Glomerular and Vascular Atrial Natriuretic Factor Receptors in Saralasin-Sensitive and -Resistant Two-Kidney, One-Clip Hypertensive Rats

Raul Garcia, Guillemette Gauquelin, Marc Cantin, and Ernesto L. Schiffrin

We have investigated whether there is a relation between renin dependency of two-kidney, one-clip (2K1C) hypertensive rats and the density of renal glomerular and vascular atrial natriuretic factor (ANF) receptors. Conscious 2K1C rats with blood pressure of 150 mm Hg or higher were classified according to their sensitivity to the blood pressure-lowering effect of the angiotensin II antagonist saralasin. Both hypertensive groups had lower body weights and greater relative heart weights than normotensive controls. Hematocrit was lower and plasma volume higher in saralasin-resistant animals than in either saralasin-sensitive or control rats. Plasma renin activity was higher in the saralasin-sensitive group than in the resistant rats. Plasma ANF concentration was greater in saralasin-resistant than in either not hypertensive or saralasin-sensitive animals. ANF was reduced in both atria of saralasin-resistant 2K1C animals but only in the left atrium of the sensitive group. Both hypertensive groups showed an increased ventricular ANF concentration. The number of glomerular ANF binding sites was significantly lower in the clipped kidney of both hypertensive groups. This lower density of binding sites was accompanied by an increased affinity. In saralasin-sensitive rats, the density of glomerular ANF receptors in the nonclipped kidney was significantly higher than in the controls. Saralasin-resistant rats exhibited a decreased number of vascular ANF binding sites in both mesenteric arteries and aorta. We conclude that through modulation of its glomerular and vascular receptors, ANF may contribute to the differential sodium handling of saralasin-sensitive and -resistant 2K1C hypertensive rats and to the reduced vascular responsiveness to ANF observed in the saralasin-resistant hypertensive rats. (Circulation Research 1988;63:563–571)

During the initial phase (2–3 weeks) of development of two-kidney, one-clip (2K1C) hypertension in the rat, sodium retention may1,2 or may not3 occur. However, if the blood pressure (BP) level rises over 180 mm Hg, a negative sodium balance is usually present.1,2,4 Because this model of experimental hypertension is associated with stimulation of renin secretion,1,2 the loss of sodium is accompanied by a further stimulation of the renin-angiotensin system. The increased activity of the renin-angiotensin system may represent an important mechanism for the maintenance of elevated BP under conditions of sodium depletion. Renin dependency in this model of experimental hypertension seems to be a changing process during the development of high BP.5,6 As the hypertension becomes more chronic, the frequency of apparent renin dependency is reduced.6 2K1C rats that do not develop severe hypertension may have an increased blood volume,1 but no clear evidence of sodium retention has been reported at this stage.7,8 Physiologically regulated specific receptors for atrial natriuretic factor (ANF) have been demonstrated in rat renal glomeruli.9,10 Because the density of glomerular ANF receptors in the clipped and nonclipped kidney varies during the development of 2K1C hypertension (G. Gauquelin, E.L. Schiffrin, M. Cantin, and R. Garcia, unpublished observations) and sodium excretion by the clipped kidney is reduced,11 we investigated whether the number of glomerular ANF receptors in the clipped and non-
clipped kidney is related to the renin dependency of 2K1C animals. This would help to explain, at least in part, the different sodium handling by each kidney. We recently demonstrated that in one-kidney, one-clip (1K1C) animals, which have a positive sodium balance, the number of glomerular receptors for ANF is significantly decreased. Consequently, glomerular receptors in the clipped and nonclipped kidney were assessed in the present study.

Vascular ANF receptors in the blood vessels of 1K1C and deoxycorticosterone-salt hypertensive rats may be inversely correlated with plasma ANF levels. In each of these models of experimental hypertension, the density of vascular ANF receptors is decreased, the potency of ANF to relax norepinephrine-contracted vascular strips is diminished, and ANF has reduced BP-lowering effects. Because 2K1C hypertensive rats, depending on whether they are saralasin-sensitive, may respond to a chronic infusion of ANF with a reduction in BP, we investigated whether this hypotensive response is related to changes in the density or affinity of vascular ANF receptors. Plasma, atrial, and ventricular ANF concentrations as well as plasma and blood volume were measured in this study.

Materials and Methods

Animal Experiments

2K1C hypertension was produced in male Sprague-Dawley rats (130-150 g; Charles River, St-Constant, Quebec, Canada) under sodium pentobarbital anesthesia (60 mg/kg i.p.) by partial constriction of the left renal artery with a rigid silver clamp of 0.20 mm i.d.; the contralateral kidney was left untouched. Animals subjected to a sham operation served as normotensive controls. All groups were maintained on regular rat chow and tap water ad libitum. Systolic BP was monitored twice a week or more. Animals were killed when their BP became stable for 30 minutes. When their BP became stable for 30 minutes, a second injection of angiotensin II was administered through the intrafemoral vein catheter at a rate of 4 µl/min/rat for a total of 30 minutes. After the first 15 minutes, a second injection of angiotensin II was administered through the previously washed intravenous catheter. Animals with a BP decline of 25 mm Hg or more (maximal response) were considered saralasin-sensitive; those whose BP dropped by 10 mm Hg or less were classified as saralasin-resistant. Animals that reacted with a fall in BP ranging between 10 and 25 mm Hg were eliminated from the experiment. Both catheters were removed on termination of the saralasin infusion.

The second part of the experiments started no later than 4 days after the animals were classified.

Biochemical Methods

Trunk blood was collected immediately after decapitation. Plasma renin activity (PRA) was measured by radioimmunoassay (RIA) of generated angiotensin I.

Blood for ANF measurement was collected in tubes containing the following protease inhibitors at final concentrations of 1.0 x 10^-5 M EDTA, 5 x 10^-6 M pepstatin, and 3 x 10^-5 M phenylmethylsulfonyl fluoride (PMSF). The samples were immediately centrifuged at 2,000g for 10 minutes at 4°C. ANF was extracted from plasma with Vycor glass beads (Corning Glass Works, Corning, New York) and measured by RIA, as described elsewhere. The detection limit of the assay was 0.75 pg/tube. The interassay and intra-assay coefficients of variance were below 14%, and the recovery of added 125I-ANF was 75%.

Hearts, kidneys, thoracic aortas, and mesenteric vascular beds were rapidly removed after the rats were killed. The right and left atria were dissected separately, and atrial ANF concentrations were measured by RIA. Briefly, the atria were homogenized for 1 minute with a Polytron (set at 8) in 2 ml 0.1 M acetic acid containing the same protease inhibitors as described above. The ventricles were carefully dissected, minced, and boiled in 1 M acetic acid for 5 minutes (10 ml acetic acid/g tissue); they were then cooled to room temperature and homogenized in a Polytron (30 seconds, set at 8). The homogenates were then centrifuged at 15,000 rpm for 10 minutes, and the supernatant was applied to a C18 Sep-Pak cartridge (Waters Associates, Milford, Massachusetts), which had been previously activated with 5 ml of 100% acetonitrile. The cartridges were washed twice with 5 ml of 0.1% trifluoroacetic acid in H2O, and the ANF adsorbed on the columns was eluted with 80% acetonitrile in 0.1% trifluoroacetic acid. The material was lyophilized in a Speed-Vac and kept at -70°C until RIA. The detection limit of the assay was 3 pg/tube. The interassay and intra-assay coefficients of variance were below 16%, and the recovery of added 125I-ANF was 85%. Atrial and ventricular protein content was assessed by a modification of Bradford's method. Plasma ANF levels and tissue ANF content were not corrected for recoveries.

5'-Nucleotidase was measured by a modification of the method of Song and Bodansky.
**Preparation of Glomerular Membranes**

The kidneys were excised from the renal capsules, placed in ice-cold 0.9% NaCl, and dissected longitudinally, and the medulla and papilla were eliminated. They were then homogenized by passing the cortical tissue through a 0.5-mm grid. The mush was diluted with 0.9% NaCl and filtered through a 200-, 50-, 100-, and 150-μm mesh nylon sieve. Glomeruli retained in the sieve were collected in a 50-ml centrifuge tube and washed by centrifugation (4°C, 2,000g). After the last centrifugation, they were suspended in 0.05 M Tris-HCl (pH 7.2) and kept at -70°C. The purity of the glomerular suspension was assessed by light microscopy and was estimated to be about 95% at the end of the preparation. This glomerular suspension was homogenized for 1 minute with a Polytron (set at 6), centrifuged at 30,000g for 30 minutes, and resuspended in 1 ml 0.05 M Tris-HCl (pH 7.2). An aliquot was taken for protein determination by a modification of Bradford’s method.19

**Glomerular Membrane Binding Assay**

The binding assay was performed as follows: aliquots (35 g) of glomerular membrane protein from both clipped and nonclipped kidneys were incubated in duplicate for 60 minutes at 22°C, as described by Carrier et al,21 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 assay buffer and rapid filtration through polyethylenimine-treated Whatman GF/C filters, which were then rinsed three times with 3 ml Tris-HCl (pH 7.2), allowed to dry, and counted in a LKB Gamma Counter (Turku, Finland) with 65% efficiency. 125I-ANF was prepared by the lactoperoxidase method.20 It had a specific activity of approximately 1,000 Ci/mmol. Rat ANF (Ser 99-Tyr 126) was purchased from BioMega (Laval, Quebec, Canada).

The glomerular ANF receptor binding assay had been previously validated. Binding equilibrium was achieved after 60 minutes of incubation. No degradation of ANF was detected by high-pressure liquid chromatography after incubation.21

**Preparation of Vascular Membranes**

The mesenteric vascular bed was isolated as described elsewhere.22 The thoracic aorta was dissected, and the first 0.5 cm of the proximal end was discarded. The tissues were then immersed in 0.25 M sucrose solution, finely minced with scissors, and homogenized in a Polytron (set at 8; two 10-second homogenizations). The homogenate was then centrifuged at 1,500g for 10 minutes at 4°C; the supernatant was decanted and recentrifuged. The final supernatant was filtered through cheesecloth, and then centrifuged at 104,000g for 30 minutes. The pellet was resuspended in a 0.05 M Tris-HCl buffer (pH 7.4) containing 5 mM MgCl2, 0.5 mM PMSF, 0.1% bacitracin, and 1 μM aprotinin. Proteins were measured by a modification of Bradford’s method.19 Bovine serum albumin was then added at a concentration of 0.2%, and the membranes were diluted to a protein concentration of 0.5 mg/ml for mesenteric arteries and 2 mg/ml for aorta in the Tris-HCl buffer containing 0.2% albumin (assay buffer).

**Vascular Membrane Binding Assay**

The binding assay was performed as described previously.23 Briefly, 30 pM labeled ANF and unlabeled ANF (10^-12 to 10^-6 M) was used in competition experiments with 50 μg protein/tube for mesenteric arteries and 200 μg protein/tube for aortas at 4°C for 60 minutes. All assays were performed in duplicate. Separation of bound and free radioactivities was achieved by rapid filtration through polyethylenimine-treated Whatman GF/C filters soaked with 0.5 ml of assay buffer. The filters were washed twice with 3 ml 0.9% NaCl, allowed to dry, and counted in a LKB Gamma Counter.

**Plasma and Blood Volume**

Plasma and blood volumes were measured as described previously.24 The animals were anesthetized with sodium pentobarbital (60 mg/kg body wt i.p.), and right intracarotid artery and intrajugular vein catheters were installed. Once the animals were conscious (1–2 hours later), plasma volume was estimated with Evans blue.25 Two hundred μl of a solution containing 3 mg Evans blue was

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**Table 1. Blood Pressure, Body and Heart Weights, Hematocrit, and Plasma Renin Activity in Saralasin-Sensitive and -Resistant 2K1C Hypertensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>BP (mm Hg)</th>
<th>Body weight (g)</th>
<th>Heart weight (mg/100 g body wt)</th>
<th>Hematocrit (%)</th>
<th>PRA (ng A/μl/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (n = 24)</td>
<td>111 ± 3</td>
<td>338 ± 6</td>
<td>343 ± 5</td>
<td>44.7 ± 1.0</td>
<td>2.35 ± 0.32</td>
</tr>
<tr>
<td>Saralasin-sensitive 2K1C (n = 11)</td>
<td>178 ± 5</td>
<td>295 ± 12*</td>
<td>452 ± 20*</td>
<td>45.1 ± 1.0</td>
<td>14.31 ± 2.75*</td>
</tr>
<tr>
<td>Saralasin-resistant 2K1C (n = 16)</td>
<td>168 ± 5</td>
<td>305 ± 7*</td>
<td>456 ± 17*</td>
<td>41.8 ± 1.0†</td>
<td>7.44 ± 1.42†</td>
</tr>
</tbody>
</table>

BP, mean direct blood pressure; PRA, plasma renin activity; Al, angiotensin I; 2K1C, two-kidney, one-clip.

*p<0.01 vs. sham-operated controls; †p<0.05 vs. sham-operated controls; ‡p<0.05 vs. saralasin-sensitive 2 rats. Values are mean ± SEM.
administered through the venous catheter washed with 150 μl saline. Ten minutes later, 1.0 ml blood was withdrawn through the arterial catheter and centrifuged. The Evans blue concentration in plasma samples was determined by spectrophotometry, and standard formulae were applied to calculate plasma and blood volumes.25

**Analysis of Data**

The results are expressed as mean±SEM. The data were evaluated by one-way analysis of variance, and an a posteriori contrast test according to Bonferroni’s method was applied whenever a level of significance was found (p<0.05). The binding data were analyzed with the computer-based LI-GAND program,26 after preliminary treatment of data with the EDBA program,27 to determine the density and affinity of binding sites in competition experiments. These results were analyzed by one-way analysis of variance, an a posteriori test according with Bonferroni’s method, and were considered significant at p<0.05.

**Results**

Table 1 depicts the BP, body and heart weights, hematocrit, and PRA in sham-operated and saralasin-sensitive and -resistant 2K1C rats. Saralasin-sensitive 2K1C animals tended to have a slightly higher BP than saralasin-resistant rats, but this difference was not significant. Both hypertensive groups had lower body weights and higher relative heart weights than the controls. Hematocrit was significantly lower (p<0.05) in saralasin-resistant animals than in either saralasin-sensitive or normotensive controls; no difference in hematocrit was found between the last two groups. Each hypertensive group presented higher PRA values than sham-operated animals, and this parameter was higher in the saralasin-sensitive than in the saralasin-resistant group (p<0.05).

| **Table 2. Glomerular Atrial Natriuretic Factor Receptors in Saralasin-Sensitive and -Resistant 2K1C Hypertensive Rats** |
|------------------|------|-----------------|
| **Group**        | **Kd** (pM) | **Bmax** (fmol/mg protein) |
| Right unclipped kidney |        |                          |
| Saralasin-sensitive 2K1C | 49 ± 3 | 304 ± 16*           |
| Saralasin-resistant 2K1C | 44 ± 10 | 79 ± 11*          |
| Sham-operated       | 61 ± 4  | 140 ± 4            |
| Left clipped kidney  |        |                          |
| Saralasin-sensitive 2K1C | 28 ± 81 | 71 ± 15*           |
| Saralasin-resistant 2K1C | 14 ± 11 | 43 ± 4*            |
| Sham-operated       | 112 ± 7 | 273 ± 28           |

Bmax, maximum density of binding sites; Kd, dissociation constant; 2K1C, two-kidney, one-clip.
*p<0.01, tP<0.05 vs. sham-operated.

Each value represents the mean of two experiments for hypertensive animals and four experiments for sham ± SD. Membranes were obtained from 10 rats and used in each experiment.
Figure 1 illustrates plasma ANF (upper panel), atrial and ventricular ANF (middle panel) and the number of specific binding sites in renal glomeruli \((B_{\text{max}}\text{ lower panel})\). Plasma ANF was significantly higher in saralasin-resistant 2K1C rats \((79.21 \pm 8.42 \text{ fmol/ml plasma})\) than in either normotensive controls \((46.00 \pm 3.00 \text{ fmol/ml plasma}, p<0.01)\) or saralasin-sensitive animals \((57.44 \pm 6.68 \text{ fmol/ml plasma}, p<0.05)\). The latter group tended to have higher levels of plasma ANF than the sham-operated rats, but this apparent difference was not statistically significant. Saralasin-resistant 2K1C animals had lower ANF concentrations \((\mu\text{g/mg protein})\) in both atria when compared with the normotensive controls, whereas this difference was only evident in the left atrium of saralasin-sensitive rats. Each hypertensive group had higher ventricular ANF concentrations \((\text{ng/mg protein})\) than the normotensives.

The number of glomerular ANF binding sites \((B_{\text{max}}\text{ fmol/mg protein})\) was significantly lower in the clipped kidney of each hypertensive group (Figure 1, lower panel, and Table 2). This lower binding site density was accompanied by an increased affinity \((K_a\text{ Table 2})\). The nonclipped right kidney behaved differently in each hypertensive group. While the density of binding sites was significantly higher in saralasin-sensitive rats than in the controls, it was significantly lower in saralasin-resistant 2K1C animals. No affinity differences were noted in ANF binding sites in the right kidney between hypertensives and controls. Figure 2 shows representative binding curves for the right and left kidneys of the controls and saralasin-sensitive and -resistant groups.

The density of vascular ANF binding sites, in both mesenteric arteries and aortas, was significantly lower in saralasin-resistant 2K1C rats compared with either saralasin-sensitive or control animals (Table 3 and 4). No differences in the affinity of the binding sites were observed between the experimental groups. Figure 3 depicts representative binding curves for mesenteric artery membranes in the controls and both hypertensive groups.

Plasma and blood volumes were significantly higher in saralasin-resistant 2K1C hypertensive rats than in either saralasin-sensitive or normotensive animals (Figure 4). No difference was detected between the last two groups.

**Discussion**

The constriction of one renal artery, leaving the contralateral kidney intact, results in early sodium retention, which may be followed by sodium loss and a negative sodium balance. Rats developing a "malignant" form of hypertension have higher BP and PRA levels than those developing a "benign" form of hypertension in which, however, an increased blood volume may be found. These two types of 2K1C hypertension could be more or less

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**Table 3. Atrial Natriuretic Factor Binding to Particulate Fraction From Mesenteric Arteries**

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>(B_{\text{max}}) (fmol/mg protein)</th>
<th>(K_d) (nM)</th>
<th>Protein (mg)</th>
<th>5'-Nucleotidase ((\mu\text{mol Pi/mg protein/hr}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>155 ± 64</td>
<td>0.31 ±0.04</td>
<td>1.5 ± 0.4</td>
<td>12.3 ± 1.8</td>
</tr>
<tr>
<td>Saralasin-sensitive</td>
<td>5</td>
<td>113 ± 38</td>
<td>0.38 ±0.10</td>
<td>1.9 ± 0.6</td>
<td>14.2 ± 1.5</td>
</tr>
<tr>
<td>Saralasin-resistant</td>
<td>6</td>
<td>49 ± 17*</td>
<td>0.27 ±0.07</td>
<td>1.8 ± 0.4</td>
<td>13.8 ± 1.5</td>
</tr>
</tbody>
</table>

\(n\), number of binding experiments (membranes obtained from four or five rats were used for each group in each experiment); \(B_{\text{max}}\), maximum density of binding sites; \(K_d\), dissociation constant; 2K1C, two-kidney, one-clip.

\(*p<0.01\) versus other groups. Values are mean ± SEM.
TABLE 4. Atrial Natriuretic Factor Binding to Particulate Fraction From Aortas

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>(B_{\text{max}}) (fmol/mg protein)</th>
<th>(K_a) (nM)</th>
<th>Protein (mg)</th>
<th>5'-Nucleotidase (amol Pi/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>42.1 ± 18.6</td>
<td>0.36 ± 0.18</td>
<td>2.2 ± 0.4</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>Saralasin-sensitive</td>
<td>2KIC</td>
<td>46.6 ± 8.6</td>
<td>0.39 ± 0.24</td>
<td>1.9 ± 0.5</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td>Saralasin-resistant</td>
<td>2KIC</td>
<td>28.2 ± 3.1*</td>
<td>0.24 ± 0.06</td>
<td>2.4 ± 0.4</td>
<td>3.3 ± 1.3</td>
</tr>
</tbody>
</table>

*\(p<0.05\). Values are mean ± SEM.

\(n\), number of binding experiments (membranes obtained from four rats were used for each group in each experiment); \(B_{\text{max}}\), maximum density of binding sites; \(K_a\), dissociation constant; 2KIC, two-kidney, one-clip.

equated with saralasin-sensitive and -resistant 2KIC animals, the former having higher BP and PRA levels, as in the "malignant" form. Thus, the renin-angiotensin system seems to play an important role in maintaining high BP in saralasin-sensitive 2KIC rats. Whether sodium retention is one of the main mechanisms maintaining hypertension in the resistant group is controversial. The mechanism responsible for sodium loss has not been clearly established, but pressure natriuresis by the nonclipped kidney has been suggested to play a role. This mechanism could be operant in acute experiments, but another factor(s) may be involved in 2KIC hypertension where the clipped kidney exhibits chronically decreased sodium excretion in the presence of perfusion pressure distal to the arterial clamp that is identical to that seen in normotensive animals.

Saralasin-sensitive (renin-dependent) hypertensive animals have higher BP and PRA levels than those resistant to saralasin infusion. In the present study, we confirmed our previous results showing that saralasin-sensitive rats had higher PRA values than either normotensive and saralasin-resistant animals and tended to have a higher BP than the latter group (Table 1). However, the hypertensive groups had a similar degree of cardiac hypertrophy.

Saralasin-resistant rats presented lower hematocrit values, suggesting an increased plasma volume. This was substantiated by the actual plasma volume measured. These data agree with the findings of Mohring et al., who, with a similar method, found an increased plasma volume in "benign" hypertensive 2KIC animals. Even if plasma ANF tended to be higher in saralasin-sensitive rats, it is significantly different from that in the controls only in saralasin-resistant animals. The absence of elevated ANF levels in saralasin-sensitive rats was unexpected because the concentration of ANF in plasma is high in spontaneously hypertensive rats, a non-volume-dependent form of hypertension. A possible explanation could be that blood samples were collected in conscious animals by decapitation and because acute stress may increase plasma ANF levels, an elevation of basal values may mask any difference we find between controls and saralasin-sensitive animals. On the other hand, ANF plasma concentrations are clearly increased in the saralasin-resistant hypertensive rats, presumably because release may be further stimulated in these hypertensive rats because of their larger blood volume (present study and references 34 and 35).

The decrease in the concentration of ANF in both atria of saralasin-resistant 2KIC rats follows a pattern similar to that previously reported in 1KIC animals, where volume expansion is believed to play an important role in the development and maintenance of hypertension. Saralasin-sensitive animals present such a decrease only in the left atrium, as has also been reported in spontaneously hypertensive rats. This diminution in atrial ANF is probably related to intra-atrial changes that result in atrial stretch and enhanced release of the peptide. In each hypertensive group, ventricular ANF content is higher than in the normotensive controls. A similar increase has been reported in cardiomyopathic hamsters and a significant correlation has been found between plasma ANF and cardiac hypertrophy, but...
whether ventricular cardiocytes may contribute to the release of ANF into the circulation remains to be established.

We have previously noted (G. Gauquelin, E.L. Schiffmann, M. Cantin, and R. Garcia, unpublished observations) that during the development of 2K1C hypertension, the density of glomerular ANF receptors is increased in the nonclipped kidney at 3 weeks after clipping. At 5 and 7 weeks, the number of receptors is decreased in both clipped and nonclipped kidneys. Because the early stage of 2K1C hypertension is characterized by a higher frequency of renin dependency,6 we have now classified 2K1C hypertensive animals according to their saralasin sensitivity before investigating their glomerular ANF receptors. We have now demonstrated (Figure 1 and Table 2) that the density of glomerular ANF receptors is significantly decreased in the left clipped kidney in both saralasin-sensitive and -resistant animals with a concomitant decrease in $K_d$. The right nonclipped kidney behaves in a different way; whereas there is a significant increase in number of glomerular ANF binding sites in saralasin-sensitive animals, a decreased number of such sites are observed in the saralasin-resistant group. No alterations in binding affinity are observed in either group. Because the ANF concentration in blood reaching both kidneys is the same and the number of glomerular ANF receptors in clipped and nonclipped kidneys differs in saralasin-sensitive animals, factors other than plasma ANF may modulate glomerular ANF receptors. Whether hemodynamic differences between kidneys play a role in this different behavior of ANF receptors in clipped and nonclipped kidneys remains to be elucidated.

Split renal function studies on 2K1C hypertensive rats have demonstrated that sodium excretion by the nonclipped kidney is always higher, not only in comparison with the clipped organ but also with a normal kidney.11 On the other hand, sodium excretion by the clipped kidney is always reduced relative to the kidneys of intact rats, and the difference is evident even when renal blood flow11 and perfusion pressure are not diminished.29,30 The whole kidney glomerular filtration rate is lower in the clipped kidney of 2KIC animals than in either the nonclipped 2KIC rats or untouched kidney of normotensive rats.38 The higher glomerular filtration rate in the nonclipped kidney is only maintained at an elevated BP level.39 The renin-angiotensin system has been invoked as one of the factors that may be involved in the hemodynamic and functional changes observed in the nonclipped kidney of 2KIC hypertensive animals,38,39 but the inhibition of this system induces inconsistent responses in sodium excretion,39 which could be partially attributed to the use of anesthesia (hence, stimulated renin secretion) and of nonselected animals (saralasin-sensitive vs. saralasin-resistant).

The natriuretic effect of ANF may be attributable to an enhanced glomerular filtration rate, increasing the absolute and fractional excretion of sodium,40-42 but experimental evidence has been presented to indicate that other factors, such as inner medullary washout, could be equally important.43

We have now presented evidence that in the nonclipped kidney of saralasin-sensitive 2KIC animals, which usually exhibit increased natriuresis,1 the number of glomerular ANF receptors is augmented, whereas in both kidneys of saralasin-resistant rats, in which blood volume is increased, these receptors are decreased in number. Such findings suggest that through the regulation of renal glomerular ANF receptors, ANF may be one of the factors involved in the differential renal handling of sodium by saralasin-sensitive and -resistant groups. Moreover, increased levels of plasma ANF are apparently not necessary for its biological effect,14,24 which, in the case of high-renin, saralasin-sensitive 2KIC rats, may be potentiated.44 Acute administration of ANF to 2KIC animals induces a more pronounced natriuresis and diuresis45,46 than in 1KIC animals, in which a reduced density of glomerular ANF receptors are found.12

Using a mixed group of 2KIC animals (non-saralasin selected), we found that chronic infusion of low doses of ANF produces a variety of hypotensive responses (R. Garcia, unpublished observations); later, it was demonstrated that only renin-dependent 2KIC animals respond with a substantial lowering of BP.16 The density of ANF receptors in cultured vascular smooth muscle47 and in blood vessels of 1KIC13 and deoxycorticosterone-salt14,15 hypertensive rats varies inversely with the ambient concentrations of ANF. Moreover, these models of hypertension with down-regulated vascular ANF receptors present a poor48 or nonexistent49 hypotensive response to chronically infused ANF.

Vascular ANF receptor density in nonselected 2KIC animals tended to be lower than in the controls, but no significant difference was found, although aortic relaxation responses to ANF were...
decreased in some of the animals examined. Because the density of vascular ANF receptors in some models of hypertension appears to be correlated with the hypotensive response to chronic ANF infusion, we reinvestigated whether there was any difference in the density of vascular ANF binding sites in saralasin-sensitive and -resistant 2KIC rats. We have now demonstrated that saralasin-sensitive animals present a density of vascular ANF receptors, in both mesenteric arteries and aortas, similar to those in normotensive controls. In contrast, the density of vascular ANF receptors in both vascular tissues is significantly decreased in saralasin-resistant animals. These results may explain the lack of response of saralasin-resistant animals to chronic ANF infusion and the ambiguous binding and relaxation results reported previously in noneselected 2KIC animals. The present study also corroborates previous results demonstrating that an increased concentration of plasma ANF may down-regulate vascular ANF receptors. The level of BP does not seem to play a role in this down-regulation because the degree of hypertension in both groups was not significantly different. In summary, our data indicate that in saralasin-sensitive 2KIC rats, the number of glomerular ANF receptors is higher in the nonclipped kidney and lower in the clipped kidney and the density of vascular ANF receptors is similar to that found in normotensive controls. Saralasin-resistant 2KIC rats present a significant reduction in the number of glomerular ANF binding sites in both the clipped and nonclipped kidney and significantly decreased vascular ANF receptors. These findings may explain in part the different sodium handling and vascular responses to exogenous ANF in both groups.

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