Involvement of Brain Mineralocorticoid Receptor in Salt-Enhanced Hypertension in Spontaneously Hypertensive Rats

Kamal Rahmouni, Mariette Barthelmebs, Michèle Grima, Jean-Louis Imbs, Wybren De Jong

Abstract—We recently showed that brain mineralocorticoid receptors (MRs) are involved in blood pressure and kidney function control in normotensive Wistar rats. We now assessed the involvement of brain MRs in spontaneously hypertensive rats (SHR), in which the presence of adrenocorticoids has been shown to be required for the development of hypertension. The effect of a single intracerebroventricular (ICV) injection of an MR antagonist (RU28318) on systolic blood pressure (SBP) and renal function was examined in conscious adult SHR and Wistar-Kyoto rats (WKY) maintained on a standard-sodium diet (0.4% Na⁺). In WKY, a long-lasting decrease in SBP was caused by the ICV injection of 10 ng RU28318 as previously reported in Wistar rats, associated with increased urinary excretion of water and electrolytes. In SHR maintained on the standard diet, the ICV injection of RU28318 (10 or 100 ng) had no effect on cardiovascular and renal functions. However, the ICV injection of 10 ng RU28318 in SHR after 3 weeks of high sodium intake (8% Na⁺) caused a long-lasting decrease in SBP. The effect was present at 8 hours (ΔSBP 34±2 mm Hg), persisted at 24 hours (ΔSBP 29±1 mm Hg), and disappeared at 48 hours after the injection. The hypotension was not associated with changes in heart rate, urinary excretion of water and electrolytes, and plasma renin activity, whereas renal denervation did not affect the decrease in SBP. A more pronounced decrease in SBP (49±3 mm Hg at 8 hours) was observed with 100 ng RU28318. This dose of the antagonist was without effect after subcutaneous administration. Thus, brain MRs appear to participate in the maintenance of hypertension in conscious adult SHR sensitized by sodium loading. (Hypertension. 2001;38:902-906.)

Key Words: brain | mineralocorticoids | rats, inbred SHR | sodium | rats, inbred WKY

Several lines of evidence suggest that brain mineralocorticoid receptors (MRs) are involved in blood pressure control in hypertension induced by mineralocorticoids but also appear to play a role in cardiovascular control in normotensive animals.1–4 In normotensive Wistar rats, we recently showed that selective blockade of brain MRs induced a long-lasting decrease in blood pressure associated with increased diuresis and augmented urinary excretion of electrolytes.4 The involvement of brain MRs in hypertension was demonstrated in deoxycorticosterone (DOCA)-salt rats as well as in genetic hypertension in Dahl salt-sensitive rats. Intracerebroventricular (ICV) infusion of an MR antagonist inhibited the rise in blood pressure induced by DOCA-salt as well as the development of salt-dependent hypertension in Dahl salt-sensitive rats.2,5 For spontaneously hypertensive rats (SHR), data are lacking regarding a role of brain MRs in the development or maintenance of hypertension, except for a brief report suggesting refractoriness to high doses of an MR antagonist administered ICV in adult SHR.4

The pathophysiology of the development of hypertension in SHR so far has not been resolved. However, evidence is substantial for a role of brain mechanisms that cause an early increase in sympathetic nervous activity.7–9 SHR show enhanced pressor responses to a variety of centrally acting stimuli and to different kinds of stress.9,10 A contribution of the kidneys to the early rise of blood pressure in SHR has been suggested by several investigators.8,11 The importance of the sympathetic innervation of the kidneys of SHR was demonstrated by renal denervation that delayed and attenuated the development of hypertension associated with reduced sodium retention.12,13 Additional evidence for a role of the kidneys translates from renal cross-transplantation studies in SHR with normotensive control animals.14 SHR exposed to a high sodium intake generally respond with a moderate enhanced development of hypertension.15,16 Interestingly, increased renal sympathetic nerve activity (RSNA) is a characteristic of SHR, and the RSNA of SHR appears to be enhanced by high sodium intake.8,13,17–19 Finally, in SHR, adrenocortical steroids are required for the development of hypertension, probably via the permissive action of both mineralocorticoid and glucocorticoid activities.20,21

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From the Institut de Pharmacologie, Faculté de Médecine, Université Louis Pasteur (K.R., M.B., M.G., J.-L.I., W. De J.), Strasbourg, France; and Service d’Hypertension Artérielle, Maladies Vasculaires et Pharmacologie Clinique, Hôpitaux Universitaires de Strasbourg (M.G., J.-L.I.), France. Correspondence to Kamal Rahmouni, PhD, University of Iowa, 524 MRC, Iowa City, IA 52242. E-mail kamal-rahmouni@uiowa.edu

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In the present study, we examined the contribution of brain MRs in the maintenance of high blood pressure in SHR. For this purpose, we assessed the effect of single ICV administration of the selective MR antagonist RU28318 on cardiovascular and renal functions of normotensive Wistar-Kyoto (WKY) and adult SHR that were fed a standard- or high-sodium diet.

**Methods**

Male WKY (9 weeks of age, n=11) and SHR (9 to 10 weeks of age, n=80) (Janvier, Le Genest Saint Isle, France) were used. The animals were housed with conditions of a standard light/dark cycle (12 hour/12 hour) and temperature (21°C) with free access to tap water and rat chow. All procedures were in accordance with the guidelines of the French government and the European Community concerning the use of animals for scientific research.

One experiment was performed in WKY and 4 experiments were performed in SHR as follows. In protocol 1, we examined the effect of ICV injection of 10 ng RU28318 on cardiovascular and renal parameters in WKY (n=11) fed a standard diet. In protocol 2, we examined the effect of ICV injection of 10 or 100 ng RU28318 on cardiovascular and renal parameters in SHR (n=28) after 3 weeks on an 8%-sodium diet (UAR). In addition, basal cardiovascular and renal excretory parameters and plasma renin activity (PRA) were compared between SHR on standard- and high-sodium diets. In protocol 3, we examined the effect of ICV injection of 10 or 100 ng RU28318 on cardiovascular and renal parameters in SHR (n=28) after 3 weeks on an 8%-sodium diet (UAR). In addition, basal cardiovascular and renal excretory parameters and plasma renin activity (PRA) were compared between SHR on standard- and high-sodium diets. In protocol 4, we examined the effect of ICV injection of 100 ng RU28318 on cardiovascular and renal parameters in bilaterally kidney-denervated and sham-operated SHR after 3 weeks on an 8%-sodium diet (n=12).

In all 5 protocols, cardiovascular and renal parameters were assessed the last day before and 0 to 48 hours after ICV injection of the MR antagonist. All rats were accustomed to the conditions of indirect systolic blood pressure (SBP) measurement and of metabolic cages as described previously.4 The ICV cannula was implanted in the left lateral ventricle in rats 1 week before experimentation. Renal denervation or sham operation was performed as described previously. Each rat received only 1 ICV or SC injection administered in a volume of 2 µL ICV or 0.2 mL SC. Controls received the vehicle (2% ethanol-saline) via the same route.

At the end of the experiment, an Evans blue solution was injected ICV to ascertain the position of the ICV cannula. In 1 experiment, blood was collected after decapitation for PRA assay assessed by radioimmunoassay.4 In the kidney-denervated and sham-operated groups, the kidneys were removed, and the norepinephrine content was determined by HPLC.4 Sodium, potassium, and chloride levels in the urine were measured with an indirect potentiometric method (Synchrop EL-ISE; Beckman).

All data are expressed as mean±SEM. Statistical analysis was performed using Student’s t test or 2-way ANOVA on repeated measures and using the Student-Newman-Keuls or Bonferroni test for comparison among groups at any times. A value of P<0.05 was considered significant.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

**Results**

**Effect of ICV Administration of RU28318 in WKY and SHR on Standard-Sodium Diet**

Basal values of cardiovascular and renal function parameters did not differ in the 2 groups of WKY. A long-lasting decrease in SBP was observed after the ICV injection of 10 ng of the MR antagonist, without an effect on heart rate (HR) (Table 1). The decrease in SBP was ~23 mm Hg at 8 hours (P<0.01). The effect persisted at 24 hours after treatment (treated 97±2, control 114±4 mm Hg, P<0.01) and disappeared at 48 hours as SBP returned to baseline values. As shown in Table 1, increased diuresis was observed in the RU28318-treated group at 0 to 8 hours (~213% of the control group, P<0.01). In the same period, a significant increase in the urinary excretion of electrolytes (sodium, potassium, and chloride) was observed in the treated group. During the periods of 8 to 24 and 24 to 48 hours, there were no differences in urinary excretion of water and electrolytes between RU28318- and vehicle-treated WKY.

The ICV injection of 10 ng or a 10-fold higher dose (100 ng) of the MR antagonist did not affect cardiovascular functions in SHR fed the standard-sodium diet. SBP did not change at 8 (Table 1), 24, or 48 hours after RU28318 ICV injection. There were no significant changes in the renal functions (urinary excretion of water and electrolytes) in

### Table 1. Effect of ICV Administration of RU28318 on Cardiovascular and Renal Parameters in Normotensive WKY and SHR Compared With Vehicle-Treated Control Animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY Vehicle (n=5)</th>
<th>SHR Vehicle (n=6)</th>
<th>WKY RU28318 (n=14)</th>
<th>SHR RU28318 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP, mm Hg</strong></td>
<td>114±2</td>
<td>91±3†</td>
<td>171±2</td>
<td>170±3</td>
</tr>
<tr>
<td><strong>HR, bpm</strong></td>
<td>421±19</td>
<td>423±16</td>
<td>384±7</td>
<td>376±5</td>
</tr>
<tr>
<td><strong>Diuresis, µL · h⁻¹ · 100 g⁻¹</strong></td>
<td>216±80</td>
<td>460±23†</td>
<td>386±52</td>
<td>329±52</td>
</tr>
<tr>
<td><strong>Na⁺, µmol · h⁻¹ · 100 g⁻¹</strong></td>
<td>22±9</td>
<td>64±2²</td>
<td>24±4</td>
<td>28±2</td>
</tr>
<tr>
<td><strong>K⁺, µmol · h⁻¹ · 100 g⁻¹</strong></td>
<td>29±7</td>
<td>59±7*</td>
<td>25±4</td>
<td>35±4</td>
</tr>
<tr>
<td><strong>Cl⁻, µmol · h⁻¹ · 100 g⁻¹</strong></td>
<td>25±8</td>
<td>61±3*</td>
<td>26±4</td>
<td>28±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SBP and HR were measured 8 hours after the ICV injections. The electrolytes were measured in the urine collected at 0 to 8 hours. *P<0.05, †P<0.01 vs vehicle.
Effect of ICV administration of RU28318 (10 and 100 ng) on SBP (A) and HR (B) of conscious SHR on an 8%-sodium diet for 3 weeks compared with the vehicle-treated rats. Data are mean±SEM for 6, 8, and 14 animals in the 10 ng, 100 ng, and vehicle groups, respectively. Time is indicated in hours after ICV injection; t=0 indicates preinjection values. *P<0.001 vs vehicle; #P<0.05 vs 10 ng.

treated SHR at 0 to 8 (Table 1), 8 to 24, and 24 to 48 hours (data not shown).

Effect of ICV Administration of RU28318 in SHR on High-Sodium Diet

After 3 weeks on the 8%-sodium diet, SBP in the SHR (n=14) was elevated (207±3 mm Hg) compared with that in the SHR (n=6) maintained on a standard-sodium diet (165±3 mm Hg, P<0.001). HR did not differ significantly. The high-sodium diet increased water intake (4-fold), urinary volume (8-fold), and urinary excretion of sodium and chloride (~10-fold), whereas body weight was decreased (29%). Basal renal functions of the 3 groups of rats on the high-sodium diet receiving ICV injections did not differ (data not shown).

As depicted in the Figure, A, the ICV injection of 10 ng RU28318 in SHR on an 8%-sodium diet induced a long-lasting decrease in SBP. The effect was present at 8 hours (ΔSBP 34±2 mm Hg, P<0.001), persisted at 24 hours (ΔSBP 29±1 mm Hg, P<0.001), and disappeared at 48 hours. HR did not change significantly in the RU28318-treated SHR compared with the vehicle-treated rats (Figure, B). There was no significant change in urinary excretion of water and electrolytes after ICV injection of RU28318 (Table 2). PRA measured at the end of the experiment also showed no difference between RU28318- and vehicle-treated groups (6.9±2 versus 5.9±2 μg·mL⁻¹·h⁻¹, respectively).

A 10-fold higher dose of RU28318 (100 ng) induced a more pronounced decrease in SBP (Figure, A). At 8 hours after ICV injection, the decrease in SBP was 49 mm Hg; at 24 hours, it was 32 mm Hg; and it disappeared at 48 hours. Despite the pronounced decrease in SBP, no significant changes in HR (Figure, B) and urinary excretion of water and electrolytes were observed in the rats treated with 100 ng RU28318 ICV (Table 2). The SC administration of 10 ng RU28318 in SHR on an 8%-sodium diet, compared with the vehicle-treated SHR, failed to affect SBP (the values at 8 hours after administration were 207±4 and 204±3 mm Hg, respectively), HR, or renal functions (diuresis and urinary excretion of electrolytes) (data not shown).

Effect of ICV Administration of RU28318 in Kidney-Denervated SHR on High-Sodium Diet

Bilateral kidney denervation performed 1 week before ICV injection slightly affected basal values of SBP compared with the sham-operated group (198±2 versus 207±3 mm Hg, P<0.05). HR and renal function parameters were not different in denervated and sham-operated groups. The ICV injection of 10 ng RU28318 at 8 hours induced a similar decrease in SBP in sham-operated (ΔSBP 31±3 mm Hg) and kidney-denervated (ΔSBP 29±3 mm Hg) SHR on a high-sodium diet. The SBP fell at 24 hours was 16±2 mm Hg in the sham-operated group and 8±3 mm Hg in the kidney-denervated group (P=0.07). At 48 hours, the SBP returned to baseline values in both groups. HR and urinary excretion of water and electrolytes did not change after ICV injection of RU28318 in the sham-operated and kidney-denervated groups (data not shown). The ICV injection of vehicle had no effect on cardiovascular and renal functions in the sham-operated and kidney-denervated SHR. Renal tissue norepinephrine concentration in the sham-operated SHR was 251±31 ng/g tissue and decreased to 17±2 ng/g tissue in the kidney-denervated group.

Discussion

Our results show that in SHR with a standard sodium intake, there was no response to blockade of brain MRs. This was the case for both arterial blood pressure and renal functions. In contrast, in normotensive WKY, the ICV administration of 10 ng RU28318 caused a decrease in SBP that lasted >24 hours. During the first 8 hours, this decrease in SBP was associated with a brisk diuresis and enhanced urinary excretion of sodium, potassium, and chloride. These effects observed in WKY mimic the effects of an ICV injection of RU28318 on SBP and renal functions of normotensive Wistar rats.

### Table 2. Effect of ICV Administration of RU28318 (10 and 100 ng) on Diuresis and Urinary Excretion of Electrolytes in the Period of 0 to 8 Hours in SHR on an 8%-Sodium Diet for 3 Weeks Compared With Vehicle-Treated Control Animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle (n=14)</th>
<th>RU28318 10 ng (n=8)</th>
<th>RU28318 100 ng (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis, μL·h⁻¹·100 g⁻¹</td>
<td>303±69</td>
<td>391±89</td>
<td>300±65</td>
</tr>
<tr>
<td>Na⁺, μmol·h⁻¹·100 g⁻¹</td>
<td>70±16</td>
<td>67±16</td>
<td>61±16</td>
</tr>
<tr>
<td>K⁺, μmol·h⁻¹·100 g⁻¹</td>
<td>8±1</td>
<td>8±2</td>
<td>10±2</td>
</tr>
<tr>
<td>Cl⁻, μmol·h⁻¹·100 g⁻¹</td>
<td>64±14</td>
<td>79±23</td>
<td>58±16</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
thermore, the present study shows that after a period of 3 weeks of high sodium intake, SHR responded to the ICV administration of RU28318 with a decrease in SBP that lasted >24 hours but had no effect on renal functions. It is of interest to note that the ICV infusion of RU28318 that blocked brain MRs in Dahl salt-sensitive rats inhibited the development of hypertension in these genetically hypertension-prone rats in which the development of high blood pressure is to a large degree dependent on high sodium intake. In normotensive rats, a high-sodium diet did not affect the decrease in blood pressure induced by ICV RU28318 (K.R., M.B., M.G., J.-L.I., W. De J., unpublished data). In contrast to the blockade of the renal response to ICV RU28318 observed in normotensive Wistar rats, in SHR on an 8%-sodium diet, there was no significant effect of renal denervation. Renal denervation, however, tended to inhibit the hypotensive action of RU28318 in SHR at 24 hours, although this effect did not reach statistical significance. A central site of the hypotensive action of RU28318 in SHR on high sodium intake was revealed by the absence of response to SC administration of the MR antagonist. Similar conclusions regarding the site of action were reached previously on the basis of results obtained in normotensive rats on a standard-sodium diet treated with RU28318.3,4 In contrast to the hypotensive effect of a single ICV injection of RU28318 during the first 24 hours in normotensive WKY, the longer-term ICV infusion of RU28318 in normotensive Sprague-Dawley rats in which blood pressure was not measured during the first 48 hours had no effect on blood pressure.22 It may be that under these chronic conditions, compensatory mechanisms played a role. A difference between our study in WKY and those performed in Sprague-Dawley rats2,22 is the use of a higher dose of RU28318 in the latter studies (ie, a daily infusion in the microgram range compared with a single 10-ng injection).

The blockade of brain MRs has been suggested to decrease sympathetic output, resulting in a decrease in blood pressure and the withdrawal of sympathetic nervous activation of renal tubular α-adrenoceptors.3,4 This latter action resulted in diuresis and enhanced urinary excretion of electrolytes.5,13 Our present findings suggest that brain MRs or postreceptor mechanisms of SHR differ from those of WKY. Unfortunately, there are very limited literature data available regarding the brain MRs of SHR. Gomez et al23 found no difference in brain hippocampal MR mRNA of 5 different strains of rats, including SHR and WKY. A recent report found no difference in MR number in the heart and kidney of SHR compared with those of WKY.24 In vitro binding of MRs obtained from the heart and kidney of SHR at 14 weeks of age to DNA cellulose was increased compared with that of WKY. The relevance of this observation remains to be determined. Clearly, any conclusion regarding possible dysfunction of brain MRs or related postreceptor mechanisms in SHR awaits future studies regarding these aspects.

Although multiple differences have been reported in cardiovascular control mechanisms at different levels of the neuraxis and in the periphery in SHR,9,13 these are in general relative (decreased or increased responses) compared with normotensive control animals. Such differences do not appear to provide an explanation for the complete lack of response to central MR blockade in SHR with standard sodium intake. In view of the absence of a renal response in SHR on standard or high sodium intake to ICV MR blockade, we also considered a role of a potential defect at the level of the kidney. Typically, SHR at an early age have a high renal vascular resistance, and this is further enhanced by a high sodium intake (see the introduction). The absence of a response to central MR blockade may also be caused by a dysfunction in SHR brain. A brain region critically involved in the central effects of aldosterone and of MR antagonists is the anteroventral third ventricle (AV3V) area. This complex brain region is situated around the base of the third cerebral ventricle and encompasses several brain nuclei.25,26 The AV3V area coordinates neural control of body fluid homeostasis and of cardiovascular functions, and it integrates information from the kidneys received via the renal afferent nerves.25,26 The AV3V area seems to function differently in SHR. As shown by Brody and colleagues,25,27,28 lesions placed in this region interfered with several different forms of hypertension in rats (DOCA-salt, Goldblatt, and Dahl salt-sensitive rats) but, by contrast, failed to affect the development or maintenance of hypertension in SHR. In SHR, the lesion of the AV3V region caused adipsia and the loss of pressor and dipsogenic responses to centrally administered angiotensin II, similar to what is observed in other rat strains. However, electrical stimulation of the AV3V region in intact SHR caused smaller vasoconstrictor and vasodilator responses than observed in WKY, with a much smaller pressor response in SHR than in WKY.27,28 Brody et al28 suggested that the interrupted reflex arc that originates with renal afferent activation may be the base of the prevention of different forms of hypertension by the AV3V lesion. We postulate that the observed absence of response to central MR blockade in SHR depends on a dysfunction of the AV3V region.

An intriguing observation in our present study concerns the occurrence of a hypotensive response to ICV administration of RU28318 in SHR on a high-sodium diet, whereas no effect on renal function was observed. High sodium intake of the magnitude used (8% Na+ for 3 weeks) is a severe challenge of homeostasis. This is reflected by the increased water intake, augmented diuresis, and decreased body weight. Although we have no data to explain the absence of a renal response to ICV administration of RU28318, it may be that the markedly enhanced RNSA of SHR on an 8%-sodium diet (see Introduction) prevented a sufficient and selective withdrawal of RNSA.

In summary, our results in WKY confirm the effects of acute brain MR blockade by RU28318 in normotensive Wistar rats, causing a decrease in SBP and a short-lasting enhanced diuresis and increased urinary excretion of electrolytes. SHR failed to respond to the same and a 10-fold higher ICV dose of RU28318, putatively due to a defect in the hypothalamic AV3V region that involves MR functioning. A high-sodium diet (8% Na+) resulted in enhanced hypertension in SHR and restored the hypotensive response to brain MR blockade but not the change in renal function. Our working hypothesis is that the high-sodium diet restored the hypotha-
lamic responsiveness to MR blockade while enhanced RNSA prevented the effects on kidney function.

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References

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