Role of central mineralocorticoid binding sites in development of hypertension

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JANIAK, PHILIP C., STEPHEN J. LEWIS, AND MICHAEL J. BRODY. Role of central mineralocorticoid binding sites in development of hypertension. Am. J. Physiol. 259 (Regulatory Integrative Comp. Physiol. 28): R1025–R1034, 1990.—The possibility that central mineralocorticoid binding sites are involved in the development of mineralocorticoid hypertension was examined using chronic blockade of these sites with a specific mineralocorticoid receptor antagonist RU 28318 administered by intracerebroventricular (icv) infusion. The antagonist significantly attenuated the development of deoxycorticosterone acetate (DOCA)-salt hypertension, but the development of one-kidney, one-clip renal hypertension was not affected. This antihypertensive action was attributable to a central action, since intraperitoneal infusion of the same dose of mineralocorticoid antagonist did not alter the peak development of DOCA-salt hypertension. The icv infusion of RU 28318 did not change either the increase of fluid intake induced by DOCA-salt treatment or the pressor reactivity to centrally or peripherally injected arginine vasopressin and angiotensin II and peripherally administered phenylephrine. The antihypertensive action of icv infusion of the mineralocorticoid antagonist was associated with a reduction of neurogenic vasomotor tone and a restoration of impaired arterial baroreflexes. We conclude that functional integrity of central mineralocorticoid binding sites is required for the full development of DOCA-salt hypertension.

BRODY, R. Role of central mineralocorticoid binding sites in development of hypertension. Am. J. Physiol. 259 (Regulatory Integrative Comp. Physiol. 28): R1025-R1034, 1990.—The possibility that central mineralocorticoid binding sites are involved in the pathogenesis of hypertension in the one-kidney, one-clip model, was also investigated. To accomplish these goals, the specific competitive mineralocorticoid receptor antagonist RU 28318 was infused chronically via the lateral cerebral ventricle of both normotensive and hypertensive rats.

IN THE PAST DECADE, the contribution of the central nervous system to the development of mineralocorticoid-induced hypertension, e.g., deoxycorticosterone acetate-salt (DOCA-salt) model, has been examined extensively. The anteroventral region of the third ventricle (AV3V) (2), the septal area of the limbic system (26), the area postrema (10), and the paraventricular nucleus of the hypothalamus (22) have been shown to play an important role in the development of this form of hypertension, since lesions of these regions abolished or reduced the increase in arterial pressure produced by DOCA-salt treatment. The contribution of the brain to the development of this hypertension appears to involve arginine vasopressin (AVP) and the sympathetic nervous system (2, 5, 6).

Several autoradiographic and immunohistochemical studies have revealed the presence of specific binding sites for aldosterone in several regions of the brain, including hypothalamus, hippocampus, amygdala, and septum (1, 3, 8, 19, 34); many of these regions are involved in central control of the circulation. Direct evidence for these binding sites in cardiovascular control has been reported recently. Chronic intracerebroventricular (icv) infusion of aldosterone (11) or DOCA (27) produced hypertension. Direct administration of aldosterone into the subcommissural organ elicited natriuresis and an increase in plasma epinephrine (9). More recently, our laboratory demonstrated that a 2-day icv infusion of aldosterone selectively attenuated the pressor response evoked by icv injection of arginine vasopressin (AVP) (14) at a dose that was ineffective when administered systemically.

The capacity of lipophilic mineralocorticoids to enter the brain from the periphery and the direct evidence for involvement of central mineralocorticoid binding sites in control of the circulation prompted us to examine the role of these binding sites in the development of DOCA-salt hypertension. As a control, the potential contribution of these central binding sites in the pathogenesis of another non-renin-dependent form of hypertension, the one-kidney, one-clip model, was also investigated. To accomplish these goals, the specific competitive mineralocorticoid receptor antagonist RU 28318 was infused chronically via the lateral cerebral ventricle of both normotensive and hypertensive rats.

Our findings indicate that chronic selective blockade of central mineralocorticoid binding sites significantly reduced DOCA-salt hypertension by mechanisms involving a restoration of the arterial baroreflex and a decrease in neurogenic vasomotor tone. In contrast, the development of hypertension in the one-kidney, one-clip model was unaltered by the chronic central blockade of mineralocorticoid binding sites. A preliminary report of these results has been published (FASEB J. 2: A1280, 1988).

MATERIALS AND METHODS

DOCA-salt model. For this study, male Sprague-Dawley rats weighing 200–250 g were used. Each day for 1 wk the systolic blood pressure of the rat was measured by tail-cuff method, using a photoelectric pulse transducer (ITTC, Woodland Hills, CA) coupled to an automatic cuff inflator (Narco, Houston, TX). During this procedure, the animals were restrained in plastic cylinders and were warmed to 26°C. They were allowed to stay in these conditions for ≤30 min. The last three measurements were averaged and taken as the control value before the commencement of the treatment. The diet was composed of regular rat chow and for fluid...
intake a solution of 1% NaCl and 0.2% KCl. KCl was added to the saline solution to reduce the depletion in potassium induced by DOCA treatment. This solution was given to the rats before the implantation of DOCA. Rats not treated with DOCA (sham vehicle) were allowed to drink tap water. The fluid intake and the systolic blood pressure were evaluated every 2 days and were averaged over a period of 4 days.

One day before the surgery, injectors (25 gauge, 10.5-mm length) for osmotic minipumps were sterilized using ethylene oxide. On the day of surgery, the rats were anesthetized with a mixture of ketamine (140 mg/kg) - acepromazine (14 mg/kg) injected intraperitonely. The antibiotic, oxytetracycline, was administered intraperitoneally after surgery. One kidney was removed, with care taken to maintain the integrity of the adjacent adrenal gland. Silicone rubber pellets impregnated with DOCA (200 mg/kg) were implanted subcutaneously in two different sites. In sham operated animals with no DOCA-salt treatment, the kidney was exposed for 3 min, and nonimpregnated silicone rubber pellets were implanted. The animals were then placed in a stereotaxic apparatus (Kopf) for implantation of a guide cannula (20 gauge, 10-mm length) in the left lateral cerebral ventricle. The minipumps (Alzet model 2ML4) for icv infusion of a competitive mineralocorticoid antagonist RU 28318 [7β-hydroxy-3-oxo-7α-propyl[11α]-pregn-4-ene-21 potassium carboxylate] (a generous gift provided by Dr. M. Worcel of Roussel-UCLAF, Romainville, France) or vehicle (saline) were prepared 1 day before implantation as recommended by manufacturer and the filling of the minipump was performed through a micropore to enable the use of a sterile solution. The rate of infusion of either vehicle or antagonist was 2.5 μl/h and lasted for 4 wk. Once the minipump was implanted sc, the injector was secured in the guide cannula by covering it with dental cement. For chronic intraperitoneal infusion only the reservoir of the pump and the fluid modulator were filled with sterile solution of RU 28318 before they were connected to each other. The pump was then implanted into the peritoneal cavity. All surgery was performed under aseptic conditions.

The animals were divided into five groups: 1) DOCA-salt-treated rats receiving icv infusion of saline (DOCA/saline icv); 2) DOCA-salt-treated rats receiving icv infusion of 3 μg/h RU 28318 (DOCA/RU 28318 icv); 3) DOCA-salt-treated rats receiving intraperitoneal infusion of 3 μg/h of RU 28318 (DOCA/RU 28318 ip); 4) sham-operated and -treated rats receiving icv infusion of saline (Veh/saline icv); and 5) sham-operated and -treated rats receiving icv infusion of 3 μg/h RU 28318 (Veh/RU 28318 icv).

Systolic blood pressure and fluid intake were evaluated every 2 days for 23 days, and the weight of rats was checked. On day 24 of the experiment the rats were anesthetized by intraperitoneal injection of ketamine-acepromazine as described above. The femoral artery and vein were catheterized for blood pressure measurement and drug administration respectively. The catheters were made of PE-10 connected to PE-50 (Clays-Adams). Then by use of the stereotaxic apparatus, a guide cannula (20 gauge, 15-mm length) was placed into the right lateral cerebral ventricle.

After a recovery period of 2 days, basal heart rate (HR) and mean arterial pressure (MAP) were measured in the five groups. Pressor responses to icv injection of AVP (50 ng) and angiotensin II (ANG II, 150 ng) were evaluated as well as the effects of intravenous administration of AVP (100 ng/kg) and phenylephrine hydrochloride (PE, 5 μg/kg). Intravenous and icv administration of AVP, ANG II, and PE was made in bolus doses. The vasopressinergic and sympathetic contributions to the maintenance of arterial pressure were investigated by successive intravenous injections of an AVP V₁-receptor antagonist [d(CH₂)₅Tyr(Me)]ATP (AVP-X, 10 μg/kg, Peninsula Laboratories) and a ganglionic blocker trimethaphan camysylate (10 mg/kg). As controls, cardiovascular effects of saline injected by the intravenous (0.3 ml) and icv (3 μl) routes were measured. One day after the direct recording of MAP, the rats were decapitated and the central sites of injection and infusion were checked postmortem by injection of 1 μl of Evans blue dye through the cannula.

One-kidney, one-clip model. Male Sprague Dawley rats (250–300 g) were anesthetized with intraperitoneal injection of ketamine-acepromazine. The femoral artery and vein were catheterized as described above. The venous and arterial catheters were filled with heparinized saline (50 ml/ml) and tunneled subcutaneously to exit at the back of the neck where they were exteriorized and sutured to the skin. The catheters were flushed every 2 days with heparinized saline solution (50 mg/ml). After surgery, each rat was placed in an individual cage and had free access to chow (Teklad) and water. After 2 days of recovery, basal MAP and HR were recorded and the cardiovascular reactivity to intravenously injected PE (1, 2, 4, and 5 μg/kg) and AVP (5, 10, and 25 ng/kg) was evaluated. As with the DOCA-salt model, the vasopressinergic and sympathetic components of arterial pressure were measured by successive intravenous administration of the AVP V₁-receptor antagonist and trimethaphan. At the end of the experiment, the animals were anesthetized by intraperitoneal administration of ketamine-acepromazine and a unilateral nephrectomy was performed. The renal artery of the contralateral kidney was dissected free and a silver clip (0.3 ID) was adjusted around it. The rat was then placed in a stereotaxic apparatus (Kopf) for implantation of a guide cannula (20 gauge, 10.5 mm length) in the lateral cerebral ventricle. An osmotic minipump (Alzet model 2002) was then introduced sc and connected to the guide cannula in the lateral ventricle for icv infusion of the mineralocorticoid receptor antagonist RU 28318, or saline. The rate of infusion was 0.5 μl/h, and the animals were treated for a period of 12 days.

Basal MAP and HR were measured after 4, 8, and 12 days of central infusion of RU 28318 or saline in conscious freely moving rats. On day 12, the cardiovascular reactivity to intravenous injection of PE and AVP were again examined, as well as the vasopressinergic and sympathetic components of arterial pressure determined by intravenous administration of AVP-X and trimethaphan. At the end of the experiment the animals were killed and the central site of infusion was verified postmortem by injection of dye. The position of the clip around the renal artery was also checked.
CENTRAL MINERALOCORTICOIDS AND HYPERTENSION

Systolic Blood Pressure (mm Hg)

Days

DOCA sc/Saline icv (n=7)
DOCA sc/RU 28318 icv (n=13)
DOCA sc/RU 28318 ip (n=11)
VEH sc/Saline icv (n=7)
VEH sc/RU 28318 icv (n=5)

Statistical analysis was carried out by 1-way ANOVA; post hoc test was a Student's modified t test with Bonferroni adjustment. See Fig. 1 for abbreviations.

The recording of the cardiovascular parameters was carried out using a Century Technology pressure transducer (model CP-01) connected to a Beckman R611 Dynograph recorder. HR was measured by a Beckman 9857B cardiotachometer providing the derivative function of the arterial pulse pressure.

Statistical analysis. The original data sets and the derived data sets (e.g., maximum change from baseline) were examined to ensure that they were normally distributed and that each of the subsets (e.g., group or each time within a group) were of approximately equal variance (32). Once this had been established a repeated measures analysis of variance (ANOVA) was applied. The error mean square term from the ANOVA was incorporated into the Student’s modified t test with the Bonferroni adjustment for multiple comparisons between means (30).

RESULTS

DOCA-salt hypertension model. As shown in Fig. 1, chronic DOCA-salt treatment produced an increase in systolic blood pressure. The magnitude of this hypertension was significantly reduced by icv infusion of the mineralocorticoid antagonist RU 28318. This action of RU 28318 was statistically significant from day 7 and was maintained over the 23 days of the experiment. Intraperitoneal infusion of RU 28318 did not significantly alter the peak development of DOCA-salt hypertension; however, the increase in arterial pressure appeared to be delayed. Central infusion of the aldosterone antagonist did not change the systolic blood pressure in vehicle-treated animals.

These results were confirmed on day 26 by direct measurement of MAP and HR (Fig. 2). DOCA-salt treatment raised arterial pressure to the hypertensive level of 185 ± 8 mmHg. Icv infusion of RU 28318 prevented pressure from reaching these levels, with rats averaging 149 ± 3 mmHg. In contrast, the same dose of RU 28318 infused intraperitoneally did not significantly alter the final level of pressure induced by DOCA-salt treatment (175 ± 6 mmHg). In normotensive animals, 26-day icv infusion of RU 28318 did not modify the MAP. HR was similar within the five groups.

The effect of central infusion of RU 28318 on the development of DOCA-salt hypertension was confirmed in another study using only direct measurement of MAP and HR (Fig. 3). After 13 days of treatment the DOCA-salt-treated rats exhibited hypertension (169 ± 7 mmHg), which was significantly reduced by the icv infusion of RU 28318 (126 ± 3 mmHg), although these rats showed
a small but significant increase in MAP.

The effects on fluid intake are summarized in Fig. 4. Chronic subcutaneous administration of DOCA produced a marked increase in fluid intake; however, neither icv nor intraperitoneal infusion of RU 28318 changed the increase in fluid intake induced by treatment with DOCA. Chronic icv infusion of mineralocorticoid agonist also did not alter the water intake in the vehicle-treated animals.

The cardiovascular responses produced by icv administration of AVP and ANG II were investigated after 26 days of treatment in each group. The data are summarized in Fig. 5. Central injection of AVP in DOCA-salt treated rats produced an increase in MAP associated with tachycardia. This response was not significantly changed by chronic icv infusion of the mineralocorticoid agonist. The pressor response to central AVP was greater in the DOCA/RU 28318 ip group, but this enhanced pressor action is probably not attributable to a greater responsiveness to the centrally administered peptide, since “barrel rotation” also occurred in many animals of this group. The occurrence of this motor disturbance (well known to be associated with central administration of AVP) was also more frequent in the Veh/saline icv and Veh/RU 28318 icv groups.

Intracerebroventricular injection of ANG II evoked a pressor response generally associated with bradycardia. The cardiovascular reactivity to central administration of this peptide was similar between the five groups. Although the pressor reactivity to intravenous injection of PE was not different between the five groups (Fig. 6), alterations in reflex-mediated bradycardia were found. In the DOCA/saline icv group, the bradycardia was significantly attenuated compared with that found in the normotensive Veh/saline icv group. In contrast, the reflex bradycardia induced by intravenous injection of PE was significantly greater in the DOCA/RU 28318 icv and DOCA/RU 28318 ip groups than in the DOCA/saline icv group. Furthermore, the bradycardia in the two DOCA/RU 28318-treated groups was similar to the one in the Veh/saline icv group. In the sham-operated normotensive rats, icv infusion of RU 28318 did not change reflex bradycardia.

The pressor response to intravenous administration of AVP was not significantly modified by central infusion of RU 28318 in DOCA-salt-treated rats but was increased in the DOCA/RU 28318 ip group. Similar to the results found with PE, the bradycardia evoked by intravenous
injection of AVP, although not different statistically, was greater in the DOCA groups receiving RU 28318 by either the icv or intraperitoneal routes.

Figure 7 summarizes the effects of the AVP V₁ receptor antagonist and trimethaphan on HR and MAP. The antagonist of AVP did not significantly change the IHR or MAP in any groups. Trimethaphan produced a significantly greater decrease in MAP and HR in the DOCA/saline icv group than in the Veh/saline icv group. The fall in MAP produced by the ganglionic blocker was significantly smaller in both DOCA/RU 28318 icv and ip groups than in the DOCA/saline icv group, when the data were analyzed by ANOVA. In the DOCA/saline icv group, trimethaphan produced a significant bradycardia compared with the DOCA/saline icv group in which HR was not altered by the ganglionic blockade. The nonvasopressin humoral contribution to the maintenance of arterial pressure, estimated by the level of MAP remaining after intravenous injection of AVP-X and trimethaphan, was not significantly different among the five groups.

**One-kidney, one-clip hypertensive model.** The one-kidney, one-clip procedure produced a rapid increase in MAP with no significant change in HR (Fig. 8). The development of this hypertension was not significantly altered by chronic icv infusion of the mineralocorticoid receptor antagonist RU 28318.

The effects of central infusion of the mineralocorticoid antagonist on peripheral cardiovascular reactivity, the baroreflex, and the vasopressinergic and neurogenic components of arterial pressure are summarized in Figs. 9–11. The most consistent and expected findings in these studies were that one-kidney, one-clip hypertension impaired the baroreflex and enhanced the neurogenic component of arterial pressure. The effects on the baroreflex were confirmed when the data were expressed as AHR/AMAP vs. doses of PE or AVP. Twelve-day central infusion of RU 28318 produced a further impairment of the baroreflex (Figs. 9 and 10) but did not affect the...
enhancement of neurogenic tone associated with the hypertension (Fig. 11).

**DISCUSSION**

Evidence for a major participation of the central nervous system in the pathogenesis of DOCA-salt hypertension, for the existence of central mineralocorticoid binding sites, and for cardiovascular effects of mineralocorticoid administered selectively to the brain prompted us to hypothesize that the development of mineralocorticoid-induced hypertension might depend on direct central actions of this class of steroid. Specifically, we suggested that the traditional peripheral administration of DOCA might produce hypertension in toto or in part by 1) penetration of DOCA into the brain, 2) interaction with central binding sites in any of several regions associated with cardiovascular control, and finally 3) central activation by DOCA of the enhanced sympathetic nervous system activity known to be associated with this model of hypertension. The hypothesis was tested with the use of a selective competitive antagonist of mineralocorticoid binding sites (RU 28318) administered to the central nervous system in a dose too low to exert effects when given peripherally.

The major finding of this study was that chronic blockade of central mineralocorticoid binding sites significantly attenuated the development of DOCA-salt hypertension. The magnitude of the hypertension was lower and the latency for the rise in arterial pressure was markedly longer. These data indicate that DOCA-salt hypertension is in fact dependent in part on a central action of the steroid. The residual increase in arterial pressure found in the DOCA/RU 28318 icv-treated group may well be attributable to peripheral actions of mineralocorticoids.

The inhibitory effect of centrally infused RU 28318 appears not to be due to a leak from the brain to the periphery, since chronic intraperitoneal infusion of the same dose of mineralocorticoid antagonist did not reduce the peak level of the hypertension. These findings are consistent with the results of Moura and Worecl (21), who found that a large dose of RU 28318 (at least 5 mg kg\(^{-1}\) day\(^{-1}\) po) was needed to abolish the development of DOCA-salt hypertension. It should be noted, however, that the appearance of the hypertension was delayed by \(-1\) wk. The mechanism by which intraperitoneal infusion of RU 28318 delayed the development of DOCA-salt hypertension is unclear. It is interesting to note that, after 26 days of intraperitoneal infusion, RU 28318 re-established normal baroreflex activity despite persistent hypertension. We can postulate that early protection against impairment of the baroreflex might have been sufficient to buffer against early development of miner-

**FIG. 7.** Depressor and heart rate responses to intravenous administration of vasopressin V\(_1\)-receptor antagonist (AVP-X, \(\Delta\)) and trimethaphan after AVP-X injection (B) in rats receiving 26 days of treatment. AMAP and AHR, change in mean arterial pressure and heart rate, respectively. Statistical analysis was performed by a repeated-measures ANOVA; post hoc test was Student's modified t test with Bonferroni adjustment for multiple comparison between means. SE from ANOVA is reported on 1st bars of graphs. * P < 0.05. See Fig. 1 for abbreviations.

**FIG. 8.** Effect of icv infusion of aldosterone antagonist RU 28318 or saline on mean arterial pressure (MAP) and heart rate (HR) in 1-kidney, 1-clip operated rats. Statistical analysis was performed by a 2-way ANOVA with repeated measures; post hoc test was a Student's modified t test with Bonferroni adjustment for multiple comparisons between means. SE from ANOVA is shown at day 8. n = 8 animals in each group.
alocorticoid hypertension. After day 11, this putative protective effect might be overwhelmed by the primary mechanisms of DOCA-salt hypertension. Despite the central antihypertensive action of RU 28318 in DOCA-salt hypertensive rats, chronic blockade of central mineralocorticoid binding sites did not reduce arterial pressure in normotensive animals.

Because the central infusion of the antagonist only attenuated DOCA-salt hypertension, there was either incomplete block of central mechanisms involving AVP and the sympathetic nervous system (2, 5, 6) or several potential peripheral mechanisms were left relatively unaffected. Excess of mineralocorticoid enhances Na+ reabsorption in distal tubules and increased plasma Na+ concentration has been found in DOCA-salt hypertensive animals (29), perhaps linked to early volume expansion and increased fluid intake. Another peripheral target for mineralocorticoids is vascular smooth muscle, where AVP is thought to have an additive or permissive interaction with aldosterone. The fact that peripheral infusion of RU 28318 failed to alter the magnitude of the hypertension suggests that partial blockade of central mechanisms rather than the contribution of peripheral actions explains the attenuation produced by central infusion.

DOCA-salt treatment progressively increases fluid intake. Although the development of the hypertension was significantly reduced by the chronic infusion of RU 28318, the increased fluid intake remained unchanged. The protective effect of RU 28318 on DOCA-salt hypertension was clearly independent of the increase in saline intake and thus may also be independent of the alteration in fluid-electrolyte homeostasis such as sodium and water retention associated with the early stages of DOCA-salt hypertension. Further studies would be necessary how ever to rule out whether central RU 28318 changes any of these parameters.

Although mineralocorticoids have been reported to increase the number of central ANG II receptors (15, 31), the pressor effects induced by the central administration of this peptide were not enhanced by DOCA-salt treatment. In fact, central inhibition of mineralocorticoid binding sites did not alter the ANG II response in either hypertensive or normotensive animals. The dipsogenic effect induced by icv administration of ANG II was not investigated in this experiment (i.e., no water was present in the cage), since drinking behavior alone can modify the pressor response produced by the peptide.

These data differ from those reported in the literature, but the discrepancies could arise from the different protocols used in the various other studies. Wilson et al. (31) demonstrated that DOCA-salt treatment (8–10 wk) enhanced the pressor and drinking responses elicited by icv administration of ANG II. They used female rats, and it is known that in estrogen-treated rats both the

![Figure 9](http://ajpregu.physiology.org/)

**FIG. 9.** Effects of icv infusion of RU 28318 and saline on pressor and heart rate responses produced by graded doses of phenylephrine (PE) administered intravenously in 1-kidney, 1-clip operated rats before (saline or RU 28318 icv day 0) and after 12 days (saline or RU 28318 icv day 12) of treatment. A MAP and ΔHR, change in mean arterial pressure and heart rate, respectively. Slopes were analyzed by orthogonal partition and compared by a repeated measures ANOVA; post hoc test was a Student’s modified t test with Bonferroni adjustment for multiple comparisons between means. SE from ANOVA is shown at 4.0 μg/kg, n = 8 animals in each group. * P < 0.05.

![Figure 10](http://ajpregu.physiology.org/)

**FIG. 10.** Effects of icv infusion of RU 28318 and saline on pressor and heart rate responses produced by graded doses of AVP administered intravenously in 1-kidney, 1-clip rats before (saline or RU 28318 icv day 0) and after 12 days (saline or RU 28318 icv day 12) of treatment. A MAP and ΔHR, change in mean arterial pressure and heart rate, respectively. Slopes were analyzed by orthogonal partition and compared by repeated measures ANOVA; post hoc test was a Student’s modified t test with Bonferroni adjustment for multiple comparison between means. SE from ANOVA is shown at 25 ng/kg, n = 8 animals in each group. * P < 0.05.
dipsogenic response to ANG II and ANG II receptor binding are reduced. Therefore the presence of gonadal steroids or the sex of the animal could alter the response to centrally injected ANG II. The dose of DOCA given was much smaller than the one we used. The DOCA treatment did not significantly modify basal arterial pressure, whereas our DOCA-salt-treated rats exhibited marked hypertension. Their rats were not uninephrecomized, and no potassium was added to the saline drinking solution to reduce potassium depletion usually induced by the DOCA treatment. Finally, it was not stated whether the rats had free access to water when the pressor response to icv-injected ANG II was monitored, a condition that enhances the centrally evoked pressor response.

King et al. (15) indicated that salt appetite and brain binding of ANG II were increased after 4 days of DOCA treatment (500 µg/day). This short treatment would probably not modify the basal arterial pressure, although the authors did not report any data on this parameter.

Gutkind et al. (12) used a protocol similar to the one we describe. They reported that after 4 wk of DOCA-salt treatment (no potassium was supplied in the drinking solution) the rats exhibited significant hypertension with an increase in central ANG II binding. However, they did not investigate whether the pressor and/or the dipsogenic response to centrally injected ANG II was enhanced.

Another peptide, vasopressin, has been shown to play a critical role in the development of DOCA-salt hypertension (2, 5). Indirect evidence for the role of AVP was obtained in Brattleboro rats, in which this form of hypertension failed to develop due to a genetically based absence of AVP synthesis (5). Plasma AVP has also been indicated to increase in DOCA-salt-treated rats, and an antihypertensive effect can often (5) but not always (24, 25) be produced by antagonists of AVP. Berecek et al. (2) demonstrated that vascular reactivity to AVP and norepinephrine was significantly enhanced in DOCA-salt-treated animals. Recently, we showed that a 2-day icv infusion of aldosterone attenuated the pressor response induced by icv injection of AVP (14). This interaction was mediated through central mineralocorticoid binding sites, since their blockade abolished it. We therefore examined whether the pressor action produced by central injection of AVP was also reduced in DOCA-salt hypertensive animals weeks rather than days after beginning administration of mineralocorticoid.

Our data indicated first that in DOCA/RU 28318 icv group icv injected AVP produced a marked pressor effect associated with tachycardia. Central infusion of mineralocorticoid antagonist in DOCA-salt rats did not modify the pressor action of AVP. When RU 28318 was infused intraperitoneally the increase in arterial pressure produced by icv injection of AVP appeared to be increased. However, it is important to take into account that in this latter group barrel rotation, which is a vestibular motor disturbance evoked by icv administration of AVP (33), was much greater than in the two other DOCA-treated groups. Despite the fact that the increase in arterial pressure occurs before the onset of the barrel rotation, we cannot rule out the possibility that this motor disturbance did not affect the pressor action of centrally injected AVP.

The cardiovascular reactivity to intravenously administered PE was unchanged among the five groups. Reactivity to α-adrenergic agonists has been reported to be enhanced in DOCA-salt hypertension (2); however, it must be taken into consideration that many studies on vascular reactivity are performed in vitro on isolated vessels. An underlying increase in vascular reactivity to a certain pressor agent might not be revealed in conscious unrestrained animals as an enhanced increase in arterial pressure due, for example, to the buffering action of the baroreflexes. We believe that central blockade of mineralocorticoid binding sites reduced the development of DOCA-salt hypertension by mechanisms other than decreasing the cardiovascular reactivity to systemically administered α adrenergic receptor agonists.

Baroreflex activity, estimated by the reflex bradycardia produced by intravenous injection of PE, was abolished in the DOCA/saline icv group. These data confirm the observations made originally by Matsuguchi and Schmid (18). Chronic icv infusion of RU 28318 restored the baroreflex activity in DOCA-salt-treated rats. This reversal of the impaired baroreflex could be one of the mechanisms by which central blockade of mineralocorticoid binding sites attenuates the development of DOCA-salt hypertension. It is interesting to note, however, that chronic intraperitoneal infusion of the mineralocorticoid antagonist also reestablished normal baroreflex activity in the rats with DOCA-salt treatment but...
without affecting the development of the hypertension. The effect on the baroreflex appeared to be specific for the hypertensive state, since in normotensive rats iv infusion of RU 28318 did not alter baroreflex activity.

The cardiovascular reactivity to systemic AVP differed from that of PE. The pressor effect of intravenous administration of AVP was not different between the DOCA/saline icv and DOCA/RU 28318 icv groups but was potentiated in the DOCA/RU 28318 ip group. Lariviere et al. (17) reported in DOCA-salt hypertensive animals a decrease in binding capacity for AVP in the mesenteric vasculature with no change in affinity. However, the vascular reactivity to AVP was increased, presumably due to enhanced responsiveness of postreceptor mechanisms. This study was performed on isolated mesenteric arteries, and, as we suggested above, cannot necessarily be extrapolated to conscious intact animals.

Despite the impairment of the baroreflex, intravenous injection of AVP still produced a significant bradycardia in DOCA-salt hypertensive rats. This effect could be explained by the fact that AVP produces a bradycardia by two mechanisms: an activation of the arterial baroreceptor reflex and a central action involving the area postrema and forebrain structures such as the median preoptic nucleus (23). AVP also causes a greater decrease in HR for a given increase in MAP than other vasoconstrictor agents such as α-adrenergic agonists or ANG II (20). As with PE, the bradycardia induced by intravenous injection of AVP seemed to be potentiated by the infusion of mineralocorticoid antagonist given either icv or intraperitoneally in DOCA-salt-treated rats.

Bolus intravenous injection of an AVP V1-receptor antagonist has been reported to slightly lower arterial pressure and increase heart rate in DOCA-salt hypertensive rats. In our experiments, peripheral blockade of AVP V1 receptors decreased the MAP in some animals; however, most of the DOCA-treated animals did not respond to intravenous injection of the AVP receptor antagonist, and the overall difference was statistically significant. Furthermore, the cardiovascular effect of the AVP antagonist was not affected by the central infusion of mineralocorticoid antagonist, suggesting that the prevention of development of DOCA-salt hypertension cannot be attributed to a change in vasopressinergic component of the arterial pressure. Despite the evidence of elevated plasma AVP level in DOCA-salt hypertensive animals, the antihypertensive effect of AVP antagonists has been the object of disagreements in the literature (24). According to Rascher et al. (25), the failure to lower MAP in DOCA-salt hypertension is due to the fact that the fall in vascular resistance mediated by the blockade of AVP V1 receptors is compensated by an increase in cardiac output leading to no change in MAP.

Evidence for increased sympathetic outflow has been reported in DOCA-salt hypertension (6, 7). In our study intravenous injection of trimethaphan, a ganglionic blocker, was used to evaluate the neurogenic component of arterial pressure. As expected the neurogenic contribution to the maintenance of arterial pressure was increased by the DOCA-salt treatment. Chronic central blockade of mineralocorticoid binding sites slightly but significantly reduced the fall in MAP produced by intravenous injection of trimethaphan. The reduction of the neurogenic component cannot be fully responsible for the antihypertensive action mediated by chronic iv infusion of mineralocorticoid antagonist in DOCA-salt treated rats, because it was only slightly attenuated by ganglionic blockade and because intraperitoneonal administration of RU 28318 also reduced neurogenic tone, but without altering the development of the hypertension. The nonvasopressin humorally derived components of arterial pressure, estimated from the level of MAP remaining after intravenous injection of AVP-X and trimethaphan, was not different between the DOCA-salt treated groups. These data indicate that iv infusion of the mineralocorticoid antagonist did not attenuate the development of mineralocorticoid hypertension by altering humoral contributions to the maintenance of arterial pressure.

Together these data suggest that the protective effect against the development of DOCA-salt hypertension produced by chronic blockade of central mineralocorticoid binding sites potentially involves at least two mechanisms: a reduction of the neurogenic tone and a restoration of the impaired baroreflex activity. Interestingly, similarities can be found between the central renin-angiotensin system and central mineralocorticoid binding sites for their role in the pathogenesis of DOCA-salt hypertension. The specific blockade of the brain converting enzyme, at a dose that was ineffective when given systemically, significantly reduced the elevation of MAP in DOCA-salt-treated animals. This action was mediated by a decrease of fluid intake, prevention of the increase of plasma AVP, and a restoration of the impaired baroreceptor reflex (13). Several lines of evidence suggest that the brain renin-angiotensin system and the central mineralocorticoid binding sites could interact with each other in mineralocorticoid hypertension. Wilson et al. (31) showed that mineralocorticoids increase the number of central ANG II receptors in rats, and Brooks and Malvin (4) showed that central administration of ANG II inhibited aldosterone secretion by a central mechanism.

The contribution of central mineralocorticoid binding sites was specific for mineralocorticoid hypertension, since the blockade of these sites did not affect the development of one kidney, one-clip hypertension. As with DOCA-salt hypertension, one-kidney, one-clip hypertension is characterized by volume expansion, an increase in sympathetic outflow and in plasma AVP concentration associated with a reduction of the density of AVP binding sites with no change in affinity (16, 17). In contrast, these two models of hypertension differ by an increase in vascular reactivity to AVP and elevated plasma sodium concentration in DOCA-salt hypertension, whereas in this model of renal hypertension these parameters remain unchanged. Despite the absence of an effect of the mineralocorticoid antagonist in one kidney, one-clip hypertension, we cannot rule out the involvement of mineralocorticoid binding sites in the pathogenesis of other models of hypertension. For example, it is possible that these central sites could participate in the development of another type of renal hypertension, the two-kidney, one-clip model. This latter model exhibits high plasma renin activity, is renin dependent, and is associated with elevated plasma aldosterone.
terone levels, which could activate the central mineralocorticoid binding sites.

In conclusion, our findings suggest that the complete development of DOCA-salt hypertension requires the functional integrity of central mineralocorticoid binding sites. These central sites appear to be involved specifically in the pathogenesis of mineralocorticoid hypertension, since their blockade did not alter a non-renin-dependent "volume expanded" form of renal hypertension. The mechanisms by which the chronic blockade of central mineralocorticoid binding sites exerts an anti-hypertensive action in the DOCA-salt model were not determined fully. However, the anti-hypertensive effects were associated with a reduction of neurogenic vasomotor tone and a restoration of the impaired baroreflex.

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Received 12 October 1989; accepted in final form 2 July 1990.

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