sensitive; those with a decrease of 10 mm Hg or less were considered saralasin-resistant. The animals were then housed in metabolic cages. Groups of sham-operated normotensive, saralasin-sensitive or saralasin-resistant two-kidney, one clip (2K1C) rats were infused with atrial natriuretic factor (Arg 101–Tyr 126), 100 ng/hr per rat, for 6 days. Corresponding control groups were sham-infused. Blood pressure was initially higher in the saralasin-sensitive groups (176 ± 6 and 181 ± 1 mm Hg, respectively) than in the saralasin-resistant groups (160 ± 4 and 169 ± 4 mm Hg, respectively). Atrial natriuretic factor infusion produced a gradual decline in blood pressure to 128 ± 5 mm Hg, but only in saralasin-sensitive 2K1C animals. Urinary volume, initially higher in saralasin-sensitive hypertensive than in normotensive rats, was depressed during atrial natriuretic factor infusion. Urinary sodium excretion and water intake showed the same tendency, but the changes were not significant. No such modifications were observed in saralasin-resistant or sham-operated rats infused with atrial natriuretic factor. Body weight, which was higher in normotensive animals, was unchanged during atrial natriuretic factor infusion. Saralasin-sensitive, noninfused 2K1C rats were the only group with higher plasma renin activity than sham-operated, normotensive controls. Plasma aldosterone was higher in the former than in the other five groups. Saralasin-resistant 2K1C rats, infused or not, or sham-operated rats infused with atrial natriuretic factor, had plasma aldosterone levels no different from those of normotensive controls. A positive correlation was noted between plasma renin activity and plasma aldosterone. Plasma renin activity and concentration of immunoreactive atrial natriuretic factor were similar in all groups. These results suggest that the hypotensive response observed in saralasin-sensitive 2K1C rats during chronic atrial natriuretic factor infusion could be secondary to an inhibitory effect on renin release and that a decrease in the latter could play a role in the depression of plasma aldosterone.

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KEY WORDS • atrial natriuretic factor • two-kidney, one clip hypertension • saralasin • plasma aldosterone • plasma renin activity

We have recently reported1 that chronic infusion of atrial natriuretic factor (ANF) reduces blood pressure (BP) in two-kidney, one clip (2K1C) hypertensive rats. This decline in BP is accompanied by decreased plasma renin activity (PRA), in contrast with acute experiments on the same model of hypertension, in which PRA increased after acute ANF administration.2 What remained doubtful in our earlier investigation was whether the normalization of BP in 2K1C animals was a consequence of ANF-induced hemodynamic changes or of PRA inhibition.

The pathogenesis of 2K1C hypertension in the rat has been ascribed to enhanced renin release by the ischemic kidney. However, during the different stages of the development of hypertension, not all 2K1C hypertensive animals are renin-dependent.3,4 Since we have found (R. Garcia et al., unpublished observations, 1986) that not all 2K1C hypertensive rats respond to chronic ANF infusion with similar BP reductions, we decided to investigate whether the
responsiveness to ANF of this model of experimental hypertension is secondary to renin dependency.

Materials and Methods

The 2K1C model of hypertension was produced in male Sprague-Dawley rats (weight, 180–200 g) under sodium pentobarbital anesthesia (60 mg/kg body weight i.p.) by partial constriction of the left renal artery with a silver clamp of 0.20 mm internal diameter; the contralateral kidney was left untouched. Animals subjected to sham operation, in which the left kidney was exposed and the left renal artery was stripped, served as normotensive controls.

Systolic BP was measured indirectly twice a week by means of a tail cuff, as described elsewhere.1 When the BP of the 2K1C rats was 150 mm Hg or higher for 2 or 3 consecutive weeks, the animals were anesthetized with sodium pentobarbital and intrafemoral vein and artery catheters were installed. The intra-arterial catheter was connected to a Gould Statham P23ID pressure transducer (Saddle Brook, NJ, USA) as soon as the rats were fully awake, and their direct mean arterial BP was thus monitored continuously. When their BP became stable for 30 minutes, the animals were challenged with a 50-ng bolus injection of angiotensin II (Hypertensin, CIBA, Dorval, Quebec, Canada) administered through the intrafemoral vein catheter at a rate of 4 µg/min per rat, which was immediately followed with 200 µl of 5% dextrose in water. A continuous infusion of saralasin ([Sar-1-Ala8]angiotensin II, Peninsula Laboratories, Belmont, CA, USA) in 5% dextrose through the intrafemoral vein catheter at a rate of 4 µg/min per rat, was then given for a total of 60 minutes. After the first 30 minutes, a second injection of angiotensin II was administered through the previously washed intrafemoral catheter. Animals with a BP decline of 30 mm Hg or more (as a maximal response) were considered saralasin-sensitive; those whose BP fell by 10 mm Hg or less were categorized as saralasin-resistant.3 Rats that reacted with a decrease in BP ranging between 10 and 30 mm Hg were not included in the experiment. Both catheters were removed on termination of the saralasin infusion. The animals then were housed in individual metabolic cages for 3 to 4 days to allow them to adapt to their new environment.

The second part of the experiment was started not later than 4 days after the animals were classified. They were maintained on regular rat chow and tap water ad libitum. Twenty-four hours after this initial period, they were separated into six experimental groups. One group each of sham-operated, saralasin-sensitive or saralasin-resistant 2K1C rats was implanted in the neck, under ether anesthesia, with osmotic minipumps (Model 2001: Alza, Palo Alto, CA, USA) containing synthetic ANF (Arg 101–Tyr 126, Institute Armand Frappier, Laval, Quebec, Canada) dissolved in 0.1 M acetic acid and volume-completed with 0.9% NaCl; release of the peptide was calculated at 100 ng/hr (35 pmol/hr). The pumps were connected to the left jugular vein by a polyethylene catheter (PE-60). Matching groups of sham-operated, saralasin-sensitive or saralasin-resistant 2K1C rats were likewise anesthetized, and a piece of plastic tubing, of the same size as that used for the minipumps, was implanted subcutaneously; the left jugular was cannulated with a blind catheter.

Urinary volume (UV) and water intake were quantified daily starting 24 hours before the pumps were implanted. Systolic BP was measured indirectly every day from the day the pumps were installed (Day 0). Body weight was recorded daily. Urinary sodium was assessed by flame photometry.

The animals were killed by decapitation 6 days after the pumps were installed. Blood was collected, the heart was excised and weighed, and both atria were dissected separately. The PRA was measured by radioimmunoassay of generated angiotensin I.5 Plasma aldosterone was determined by radioimmunoassay after extraction and paper chromatography.6 The atria were homogenized in 2 ml of 0.1 M acetic acid for 1 minute and centrifuged at 12,000 g for 20 minutes. The supernatant was stored at −70°C, then thawed and centrifuged a second time. The pellet was discarded, and the supernatant was stored at −70°C until assayed. The ANF then was measured by radioimmunoassay.7 Protein concentration was ascertained by a modification of Bradford’s method.8

The results are expressed as means ± SEM. The data presented in Figures 1 to 5 were analyzed by two-way analysis of variance with repeated measures to globally test the time effect, the group effect, and the group interaction by time. One-way analysis of variance was employed with repeated measures for each group to globally test the time effect. Dunnett’s test was applied wherever a level of significance was found ($p<0.05$).

The data in Tables 1 and 2 were analyzed by one-way analysis of variance for each variable, followed by the Student-Newman-Keuls test. When the variability was not the same for the different groups, the data were subjected to logarithmic transformation.

Results

Figure 1 depicts BP in the sham-operated normotensive group, the saralasin-sensitive 2K1C group, and the saralasin-resistant 2K1C group. Before the ANF infusion was started, basal BP (Time 0) was higher ($p<0.05$) in saralasin-sensitive than in saralasin-resistant 2K1C animals (176 ± 6 mm Hg in saralasin-sensitive, ANF-infused rats vs 181 ± 6 mm Hg in saralasin-sensitive, noninfused animals). The BP of ANF-infused and noninfused saralasin-resistant rats was 160 ± 4 and 169 ± 4 mm Hg, respectively. Of the three groups infused with ANF, only the saralasin-sensitive 2K1C animals showed a maximal BP decline to 128 ± 5 mm Hg at Day 5. No BP changes were observed in either normotensive or saralasin-resistant, hypertensive animals.
Figure 1. Effect of chronic administration of ANF on blood pressure in sham-operated normotensive (a) and saralasin-sensitive (b) and saralasin-resistant (c) 2K1C hypertensive rats.

Figure 2 illustrates the sodium excretion rate in all experimental animals. Although the hypertensive group tended to have a higher urinary sodium excretion rate than the normotensive rats, no difference between them was manifested. Saralasin-sensitive, ANF-infused 2K1C animals also displayed a slightly lower sodium excretion rate, but again this difference was not significant. Basal UV was significantly higher (p<0.05) in both saralasin-sensitive 2K1C groups than in normotensive animals (Figure 3). A significant drop in UV, noted in saralasin-sensitive, ANF-infused 2K1C animals, coincided with the decline in BP. No such change was seen in either ANF-infused normotensive or saralasin-resistant 2K1C animals.

As with the urinary sodium excretion rate, water intake tended to be higher among hypertensive than among normotensive animals, but no statistical significance was found (Figure 4). The slight decrease in water intake observed in saralasin-sensitive, ANF-infused 2K1C rats was also not significant.

Body weight was higher in the normotensive than in the hypertensive groups. None exhibited weight changes during ANF infusion (Figure 5).

Saralasin-sensitive, noninfused 2K1C rats represented the only group with a higher PRA than that of sham-operated animals (Table 1). Plasma aldosterone in this group was significantly higher than in the other five groups. Because of the wide range of values of plasma aldosterone in saralasin-resistant 2K1C animals, this parameter showed no difference between ANF-infused and noninfused rats. When values for all groups were pooled, a positive and significant correlation was evident between PRA and plasma aldosterone (r = 0.64, p < 0.001). The heart weight of all hypertensive animals was greater than that of normotensive rats. However, in saralasin-sensitive, ANF-infused hypertensive rats, heart weight was higher than in sham-operated controls but significantly lower (p < 0.05) than in saralasin-sensitive, noninfused 2K1C animals. No differences in hematocrit were discerned between the various groups.

The total atrial content or concentration of atrial ANF revealed no differences between either normotensive and hypertensive or ANF-infused and noninfused animals (Table 2).

Discussion

We have demonstrated previously that chronic infusion of ANF into 2K1C hypertensive rats decreases BP and PRA, but we could not elucidate whether the decrease in BP was a consequence of the decrease in PRA. Repeated experiments have shown, however, that not all 2K1C rats respond to ANF infusion with the same degree of hypotension (R. Garcia et al., unpublished observations, 1986). Moreover, the chronic effect of ANF on plasma aldosterone in the same model of hypertension was not clearly defined. We have now determined that chronic infusion of ANF decreases BP, PRA, and plasma aldosterone in 2K1C hypertensive rats only when they are renin-dependent. As reported earlier, the BP reduction was accompanied by a fall in UV, and a similar tendency was evident in urinary sodium excretion and water intake. Furthermore, the BP decrease was reflected by a partial regression of cardiac hypertrophy. No such changes
were evident in ANF-infused, saralasin-resistant 2K1C or sham-operated rats.

The pathogenesis of hypertension in the 2K1C model classically has been attributed to renin overproduction by the ischemic kidney. However, several investigators\(^3\,^4\) have demonstrated that, whatever the stage of development of hypertension in 2K1C rats, a certain percent are not renin-dependent. Whether the mechanism of hypertension in the latter is related to a positive sodium balance or to unknown factor(s) remains to be elucidated.\(^10\,^12\)

Higher BP readings in saralasin-sensitive than in saralasin-resistant hypertensive rats seem to be frequent findings.\(^3\) The finding that chronic administration of ANF decreases BP in 2K1C hypertensive animals only when they are renin-dependent suggests that the hypotensive effect of ANF in this model of experimental hypertension is related to the inhibition of renin release and not to a direct hemodynamic action, as observed during acute experiments.\(^13\) By depressing renin release and, consequently, plasma angiotensin II, ANF could indirectly diminish vascular peripheral resistance. However, since ANF inhibits the in vitro contraction induced in vascular smooth muscle by various agonists, including angiotensin II,\(^14\,^15\) the possibility of a direct inhibitory effect in the effector site—in this case the vascular smooth muscle—cannot be excluded.

The action of ANF on PRA is a controversial subject. Acute experiments on anesthetized animals\(^16\,^18\) with stimulated renin release have demonstrated that ANF administration depresses the PRA and renin se-
cretory rate. These findings have been interpreted as the result of either an increased sodium load to the macula densa\textsuperscript{16,17} or a direct inhibitory effect on juxtaglomerular cells.\textsuperscript{17,18} The fact that renin, released by kidney slices, can also be suppressed by ANF\textsuperscript{19} lends added credibility to the latter explanation. The present finding of decreased renin in the absence of increased urinary sodium excretion indicates a similar trend. No significant PRA changes were observed in either saralasin-resistant 2K1C or sham-operated rats infused with ANF, suggesting that renin has to be previously stimulated to be inhibited. Suppression of a preactivated adenylate cyclase-cyclic adenosine 3'5'-monophosphate system could be an explanation.\textsuperscript{18,19} The PRA was also not modified when a volume-dependent model of experimental hypertension, such as the one-kidney, one clip rat, was infused with ANF.\textsuperscript{20} On the other hand, acute administration of ANF to renin-dependent 2K1C rats has been reported to produce a further elevation of already high PRA.\textsuperscript{2} It is possible that, since acute administration of ANF evokes profuse natriuresis, a reduction in blood volume, secondary to sodium depletion, could decrease renal perfusion pressure, stimulating renin release.

Another characteristic of ANF is its inhibitory influence on aldosterone secretion induced by angiotensin II-stimulated, adrenocorticotropic hormone-stimulated, or K\textsuperscript+-stimulated adrenal cells "in vitro."\textsuperscript{21-23} and by angiotensin II "in vivo."\textsuperscript{22} Our present data demonstrate that plasma aldosterone is significantly higher in saralasin-sensitive, noninfused rats than in saralasin-sensitive, ANF-infused 2K1C animals; levels in the latter were not different from those in sham-operated controls. A similar tendency was noted in ANF-infused, saralasin-resistant rats, but because of intra-group variations it did not reach significant levels. Whether the lower plasma aldosterone found in renin-dependent hypertensive animals infused with ANF is due to a direct inhibitory effect of the peptide on adrenal cells — as seen "in vitro"\textsuperscript{21-23} — or to the lower plasma renin levels could not be elucidated from the present experiments. The correlation between PRA and plasma aldosterone could be an indication of the latter possibility. Others\textsuperscript{2} have reported a dissociation between PRA and plasma aldosterone after acute ANF administration to renin-dependent 2K1C rats, which suggests that, in this case, ANF probably acts directly on aldosterone production by the adrenal cells.
The atrial content and concentration of atrial ANF were similar in all presently investigated groups, in contrast to other models of spontaneous and non-spontaneous hypertension, in which a lower immunoreactive atrial ANF content was found, indicating that BP was not the only factor involved. However,
the atrial content of ANF is not a good predictor of its plasma concentration, at least during chronic hypertension (R. Garcia et al., unpublished observations, 1986). The ANF infusion did not modify atrial ANF content in the present experiments.

In summary, we have demonstrated that chronic ANF infusion in 2K1C rats decreases BP, PRA, and plasma aldosterone only when the hypertensive animals are renin-dependent. The hypotensive and aldosterone inhibiting effects could be mediated by suppression of renin release and might not have a direct impact on the effector organs. The possibility of using an ANF infusion test to discern renin-dependent human renovascular hypertension remains open.

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References


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