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SPECIFIC BINDING OF ATRIAL NATRIURETIC FACTOR TO RENAL GLOMERULI IN DOCA- AND DOCA-SALT-TREATED RATS CORRELATION WITH ATRIAL AND PLASMA LEVELS

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ABSTRACT: Since volume expansion and high blood pressure (BP) are known stimuli of atrial natriuretic factor (ANF) release, and since this peptide may be involved in mineralocorticoid escape, we investigated the effects of chronic deoxycorticosterone (DOCA) and DOCA-NaCl treatment on renal glomerular ANF receptor density and affinity in relation to atrial and plasma ANF levels. An increase in plasma immunoreactive ANF (IR-ANF) was observed both after two and four weeks of treatment. IR-ANF concentrations were elevated in the left atrium only in four-week DOCA treated rats. Administration of the mineralocorticoid alone resulted in a decreased density of glomerular ANF receptors in both time periods investigated. DOCA-NaCl-treated animals presented an increased receptor density during the pre-hypertensive stage (2 weeks) and a reduced density in the later hypertensive period (4 weeks). Receptor affinity in both groups was identical to that in the controls after 2 weeks and was augmented after 4 weeks of treatment. Our data suggest that the down-regulation of renal glomerular ANF receptors during chronic DOCA-NaCl administration may play a role in the maintenance of high BP in this model of volume-expanded hypertension.

Mammalian atrial myocytes secrete a family of potent natriuretic and diuretic peptides (1, 2) which are known as atrial natriuretic factor (ANF). The circulating form of ANF in the rat is a 28-amino acid peptide ANF (Ser 99 - Tyr 126) (3). It has been speculated that ANF is involved in the regulation of extracellular fluid volume, electrolyte balance and blood pressure (BP) (4, 5). Decreased atrial ANF levels and increased plasma ANF

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concentrations are observed in spontaneously-hypertensive (SHR) (6) and hypertensive one-kidney, one-clip rats (7). Chronic infusions of low doses of ANF lower arterial BP in different models of hypertension, including SHR, two-kidney, one-clip and one-kidney, one-clip rats (8, 9). It has recently been suggested that the renal sodium retention escape observed during mineralocorticoid administration, is mediated by ANF (10).

Binding studies have demonstrated the presence of specific ANF receptors in the kidney, adrenal cortex and aorta (11-13). The glomeruli have been identified as the most important localization of binding sites in the kidney (14). Vascular ANF receptors in the rat (13) have been reported to be decreased in volume-expanded models of hypertension (15) particularly in the DOCA-NaCl hypertensive rat (16). In the present study, we have examined the binding site characteristics of ANF in glomeruli during mineralocorticoid administration and DOCA-NaCl-induced hypertension in the rat.

MATERIALS AND METHODS

Male Sprague-Dawley rats (160-180 g; Charles River, St. Constant, Qué.) were maintained at a constantly controlled room temperature with a 12:12 h dark:light periodicity. They were fed regular pelleted rat chow and tap water "ad libitum" for 1 week prior to the experiment. Deoxycorticosterone (DOCA)-NaCl hypertension was induced by the method of Ormsbee and Ryan (17). The rats were uninephrectomized under ether anesthesia, after which silicone rubber impregnated with 100 mg DOCA acetate per rat was implanted and the animals were offered 1% saline to drink. The DOCA-treated group was similarly prepared but was offered tap water. The controls were subjected to left nephrectomy and silicone rubber not impregnated with DOCA was implanted. The rats were killed under sodium pentobarbital anesthesia 2 weeks and 4 weeks after the initiation of treatment. To exclude any possible effects of diurnal variation, all the animals were sacrificed between 8 and 10 A.M. BP was measured indirectly by means of the tail-cuff method under light ether anesthesia.

To determine plasma immunoreactive ANF (IR-ANF) concentrations, blood was withdrawn by jugular vein puncture under sodium pentobarbital anesthesia (60 mg/kg body weight, i.p.). The blood samples were collected in ice-chilled plastic tubes containing the following protease inhibitors: EDTA (10^{-5} M), pepstatin (10^{-5} M), and phenylmethylsulfonyl fluoride (PMSF, 3×10^{-5} M). Plasma was immediately separated from cells, extracted on Vycor glass and measured by a specific radioimmunoassay (RIA) (18). Micro-hematocrit and serum Na^+ were also measured. The heart and kidneys were removed immediately after sacrifice. The former was weighed, and both atria were processed separately and stored at -70°C until assayed. Atrial ANF was measured by RIA (19). Plasma and atrial IR-ANF concentrations were determined twice, and the mean of the two values was recorded. To determine glomerular ANF receptor density and affinity, glomeruli were isolated from the kidneys as described previously (20).

Aliquots (35 µg) of glomerular membrane protein were incubated in duplicate for 60 minutes at 22°C, as described by Carrier et al. (20), in the presence of increasing concentrations of unlabeled ANF (10^{-13}-10^{-7} M) and 20 pM ^{125}I-ANF in a final volume of 1 ml. The reaction was stopped by dilution with 3.5 ml of Tris-HCl pH 7.2 and by rapid filtration through polyethylenimine-treated Whatman GF/C filters, which were rinsed 3 times with 3 ml of Tris-HCl pH 7.2, allowed to dry, and counted in a LKB Pack Gamma Counter (Turku, Finland) with 65% efficiency. The ANF employed was rANF (Ser 99 - Tyr 126) (BioMega Inc., Laval, Quebec).

The results, expressed as means ± SEM, were evaluated by two-way analysis of variance. "A posteriori" comparisons were performed according to the method of Bonferroni. The binding data were analyzed with the computer-based LIGAND program (21) to determine the density and affinity of binding sites in the competitive experiments.

RESULTS

Two-week treatment

BP was higher in the DOCA-NaCl group than in either DOCA-treated or control rats (Table 1; $p < 0.01$). Plasma ANF was increased only in DOCA-NaCl animals (Figure 1; $p < 0.01$). A correlation between plasma ANF and BP ($r=0.63$; $p < 0.001$) and between ANF and heart weight ($r=0.49$; $p < 0.001$) was noted. Table 1 demonstrates that heart weight was higher and hematocrit and serum Na^+ lower in treated than in control animals. Body weight was decreased only in DOCA-NaCl rats.

TABLE 1

EFFECTS OF 2-WEEK DOCA AND DOCA-NACL TREATMENT ON

BLOOD PRESSURE, BODY AND HEART WEIGHTS, HEMATOCRIT AND SERUM NA^+

Group	Blood Pressure (mmHg)	Body weight (g)	Heart weight (mg/100 g body weight)	Hematocrit (%)	Na^+ (mmol/l)
Control n=10	97 ± 2	275 ± 7	299 ± 8	43 ± 0.7	140 ± 0.6
DOCA n=10	97 ± 1	262 ± 4	352 ± 12 *	40 ± 0.5 **	137 ± 0.9 *
DOCA-NaCl n=10	144 ± 2 **	192 ± 13 **†	369 ± 10 **	38 ± 0.7 **	137 ± 0.5 *

Values are means ± SEM.

* $p < 0.05$ vs control group

** $p < 0.01$ vs control group

† $p < 0.01$ DOCA vs DOCA-NaCl

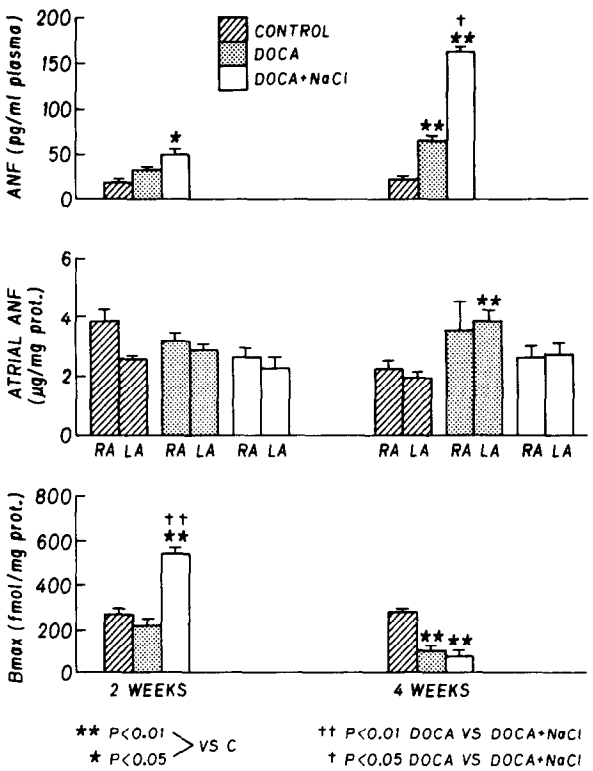

Figure 1. Effects of DOCA and DOCA-NaCl treatment for 2 or 4 weeks on plasma and atrial IR-ANF and on renal glomerular ANF receptors. (RA = right atrium; LA = left atrium). Results are means ± SEM.

Total atrial ANF content (µg/atrium; not shown) and atrial ANF concentration (µg/mg.protein) were unchanged. The characteristics of ^{125}I-ANF-binding to glomeruli from controls, DOCA and DOCA-NaCl animals were examined. Representative competition binding curves are illustrated in Figure 2A. There was a minor difference in the binding profiles of controls and DOCA-treated rats. ANF receptor density was slightly lower in DOCA and higher in hypertensive DOCA-NaCl animals than in the controls (Table 3). No change was observed in the affinity of glomerular ANF receptors.

Four-week treatment

The results of this experiment are illustrated in Table 2 and Figures 1 and 2. After 4 weeks of treatment, BP was higher in the DOCA-NaCl group than in either the controls or DOCA rats (170 ± 2 mmHg, 110 ± 2 mmHg and 118 ± 6 mmHg, respectively). Serum Na^+ and hematocrit were unchanged. Heart weight

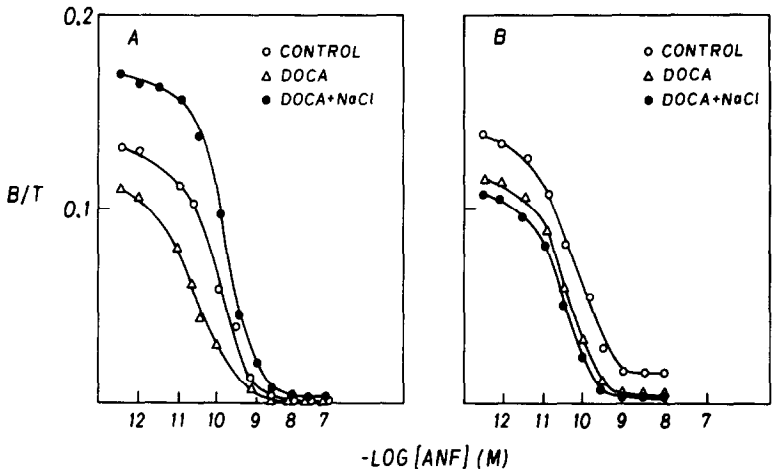

Figure 2. Representative competition curves of ^{125}ANF (20 pM) in the presence of increasing concentrations of unlabeled ANF, expressed as bound/total vs total (unlabeled ANF). Analysis of the binding curves was performed with the computerized LIGAND program (21) to determine the density and affinity of binding sites. Panel A controls (O), Bmax=261 fmol/mg.protein, Kd=56 pM; DOCA (Δ), Bmax=197 fmol/mg.protein, Kd=83 pM; DOCA-NaCl (•), Bmax=560 fmol/mg.protein, Kd=74 pM. Panel B controls (O), Bmax=351 fmol/mg.protein, Kd=100 pM; DOCA (Δ), Bmax=128 fmol/mg.protein, Kd=29 pM; DOCA-NaCl (•), Bmax=91 fmol/mg.protein, Kd=20 pM.

was greater in both treated groups, whereas body weight was lower in hypertensive DOCA-NaCl animals than in control rats.

TABLE 2

EFFECTS OF 4-WEEK DOCA AND DOCA-NACL TREATMENT ON BLOOD PRESSURE, BODY AND HEART WEIGHTS, HEMATOCRIT AND SERUM NA^+

Group	Blood Pressure (mmHg)	Body weight (g)	Heart weight (mg/100 g body weight)	Hematocrit (%)	Na^+ (mmol/l)
Control n=10	110 ± 2	365 ± 11	302 ± 6	41 ± 0.8	143 ± 2
DOCA n=10	118 ± 6	374 ± 16	374 ± 25 **	39 ± 0.5	143 ± 0.5
DOCA-NaCl n=10	170 ± 2 ** ††	271 ± 12 ** †	417 ± 10 ** †	41 ± 0.5	143 ± 0.5

Values are means ± SEM.

* $p < 0.05$ vs control group

** $p < 0.01$ vs control group

† $p < 0.05$ DOCA vs DOCA-NaCl

†† $p < 0.01$ DOCA vs DOCA-NaCl

TABLE 3

ANF RECEPTORS IN RAT RENAL GLOMERULI AFTER TWO- AND FOUR-WEEK DOCA AND DOCA-NACL TREATMENT

Group	Kd (pM)	Bmax (fmol/mg protein)
Control		
Two weeks	57 ± 4	260 ± 10
Four weeks	92 ± 5	355 ± 5
DOCA		
Two weeks	80 ± 5	220 ± 15 *
Four weeks	30 ± 1 **	130 ± 2 **
DOCA-NaCl		
Two weeks	76 ± 5	570 ± 20 **
Four weeks	32 ± 8 **	104 ± 10 **

Bmax = maximum density of binding sites.

Kd = dissociation constant.

* $p < 0.05$ vs control.
** $p < 0.01$ vs control.

Each value represents the mean of 2 experiments ± SEM.

Plasma IR-ANF was 25 ± 6 pg/ml in the controls, 64 ± 10 pg/ml in DOCA rats, and 164 ± 35 pg/ml in DOCA-NaCl animals ($p < 0.01$ for both treated groups). Only in the left atrium of the DOCA group was a higher ANF concentration found. Glomerular ANF receptor density averaged 355 ± 5 fmol/mg glomerular protein in the controls compared with 130 ± 2 fmol/mg in DOCA ($p < 0.01$) and 104 ± 10 in hypertensive DOCA-NaCl ($p < 0.01$) rats. Glomerular ANF receptor affinity was higher in DOCA (Kd=30 ± 1 pM) and DOCA-NaCl animals (Kd=32 ± 8 pM) than in the controls (Kd=92 ± 5 pM). Representative binding curves are shown in Figure 2.

DISCUSSION

Our study was designed to quantify the modifications of glomerular ANF-binding sites during the evolution of DOCA-NaCl hypertension and to correlate

them with changes in ANF concentrations. Two weeks of DOCA-salt treatment were chosen, since systolic BP in these rats was slightly higher than in the controls but still below the levels observed in animals with established hypertension. After 4 weeks, the hypertension was more severe.

The first set of results demonstrated that after 2 weeks of DOCA administration, the secretion of IR-ANF was not significantly increased. These data are consistent with the findings of Luft et al. (22) after 72-h DOCA treatment. Following 4 weeks of DOCA administration, the plasma and atrial IR-ANF concentrations were significantly elevated, indicating a stimulatory effect on the synthesis and secretion of ANF. This chronic volume-expansion experiments with DOCA, may have directly stimulated ANF release. However, this possibility is unlikely, since no mineralocorticoid cardioreceptors have been detected (23).

The present findings confirmed the work of others showing the presence of ANF-binding sites in glomeruli (14). Our results also demonstrated changes in ANF receptors before plasma IR-ANF concentrations rose significantly. A small increase in plasma IR-ANF levels decreased Bmax. This inverse relationship between plasma ANF concentrations and the number of ANF receptors after 2-week DOCA treatment suggested a down-regulation of binding sites. Receptor affinity was unchanged, and our computerized analysis revealed the presence of only one population of high-affinity sites.

In the pre-hypertensive stage, plasma IR-ANF was higher in DOCA-salt animals than in the controls without changes in atrial ANF content, suggesting an increase in synthesis and secretion. The difference in plasma and atrial ANF between DOCA and DOCA-NaCl rats after 2-week treatment was not significant. The lower values of hematocrit in both groups after 2 weeks indicated plasma volume expansion. These results confirmed the findings of previous investigations on plasma IR-ANF in other models of hypertension (6, 7). After 4 weeks of treatment, plasma IR-ANF concentrations were higher in both the DOCA and DOCA-NaCl groups, and atrial IR-ANF levels were either

unchanged or higher than in the controls, suggesting that, at this stage, there was also an increase in the synthesis and secretion of ANF.

The density of glomerular ANF-binding sites in hypertension is not well-documented. Saito et al. (24) have recently reported different binding profiles in renal cortex membranes of 14-week-old SHR compared with WKY, showing a decrease in the number and affinity of ANF-binding sites in SHR, but since this study was performed on basolateral membranes, it is not comparable with our present results. Similar data have been obtained, however, in crude renal membranes of 12-week-old SHR (25). Our present findings demonstrate that, after 2 weeks of treatment, when BP is 144 ± 2 mmHg, the DOCA-NaCl rats exhibit an increase in glomerular Bmax compared to the controls. After 4 weeks of treatment, when BP is 170 ± 2 mmHg, a decrease in glomerular Bmax is observed. Several studies have suggested that ANF may act directly on the kidney, probably by enhancing the glomerular filtration rate (26-28), to increase the absolute and fractional excretion of sodium. In the DOCA-NaCl model of hypertension, the secretion of renin is inhibited. The increase of ANF receptors would tend to reduce sodium retention. During the pre-hypertensive stage, this expansion in the number of binding sites is perhaps a compensatory response either to sodium retention (and hence volume expansion) or BP elevation. After 4 weeks of treatment, plasma IR-ANF concentrations are much higher than in the controls, and the ANF receptors are down-regulated such as it has been previously described in vascular tissue (16). The Kd of glomerular ANF-binding sites from DOCA-NaCl rats is lower than in control animals, indicating an increased affinity of receptors in DOCA-NaCl hypertensives, which is also seen in normotensive DOCA-treated rats. Diuresis and natriuresis are exaggerated in SHR following exogenously-administered ANF (29). Although these enhanced responses have not been noted in DOCA-NaCl-treated rats, the increased affinity of ANF glomerular receptors would suggest a decreased threshold for the response to ANF in these rats, but associated with a decrement in the maximum

natriuretic response to ANF, in agreement with the reduced number of receptors.

In conclusion, our data indicate that the density and affinity of glomerular ANF receptors during DOCA and DOCA-NaCl administration may depend on the length of treatment and that once hypertension is well-established in DOCA-NaCl rats, receptor down-regulation is clearly evident.

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