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European Journal of Pharmacology 436 (2002) 207–216

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Effects of brain mineralocorticoid receptor blockade on blood pressure and renal functions in DOCA–salt hypertension

Kamal Rahmouni [a,*], Rosana M. Sibug [b], E. Ronald De Kloet [b], Mariette Barthelmebs [a], Michèle Grima [a,c], Jean-Louis Imbs [a,c], Wybren De Jong [a,1]

[a] *Institut de Pharmacologie, Faculté de Médecine, Université Louis Pasteur, Strasbourg, France*
[b] *Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research and Leiden University Medical Center, Leiden, The Netherlands*
[c] *Service d'Hypertension Artérielle, Maladies Vasculaires et Pharmacologie Clinique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France*

Received 24 October 2001; accepted 7 December 2001

Abstract

In normotensive rats, we have previously demonstrated a role of brain mineralocorticoid receptors in blood pressure and renal function control. In the present study, the coordinate cardiovascular and renal effects of brain mineralocorticoid receptor blockade were examined by intracerebroventricular (i.c.v.) administration of a selective mineralocorticoid receptor antagonist (RU28318; 3,3-oxo-7 propyl-17-hydroxy-androstan-4-en-17yl-propionic acid lactone) in rats with hypertension induced by deoxycorticosterone acetate (DOCA) and salt. DOCA pellets were implanted s.c. in male Wistar rats given 0.9% NaCl as drinking solution 3 or 5 weeks before assessment of the effects of i.c.v. injection of RU28318 on cardiovascular and renal functions. Changes in expression of brain angiotensinogen, atrial natriuretic peptide (ANP) and mineralocorticoid receptor mRNA in specific brain areas in 3-week DOCA–salt rats were evaluated by in situ hybridization. The rise in systolic blood pressure induced by DOCA–salt treatment was most marked during the first 3 weeks. At 3 and 5 weeks after implantation of the DOCA-pellets a single i.c.v. injection of 10 ng of RU28318 significantly decreased systolic blood pressure during 24 h as assessed at 2, 8 and 24 h, while heart rate was not altered. Increased urinary excretion of water and electrolytes was observed in 3- and 5-week DOCA–salt rats during the period 0–8 h after i.c.v. injection of RU28318 while the suppressed plasma renin activity was not affected. The expression of brain angiotensinogen, ANP and mineralocorticoid receptor mRNA was not altered by 3-week DOCA–salt treatment, but 3 h after i.c.v. injection of RU28318, mineralocorticoid receptor mRNA expression in hippocampal cell fields responded with an increase of about 40%. In conclusion, these results demonstrate that in rats with hypertension induced by DOCA–salt, brain mineralocorticoid receptor blockade affects renal function and blood pressure regulation.

Keywords: Mineralocorticoid hypertension; RU28318; Brain; Diuresis; Angiotensinogen

1. Introduction

A common feature of mineralocorticoid hypertension caused by excess mineralocorticoids is increased blood pressure, initial sodium and water retention, hypokalemia and suppression of the peripheral renin–angiotensin system (Biglieri, 1991). In animals, deoxycorticosterone-acetate (DOCA)–salt hypertension is a frequently used model to study the role of centrally acting factors in the pathogenesis of mineralocorticoid hypertension. One of these factors is the brain renin–angiotensin system as indicated by the attenuation of the development of DOCA–salt hypertension in response to intracerebroventricular (i.c.v.) infusion of an inhibitor of angiotensin-converting enzyme (Itaya et al., 1986) and also of an angiotensin II receptor antagonist (Nishimura et al., 1998). Furthermore, an activated brain renin–angiotensin system and increased density of angiotensin II receptors in brain areas related to cardiovascular and body fluid control was observed in rats with DOCA–salt hypertension (Wilson et al., 1986; Gutkind et al., 1988). A role of brain atrial natriuretic peptide (ANP) was suggested (Geiger et al. (1989) because of the increased ANP content of some brain nuclei in DOCA–salt hypertensive rats.

Direct action of mineralocorticoids in the brain resulting in cardiovascular effects has been demonstrated by several investigators. Gómez Sanchez (1988) and Chen et al.

* Corresponding author. University of Iowa 524 MRC, Iowa City, IA 52242, USA. Fax: +1-319-335-8547.
E-mail address: kamal-rahmouni@uiowa.edu (K. Rahmouni).

[1] The untimely death of Wybren de Jong (April 29, 2001) was a great shock.

0014-2999/02/$ - see front matter
PII: S0014-2999(01)01586-2

(1989) showed that continuous i.c.v. administration of small amounts of aldosterone in rats produced a significant increase in arterial blood pressure. A key contribution of brain mineralocorticoid receptors in mineralocorticoid-induced hypertension in rats was demonstrated by chronic i.c.v. infusion of a low dose (ineffective when given systemically) of a mineralocorticoid receptor antagonist (3,3-oxo-7 propyl-17-hydroxy-androstan-4-en-17yl-propionic acid lactone, RU28318) that inhibited development of this type of hypertension (Gómez Sanchez et al., 1990; Janiak et al., 1990).

The exposure to excess mineralocorticoids and salt may affect kidney function directly and/or via brain mechanisms and humoral agents. Increased renal vascular reactivity to pressor agents was observed in rats 4 days after DOCA implantation, before a rise in arterial blood pressure occurred and thus suggesting that renal vascular function change could be a pathogenic factor in the development of DOCA–salt hypertension (Berecek et al., 1980b). Destruction of catecholaminergic neurons in the brain by 6-hydroxydopamine prevented the enhanced vascular reactivity to pressor agents, suggesting that central mechanisms are at the origin of vascular changes in the kidney (Berecek et al., 1980a). Evidence for participation of the renal nerves in DOCA–salt hypertension is derived from denervation experiments. Renal denervation delayed the onset of high blood pressure in DOCA–salt rats (Katholi et al., 1980; DiBona and Kopp, 1997). Several brain cell groups located in the pons-medulla and hypothalamus appear to be involved in regulation of renal function and are thought to do so by affecting sympathetic outflow to the kidney (Akpogomeh and Johns, 1991; DiBona and Kopp, 1997). Activation of neurons in the paraventricular nucleus of the hypothalamus in anesthetized rats caused an increase in mean arterial blood pressure associated with a decrease in glomerular filtration rate, renal plasma flow, diuresis and urinary sodium excretion (Haselton and Vari, 1998). Electrical stimulation of the anteroventral part of the third ventricle (AV3V) in anesthetized rats produced a frequency-dependent increase in renal and mesenteric vascular resistance and a reduced renal blood flow together with vasodilation of the hindlimb (Fink et al., 1978). Finally, lesions of the paraventricular nucleus or the AV3V region inhibited the development of DOCA–salt hypertension in rats (Nakata et al., 1989; Brody et al., 1978).

In conscious normotensive Wistar rats, we recently showed that brain mineralocorticoid receptors are a critical determinant in the control of renal function, which is linked to the function of these receptors in mediating the aldosterone effects on cardiovascular regulation (Rahmouni et al., 1999a). One-shot blockade of these receptors by the injection of the selective mineralocorticoid antagonist RU28318 decreased systolic blood pressure and increased urinary excretion of water and electrolytes. Previously, we found a similar decrease of systolic blood pressure induced by RU28318 in DOCA–salt treated hypertensive rats (Van den Berg, 1994b). In the present study, we examined the involvement of brain mineralocorticoid receptors in the coordinate control of cardiovascular and renal functions of DOCA–salt hypertensive rats using the same approach as applied in the normotensive rats (Rahmouni et al., 1999a). We hypothesized that, like in normotensive rats, the decrease in systolic blood pressure induced by brain mineralocorticoid receptor blockade in DOCA–salt rats will be associated with an increase in urinary excretion of water and electrolytes. We assessed the effects of a single i.c.v. administration of the selective mineralocorticoid receptor antagonist (RU28318) on systolic blood pressure and heart rate, and on renal excretion of water and electrolytes after 3 and 5 weeks DOCA–salt treatment. In addition, to investigate the potential central pathways involved in the effects of brain mineralocorticoid receptor blockade, we assessed the gene transcription of angiotensinogen, ANP and mineralocorticoid receptor in some brain regions of 3-week DOCA–salt rats.

2. Materials and methods

2.1. Animals

Adult male normotensive Wistar rats (Janvier, Le Genest Saint Isle, France) were used. Animals were housed under constant temperature (20 °C) and 12-h light/dark cycle. Food and drinking solution (water or 0.9% saline) were available ad libitum throughout the study. All experiments were conducted in accordance with guidelines for the care and use of animals of the French government and the European Community.

The effects of i.c.v. injection of RU28318 on cardiovascular and renal parameters and plasma renin activity were studied in 3-week (development phase) and 5-week (maintenance phase) DOCA–salt rats. Furthermore, we evaluated changes in expression of brain angiotensinogen, ANP and mineralocorticoid receptor mRNA in specific brain areas in 3-week DOCA–salt rats after i.c.v. injection of RU28318. The influence of 3-week DOCA–salt treatment on basal values of these latter parameters was assessed in a separate experiment.

2.2. Experimental protocol

Three or five weeks before i.c.v. injection, two pellets of DOCA (about 25 mg each) were implanted s.c. in rats under ether anesthesia and 0.9% saline was provided as drinking solution. Sham operated rats were subjected to the same procedure but did not receive DOCA pellets and had tap water to drink. Water drinking rats were chosen as a control group because salt intake did not affect the cardiovascular and renal effects of brain mineralocorticoid receptor blockade (Rahmouni et al., unpublished data). Rats assigned to study the effects of brain mineralocorticoid receptor block-

ade on cardiovascular and renal function as well as on the expression of brain angiotensinogen, ANP and mineralocorticoid receptor mRNA were exposed to systolic blood pressure measurement procedure during 2 weeks before i.c.v. injection as previously described (Rahmouni et al., 1999a). For i.c.v. administration, a permanent polyethylene cannula was implanted under pentobarbital anesthesia (50 mg/kg, i.p.) in the left lateral ventricle at least 1 week before injections (Rahmouni et al., 1999a). To assess urine and electrolyte output, fluid consumption and food intake, the rats were placed in metabolic cages two days before i.c.v. injection and allowed to become accustomed to the cage for the first day with subsequent measurement of the different parameters over a 24-h interval prior to i.c.v. administration. Intracerebroventricular injections were performed only once; each rat received 10 ng of the mineralocorticoid receptor antagonist or vehicle (2% ethanol-saline) in a volume of 2 μl between 0900–1100 h. Systolic blood pressure and heart rate were determined at 2, 8, 24 and 48 h after injection. Fluid consumption, food intake and urinary excretion of water and electrolytes were measured during the periods 0–8, 8–24 and 24–48 h after RU28318 i.c.v. administration. In the experiment where expression of angiotensinogen, ANP and mineralocorticoid receptor mRNA was studied, only fluid consumption, food intake and renal parameters were measured in the period 0–3 h after i.c.v. injection of RU28318 or vehicle, in order to check effectiveness of i.c.v. administration of the mineralocorticoid receptor antagonist. Brains of these rats were frozen and stored at −80 °C for further analysis. All rats were killed at the end of the experiment by decapitation and trunk blood was collected. To check cannula placement, an Evans blue solution was injected i.c.v. before decapitation and dye diffusion in the brain was verified except for the experiment in which mRNA expression was measured.

2.3. Assays

Blood was collected in sodium-EDTA coated tubes and promptly centrifuged. Plasma was frozen at −80 °C for renin activity determination by radioimmunoassay procedure. Plasma renin activity was expressed as nanograms of angiotensin I generated per millilitre of plasma per hour (ng Ang I/ml/h). An indirect potentiometric method using selective electrodes (Synchron EL-ISE, Beckman, Gagny, France) was used to determine the concentration of the different electrolytes (Na^+, K^+ and Cl^-) in the urine.

2.4. In situ hybridization

2.4.1. Riboprobes

PCR-script plasmids, containing either a 307-basepair insert of the rat angiotensinogen mRNA located at the amino terminal part or 441-basepair insert of the rat pro-ANP at the carboxy terminal end, were used as templates to generate poly-chain reaction (PCR) products for in vitro transcription. Two sets of primers with each set containing a primer specific for the promotor site and another at the angiotensinogen or ANP insert, were used to produce the two orientations. Sense and antisense 35S-UTP labeled probes were generated by in vitro transcription using T3 and T7 RNA polymerase according to a standard protocol (Boehringer, Mannheim). The mineralocorticoid receptor probe was transcribed from a 513-basepair rat brain cDNA fragment, which encodes for the last 30 amino acids at the C-terminus of mineralocorticoid receptor plus the adjacent highly specific 3′ untranslated region (courtesy of J.L. Arriza, USA). The labeled antisense and sense probes for the mineralocorticoid receptor were generated by in vitro transcription using SP6 polymerase and T7 polymerase respectively, using the same protocol as used for angiotensinogen and ANP.

2.4.2. Hybridization

Cryostat sections (20 μm) of the AV3V, paraventricular nucleus, dorsal hippocampus, heart and liver were cut at −20 °C, thaw-mounted on 0.01% poly-L-lysine coated slides and stored at −80 °C until hybridization. Sections were fixed in freshly prepared 4% paraformaldehyde in phosphate buffered saline (PBS) (pH 7.2) for 60 min at room temperature just before hybridization. The sections were then washed twice in PBS (5 min each), permeabilized by proteinase K treatment (100 μg/100 ml in 0.1 M Tris, pH 8.0) for 10 min at 37 °C, rinsed briefly with diethylpyrocarbonate-treated water, acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0), washed with 2× saline sodium citrate (SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) for 10 min, dehydrated in an increasing graded ethanol series and then air-dried.

Table 1
Effect of 3- and 5-week DOCA–salt treatment on different parameters as compared to sham operated rats

Parameters	3-week		5-week	
	Sham	DOCA–salt	Sham	DOCA–salt
Body weight (g)	387 ± 8	371 ± 4[a]	409 ± 9	387 ± 5[a]
SBP (mm Hg)	116 ± 4	158 ± 2[b]	120 ± 2	168 ± 2[b]
HR (bpm)	367 ± 8	324 ± 4[b]	369 ± 10	341 ± 9[a]
Food (μg/h/100 g)	244 ± 12	260 ± 10	230 ± 38	226 ± 18
Drinking (μl/h/100 g)	206 ± 32	988 ± 81[b]	232 ± 33	1082 ± 149[c]
Diuresis (μl/h/100 g)	105 ± 9	545 ± 72[b]	105 ± 5	761 ± 101[b]
U_{Na+} (μM/h/100 g)	18 ± 2	88 ± 13[c]	16 ± 3	120 ± 15[b]
U_{K+} (μM/h/100 g)	23 ± 2	46 ± 9	25 ± 3	57 ± 13
U_{Cl-} (μM/h/100 g)	17 ± 2	58 ± 7[b]	21 ± 3	117 ± 11[b,d]

Data are means ± S.E.M. of 6, 16, 9 and 19 rats per group, respectively, in 3-week sham, 3-week DOCA–salt, 5-week sham and 5-week DOCA–salt. Abbreviations: SBP, systolic blood pressure; HR, heart rate; U_{Na^+}, U_{K+} and U_{Cl-}, urinary excretion of Na^+, K^+ and Cl^-.

[a] $P < 0.05$ as compared to the corresponding sham group.

[b] $P < 0.001$ as compared to the corresponding sham group.

[c] $P < 0.01$ as compared to the corresponding sham group.

[d] $P < 0.05$ as compared to 3-week DOCA–salt rats.

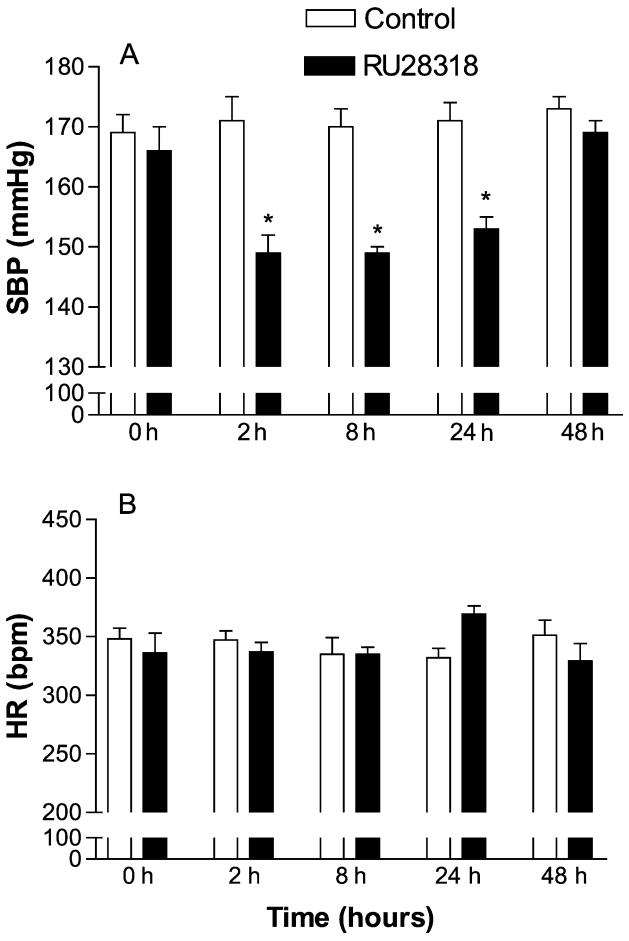

Fig. 1. Effect of i.c.v. administration of 10 ng of RU28318 on (A) systolic blood pressure (SBP) and (B) heart rate (HR) of 3-week DOCA–salt rats. The control group corresponds to the vehicle-treated rats. Data are means ± S.E.M. of 8 animals per group. Time is indicated in hours after i.c.v. injection; time 0 indicates pre-injection value. * $P < 0.001$ as compared to the control group.

Angiotensinogen and ANP hybridization was performed as follows: the hybridization mix consisted of 70% formamide, 10% dextran sulfate, 2 × SSC, 1 × Denhardt's solution, 10 mM dithiothreitol, 0.1 mg/ml yeast tRNA and 0.1 mg/ml sheared salmon sperm DNA, to which 2.0×10^6 dpm/ml labeled riboprobe was added. A 100-μl portion of this mix was then pipetted on each slide. The slides were then covered with 24 × 50 mm microscopic coverslips, stacked and sealed in slide boxes, placed inside a moist chamber and hybridized overnight at 53 °C. The following morning the coverslips were removed and the slides were washed twice in 2 × SSC at room temperature each time for 10 min. Thereafter, the slides were treated with RNAse A (2 mg/100 ml in 0.5 M NaCl, pH 7.5) at 37 °C for 10 min, washed three times at 60 °C in 2 × SSC/50% formamide (15 min each) and finally in 2 × SSC (5 min). The slides were then dehydrated in a graded alcohol series, air-dried and exposed to Kodak X-OMAT AR film for 4–14 days, depending on the tissue used.

The mineralocorticoid receptor hybridization procedure was adapted from Van Eekelen et al. (1988) with some modifications. Briefly, 100 μl hybridization mix (2.5×10^6 dpm) was pipetted on each slide and incubated overnight in a moist chamber at 45 °C. The coverslips were removed in 2 × SSC and washed thrice in 2 × SSC at room temperature. Thereafter, they were washed twice in 2 × SSC/50% formamide at 55 °C (15 min each) and rinsed briefly in 2 × SSC. They were then treated with RNAse A (2 mg/ml) for 30 min, dipped briefly in 2 × SSC and washed three times in 2 × SSC/50% formamide at 55 °C (15 min each) and subsequently washed twice in 2 × SSC (5 min each). Finally, they were dehydrated in a graded series of alcohol, air-dried and exposed to Kodak X-OMAT AR films, 4–21 days, depending on the tissue used.

First, we compared, by in situ hybridization, the brain expression of angiotensinogen, ANP and mineralocorticoid receptor genes between 3-week sham and DOCA–salt treated rats. In this experiment, the expression of angioten-

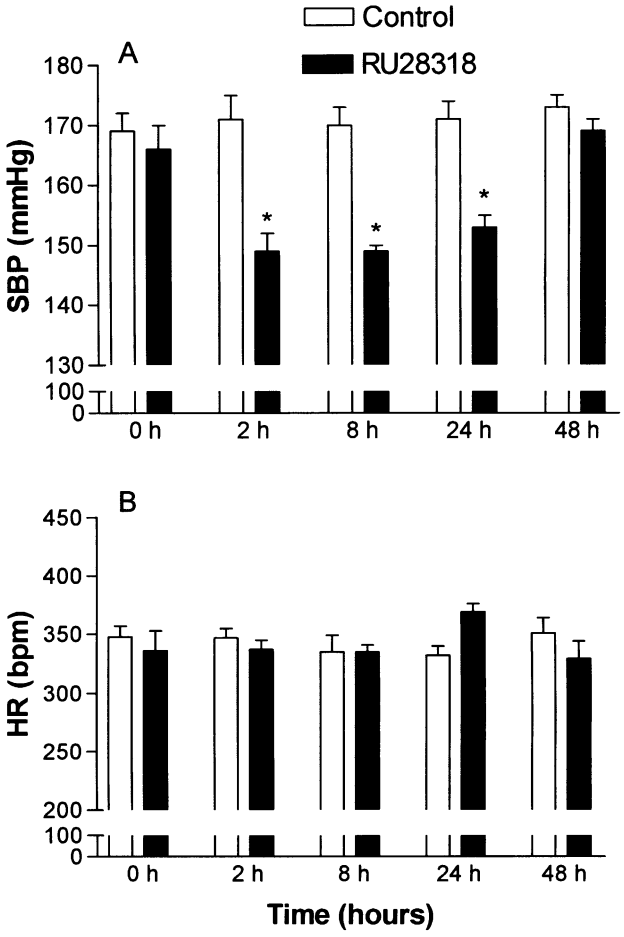

Fig. 2. Effect of i.c.v. administration of 10 ng of RU28318 on (A) systolic blood pressure (SBP) and (B) heart rate (HR) of 5-week DOCA–salt rats. The control group corresponds to the vehicle-treated rats. Data are means ± S.E.M. of 9–10 animals per group. Time is indicated in hours after i.c.v. injection; time 0 indicates pre-injection value. * $P < 0.001$ as compared to the control group.

sinogen and ANP were also assessed, respectively, in the liver and atria as a control of the efficacy of the DOCA–salt treatment. However, in 3-week DOCA–salt rats treated intracerebroventricularly with either vehicle or RU28318 the expression of angiotensinogen, ANP and mineralocorticoid receptor genes were compared only in the brain. Liver and heart were not collected, because we did not expect changes after central RU28318 administration.

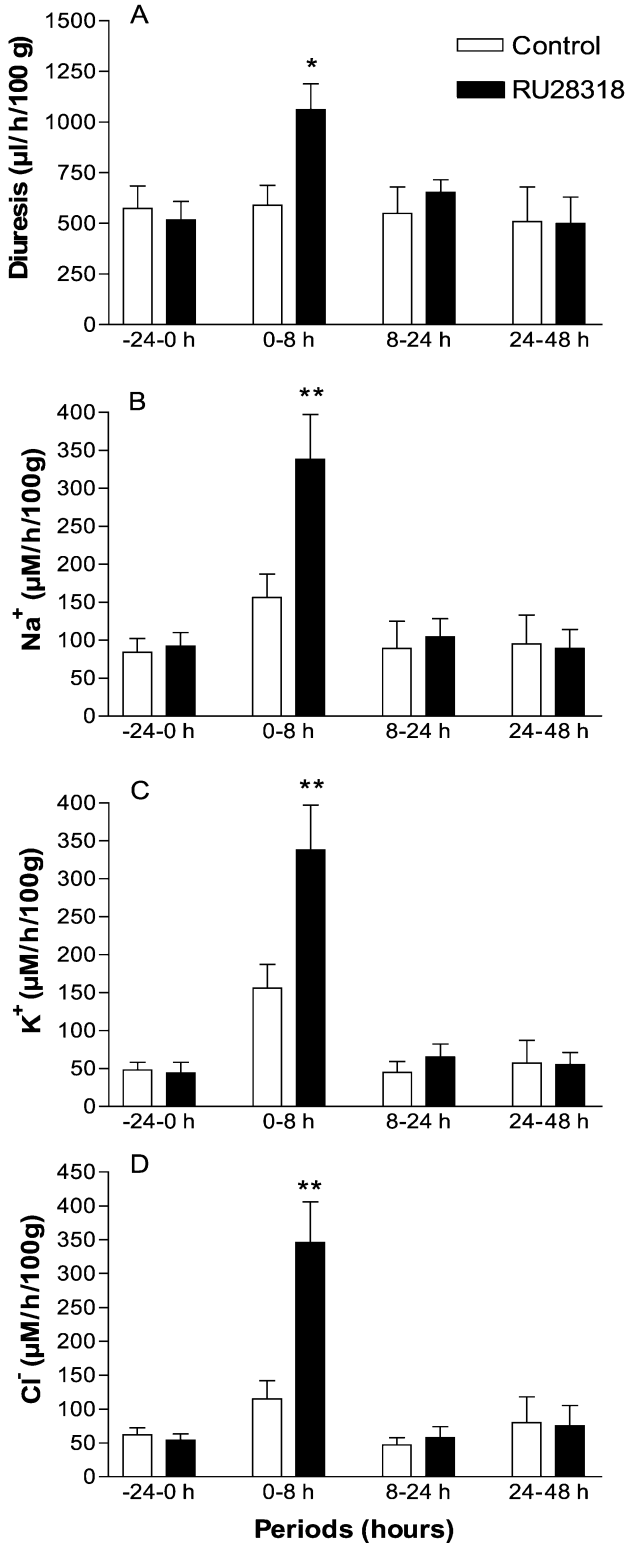

Fig. 3. Effect of i.c.v. administration of 10 ng of RU28318 on diuresis (A) and urinary excretion of Na^+ (B), K^+ (C) and Cl^- (D) in 3-week DOCA–salt rats. The control group corresponds to the vehicle-treated rats. Data are means ± S.E.M. of 8 animals per group. Periods are indicated in hours after i.c.v. injection; period (−24–0 h) indicates pre-injection value. * $P<0.01$, ** $P<0.001$ as compared to the control group. At 8 h, the body weights in vehicle- and RU28318-treated groups were 3589 ± 6 and 3659 ± 5, respectively, and absolute urine volumes (period 0–8 h) were 12.7 ± 2.1 and 23 ± 2.6 ml ($P<0.01$ by t test).

2.4.3. Densitometric analysis

The autoradiograms were quantified using an Olympus image analysis system with appropriate software (Paes Nederland, The Netherlands). A shading correction was first performed and the images were further corrected for film background. The labeling intensities of the AV3V, paraventricular nucleus and liver were measured for angiotensinogen expression; AV3V and hippocampus for mineralocorticoid receptor expression; AV3V and heart for ANP expression. Results were expressed in arbitrary units.

2.5. Statistical analysis

Results are expressed as mean ± standard error of the mean (S.E.M.). One-way analysis of variance (ANOVA) was used to compare the effects of 3- and 5-week DOCA–salt treatment to the sham operated groups, while the effects of RU28318 in DOCA–salt rats were analyzed using two-way ANOVA on repeated measurements. Student–Newman–Keuls or Bonferroni tests were used for comparison among groups at any times. For mRNA levels of angiotensinogen, mineralocorticoid receptor and ANP values of three to five sections for each brain area per animal were pooled and analyzed for the different treatment groups using Student's t-test. A value of $P<0.05$ was considered significant.

3. Results

3.1. Effect of DOCA–salt treatment

Before DOCA implantation there was no difference between DOCA–salt and sham groups in systolic blood pressure (115 ± 2 vs. 116 ± 2 mm Hg) and heart rate (421 ± 5 vs. 418 ± 8 bpm). Most of the rise in blood pressure induced by DOCA–salt was marked during the first 3 weeks (Table 1). Heart rate was significantly decreased. Five-week DOCA–salt treatment did not substantially increase hypertension further, while the difference in heart rate level between DOCA–salt and sham groups remained. As shown in Table 1, the development of hyper-

tension after DOCA implantation was associated with decreased body weight and increased fluid intake, diuresis and urinary excretion of Na^+ and Cl^-. In 3- and 5-week DOCA–salt rats, fluid intake reached about five times the value of fluid intake of sham operated rats. The changes in urinary excretion of water, Na^+ and Cl^- induced by DOCA–salt showed a similar pattern. The increase in urinary excretion of Cl^- was more pronounced ($P<0.05$) in 5-week than in 3-week DOCA–salt rats.

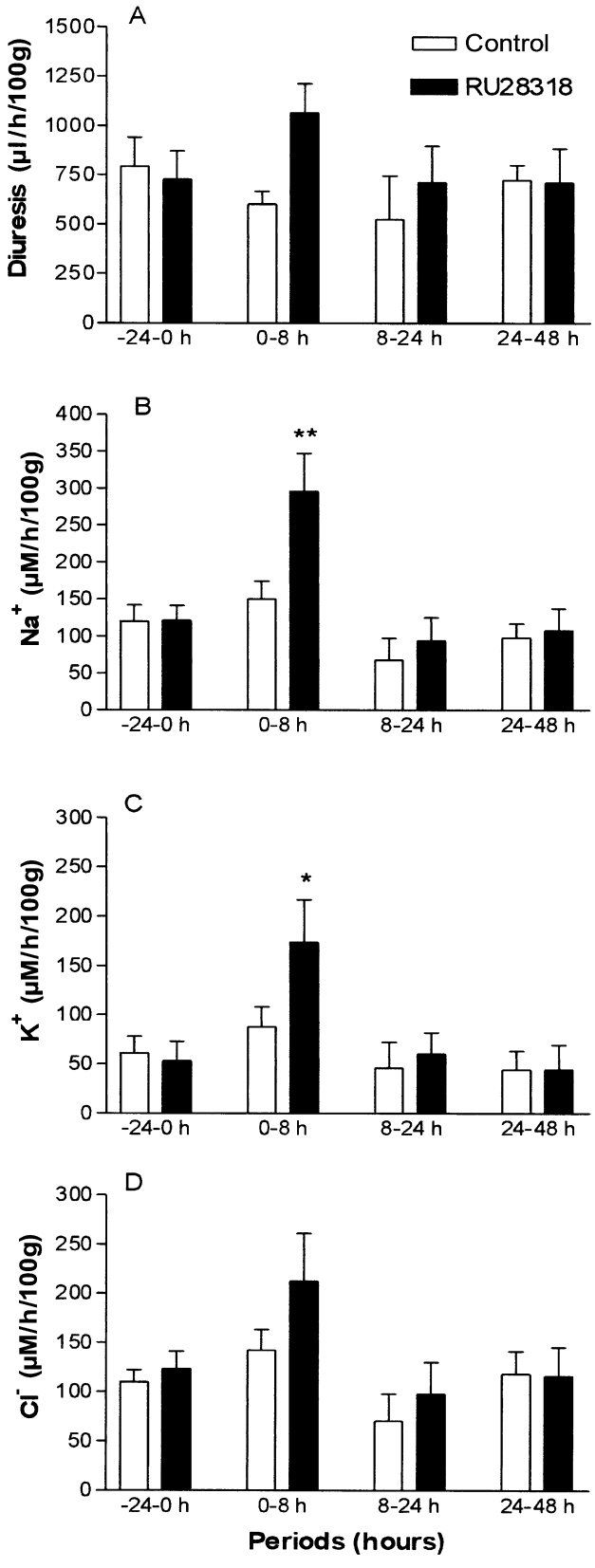

Fig. 4. Effect of i.c.v. administration of 10 ng of RU28318 on diuresis (A) and urinary excretion of Na^+ (B), K^+ (C) and Cl^- (D) in 5-week DOCA–salt rats. The control group corresponds to the vehicle-treated rats. Data are means ± S.E.M. of 9–10 animals per group. Periods are indicated in hours after i.c.v. injection; period (−24–0 h) indicates preinjection value. * $P<0.05$, ** $P<0.001$ as compared to the control group. At 8 h, body weights in vehicle- and RU28318-treated rats were 379 ± 29 and 381 ± 9 g, respectively, and absolute urine volumes in the period 0–8 h: 13.7 ± 4.6 and 22.8 ± 3.2 ml ($P<0.05$).

3.2. Cardiovascular effects of i.c.v. RU28318 in DOCA–salt rats

The effects of mineralocorticoid receptor antagonist and vehicle on cardiovascular function are shown in Figs. 1 and 2. A single i.c.v. injection of 10 ng of RU28318 markedly affected systolic blood pressure both in 3- and 5-week DOCA salt rats. Systolic blood pressure started to decline at 2 h after the i.c.v. injection of the mineralocorticoid receptor antagonist. The decrease in systolic blood pressure at 2 h of about 13 mm Hg in 3-week DOCA–salt rats was less pronounced than in 5-week DOCA–salt rats (about 22 mm Hg). In 3-week DOCA–salt rats, the decrease in systolic blood pressure was maximal at 8 h (144 ± 2 vs. 166 ± 2 mm Hg, $P<0.001$). The effect persisted at 24 h (151 ± 3 vs. 164 ± 2 mm Hg, $P<0.001$) and had disappeared at 48 h after i.c.v. injection. In 5-week DOCA–salt rats, a similar fall of systolic blood pressure, as compared to the 3-week DOCA–salt rats, occurred at 8 h (149 ± 1 vs. 170 ± 3 mm Hg, $P<0.001$) and was still present at 24 h (154 ± 2 vs. 171 ± 3 mm Hg, $P<0.001$). Blood pressure returned to the level of the vehicle-treated controls at 48 h. Heart rate did not differ significantly between RU28318 and vehicle-treated rats (Figs. 1B and 2B) during all observation times.

3.3. Renal and endocrine effects of i.c.v. RU28318 in DOCA–salt rats

As shown in Figs. 3 and 4, only during the period 0–8 h after i.c.v. injection increased urinary excretion of water and electrolytes was observed in the RU28318-treated group as compared to vehicle-treated controls. In 3-week DOCA–salt rats, diuresis was increased to 179% (as compared to vehicle-treated controls, $P<0.01$), Na^+ excretion to 217% ($P<0.001$), K^+ excretion to 263% ($P<0.001$) and Cl^- excretion to 301% ($P<0.001$). In 5-week DOCA–salt rats, a similar magnitude of increase in diuresis (167%) compared to 3-week DOCA–salt rats was observed in the

RU28318-treated group. However, urinary electrolyte excretion was less pronounced. Urinary excretion of Na^+ and K^+ increased to about 197% ($P<0.01$ and 0.05, respectively) and Cl^- excretion to only 148%. We did not measure other ions (e.g. HCO_3^-, Ca^{2+}).

No differences in saline consumption and food intake between the RU28318- and vehicle-treated groups in 3- and 5-week DOCA–salt rats were observed (data not shown). Plasma renin activity measured at the end of the experiment showed no differences between RU28318- and vehicle-treated rats of 3-week (1.2 ± 0.8 vs. 0.24 ± 0.14 ng Ang I/ml/h, respectively) and 5-week DOCA–salt rats (1.9 ± 0.15 vs. 2.0 ± 0.23 ng Ang I/ml/h, respectively). These plasma renin activity values of DOCA–salt were decreased as compared to sham operated rats (6.8 ± 1 and 12.7 ± 3 ng Ang I/ml/h, respectively, of the 3- and 5-week experiments).

3.4. Angiotensinogen, ANP and mineralocorticoid receptor transcription

Basal values of systolic blood pressure and heart rate of both groups of DOCA–salt rats used to assess mRNA expression in brain tissue and peripheral organs at 3 weeks were similar as observed in the animals used in Table 1 (data not shown). The increase in urine output in RU28318-treated DOCA–salt rats during the period 0–3 h as compared to the vehicle-treated DOCA–salt rats (respectively, 576 ± 37 and 296 ± 38 μl/h/100 g body weight, $P<0.001$) confirmed the effectiveness of i.c.v. administration of 10 ng of RU28318.

As shown in Table 2, DOCA–salt treatment did not alter the angiotensinogen mRNA level in the AV3V region and paraventricular nucleus but decreased expression level in the liver (sham 338 ± 11 vs. DOCA–salt 283 ± 18, $P<0.05$). On the other hand, ANP mRNA expression in the atria was enhanced by DOCA–salt treatment (sham 394 ± 21 vs. DOCA–salt 450 ± 12, $P<0.05$), which however did not affect the expression levels in the AV3V. In the case of mineralocorticoid receptor expression, no difference was observed in the AV3V and hippocampus of sham and DOCA–salt rats. However, among the DOCA–salt rats, i.c.v. administration of RU28318 significantly increased mineralocorticoid receptor mRNA levels in the hippocampal cell fields CA1, CA3 and dentate gyrus (Table 3), but did not affect the angiotensinogen and ANP mRNA levels in the AV3V and paraventricular nucleus.

Table 2
Effect of 3-week DOCA–salt treatment on angiotensinogen, ANP and mineralocorticoid receptor transcription

	Sham	DOCA–salt
Angiotensinogen		
AV3V	188 ± 10	203.7 ± 19
PVN	120 ± 7	132 ± 8.3
liver	338 ± 11	283 ± 18[a]
ANP		
AV3V	44 ± 6	28 ± 3.8
Atria	394 ± 21	450 ± 12[a]
Mineralocorticoid receptor		
AV3V	38 ± 3	43 ± 3
Hippocampus:		
CA1	182 ± 8	180 ± 6
CA3	109 ± 2.2	114 ± 6
DG	194 ± 4	201 ± 8

Data are means ± S.E.M. of 8 rats in each group. Values represent arbitrary units. Abbreviations: ANP, atrial natriuretic peptide; AV3V, anteroventral third ventricle; PVN, paraventricular nucleus; CA, cell groups of Ammon's horn; DG, dentate gyrus.
[a] $P<0.05$ as compared to the sham group.

Table 3
Effect of i.c.v. administration of RU28318 (10 ng) on brain angiotensinogen and mineralocorticoid receptor transcription in 3-week DOCA–salt rats

	Vehicle	RU28318
Angiotensinogen		
AV3V	170 ± 10	155 ± 7
PVN	86 ± 12	83 ± 10
ANP		
AV3V	40 ± 5	34 ± 7
Mineralocorticoid receptor		
AV3V	22 ± 4	19 ± 3
Hippocampus:		
CA1	58 ± 7	83 ± 6[a]
CA3	29 ± 2	40 ± 3[b]
DG	67 ± 7	99 ± 9[a]

Data are means ± S.E.M. of 7 rats in each group. Values represent arbitrary units. Abbreviations: ANP, atrial natriuretic peptide; AV3V, anteroventral third ventricle; PVN, paraventricular nucleus; CA, cell groups of Ammon's horn; DG, dentate gyrus.
[a] $P<0.05$ as compared to the vehicle group.
[b] $P<0.01$ as compared to the vehicle group.

4. Discussion

The present results reveal a role of brain mineralocorticoid receptor in mediating the action of mineralocorticoids on renal function of DOCA–salt hypertensive rats. As expected, acute blockade of brain mineralocorticoid receptor by 10 ng of the antagonist RU28318 increased urinary excretion of water and electrolytes during the first 8 h after i.c.v. administration of the antagonist in DOCA–salt rats, as previously was observed in normotensive rats (Rahmouni et al., 1999a). In both cases, it was accompanied by a fall in systolic blood pressure, which is in accordance with our previous observations (Rahmouni et al., 1999a; Van den Berg et al., 1990, 1994a,b). The effects of the i.c.v. administration of RU28318 suggest a centrally mediated mechanism in renal and blood pressure control, since the same or a tenfold higher dose administered s.c. were ineffective (Rahmouni et al., 1999a; Van den Berg et al., 1990, 1994b).

Although the decrease in systolic blood pressure in DOCA–salt hypertensive rats induced by RU28318 seems to be of the same magnitude, the effect has a faster onset and a longer duration than described previously for normotensive rats (Van den Berg et al., 1994b; Rahmouni et al.,

1999a). However, a definite conclusion on the enhanced sensitivity of DOCA–salt rats for mineralocorticoid receptor blockade must await further experimentation to compare the effects of i.c.v. administration of RU28318 between DOCA–salt rats and normotensive rats in the same protocol. The pathophysiological role of brain mineralocorticoid receptors in DOCA–salt hypertension has been previously documented. Janiak et al. (1990) reported that long-term i.c.v. administration of 3 μg/h of RU28318, as compared to systemic infusion of the same dose, reduced the degree of hypertension induced by a DOCA–salt regimen. Several conditions in this chronic experiment differed from our acute studies. For instance, the blood pressure was not measured during the first day, which was the time of the decrease in blood pressure in our experiment, but only after 1 day to 1 week of commencing the i.c.v. infusion of RU28318. Moreover, the higher dose of RU28318 in the aforementioned study (3 μg/h versus 10 ng) was another major difference. Nevertheless, in spite of these differences in experimental design, the effects observed after acute and chronic i.c.v. infusion of RU28318 both suggest involvement of brain mineralocorticoid receptors in the regulation of blood pressure in DOCA–salt rats.

The induction of mineralocorticoid receptor mRNA expression in hippocampus after mineralocorticoid receptor antagonist treatment clearly demonstrates the responsiveness of this particular mineralocorticoid receptor system to the steroid in the DOCA–salt model. Thus, despite the severe challenge of homeostasis induced by DOCA–salt treatment, as evidenced by the hypertension and altered renal function, brain mineralocorticoid receptors still contribute to the control of blood pressure and renal function. This finding supports the concept that brain mineralocorticoid receptors are functional also during development of mineralocorticoid hypertension. How these brain mineralocorticoid receptors operate is not precisely known, but the current evidence suggests a brain mechanism underlying neural control of kidney function and blood pressure. This evidence is based on our recent experiments demonstrating that renal denervation abolished the diuresis and the increase in urinary excretion of electrolytes induced by brain mineralocorticoid receptor blockade (Rahmouni et al., 1999a). Moreover, the renal denervation also shortened the duration of the decrease in systolic blood pressure (Rahmouni et al., 1999a).

Alteration of renal function, such as reflected in water and electrolyte retention, is known to contribute to the development of DOCA–salt hypertension. Renal denervation attenuated the magnitude of hypertension in DOCA–salt rats and was associated with an increase in urinary Na^+ excretion (Katholi et al., 1980; Koepke et al., 1986; DiBona and Kopp, 1997). Evidence has been provided that in normotensive rats as well as in DOCA–salt rats renal nerves modulate water and Na^+ reabsorption via tubular α_1-adrenoreceptors (Akpogomeh and Johns, 1991; DiBona and Kopp, 1997). The effects of central mineralocorticoid receptor blockade by 10-ng RU28318 on renal function (enhanced diuresis and excretion of electrolytes) observed in DOCA–salt rats may be explained by selective withdrawal of sympathetic tone at the tubular level similarly as was postulated to be a major mechanism in normotensive rats (Rahmouni et al., 1999a).

The overactivity of the sympathetic nervous system in DOCA–salt hypertensive rats (Reid et al., 1975; Berecek et al., 1980a; Nakata et al., 1989) would predict a marked alteration in the renal function compared to normotensive rats. In contrast, the increase in urinary excretion of water and electrolytes induced by i.c.v. administration of 10 ng of RU28318 in DOCA–salt rats were comparable to those obtained in normotensive rats (Rahmouni et al., 1999a). It could be that in our model of DOCA–salt hypertension, without uninephrectomy, the increase in renal sympathetic nerve activity is less important compared to the DOCA–salt rats with uninephrectomy. Alternatively, it could be that the suppression of the renin–angiotensin system limits the renal responses of DOCA–salt rats to i.c.v. RU28318. In support of this idea is the finding that the neurally mediated tubular responses require the presence of a minimal amount of circulating angiotensin II (Handa and Johns, 1985). Furthermore, Johns (1987) has shown that the absence of neurally mediated tubular responses in DOCA–salt rats can be restored by infusion of angiotensin II. In 5-week DOCA–salt rats, the urinary excretion of electrolytes was less affected by brain mineralocorticoid receptor blockade as compared to 3-week DOCA–salt rats, probably because at this stage alterations in circulating hormonal factors induced by DOCA–salt treatment limit the increase in urinary excretion of electrolytes.

The renal effects of brain mineralocorticoid receptor blockade may contribute to the decrease in systolic blood pressure observed in DOCA–salt rats as well as in normotensive rats. In support of this assumption. First, the concomitant presence of salidiuresis and hypotension after i.c.v. administration of RU28318 in DOCA–salt rats and normotensive rats (Rahmouni et al., 1999a). Second, abolition of salidiuresis by renal denervation shortened the duration of hypotension induced by i.c.v. injection of mineralocorticoid receptor antagonist in normotensive rats (Rahmouni et al., 1999a). Third, in Wistar female rats, in which the renal effects of brain mineralocorticoid receptor blockade were less pronounced, the decrease in systolic blood pressure was also less marked (Rahmouni et al., 1999b). The decrease in the extracellular fluid volume could be the mechanism linking the fall in blood pressure to the renal effects of i.c.v. administration of RU28318.

A number of neurotransmitter/modulator systems in the brain, such as the renin–angiotensin system and ANP, may be influenced by a systemic DOCA–salt regime. In the present study, we did not detect any change in the angiotensinogen and ANP expression in the AV3V or in the paraventricular nucleus for the angiotensinogen gene expression. Increased concentration of angiotensinogen in

the cerebrospinal fluid (Ruiz et al., 1983) and ANP content, in particular brain nuclei, including the organum vasculosum of the lamina terminalis and the subfornical organ, but not in the preoptic region (Geiger et al., 1989), has been reported in DOCA–salt rats. These effects of DOCA–salt treatment on the angiotensinogen in the cerebrospinal fluid and ANP in brain nuclei were observed 4 weeks after the start of the treatment in uninephrectomized rats. In contrast, our study was performed in intact rats after 3 weeks of DOCA–salt regime. Furthermore, a change in mRNA levels of angiotensinogen and ANP in brain regions other than the paraventricular nucleus and AV3V of DOCA–salt rats cannot be excluded. It is conceivable, therefore, that the paradoxical increase in angiotensinogen in the cerebrospinal fluid in absence of change in gene expression could reflect an altered angiotensinogen clearance or a contamination with plasma.

The absence of change in brain angiotensinogen and ANP mRNA level 3 h after i.c.v. administration of the mineralocorticoid receptor antagonist might indicate that brain mineralocorticoid receptors do not exert direct control over angiotensinogen and ANP genes expression, but suggests that other receptor systems are involved. For example, Riftina et al. (1995) reported that the decrease in angiotensinogen mRNA in forebrain areas observed after adrenalectomy was not reversed by systemic administration of aldosterone, while the selective glucocorticoid receptor agonist RU28362 was effective. Alternatively, it could be that angiotensinogen and ANP genes expression were affected by the RU28318 at other time points than used in this study.

Reports regarding effects of DOCA–salt and of mineralocorticoid receptor antagonists on brain mineralocorticoid receptor are scarce. Vallee et al. (1995) have shown that treatment with DOCA produced increased mineralocorticoid receptor binding capacity in the hypothalamus without changes in the amygdala while mineralocorticoid receptor binding was decreased in the hippocampus. In the hypothalamus, an increase in the number of mineralocorticoid receptor binding sites was associated with higher affinity values. In the present study, we used in situ hybridization and observed that mineralocorticoid receptor mRNA level in the AV3V and hippocampus were not affected by 3-week DOCA–salt treatment. Central mineralocorticoid receptor blockade with RU28318 enhanced mineralocorticoid receptor expression in the hippocampus by about 40%. Mineralocorticoid receptor blockade with spironolactone was also reported to induce 30–40% increase in mineralocorticoid receptor mRNA levels in the hippocampal subregions CA1, CA3 and dentate gyrus (McCullers and Herman, 2001). The absence of effect of RU28318 on mineralocorticoid receptor expression in the AV3V region may perhaps suggest that this is not the primary site of action of the mineralocorticoid receptor antagonist. Such a possibility clearly needs further study. Absence of change in mineralocorticoid receptor mRNA in the AV3V area does not exclude a change in the number of mineralocorticoid receptor binding sites in this area, since transcription and translation efficiency may be differently affected.

The difference in values for the mineralocorticoid receptor mRNA in the hippocampus between DOCA–salt rats, at baseline (Table 2) and after i.c.v. vehicle (Table 3), is intriguing. The decrease in the mineralocorticoid receptor mRNA level in the i.c.v. treated DOCA–salt rats could be due to the heat and restraint stress procedure, to which these rats were subjected during the 2-week training period, in order to accustom to the conditions of the systolic blood pressure measurement and the i.c.v. injection procedure 3 h prior to decapitation of the animals. Such a stress-induced decrease in mineralocorticoid receptor gene expression in the hippocampus has been reported (Herman and Watson, 1995; De Kloet et al., 1999; Fujikawa et al., 2000). The high circulating level of corticosterone induced by the stress may be responsible for this decrease in mineralocorticoid receptor mRNA level in the hippocampus since corticosterone treatment decreased the mineralocorticoid receptor gene expression in rat hippocampus (Karten et al., 1999). Whether this decrease in hippocampal mineralocorticoid receptor affects the mechanism involved in cardiovascular and volume control operating in the AV3V and brain stem regions requires further investigation.

In conclusion, our data reveal a coordinating role of brain mineralocorticoid receptor in cardiovascular regulation and renal function control in rats with hypertension induced by DOCA–salt treatment. Acute blockade of brain mineralocorticoid receptors with a selective antagonist induced a decrease in blood pressure together with an increase in diuresis and renal excretion of electrolytes, and this response was associated with increased expression of mineralocorticoid receptor gene in the hippocampus.

Acknowledgements

Kamal Rahmouni was supported by Searle, Monsanto France. Support by The Netherlands Heart Foundation to E. Ronald De Kloet and Rosana M. Sibug (project no 94-122) is gratefully acknowledged. RU28318 was a generous gift of Roussel UCLAF (Romainville, France). We are indebted to Drs. A. Berthelot and A. Gairard, Strasbourg, France, for preparing the DOCA pellets.

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