Brain Mineralocorticoid Receptor Control of Blood Pressure and Kidney Function in Normotensive Rats

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

Abstract—Brain mineralocorticoid receptors appear to contribute to mineralocorticoid hypertension and may be involved in blood pressure control in normotensive rats. We examined the effect of blockade of central mineralocorticoid receptors with the use of a selective antagonist (RU28318) on cardiovascular and renal function in conscious normotensive rats. The contribution of renal innervation was evaluated in rats with bilaterally denervated kidneys. Young adult, male Wistar rats were trained for systolic blood pressure measurement by a tail sphygmographic method and accustomed to metabolic cages for collection of urine. One week before experimentation, an intracerebroventricular cannula was implanted. Systolic blood pressure was diminished 30 minutes after an intracerebroventricular dose of 10 ng of RU28318. The effect was maximal at 8 hours and was still present after 24 hours. Blood pressure returned to the basal level by 48 hours. During the period 0 to 8 hours after intracerebroventricular injection, rats treated with the antagonist showed an increase in diuresis and urinary electrolyte excretion. No significant effect on plasma renin activity, measured 8 and 30 hours after administration of RU28318, was observed. In denervated rats, the decrease in systolic blood pressure after administration of RU28318 was reduced. The difference was statistically significant compared with controls at 2 hours but not at 8 hours, and blood pressure returned to the basal value by 24 hours. The increases in diuresis and urinary electrolyte excretion induced by RU28318 were abolished in denervated rats. These results show that brain mineralocorticoid receptors are involved in blood pressure regulation and kidney function homeostasis in conscious normotensive rats. The renal nerves appear to participate in the brain mineralocorticoid receptor control of blood pressure. (Hypertension. 1999;33:1201-1206.)

Key Words: receptors, mineralocorticoid ■ blood pressure ■ RU28318 ■ denervation ■ electrolytes ■ diuresis

Administration of deoxycorticosterone acetate (DOCA) together with an increased intake of salt in rats is a frequently used model of mineralocorticoid hypertension. Brain mechanisms and increased sympathetic outflow appear to contribute to this form of hypertension and may also be involved in blood pressure control in normotensive rats. This is based on several different observations in animals. Intracerebroventricular (ICV) administration of aldosterone increases blood pressure of conscious normotensive rats, whereas administration of a mineralocorticoid receptor (MR) antagonist decreases blood pressure. Administration of aldosterone by long-term ICV infusion results in hypertension, whereas the same dose is ineffective when given systemically. Furthermore, ICV infusion of an MR antagonist inhibits hypertension in rats induced by long-term subcutaneous infusion of a high dose of aldosterone as well as DOCA/salt hypertension. In the brain, hypothalamic structures may be involved in the central effects of mineralocorticoids. A lesion of the basal anterior hypothalamus has been reported to interfere with the development and maintenance of DOCA/salt hypertension in rats. Destruction of catecholaminergic nerve terminals in the brain by ICV injection of the neurotoxic compound 6-hydroxydopamine has been shown to inhibit the development of hypertension induced by DOCA combined with salt. On the basis of these observations, several authors suggested that a specific area of the brain plays a role in the development and maintenance of mineralocorticoid hypertension. MRs are present in the brain, in particular in the hypothalamus and some circumventricular areas that are known to have a role in the regulation of arterial blood pressure and body fluid control. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD2), which converts corticosterone and cortisol to inactive 11-dehydrocorticosterone and cortisone, respectively, is required for an aldosterone-selective MR function. This enzyme has been reported to be present in the brain and hypothalamus. Cells strongly positive for HSD2 mRNA were found in different brainstem regions, including the ventromedial hypothalamus. Long-term ICV infusion of low doses of carbenoxolone or glycyrrhizic acid, inhibitors of HSD, produced elevated blood pressure in rats.
Brain Mineralocorticoid Receptor Blockade and Kidney Function

In the central nervous system, several brain cell groups that affect different aspects of renal function have been identified. These nuclei are particularly located in the hypothalamus and medulla oblongata and are regions known to play a role in cardiovascular regulation. Afferent and efferent nerves connect the brain and the kidneys. Stimulation of intrarenal chemoreceptors with a phenol solution caused an increase in norepinephrine release in the posterior hypothalamus. Furthermore, direct electrical stimulation of afferent renal nerves altered systemic arterial blood pressure by increasing the discharge frequency of hypothalamic neurons. On the other hand, changes in efferent renal nerve activity can elicit changes in renal function. Thus, stimulation of renal nerves produced decreases in renal blood flow and glomerular filtration rate and enhanced renal tubular sodium and water reabsorption.

We wished to examine the role of brain MRs in control of cardiovascular and renal function of conscious normotensive male rats. For this purpose, the acute effects of ICV administration of a selective MR antagonist (RU28318) on systolic blood pressure (SBP) and heart rate (HR), renal excretion of water and electrolytes, and plasma renin activity (PRA) were assessed. Intact as well as denervated rats (bilateral renal denervation) were studied.

Methods

Animals
Male normotensive Wistar rats (200 to 260 g) (Janvier, Le Genest Saint Isle, France) were used. The animals were kept at 20°C with a light/dark cycle of 12 hours each (lights on at 6 AM). Animals were housed in our laboratory for at least 1 week before the experiment. Standard laboratory rat chow and tap water were provided ad libitum. All procedures used were in accordance with guidelines of the French government and the European Community concerning the use of animals.

Experimental Protocol
Four experiments were performed, three in intact rats and one in renally denervated rats as follows. Protocol 1 examined the effect of ICV injection of 10 ng of RU28318 on SBP, HR, and renal excretion of water and electrolytes (0 to 48 hours). Protocol 2 examined the effect of ICV injection of 10 ng of RU28318 on PRA. Rats were decapitated after 8 or 30 hours. These time points were used because the hypotensive effect of RU28318 reached its maximum at 8 hours and disappeared at 30 hours (preliminary observations; see also Van Den Berg et al). SBP and HR were also assessed. Protocol 3 examined the effect of ICV injection of 10 ng of RU28318 on cardiovascular and renal parameters in rats that underwent sham operation and in renally denervated rats (0 to 48 hours). Protocol 4 examined the effect of subcutaneous injection of 10 and 100 ng of RU28318 on cardiovascular and renal parameters (0 to 48 hours).

Rats were trained daily for 2 weeks before experimentation to the conditions of the blood pressure measurement with an indirect sphygmographic method on the tail as described. One week before intracerebroventricular (ICV) injection, a polyethylene cannula was implanted in the left lateral cerebral ventricle of rats anesthetized with pentobarbital (50 mg/kg IP). After the operation, animals were kept in individual cages. Two days before ICV injection, rats were placed in metabolic cages to assess water consumption, food intake, and urine output (the first day to accustom the rats to the cages and the second day to assess water consumption, food intake, and urine output during the 24 hours before treatment). Each rat received only 1 ICV injection, given between 9 and 10 AM, in a volume of 2 μL. The rats received 10 ng of the MR antagonist RU28318 (3,3-oxo-7-propyl-17-hydroxy-androstan-4-en-17yl-propionic acid lactone) or vehicle (2% ethanol in saline). Several different physiological parameters were determined before treatment and at different time intervals after ICV injection.

At the end of the experiments, the rats were decapitated and blood was collected in ice-cold tubes (with Na²⁻EDTA). Blood was promptly centrifuged (2000g for 10 minutes), and the plasma was kept in a freezer (−20°C) until assayed. The position of the ICV cannula was verified by injection of an Evans blue solution. Data from 2 rats with an incorrectly placed cannula were excluded.

Renal Denervation
One week before ICV injection and just after implantation of the ICV cannula, bilateral renal denervation was performed via bilateral flank incisions. The renal pedicle was stripped of its adventitia, and all visible nerves along the renal artery and vein were removed. The vessels were then swabbed with 10% phenol in absolute ethanol for 25 to 30 minutes. Sham denervation was accomplished by the same procedure by exposing the renal vessels while leaving the adventitia intact and applying 0.9% saline solution instead of phenol solution. Norepinephrine content of each kidney was determined by high-performance liquid chromatography coupled with an electrochemical detector. Denervation was considered effective when the renal content of norepinephrine was <10% of the content of the rats that underwent sham operation. Data of 2 rats were not used because values exceeded 10%.

Assay of Electrolytes and PRA
Sodium, potassium, and chloride in the urine and plasma were measured by an indirect potentiometric method with the use of selective electrodes. PRA was assessed by radioimmunoassay as described elsewhere. Activity was expressed as nanograms of angiotensin I generated per milliliter of plasma per hour.

Statistical Analysis
Data were mean±SEM. Results were analyzed with 2-way ANOVA on repeated measures and the Student-Newman-Keuls or Bonferroni test for comparison among groups. A value of P<0.05 was considered significant.

Results

Effect of ICV Administration of RU28318 on SBP and HR in Intact Rats
ICV injection of 10 ng of RU28318 caused a long-lasting decrease in SBP (Figure 1A). The effect was present 2 hours after ICV injection (treated, 103±2 mm Hg; control, 125±3 mm Hg; P<0.01). At 8 hours, the decrease in SBP was slightly more pronounced (97±3 versus 122±1 mm Hg, respectively; P<0.01). The effect persisted at 24 hours after administration of the compound (110±1 versus 121±3 mm Hg, respectively; P<0.05). Blood pressure returned to baseline values by 48 hours. HR was not significantly affected in the treated group as compared with the control group (Figure 1B). Injection of vehicle had no effect on these parameters.

Effect of ICV Administration of RU28318 on Kidney Function in Intact Rats
As shown in Figure 2A, ICV injection of the MR antagonist caused a significant increase in urinary water and electrolyte excretion at 0 to 8 hours. Urine volume increased to ~240% of the control volume (9.4±1 versus 3.9±1 mL and 425±47 versus 177±47 μL/h per 100 g of body weight in treated versus control rats, respectively; P<0.01). Urinary sodium excretion was increased to ~275% as compared with the control group (P<0.01; Figure 2B). Urinary potassium ex-
creteion increased to ~423% (P<0.01; Figure 2C), and urinary chloride excretion increased to ~394% (P<0.01, Figure 2D). Food intake and water consumption were not changed by ICV administration of RU28318 (data not shown).

**Effect of ICV Administration of RU28318 on PRA in Intact Rats**

Eight hours after ICV injection of RU28318, at which time the decrease in SBP was maximal, no change in PRA was observed in the treated group as compared with the control group (35±2 versus 34±11 ng/mL per hour, n=7 and 6 rats, respectively). Similarly, 30 hours after ICV administration of RU28318, no difference in PRA occurred (36±6 versus 33±3 ng/mL per hour, n=6 and 5 rats, respectively). There was no difference in plasma sodium concentration at 8 and 30 hours (data not shown).

In this experiment, cardiovascular changes up to 8 and 30 hours were similar to those described above. The decrease in blood pressure after RU28318 administration was present 30 minutes after treatment (99±2 versus 112±2 mm Hg, P<0.05). The effect was more pronounced at 8 hours (90±4 versus 107±3 mm Hg; P<0.01). No significant change in HR was observed.

**Effect of ICV Administration of RU28318 on SBP and HR in Denervated Rats**

The effect of the same dose of RU28318 (10 ng) as used to evaluate cardiovascular and kidney function was assessed in rats that underwent bilateral renal denervation. No differences in basal SBP and HR were observed between denervated rats and those that underwent sham operation (preinjection period). In the sham-operated rats, the effect of ICV administration of RU28318 on SBP (Figure 3A) did not differ from the effect described above in intact rats. In denervated rats, the effect of ICV administration of RU28318 was similar but shorter in duration. The decrease in SBP was significant compared with vehicle-treated controls at 2 hours (107±4 versus

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Figure 1. Effect of intracerebroventricular administration of 10 ng of RU28318 on (A) SBP and (B) HR in conscious normotensive rats. The control group consisted of vehicle-treated rats. Data are mean±SEM (n=7 rats per group). Time is indicated in hours after ICV injection; time 0 indicates preinjection values. * P<0.05 vs control; ** P<0.01 vs control.

Figure 2. Effect of intracerebroventricular administration of 10 ng of RU28318 on (A) diuresis (µl/h per 100 g of body weight) and urinary excretion of (B) sodium, (C) potassium, and (D) chloride (µmol/h per 100 g of body weight) in conscious normotensive rats. The control group consisted of vehicle-treated rats. Data are mean±SEM (n=7 rats per group). Time periods are indicated in hours; the period ~24–0 h indicates preinjection values. ** P<0.01 vs control.
Effect of ICV Administration of RU28318 on Kidney Function in Denervated Rats

No differences in basal renal excretion of water and electrolytes were observed between denervated rats and those that underwent sham operation (preinjection period). In sham-operated rats, the increase in diuresis in the RU28318-treated group during the first 8 hours after ICV injection of RU28318 was $9.3 \pm 2$ mL ($443 \pm 48 \mu$L/h per 100 g of body weight), compared with $4.6 \pm 0.9$ mL ($208 \pm 43 \mu$L/h per 100 g of body weight) in the vehicle-treated group ($P<0.05$; Figure 4A). In denervated rats, the increase in diuresis induced by RU28318 observed in the controls was abolished. As shown in Figure 4, renal denervation abolished the increase in urinary electrolyte excretion (sodium, potassium, and chloride) as compared with the sham-operation group.

Norepinephrine content of the kidneys averaged $152 \pm 1$ ng/g of tissue in sham-operated rats ($n=14$). Mean content of norepinephrine decreased to $3.7 \pm 1$ ng/g of tissue in denervated rats ($n=11$).

Effect of Subcutaneous Administration of RU28318 in Intact Rats

To test the systemic effect of RU28318, we assessed the effect of subcutaneous administration of 2 doses (10 or 100 ng per rat) on cardiovascular and renal function of intact rats. No effect was observed on any of the parameters measured at the different times after subcutaneous administration, which followed ICV injection (Tables 1 and 2).

Discussion

The results of this study confirm those of an earlier study\(^2\) in normotensive conscious rats, which found that acute blockade
of central MR with a selective antagonist for this receptor induces a long-lasting decrease in blood pressure. Our data show that this hypotension was associated with increased renal excretion of water, sodium, potassium, and chloride. Diuresis and increased renal excretion of electrolytes appear to contribute to the long-lasting decrease in blood pressure.

The effect on blood pressure was relatively rapid compared with the classic, more slowly occurring genomic action of corticosteroids. This genomic action occurs with a delay of 15 minutes to several hours and may persist for weeks after disappearance of the steroids. Recently, investigators found evidence of nongenomic effects of mineralocorticoids with a time course of action inconsistent with an effect via classic genomic pathways. The effects of aldosterone on intracellular Ca$^{2+}$ in vascular smooth muscle cells are immediate and reach a plateau in 3 to 5 minutes. This effect was not prevented by the MR antagonist spironolactone. Membrane, rather than intracellular steroid receptors, may be implicated in these rapid effects. So far, membrane receptors for aldosterone have been observed only outside the central nervous system. Therefore, we conclude that the observed effects of brain MR blockade are likely exerted by a genomic pathway.

The nervous system coordinates the mechanisms that underlie cardiovascular homeostasis by regulating autonomic nervous activity and secretion of hormones involved in control of the water and electrolytes balance. The mechanism by which MR blockade in the brain causes a decrease in blood pressure has not been elucidated. Janiak and Brody and Janiak et al suggested that a diminished sympathetic outflow to peripheral resistance vessels may play a role. It may be speculated that a decrease in vasopressin secretion by the posterior pituitary gland could be involved in the observed hypotension and diuresis. In mineralocorticoid-induced hypertension, increased levels of circulating vasopressin have been described. A blunted pressor response to a high dose of vasopressin (400 ng ICV) after 2 days of ICV infusion of aldosterone has been reported. After central administration of RU28318, the pressor response to vasopressin was restored. Vasopressin was not measured in the current study, but a decrease in the circulating level of the peptide may have contributed to the observed diuresis. It should be noted, however, that renal denervation prevented diuresis, and denervation of the kidneys generally does not appear to interfere with the secretion and antidiuretic effect of vasopressin.

The observed enhanced urinary excretion of potassium, sodium, and chloride associated with diuresis appears to be indicative of a specific action of brain mechanisms on renal functions. Despite the lowering of arterial blood pressure, increased renal excretion of water and electrolytes occurred. Several studies in animals have shown that stimulation of the brain (eg, by central administration of angiotensin II or carbachol) caused increased urinary excretion of potassium, sodium, chloride, and water. In these experiments, blood pressure was not changed or increased.

A change in renal sympathetic nerve activity perhaps could mediate the enhanced excretion of electrolytes and water as observed after central MR blockade by RU28318. We showed that these changes were absent in denervated rats. Renal nerves have been shown to affect water, sodium, and chloride reabsorption in the proximal tubuli and the loop of Henle. Stimulation of selective areas of the hypothalamus can either produce a decrease in PRA or an increase (lateral and posterior hypothalamus). Our data showed no change in PRA 8 and 30 minutes (anterior hypothalamus) or an increase (lateral and posterior hypothalamus). Several studies in animals have shown that stimulation of the hypothalamus can either produce a decrease in PRA or an increase (lateral and posterior hypothalamus). Several studies in animals have shown that stimulation of the hypothalamus can either produce a decrease in PRA or an increase (lateral and posterior hypothalamus). Several studies in animals have shown that stimulation of the hypothalamus can either produce a decrease in PRA or an increase (lateral and posterior hypothalamus).

### Table 1. Effect of Subcutaneous Injection of RU28318 on SBP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 h</th>
<th>2 h</th>
<th>8 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>117±4</td>
<td>118±5</td>
<td>120±4</td>
<td>119±4</td>
<td>122±4</td>
</tr>
<tr>
<td>RU28318 10 ng</td>
<td>119±2</td>
<td>123±2</td>
<td>123±3</td>
<td>127±3</td>
<td>123±3</td>
</tr>
<tr>
<td>RU28318 100 ng</td>
<td>122±3</td>
<td>122±1</td>
<td>120±3</td>
<td>123±3</td>
<td>122±3</td>
</tr>
</tbody>
</table>

Control group consists of vehicle-treated rats. Data are mean±SEM (n=6 rats per group). Periods are indicated in hours; 0 h indicates preinjection values.

### Table 2. Effect of Subcutaneous Injection of RU28318 on Diuresis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diuresis, µL/h per 100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24–0 h</td>
</tr>
<tr>
<td>Control</td>
<td>139±25</td>
</tr>
<tr>
<td>RU28318 10 ng</td>
<td>110±11</td>
</tr>
<tr>
<td>RU28318 100 ng</td>
<td>116±11</td>
</tr>
</tbody>
</table>

Control group consists of vehicle-treated rats. Data are mean±SEM (n=6 rats per group). Periods are indicated in hours; 24–0 h indicates preinjection values.
lytes. Renal innervation appears to participate in the brain MR control of blood pressure and renal function. Renal denervation abolished the increase in water and electrolyte excretion and shortened the duration of the hypotensive period.

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References


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