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Short communication

Cardiovascular and renal effects of central administration of a mineralocorticoid receptor antagonist in conscious female rats

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Abstract

In a previous study we showed that in normotensive male rats brain mineralocorticoid receptor blockade induced a long lasting decrease in blood pressure associated with increased urinary excretion of water and electrolytes. Here, we report the effect of intracerebroventricular injection of a mineralocorticoid receptor antagonist (RU28318; 3,3-oxo-7 propyl-17-hydroxy-androstan-4-en-17yl-propionic acid lactone) on cardiovascular and renal function in female rats. Compared with male rats, females are less sensitive to brain mineralocorticoid receptor blockade. Administration of RU28318 (10 ng, 100 ng) caused a significant decrease in systolic blood pressure (10–12.5%) only at 8 h after injection. An increased urinary excretion of water (about 160%) and electrolytes (about 175%) during the first 8 h after the injection was observed in the 100 ng RU28318 treated group. Heart rate, food intake and water consumption were not affected at either dose. In conclusion, in conscious female rats, brain mineralocorticoid receptors participate in blood pressure and renal function control.

Keywords: Brain; Mineralocorticoid receptor; RU28318; Female rat; Blood pressure; Renal function

1. Introduction

The incidence of hypertension and cardiovascular disease in women before menopause is lower than in men (see Bachman et al., 1991; Lange et al., 1998). Higher blood pressure levels in males than in females have also been reported in animal models of hypertension, such as spontaneously hypertensive rats and deoxycorticosterone-acetate (DOCA)–salt rats, and development of hypertension is also faster in males (Cambotti et al., 1984; Ouchi et al., 1987; Lange et al., 1998). Crofton and Share (1997) have demonstrated that testosterone enhances while estradiol attenuates the development of DOCA–salt hypertension, related perhaps to gender differences in vascular reactivity or in sympathetic nervous system regulation.

The activity of the hypothalamo-pituitary-adrenal axis and corticosteroid feedback effects on the brain differ in female and male rats (Le Mevel et al., 1978; Handa et al., 1994; Weinstock et al., 1998). Basal levels of plasma corticosterone are generally higher in females, and response to different kinds of stress is enhanced compared with males. In addition, higher basal values of circulating levels of aldosterone have been reported in female rats (Tang, 1985), and sex differences in rat brain corticosteroid receptor affinity have also been described. Markedly lower hippocampal mineralocorticoid receptor affinity has been reported in female rats as compared to males (Turner, 1992). If there is a sex difference in the affinity of mineralocorticoid receptor in the rat brain, physiological responses to blockade of this receptor may differ between female and male.

In a previous study in normotensive male rats, we showed that selective blockade of brain mineralocorticoid receptor induced a long lasting decrease in blood pressure associated with diuresis and augmented urinary excretion of electrolytes (Rahmouni et al., 1999). The enhanced urinary excretion of water and electrolytes appears to depend on a specific brain mechanism affecting kidney

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function. This renal effect, induced by brain mineralocorticoid receptor blockade, may contribute to the duration of the hypotension (Rahmouni et al., 1999). In a preliminary study in female rats, we observed no statistically significant effect of central administration of a low dose (10 ng) of a mineralocorticoid receptor antagonist (RU28318) on blood pressure and renal function (Rahmouni et al., 1998). In the present study, we further assessed the effect of brain mineralocorticoid receptor blockade in female rats. For this purpose, the effect of i.c.v. administration of two different doses of RU28318 on cardiovascular and renal function was studied in conscious female Wistar rats.

2. Material and methods

2.1. Animals

Adult female normotensive Wistar rats (190–210 g) (Janvier, Le Genest Saint Isle, France) were used. The animals were housed under standard conditions, with the lights on from 0600–1800 h. All animals received food and tap water ad libitum. The rats were housed in our laboratory for at least 1 week before the start of the experiment.

2.2. Experimental protocol

The rats were exposed daily for 2 weeks before experimentation to the conditions of systolic blood pressure and heart rate measurement by indirect sphygmomanometry. One week before i.c.v. injection, a polyethylene cannula was implanted (Rahmouni et al., 1999) in the left lateral ventricle of rats anesthetized with pentobarbital (50 mg/kg, i.p.) with the animals kept in individual cages postoperatively. To measure water consumption, food intake, urine, and electrolyte output, the rats were kept in metabolic cages (Iffa Credo, L'Arbresle, France), starting 2 days before i.c.v. injection; the first day to accustom the rats to the new environment and the second day to determine these different parameters over 24 h before treatment. Intracerebroventricular injections were given once between 0900–0100 h in a volume of 2 μl. The rats received the mineralocorticoid receptor antagonist RU28318 (3,3-oxo-7propyl-17-hydroxy-androstan-4-en-17yl-propionic acid lactone) at a single dose of 10 ng or 100 ng, or vehicle (2% ethanol–NaCl 0.9%). The various cardiovascular and renal parameters were determined before treatment and at the time intervals indicated after i.c.v. injection. The position of the i.c.v. cannula was verified by injection of an Evans blue solution at the end of the experiment.

Sodium, potassium, and chloride in the urine were measured by an indirect potentiometric method using selective electrodes (Synchron EL-ISE, Beckman, Gagny, France).

2.3. Statistical analysis

Data are expressed as means ± standard error of the mean (means ± SEM). The results were analyzed by two way repeated ANOVA and by the Bonferroni test for comparison among groups. A value of $P < 0.05$ was considered significant.

3. Results

As shown in Table 1 basal values of cardiovascular and renal parameters did not differ between treated and control groups. These values also do not differ from the basal values we obtained under the same conditions in male rats (Rahmouni et al., 1999).

Intracerebroventricular injection of 10 ng of RU28318 caused a slight but significant decrease in systolic blood pressure (treated: 101 ± 4 mm Hg vs. control: 112 ± 2 mm Hg, $P < 0.01$) only at 8 h after administration. No significant differences in heart rate and renal excretion of water, sodium, potassium, and chloride occurred with this dose of mineralocorticoid receptor antagonist.

Table 1
Cardiovascular and renal effects of intracerebroventricular administration of 10 ng and 100 ng of the mineralocorticoid receptor antagonist (RU28318) in female Wistar rats.
Data are means ± SEM of 13, six, and seven rats respectively in vehicle, 10 ng, and 100 ng RU28318 treated groups. Time and periods are indicated in hours, time zero, and period (−24–0 h) indicates preinjection values. Abbreviation: SBP, systolic blood pressure; HR, heart rate.

	Vehicle	10 ng	100 ng	Vehicle	10 ng	100 ng	Vehicle	10 ng	100ng	Vehicle	10 ng	100 ng
	0 h			*8 h*			*24 h*			*48 h*		
SBP (mm Hg)	111 ± 2	111 ± 4	115 ± 1	112 ± 2	101 ± 4[a]	98 ± 1[b]	111 ± 2	107 ± 4	108 ± 2	111 ± 2	112 ± 2	113 ± 2
HR (bpm)	410 ± 7	395 ± 8	413 ± 10	400 ± 7	392 ± 11	398 ± 12	395 ± 11	393 ± 8	397 ± 11	391 ± 6	387 ± 7	409 ± 13
Urinary excretion	*−24–0 h*			*0–8 h*			*8–24 h*			*24–48 h*		
H_2O (μl/h/100 g)	161 ± 13	154 ± 28	133 ± 11	283 ± 36	374 ± 41	449 ± 76[b]	160 ± 28	166 ± 22	167 ± 18	160 ± 20	133 ± 24	168 ± 23
Na^+ (μM/h/100 g)	20 ± 2	20 ± 6	16 ± 3	36 ± 5	32 ± 6	64 ± 7[a]	25 ± 7	30 ± 12	20 ± 3	24 ± 7	23 ± 2	19 ± 5
K^+ (μM/h/100 g)	39 ± 4	41 ± 8	32 ± 5	49 ± 6	55 ± 4	85 ± 16[a]	45 ± 12	33 ± 3	28 ± 5	46 ± 12	42 ± 4	35 ± 6
Cl^- (μM/h/100 g)	28 ± 2	32 ± 7	23 ± 4	44 ± 7	47 ± 6	77 ± 10[a]	29 ± 8	32 ± 10	21 ± 4	31 ± 8	28 ± 3	21 ± 6

[a] $P < 0.01$ compared with control by ANOVA and Bonferroni test.
[b] $P < 0.001$ compared with control by ANOVA and Bonferroni test.

Intracerebroventricular injection of 100 ng of RU28318 caused a similar decrease in systolic blood pressure similarly present at 8 h (98 ± 1 mm Hg vs. 112 ± 2 mm Hg; $P < 0.001$); 24 h after administration of the compound, the decrease in systolic blood pressure disappeared (108 ± 2 mm Hg vs. 111 ± 2 mm Hg). Blood pressure returned to baseline value after 48 h and heart rate was not significantly affected.

In the first 8 h after injection of 100 ng of RU28318, a significant increase in urinary water excretion occurred. Urine volume increased to about 160% of the value of controls ($P < 0.001$). In the same period, increased urinary electrolyte excretion was observed in the treated group, with sodium 178% ($P < 0.01$), potassium 173% ($P < 0.01$), and chloride 175% ($P < 0.01$), of control values.

Intracerebroventricular administration of the mineralocorticoid receptor antagonist (10 ng and 100 ng doses) did not affect water consumption and food intake (data not shown) compared with vehicle-treated rats.

4. Discussion

The present data show that in female rats, blockade of brain mineralocorticoid receptor induces a decrease in blood pressure while diuresis and increased urinary excretion of electrolytes was only caused by the highest dose (100 ng) of the mineralocorticoid receptor antagonist used. As in our preliminary studies (Rahmouni et al., 1998), the lower dose (10 ng) did not affect renal function, although in the present experiment this dose did decrease blood pressure. The present results corroborate our preliminary findings that there exists a sexual dimorphism of blood pressure responses to central mineralocorticoid receptor blockade, since a higher dose of i.c.v. antagonist was required to obtain similar effects on both blood pressure and renal function, albeit less than observed in male rats. The time course and magnitude of blood pressure decrease induced by central mineralocorticoid receptor blockade were also somewhat different between males and females of the same rat strain. Our results obtained in male rats (Rahmouni et al., 1999) show that i.c.v. injection of 10 ng of RU28318 caused a long lasting decrease in blood pressure with a maximum effect at 8 h (25 mm Hg), with the decrease persisting at 24 h, and disappearing at 48 h. In contrast, female rats given 10 ng or 100 ng antagonist i.c.v. showed a decrease in blood pressure of 13–14 mm Hg at 8 h, disappearing by 24 h. The increased diuresis and urinary electrolyte excretion induced in males by i.c.v. administration of 10 ng of the mineralocorticoid receptor antagonist were 1.3- to 2.4-fold higher than those observed here in females with 100 ng of the mineralocorticoid receptor antagonist i.c.v. It appears that in the female rat, the decrease in blood pressure observed following i.c.v. injection of RU28318 is not correlated to the occurrence of changes of renal function.

The effect induced by brain mineralocorticoid receptor blockade on renal function and blood pressure of male rats was not mediated by changes of the renin–angiotensin system, since no change in plasma renin activity occurred despite the decrease in blood pressure (Rahmouni et al., 1999). Renal innervation appears to mediate a part of the action of brain mineralocorticoid receptor blockade because in bilaterally denervated male rats, the increase in water and electrolytes excretion was abolished while the duration of the fall in blood pressure was shortened (Rahmouni et al., 1999). Central inhibition of efferent renal nerve activity may lead to a decrease in renal tubular sodium and water re-absorption resulting in increased urinary water and electrolyte excretion (DiBona and Kopp, 1997).

Neural regulation of the autonomic nervous system may differ in male and female rats (Crofton and Share, 1997; Weinstock et al., 1998). Autonomic nervous reflex mechanisms in females may be more effective in buffering changes in blood pressure. Normotensive female rats show a greater inhibition of lumbar sympathetic nerve activity than male normotensive rats to stimulation of cardiopulmonary receptors (see Lange et al., 1998). Furthermore, the impairment of baroreflex sensitivity is attenuated in female DOCA–salt rats compared with male DOCA–salt rats (Ouchi et al., 1987). Although it is not known if brain mineralocorticoid receptor plays a role in the enhanced baroreflex function of female rats, such attenuation may contribute to the diminished response of female rats to the i.c.v. mineralocorticoid receptor blockade we observed. Literature data regarding the role of brain mineralocorticoid receptor in cardiovascular regulation of females are very scarce. Gomez-Sanchez et al. (1992) showed that i.c.v. infusion of RU28318 inhibited the development of hypertension of female Dahl salt sensitive rats. Our present data for the first time reveal a hypotensive effect and altered renal excretory function following a single i.c.v. injection of RU28318 in female rats. Central mineralocortocoid receptors may be important in cardiovascular regulation and perhaps could play a role in the differences between females and males in this respect, but such a conclusion awaits further investigations.

In conclusion, in conscious female normotensive rats, brain mineralocorticoid receptor participates in blood pressure and kidney function control since selective blockade of this receptor induced a decrease in blood pressure, and increased diuresis and urinary excretion of electrolytes. A higher dose of the mineralocorticoid receptor antagonist was needed to obtain an effect on both blood pressure and renal function than required in male rats of the same strain.

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