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Changes of hypothalamic and plasma vasopressin in rats with deoxycorticosterone-acetate induced salt appetite

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Abstract

Mineralocorticoids play a predominant role in development of salt appetite and hypertension. Since vasoactive peptides could mediate the central effects of mineralocorticoids, we evaluated changes of immunoreactive (IR) arginine–vasopressin (AVP) in the paraventricular (PVN) and supraoptic (SON) hypothalamic nucleus during DOCA-induced salt appetite. In one model, rats having free access to water and 3% NaCl during 9 (prehypertensive stage) or 21 days (hypertensive stage) received DOCA (s.c., 10 mg/rat/in alternate days). A decrease in the IR cell area, number of IR cells and staining intensity was obtained in magnocellular PVN of rats treated during 9 days. After 21 days IR cell area and number of cells in the PVN also decreased, but staining intensity of remaining cells was normal. The same parameters were unchanged in the SON. In another model, animals treated with DOCA during 9 days had only access to 3% NaCl or water. The IR cell area in PVN and SON significantly increased in mineralocorticoid-treated and control animals, both drinking 3% NaCl. Staining intensity (PVN and SON) and number of IR cells (PVN) also augmented in DOCA-treated animals drinking salt respect of a group drinking water. Plasma AVP in rats treated with DOCA and offered salt and water, exhibited a 2–2.5 fold increase at the time of salt appetite induction. Plasma AVP was substantially higher in rats drinking salt only, while the highest levels were present in salt-drinking DOCA-treated rats. Thus, peptide depletion in the PVN may be due to increased release, because reduced levels of hypothalamic and posterior pituitary AVP were measured in this model. In rats drinking salt only the substantial increase of IR AVP in the PVN and SON, may be due to dehydration and hyperosmosis. Because DOCA-salt treated rats showed higher AVP levels in the PVN compared to untreated rats drinking salt only, it is possible that DOCA sensitized PVN cells to increase AVP production. The results suggest the vasopressinergic system could mediate some central functions of mineralocorticoids.

1. Introduction

Sodium appetite is a complex and specific response modulated by mineralocorticoids in a bimodal fashion: while low doses suppress salt intake of adrenalectomized rats [1,2] pharmacological amounts induce salt appetite in adrenalectomized animals but more notoriously in intact animals [3]. Following continued treatment with mineralocorticoids, animals develop hypertension [4–6]. In a commonly used experimental paradigm, high doses of deoxycorticosterone acetate (DOCA) increase salt intake 24–48 h after treatment [2,7–10], suggesting a receptor-mediated, genomic mechanism of action. This possibility is supported by use of steroid receptor antagonists [11], which demonstrated involvement of mineralocorticoid receptors (MR) in salt appetite induction, whereas the role of glucocorticoid receptors (GR) is less conclusive [7].

The neuroanatomical localization of MR-containing cells involved in salt appetite induction is unclear.

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Lesion and biochemical studies suggested that the anteroventral third ventricular region (AV3V), the paraventricular hypothalamic nucleus (PVN), the lateral hypothalamic area and the amygdala may be part of a complex neuroendocrine circuit in which mineralocorticoids control salt appetite [12,13]. Moreover, it is possible that salt appetite induction and development of hypertension involve the interaction of mineralocorticoids, MR, neuropeptides and enzymes synthesized in the preoptic-hypothalamic and amygdaloid areas. Among the peptide mediators, vasoactive peptidergic systems are likely candidates [14]. These comprise the family of atrial natriuretic peptides (ANP), which act in balance with the angiotensin II (Ang II) and vasopressin (AVP) systems [14–17]. AVP is also influenced by galanin, which decreases AVP biosynthesis by magnocellular neurons [18,19] although both peptides respond in parallel to hyperosmolality [20].

The present study is focused on AVP peptide expression in the hypothalamus and circulating levels of AVP of rats with DOCA-induced salt appetite. In the hypothalamus, AVP synthesized by magnocellular neurons of the PVN (mPVN) and supraoptic nucleus (SON) is transported down via the hypothalamo–hypophysial tract and released from the posterior pituitary. This system is related to osmotic control, whereas AVP produced in the parvocellular PVN (pPVN) is co-released with CRF into the portal system, both being potent ACTH secretagogues [21–23]. It has been reported that AVP is intimately involved in the development of DOCA-salt hypertension [24–28], whereas genetic hypertension is associated with a low content of AVP in the PVN [29]. Hypertensive DOCA-salt treated rats also showed increased plasma AVP concentrations and increased AVP binding sites in hypothalamus [27]. In addition to peripheral functions related to antidiuretic, pressor and natriuretic effects [24,30], AVP can modulate centrally fluid balance and blood pressure [28,31]. However, whether AVP plays a role in MC-induced salt intake has not been addressed in the above mentioned reports.

In the present report, the vasopressin system was examined in two paradigms involving salt intake. First we employed a double preference test in which tap water and concentrated saline solution were available ad libitum to animals receiving vehicle or DOCA for short or long periods (9 and 21 days). In a second paradigm, we employed rats drinking either salt solution or water only, and treated with vehicle or DOCA for 9 days. AVP peptide expression by the PVN and SON was quantitated using immunocytochemistry and computerized image analysis. In some groups, levels of AVP were measured in plasma, hypothalamus and posterior pituitary by RIA. The present work extends at the peptide level previous data on AVP mRNA in DOCA-treated rats [10].

2. Materials and methods

2.1. Salt appetite induction studies

Male Sprague–Dawley rats weighing 180–220 g were used. The rats were caged singly, with free access to tap water or 3% NaCl given in separate bottles (double preference test). After a 1-week period, during which basal ingestion of salt and water was monitored, a group of rats was given subcutaneous injections of DOCA (Sigma, St.Louis, MO, USA) (10 mg/rat/day) on alternate days over 9 days (4 injections, short treatment) or over 21 days (9 injections, chronic treatment). Control rats received vegetable oil vehicle only. A separate group of rats was given 3% NaCl only (no tap water) or tap water only, and were given subcutaneous injections of DOCA (10 mg/rat/day) or vehicle administered on alternate days over 9 days. Twenty-four hours after the last DOCA injection all rats were anesthetized by ether vapor inhalation and perfused intracardially with 0.9% NaCl. The brains were rapidly removed, frozen on dry ice and stored at −80°C until use. The protocols were approved by the Animal Care and Use Committee of the Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentine.

2.2. Immunocytochemistry for AVP in the hypothalamus

Brain cryostat sections (8 μm) were collected on glass slides subbed with gelatin. Sections were fixed for 6 min in 2% paraformaldehyde at 4°C and washed twice in PBS. After blocking the endogenous peroxidase with 1% H_2O_2 in methanol for 30 min at room temperature, sections were washed in PBS and incubated with 10% normal goat serum during 10 min at 37°C. After this step, a 1/1000 dilution of rabbit polyclonal anti-AVP serum was added (a generous gift from Dr. Ruud Buijs, Netherlands Institute for Brain Research, Amsterdam, The Netherlands) [32] in 1% normal goat serum, 0.3% Triton-X-100, and the samples incubated overnight at 4°C. Afterwards, sections were washed in PBS, and incubated with anti-IgG rabbit serum (1:200) diluted in 1% goat serum containing 0.3% Triton-X-100. This step was followed by incubation in a 1/100 dilution of avidin horseradish peroxidase (Vectastain ABC Elite, Vector Labs., Burlingham, CA, USA) in PBS and finally received 0.025% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) made in buffer 0.1 M Tris pH 7.2 containing 0.01 % H_2O_2 during 6–8 min. After two washes in distilled water, sections were dehydrated and mounted in

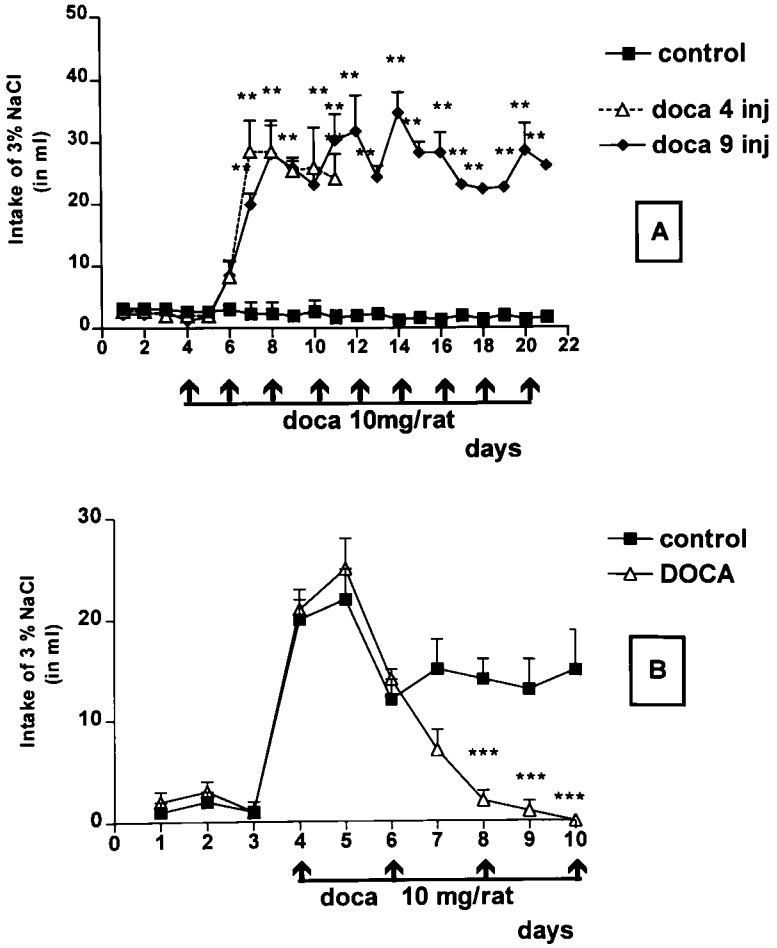

Fig. 1. NaCl 3% intake in ml during DOCA treatment (10 mg/rat/day) administered subcutaneously with vegetable oil like vehicle on alternate days (solid arrows represent DOCA-injections) in (A) rats having free access to 3% NaCl and water; 4 or 8 injections of DOCA *** $p < 0.05$. (B) rats having access only to saline solution, control rats and DOCA-treated rats, 4 injections.

Permount. Six sections per rat were analyzed containing the PVN and SON delimited according to the Paxinos and Watson atlas [33] (bregma −17 to −2.2 mm). In the case of the PVN, boundaries of each subnuclei were defined based on local cytoarchitecture [34]. Large AVP-producing cells (> 13 μm) were ascribed to the magnocellular division (mPVN), whereas small cells lying between the mPVN and the third ventricle were considered the parvicellular portion (pPVN). Expression of AVP by pPVN cells was very limited [22].

The intensity of the immunocytochemical reaction for AVP over the whole PVN, its magnocellular and parvocellular divisions and the SON was quantified by computerized image analysis, as described by Ferrini et al. [35]. The Optimas Program (Bioscan Optimas, Edmonds, WA, USA) equipped with a VT-C33ON video camera, transforms differences in color intensity into gray differences, and results were expressed as the inverse log of gray intensity per area (ILIGV/area). In this program, after images are properly decalibrated for background lighting, ILIGV/area results proportional to the unweighted average optical density, which is then used to determine the average concentration of immunoreactive peptide. This program is also able to inform the cellular area (um^2) and the number of AVP immunopositive cells.

2.3. Determination of plasma and tissue AVP

AVP in plasma was first separated from binding proteins by ethanol extraction and the concentration of immunoreactive AVP was measured using an AVP RIA kit from Nichols Institute Diagnostics B.V. (Wijchen, The Netherlands). Tissue AVP was measured according to Morris et al. [36]. Hypothalamus and posterior pituitaries were sonicated in 0.1 M HCl, diluted in 0.062 M Na_2 HPO_4, 0.013 M

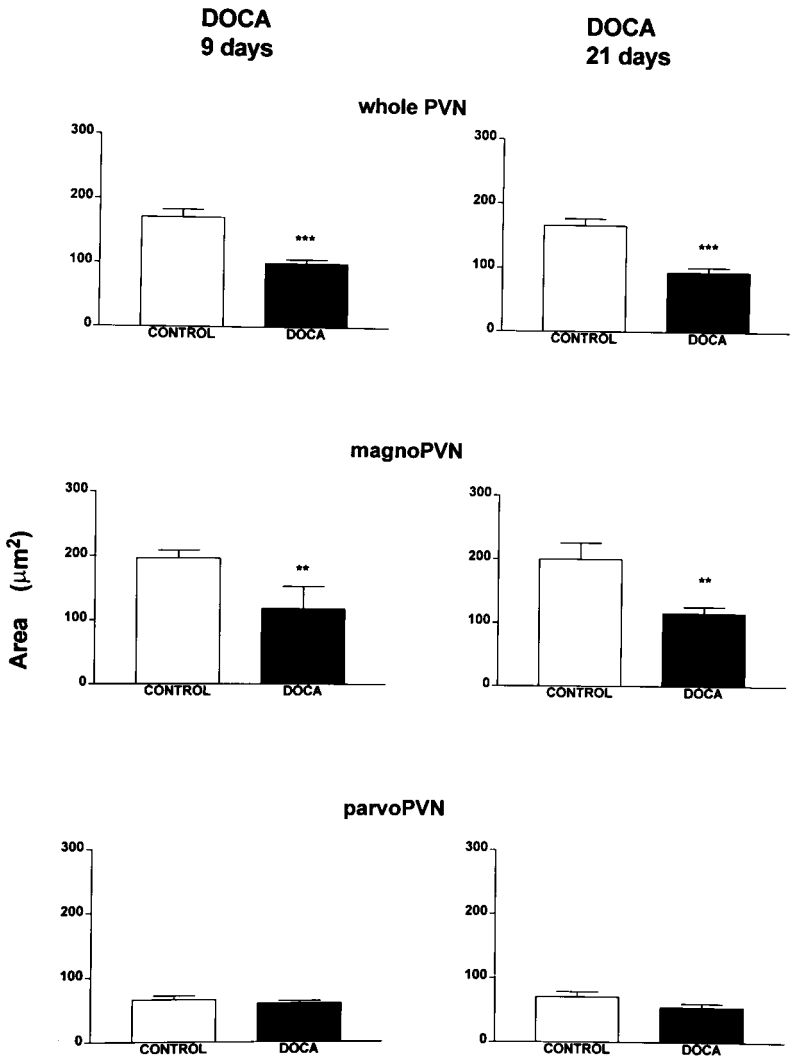

Fig. 2. Arginine–vasopressin (AVP) immunoreactive area in control rats and rats treated s.c. with 10 mg/rat/day of DOCA on alternate days during 9 days (4 injections) or 21 days (9 injections). Measurements were performed in the whole paraventricular nucleus (PVN) or in its magnocellular (magnoPVN) and parvocellular (parvoPVN) divisions. Significance: $^{***}p < 0.01$ and $^{**}p < 0.05$ vs controls, respectively.

Na_2EDTA, 0.5% bovine serum albumin and aliquots used for RIA. The antiserum anti-vasopressin (made in rabbit) and ^{125}I-AVP used were provided with the kit. The lower limit of detection of the assay was 1.3 pg/ml. Intra- and inter-assay coefficients of variation were less than 10%. The cross-reactivity is 100% for arginine–vasopressin and less than 0.1% for lysine–vasopressin, oxytocin and vasotocin.

2.4. Statistical analysis

Data are presented as mean ± S.E. The Student's *t*-test or ANOVA followed by the Newman–Keuls Multiple Comparison test were applied to compare differences between control and experimental groups. The significance level was set at $p < 0.05$ for all experiments.

3. Results

3.1. Salt appetite studies

Intake of 3% NaCl in rats injected with vehicle or DOCA over 9 days (4 injections) is shown in Fig. 1(A). In this experiment, rats were offered saline and tap water in separate bottles. In agreement with previous data [10], vehicle-treated rats did not develop a salt appetite, whereas a robust salt appetite was evident after the second DOCA administration, reaching a peak on the 8th day. When the experimental paradigm was continued for 3 weeks (Fig. 1(A)), in which case rats received 9 injections on alternate days, DOCA-treated rats maintained the increased salt appetite observed under the shorter steroid regimen. Thus, rats did not 'escape' from the natriorexigenic action of

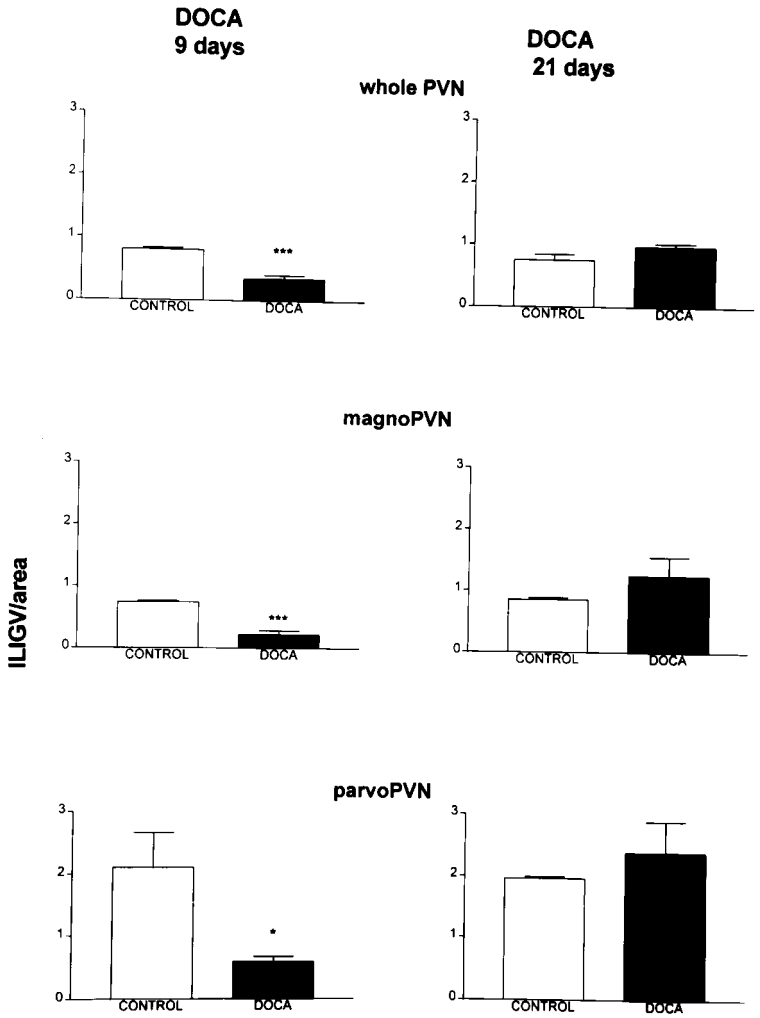

Fig. 3. Measurements of the intensity of the AVP-labelling related to cellular area (ILIGV/area) in whole PVN, magnoPVN and parvoPVN in control and DOCA-treated rats during 9 or 21 days. Significance: $^{**}p < 0.05$,$^{***}p < 0.01$ vs controls respectively.

DOCA. In this model, rats were not dehydrated as indicated by body weight measurements (controls: 252.8 ± 4.7 g; DOCA 4i: 261.4 ± 5.9 g; DOCA 9I: 267.1 ± 12.7 g, $n = 7$ rats per group).

When drinking was limited to 3% NaCl only (not tap water), increased salt ingestion developed in both vehicle and DOCA-treated rats, showing a peak 5 days after starting the experiment (Fig. 1(B)). However, concomitant with the second DOCA injection, steroid-treated rats dramatically decreased drinking, which reached a minimum at the end of the experiment. The salt-treated rats were highly dehydrated and showed a significant reduction in body weight (controls-water: 212.5 ± 9.4 g; controls-salt: 111.2 ± 5.5 g, $p < 0.001$; DOCA-water: 217.9 ± 12.7 g; DOCA-salt: 111.7 ± 7.0 g, $p < 0.001$; $n = 6$–8 rats per group).

3.2. AVP immunocytochemistry in PVN and SON

In the case of the PVN [22] AVP-immunoreactivity (IR) was particularly intense in the magnocellular division (mPVN), with few scattered cells present in the parvocellular division (pPVN). In our immunocytochemical study, three parameters were analyzed:: immunoreactive area, signal intensity (ILIGV/area) and number of cells displaying AVP-IR.

Data in Fig. 2 show that DOCA-treatment over 9 days (10 mg/rat/day, on alternate days), significantly decreased cell area in whole PVN ($p < 0.01$) and mPVN ($p < 0.05$) but not in pPVN. Furthermore, ILIGV/area (Fig. 3) was significantly attenuated by treatment with DOCA during 9 days compared with control rats in whole PVN ($p < 0.01$), mPVN ($p < 0.01$) and also in pPVN ($p < 0.05$). Although the number of cells expressing AVP-IR was not significantly affected after treatment with DOCA over 9 days in pPVN (Fig. 4), a significant reduction was obtained in the mPVN ($p < 0.05$).

When DOCA was injected over 21 days to rats with free access to tap water and salt solution, it produced

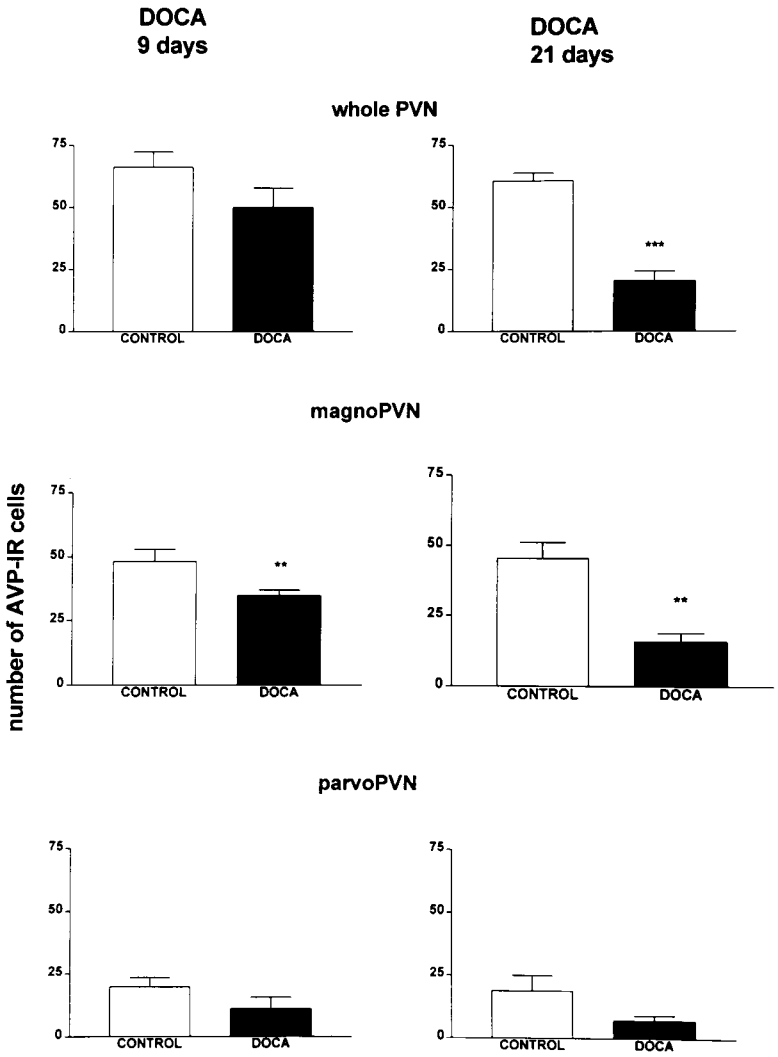

Fig. 4. Measurements of the number of AVP-immunoreactive cells in whole PVN, magnoPVN and parvoPVN in control and DOCA-treated rats during 9 or 21 days. Significance: $^{**}p < 0.05$; $^{***}p < 0.01$ vs controls, respectively.

a substantial decrease in cell area in the whole PVN and mPVN (Fig. 2, whole PVN $p < 0.01$; mPVN: $p < 0.05$). In the pPVN, however, differences were not significant. In the 21-day DOCA treatment group, ILIGV/area (Fig.3) was not significantly different from controls. Studies concerning the number of cells expressing AVP-IR (Fig. 4), established a weightly diminution in rats treated with DOCA during 21 days in whole PVN ($p < 0.01$) and mPVN ($p < 0.05$) but not in pPVN (Fig.5).To discriminate the relative roles of salt intake and steroid treatment on AVP peptide expression in the PVN, an experiment was designed in which rats were treated over 9 days with DOCA (10 mg/rat/day) on alternate days, with free access only to saline (NaCl 3%) or tap water. Rats were assigned to one of four groups: control rats drinking only water (control-WT), control rats drinking only saline (control-ST); DOCA-treated rats drinking only water (DOCA-WT) and DOCA-treated rats drinking only NaCl 3% (DOCA-ST). AVP-IR was analyzed in the mPVN and SON; group comparisons were performed by ANOVA and the Newman–Keuls post-hoc test (Fig. 6 and Table 2).The cell area expressing AVP-IR was significantly greater in control rats drinking saline solution than control rats with access to water only (Fig. 6, $p < 0.01$). The cell area with AVP-IR was also greater in DOCA-treated rats drinking saline solution than in DOCA injected rats drinking only water ($p < 0.01$). When the intensity of AVP-IR was analyzed, cells from DOCA-treated rats drinking saline solution only, showed stronger labelling than cells from DOCA-treated rats drinking water (Fig. 6, $p < 0.05$). ILIGV/Area was similar in control-WT, control-ST and DOCA-WT rats. Finally, the number of cells showing AVP-IR in mPVN was 2-fold greater in rats receiving DOCA and drinking saline solution only, the latter being significantly higher than the control-WT, control-ST and DOCA-WT groups

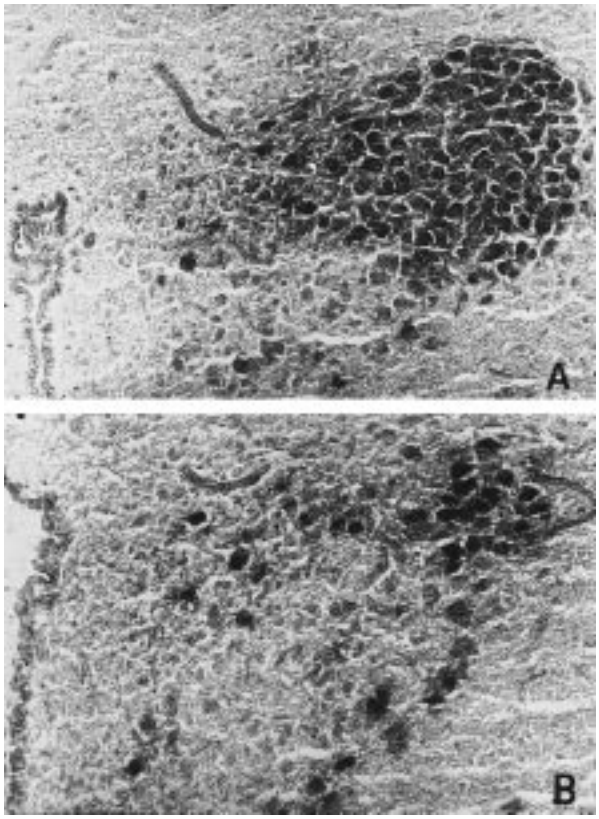

Fig. 5. Representative photomicrographs of AVP-immunoreactive cells in the PVN corresponding to control rats (A) and rats receiving DOCA treatment during 21 days (B). In this experiment, animals were offered 3% NaCl and tap water in separate bottles (double bottle preference test). Magnification: A and B 400 ×.

($p < 0.01$). In the SON, the behavior of AVP-IR peptide varied according to the experimental model. In rats offered water and 3% NaCl in separate bottles, cell area, reaction intensity and number of AVP-IR cells were not modified by 9 or 21 days of DOCA treatment (Table 2), at the time these parameters were already changing in the PVN (Fig. 2). In the second model, AVP-IR cell area in the SON significantly increased in mineralocorticoid-treated and control animals provided 3% NaCl was the only choice. Also, DOCA-ST treated rats showed significantly higher reaction intensity (ILIGV/area) than DOCA-WT in the SON, although the value did not differ from values presented by control-WT and control-ST rats (Table 2).

Table 1
Effect of salt ingestion and DOCA treatment on plasma immunoreactive vasopressin levels measured by RIA

	Treatment (days)	Plasma AVP (pg/ml)
Experiment I		
Control + water + 3% NaCl	9–21	5.10 ± 0.8
DOCA + water + 3% NaCl	9	9.24 ± 1.12[a]
DOCA + water + 3% NaCl	21	12.14 ± 1.88[b]
Experiment II		
Control + 3% NaCl	9	25.27 ± 5.3
DOCA + 3% NaCl	9	43.80 ± 4.1[c]

[a] $p < 0.05$ vs control + water + 3% NaCl.
[b] $p < 0.01$ vs control + water + 3% NaCl.
[c] $p < 0.05$ vs control + 3% NaCl.

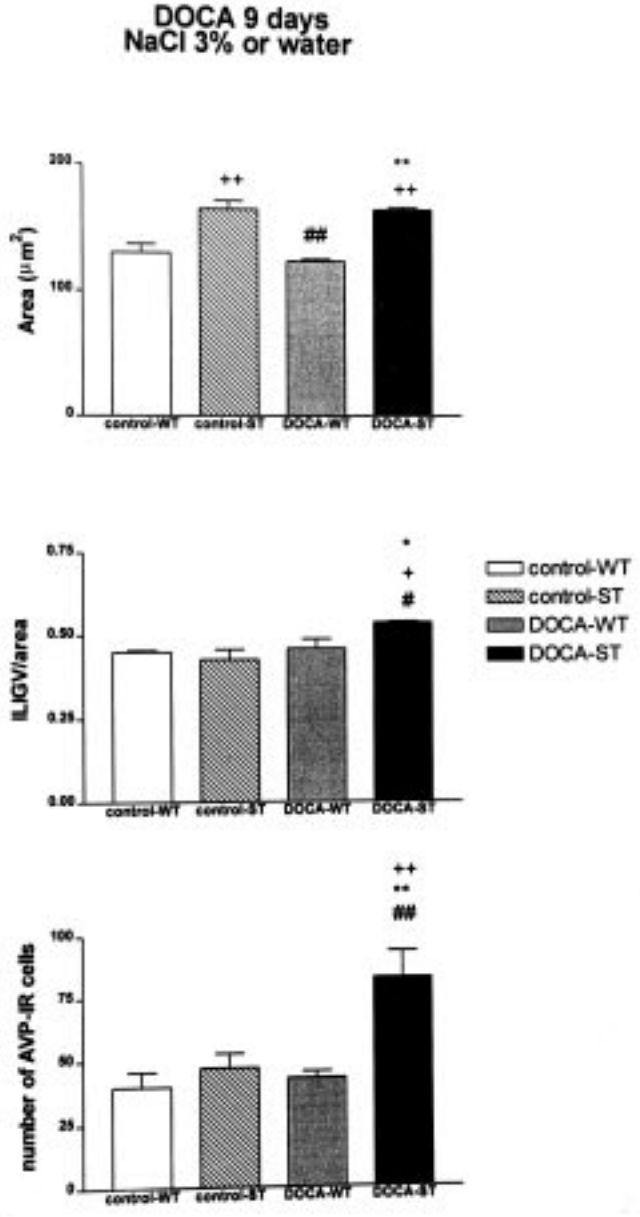

Fig. 6. AVP immunoreactive area (upper graph), immunoreaction intensity (middle graph) and number of AVP immunoreactive cells (lower graph) in control rats drinking water (control-WT), controls drinking 3% NaCl only (controls-ST), rats treated with DOCA during 9 days drinking water (DOCA-WT) or drinking 3% NaCl only (DOCA-ST). Significance: $^{+}p < 0.05$ and $^{++}p < 0.01$ vs control-WT; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs DOCA-WT; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs control-ST (ANOVA and Newman–Keuls test).

Table 2
Effect of salt ingestion and DOCA treatment on arginine–vasopressin (AVP) immunocytochemistry (ICC) in the supraoptic nucleus (SON)[a]

	Control		DOCA	
Experiment I: DOCA treatment during 9 days in rats drinking water and 3% NaCl				
Area	158.7 ± 10.9		150.3 ± 6.8	
ILIGV/area	0.532 ± 0.04		0.502 ± 0.03	
Number of cells	48.3 ± 5.0		42.6 ± 6.7	
Experiment II: DOCA treatment during 21 days in rats drinking water and 3% NaCl				
Area	162.98 ± 12.9		143.4 ± 7.8	
ILIGV/area	0.543 ± 0.03		0.487 ± 0.04	
Number of cells	45.3 ± 4.2		36.5 ± 4.9	
	Control-WT	Control-ST	DOCA-WT	DOCA-ST
Experiment III: DOCA treatment during 9 days in rats drinking water or 3% NaCl only				
Area	165.7 ± 15.6	224.5 ± 4.7*	170.7 ± 13.7	208.6 ± 9.7**
ILIGV/area	0.498 ± 0.02	0.449 ± 0.01	0.406 ± 0.01	0.483 ± 0.02+
Number of cells	50.7 ± 9.07	65.8 ± 8.1	51.2 ± 8.5	68.8 ± 6.3

[a] Area: immunoreactive cell area (μm^2); ILIGV/area: immunoreaction intensity; Number of cells: AVP immunoreactive cells per SON. Abbreviations as in legend to Fig. 6. Results represent the mean ± S.E. of 6 animals per group. Six sections per rat containing the SON were analyzed. Significance: $^{*}p < 0.05$ vs control-WT; $^{**}p < 0.05$ vs DOCA-WT; $^{+}p < 0.05$ vs DOCA-WT (ANOVA and the Newman–Keuls test).

3.3. AVP measurement in rat plasma and nervous tissues

AVP concentrations were measured in plasma from rats in the two experimental groups. First, in rats subjected to the double preference, i.e. 3% NaCl and water and given DOCA during 9 or 21 days and their vehicle-treated controls. Second, in rats drinking 3% NaCl only and receiving vehicle (control-ST) or DOCA during 9 days (DOCA-ST). In the first experiment (Table 1), AVP concentrations in plasma from DOCA-treated rats increased 2–2.5 fold in comparison with controls (control vs DOCA (9 days): $p < 0.05$ and control vs DOCA (21 days): $p < 0.01$). In the second experiment, drinking the concentrated salt solution produced a high plasma concentration of AVP in plasma, which, however, was still higher when these rats received DOCA ($p < 0.05$ vs control-ST).

Hence, increases in plasma AVP concentrations in DOCA-treated rats subjected to the double-preference test, was accompanied by a reduction of AVP-IR in cells of the mPVN. To investigate if this was due to a depletion mechanism, the content of the neuropeptide was measured in the hypothalamus and posterior pituitary from control rats and rats receiving DOCA during 9 days. We found a significant reduction of AVP content in hypothalamus (controls: 719.8 ± 82.1, DOCA: 397 ± 117.6 ng/hypothalamus, $n = 6$ rats per group, $p < 0.05$) and a borderline reduction in the posterior pituitary lobe (control: 337.7 ± 42, DOCA: 231 ± 34 ng/lobe, $p = 0.07$).

4. Discussion

In agreement with previous data [7,10] treatment of rats with DOCA induced a salt appetite after the second DOCA injection, which was maintained until the end of the study (21 days). In this animal model, we sought a potential link between salt intake and AVP peptide expression in the mPVN and SON during mineralocorticoid induced salt appetite.

First, DOCA treatment over 9 or 21 days produced similar increments in salt intake. These periods were selected because blood pressure remained normal at 9 days, but rats develop hypertension after 21 days of DOCA plus water + salt treatment [10]. Therefore, at 9 days rats were in a prehypertensive stage. At both 9 and 21 days, the PVN from DOCA-treated rats showed a similar reduction in AVP immunoreactivy, indicating peptide depletion. However, the intensity of the reaction (ILIGV/area) was reduced after 9 days but not after 21 days, suggesting that some cells in the mPVN replenished their AVP content with continued DOCA treatment. Interestingly, none of these parameters changed in the SON after DOCA treatment.

A comparison of previous data employing *in situ* hybridization (ISH) to analyze AVP mRNA levels [10] with present data on peptide expression, allow us to interpret the differences in responses of both hypothalamic nuclei. Thus, in rats with free access to water and 3% NaCl and receiving DOCA over 9 or 21 days, there is an increased expression of vasopressin mRNA levels in the magnocellular cells of the PVN and SON

[10]. It is possible that increased translation of AVP mRNA in the PVN was effected at 21 but not 9 days of continued mineralocorticoid exposure. Although regulation of AVP mRNA was similar in the PVN and SON, peptide responses seemed attenuated in the latter. This finding confirms that differences exist in homogeneity both from histological and functional point of view regarding the PVN and SON. Thus, whereas the PVN sends vasopressinergic projections to brain stem areas important for cardiovascular control in addition to those that project to the posterior pituitary, the SON's primary input is to the neurohypophysis [32]. In DOCA-salt hypertensive rats, AVP mRNA expression is increased in the PVN but not in the SON [28], whereas peptide content in the PVN but not the SON was lower in SHR rats [29]. Also, several types of stress modulated AVP mRNA exclusively in the mPVN [37].

In the second paradigm, we analyzed whether DOCA per se changed AVP peptide levels or if excess salt drinking was needed for the DOCA effect. In this situation, drinking 3% NaCl alone in the absence of DOCA already increased the immunoreactive cell area in the PVN and SON, but neither the intensity of AVP-IR per cell area nor the number of AVP positive cells was modified. In contrast, DOCA treatment superimposed to forced salt drinking in the single bottle test was a powerful stimulus for increasing in the mPVN (a) AVP immunoreactive cell area, (b) intensity of the reaction, (c) the number of immunoreactive cells. A slight increase in reaction intensity was also found in the SON of DOCA-salt treated rats respect of DOCA rats drinking water. Drinking 3% NaCl with or without DOCA treatment stimulated AVP mRNA levels in the mPVN but not the SON [10]. However, the group receiving both 3% NaCl and DOCA contained still higher AVP mRNA levels than the group receiving 3% NaCl only [10]. Because rats in the DOCA-ST group lost body weight due to dehydration, it is likely that hyperosmolality was a major contributor to the pronounced AVP-IR found in the mPVN and possibly the SON from this animal group. However, DOCA had an additional stimulatory effect in rats with exclusive 3% NaCl drinking which was not apparent in DOCA-WT rats, as shown by ISH [10] and present data on peptide expression. To explain these results, we would like to suggest that during a hyperosmotic challenge, DOCA sensitizes the biosynthesis of AVP mainly in the PVN cells rather than the SON.

Whether an osmotic effect operates in rats with DOCA-induced salt appetite is unknown. Results of plasma sodium support alternate possibilities. Previously, we found normal natremia in rats receiving 4 DOCA injections (149 mEq/l vs 147 mEq/l in controls [10]). However, hypernatremia developed after 9 DOCA injections (157 mEq/l). In the present report, concomitant to DOCA arousal of salt intake, reduced AVP-IR in the mPVN was found (Fig. 3). This paradigm also presented with high plasma levels of AVP (Table 1). Therefore, osmotic changes may not be responsible for AVP changes during salt intake induced after 4 DOCA injections, as opposed to the influence of hypernatremia in rats receiving 9 injections and in DOCA-treated rats fed the salt solution only.

To account for the changes in AVP expression reported in this and our former communication [10], we would like to propose the following hypothesis. DOCA treatment may increase AVP release from hypothalamic nuclei, the median eminence and the posterior pituitary into the blood stream. This finding was previously reported in DOCA-salt hypertensive rats [38] and rats drinking concentrated salt solutions. Direct stimulation of AVP gene transcription could be a compensatory mechanism for the continuous depletion of AVP from mPVN stores. In the case of the SON, either depletion was not as severe as in the PVN or increased biosynthesis readily compensated for the released AVP. In any case, sustained release of AVP to the peripheral circulation contributes to hypertension through its antidiuretic activity and vasoconstrictor effects [26,39]. In previous work, we showed that high blood pressure developed at 21 but not at 9 days of DOCA treatment in rats with increased salt appetite.

While the releasing effect may account in part for the hypertensinogenic actions of AVP, a different mechanism may apply to behavioral regulation. In this context, besides releasing AVP into the blood stream and the portal system, the PVN sends vasopressinergic projections to several brain areas. Among these are limbic regions, lateral septum and amygdala important for behavioral regulation [25,38]. Another projection is directed to brain stem areas involved in cardiovascular control, including the locus coeruleus, tractus solitarius and the dorsal vagal complex [32,40]. Third, there are also vasopressinergic fibers in the organum vasculosum lamina terminalis and periventricular hypothalamus, the 'AV3V region'. The AV3V region, which might be involved in fluid homeostasis also contains AVP receptors [41]. AVP is also released into the third ventricle [42] and the tissue surrounding the PVN [43]. Recently, Prakash et al. [44] detected transcripts encoding mRNA for AVP not only in axons but also in dendrites from magnocellular neurons. These authors suggest dendritic transcripts could act as templates for local AVP synthesis and subsequent release into the brain. It would be important to investigate if these projections constitute a neuroanatomical substrate accounting for the behavioral effects of AVP on salt appetite.

Thus, depletion of AVP in the mPVN cells of DOCA-treated rats exhibiting a salt appetite could be

due in part to central release of the peptide to different brain regions, resulting in hypertension as well as induction of salt intake [45]. This centrally-released AVP could become available to vasopressinergic V1 or V2 receptors. In fact, increased binding of AVP to hypothalamic receptors have been found in DOCA-treated rats [27]. Recently, we found that systemic treatment with a V1–V2 receptor antagonist attenuates DOCA-induced salt appetite (Saravia et al., in preparation).

However, the contribution of other brain peptides besides AVP to osmotic regulation is very important and possibly in close relationship with AVP. As stated in the Introduction, leading roles are played by the brain renin–angiotensin system and ANP. Ang II is a potent dipsogenic agent also involved in arousal of salt appetite [41,46]. Increased Ang II binding in several hypothalamic regions, including the PVN occurred in DOCA-salt hypertensive rats [46]. In addition to co-localization of AVP and Ang II mRNA in some hypothalamic nuclei [47], Ang II exerts powerful AVP releasing activities, pointing to a cooperation between vasopresinergic and angiotensinergic systems [17]. In contrast, ANP is an antagonist of the central actions of Ang II and AVP actions, including inhibition of salt appetite provoked by Ang II [48]. In DOCA-salt hypertensive rats, ANP receptors in some brain regions were found significantly lower than in control rats treated with salt alone [46]. Although at this stage a role for AVP in mineralocorticoid control of salt appetite is not established, future investigations may help to elucidate the biological outcome of the interaction of mineralocorticoids with vasoactive neuropeptides.

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