Regulation of Vasopressin Receptors in Deoxycorticosterone Acetate–Salt Hypertension

Deborah Trinder, Paddy A. Phillips, John Risvanis, Jayne M. Stephenson, and Colin I. Johnston

Since arginine vasopressin may play a role in mineralocorticoid hypertension, we examined the effects of deoxycorticosterone acetate (DOCA)−salt on vasopressin V₁ and V₂ receptor binding and their second messengers, inositol phosphate and adenylate cyclase, respectively, in liver and kidney to determine whether altered vasopressin receptor binding is pathogenic in mineralocorticoid hypertension. The mean arterial blood pressure of mineralocorticoid (DOCA-salt)−treated rats (163±1 mm Hg) was increased compared with control salt-treated rats (salt) (122±1 mm Hg) and water-treated rats (water, 120±1 mm Hg; p<0.001). Mineralocorticoid treatment also increased plasma sodium, osmolality, and vasopressin concentration (p<0.001). In the hypertensive animals, there was a reduction in hepatic V₁ (DOCA-salt, 91±12; salt, 132±13; and water, 145±13 fmol/mg protein; p<0.05) and renal V₂ receptor binding density (DOCA-salt, 53±5; salt, 93±9; and water, 95±9 fmol/mg protein; p<0.01), although receptor affinities remained unaltered. In contrast, the density of renal V₁ receptors was increased by mineralocorticoid treatment (DOCA-salt, 24±2; salt, 16±2; water, 18±1 fmol/mg protein; p<0.05), although the affinity was unchanged. Downregulation of V₂ receptors was associated with a decrease in maximum cyclic adenosine monophosphate levels (DOCA-salt, 19±4; salt, 49±6; water, 53±9 pmol • mg protein⁻¹ • 10 min⁻¹; p<0.05), whereas changes in V₁ receptor levels were not associated with changes in maximum inositol phosphate levels. Therefore, changes in plasma vasopressin levels rather than changes in vasopressin receptors and their maximum second messenger levels are more likely to play a role in the development of mineralocorticoid hypertension. (Hypertension 1992;20:569–574)

KEY WORDS • vasopressins • deoxycorticosterone • hypertension, mineralocorticoid

There are several lines of evidence to suggest that the hormone arginine vasopressin (AVP) plays a role in the pathogenesis of deoxycorticosterone acetate (DOCA)−salt hypertension. In this model of experimental hypertension plasma AVP is elevated,¹² and there is an increased vascular responsiveness to exogenous AVP.²⁻⁴ Also the administration of an AVP antibody reduces blood pressure.¹⁻⁵ However, the strongest evidence is obtained from experiments using the Brattleboro rat, which is unable to synthesize functional AVP and does not develop hypertension when treated with DOCA-salt.⁵⁻⁶

AVP mediates its physiological effects through two types of membrane-bound receptors that have been classified V₁ and V₂. The V₁ receptors are located in many tissues, including the vasculature, brain, liver, and kidney, are coupled to inositol phosphate turnover, and mediate the vasopressor and glycogenolytic effects of AVP. The V₂ receptors are found mainly in the kidney, are linked to adenyl cyclase and the production of cyclic adenosine monophosphate (cAMP), and are associated with antidiuresis.⁷ However, V₂ agonists also have extrarenal effects, including increased plasma factor VIII and von Willebrand factor levels⁸ and vasodilatation.⁹

The importance of V₁ receptors in DOCA-salt hypertension is unclear. The reported effects of V₁ antagonists have been contradictory; studies suggest that these peptides produce moderate reductions⁶⁻¹⁰ or no change¹¹,¹² in blood pressure in DOCA-salt−treated animals. However, V₁ receptors appear to be involved in the development of DOCA-salt hypertension since the use of a V₂ antagonist reduced blood pressure.¹³ Also, Brattleboro rats developed DOCA-salt hypertension when treated with the V₂ agonist desamino-D-AVP.⁵

In the present study, to assess further the role of V₁ and V₂ receptors in the development of mineralocorticoid hypertension, the effect of DOCA-salt on the regulation of V₁ and V₂ receptors and their second messengers, inositol phosphate and adenylate cyclase, respectively, in the liver and kidney has been investigated.

Methods

Experimental procedures used in this study were approved by the Austin Hospital Animal Research Ethics Committee according to the National Health and Medical Research Council of Australia guidelines for animal experimentation.

DOCA-Salt Hypertension

One hundred male Sprague-Dawley rats (approximately 250 g) were obtained from the Austin Hospital Animal Centre and divided into three treatment groups:
DOCA-salt, salt, and water. The DOCA-salt group received weekly injections of DOCA (Sigma Chemical Company, St. Louis, Mo.; 70 mg/kg body weight in peanut oil, i.m.) for 8 weeks with 1% NaCl solution to drink ad libitum. Salt-treated and water-treated control groups of rats received weekly injections of vehicle for 8 weeks and were given either 1% NaCl solution or water to drink ad libitum, respectively. Mean arterial blood pressure was measured weekly by the tail-cuff method.

Membrane Preparation

Rats were killed instantly by decapitation, and trunk blood was collected for measurement of plasma AVP and sodium concentration and osmolality. No anesthetic was used to prevent any anesthetic-induced effects on plasma AVP levels. The liver and kidney were weighed. Liver membranes were prepared by the method of Dickey et al. and renal medullary membranes were prepared as described previously by Marchingo et al.

Radioligands

A selective AVP V1 receptor antagonist, [1-(β-mercapto-β-cyclopentamethylene propionic acid), 7-sarcosine]-AVP ([d(CH2)5, sarcosine]-AVP) (Auspep, Melbourne, Australia), was radiosiodinated and purified as described previously. Specific activity was 2,955 Ci/mmol. A selective V2 antagonist radioligand, [Phe-3,4,5-3H]9-des-Gly-NH2-1-(β-mercapto-β-cyclopentamethylene propionic acid) ([d(CH2)5,sarcosine]-AVP) ([3H]des-Gly-NH2-d(CH2)5-d-Ileu4,l-Ileu5]-AVP), was obtained from Dupont Company, Boston, Mass. Specific activity was 66.7 Ci/mmol.

Arginine Vasopressin V1 and V2 Receptor Binding Studies

The number (Bmax) and affinity (Kd) of V1 binding sites in the liver and kidney were determined by incubating liver (60 µg) or kidney membranes (250 µg) from DOCA-salt-, salt-, and water-treated rats in a buffer containing 100 mM Tris, 10 mM MgCl2, 0.5 mg/ml bacitracin, 10 IU/ml aprotinin, and 0.1% bovine serum albumin (buffer 1, pH 7.4; Sigma) with U5 I-[3H]des-Gly-NH2-M(CH2)5[D-Ileu2,Ileu4]-AVP (0.1–2 nM) for 1 hour at 20°C. The bound and free ligands were separated by rapid filtration through Whatman GF/B filters (Maidstone, UK), and gamma radiation was measured in an LKB 1260 Multigamma II counter (Uppsala, Sweden).

Kidney medullary V1 receptor Bmax and Kd were measured by incubating 250 µg renal membranes in the buffer 1 with [3H]des-Gly-NH2-d(CH2)5-d-Ileu4,l-Ileu5]-AVP (0.1–10 nM) for 2 hours at 20°C. Membrane-bound radioactivity was collected by filtration through Whatman GF/B filters. The filters were dried and dissolved in scintillant (Filter-count, Packard Instruments Co. Inc., Downers Grove, Ill.) and beta radiation was measured in a Packard Tricarb 4530 counter.

In all cases specific binding was calculated as total binding minus nonspecific binding measured in the presence of 1 µM unlabeled AVP (Peninsula Laboratories, Belmont, Calif.). Specific binding was approximately 70% of total binding. Bmax and Kd were calculated by Scatchard analysis.

Adenylate Cyclase Activity

Renal medullary membranes (50 µg) from DOCA-salt-, salt-, and water-treated rats were preincubated in buffer 1 containing guanosine triphosphate (100 µM) and 3-isobutyl-1-methylxanthine (0.2 mM) (Sigma) in the presence (activated) or absence (basal) of AVP (1 µM) for 10 minutes at 37°C (total volume 100 µl). Activation of adenylate cyclase by NaF (10 mM) was used as a positive control. The adenylate cyclase reaction was started by the addition of adenosine triphosphate (1 mM) (Sigma) and stopped after 10 minutes of incubation at 37°C by the addition of 50 µl acetic acid (150 mM). The precipitated protein was removed by centrifugation and the supernatant was brought to pH 7.4 with 0.1 M NaOH. The cAMP produced by adenylate cyclase was measured by radioimmunoassay (Amer sham, Amersham, UK).

Inositol Phosphate Production

Liver and kidney medullary slices (approximately 5 mg) from DOCA-salt-, salt-, and water-treated rats were incubated in 250 µl Krebs buffer (mM: NaCl 120, KCl 3.5, KH2PO4 1.25, MgSO4 • 7H2O 1.2, CaCl2 • 2H2O 0.75, NaHCO3 25, glucose • H2O 10, MgCl2 5, gassed with 95% O2–5% CO2 mixture) containing 5 µCi [3H]inositol (Amersham) for 2 hours at 37°C. The tissue slices were then washed five times with Krebs buffer and incubated with 10 mM LiCl for 20 minutes following the addition of 1 µM AVP (stimulated) or Krebs buffer (basal) for 60 minutes at 37°C. Preliminary experiments showed that maximum production of tritiated total inositol phosphate was obtained after 60 minutes of stimulation with the agonist. The reaction was stopped with 10% trichloroacetic acid, the tissue was homogenized, and the precipitate was removed by centrifugation. The supernatant was brought to pH 7.4 and combined with 0.5 mM EDTA and 5 mM sodium borate and applied to a 1 ml AG-1 X8 formate column (Bio-Rad, Richmond, Calif.). The inositol phosphates were separated by the method of Berridge et al. The column was washed with 20 ml distilled water followed by 20 ml of 60 mM ammonium formate and 5 mM sodium borate to elute free inositol and glycerophosphoinositol fractions, respectively. Inositol-1-phosphate (IP1), inositol-2-phosphate (IP2), and inositol-3-phosphate (IP3) or total inositol phosphate were eluted with 20 ml of 1 M ammonium formate and 0.1 M formic acid and collected and counted for radioactivity. Total inositol phosphate was measured because of the rapid breakdown of IP3 to IP2 and IP1.

Analytical Methods

Plasma AVP was extracted using acetone and petroleum ether and measured by radioimmunoassay using a specific rabbit AVP antiserum. Plasma osmolality was determined with a Wescor Vapor Pressure Osmometer S100C (Logan, Utah), and plasma sodium was measured using an Instrumentation Laboratory System 501 ion-specific electrode (Milan, Italy). Membrane protein was measured by the method of Bradford with a gamma globulin standard.

Analysis of Data

Results were analyzed by t test or one-way analysis of variance using the Fisher post hoc comparison test as.
The mean arterial blood pressure of the DOCA-salt-treated (163±1 mm Hg) rats was significantly increased compared with the salt- (122±1 mm Hg) and water- (120±1 mm Hg; n=24; p<0.001) treated control groups (Figure 1). There was no change in the final body weight of the animals from the three treatment groups. However, there was an increase of approximately 50% in the kidney/body weight ratio of the DOCA-salt–treated rats (9.6±0.3 g/kg) compared with salt- (6.3±0.2 g/kg) and water- (6.1±0.2 g/kg; p<0.001) treated rats. By contrast, the liver weights were not affected by the DOCA-salt treatment.

The DOCA-salt–treated hypertensive animals had elevated plasma AVP levels (p<0.001). The plasma AVP was increased by approximately 70% when compared with the salt- and water-treated controls (Table 1). Plasma sodium and osmolality were also raised (p<0.05) in the DOCA-salt–treated hypertensive group compared with the salt- and water-treated control groups (Table 1).

When rat liver membranes were incubated with increasing concentrations of the selective AVP V1 receptor antagonist radioligand 125I-[d(CH2)5]sarcosine]-AVP, the number of hepatic AVP V1 binding sites in the DOCA-salt–treated animals was approximately 35% less than the salt- and water-treated animals (p<0.05).

### Table 1. Plasma Vasopressin, Sodium, and Osmolality of Water-, Salt-, and Deoxycorticosterone Acetate-Salt-Treated Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water</th>
<th>Salt</th>
<th>DOCA-salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin (pg/ml)</td>
<td>4.9±0.6</td>
<td>4.9±0.4</td>
<td>8.5±0.7*</td>
</tr>
<tr>
<td>Sodium (mM)</td>
<td>139±0.6</td>
<td>139±0.4</td>
<td>141±0.4</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>288±0.8</td>
<td>291±0.7</td>
<td>294±0.8*</td>
</tr>
</tbody>
</table>

DOCA, deoxycorticosterone acetate. Results are expressed as mean±SEM, n=24.

*p<0.001 versus salt- and water-treated rats.

The affinity of the receptor for the radioligand (K<sub>D</sub>) remained unchanged (Table 2). In contrast, the number of renal V1 binding sites was significantly increased (p<0.05) by DOCA-salt treatment (approximately 40%), but the K<sub>D</sub> again remained unaltered (Table 2).

Scatchard analysis of kidney membrane binding experiments using the selective AVP V1 antagonist radioligand ([3H]des-Gly-NH2-d(CH2)5[d-Ileu2,Ileu8]-AVP) demonstrated that the B<sub>max</sub> of the renal V1 receptors was reduced by approximately 35% (p<0.01) by DOCA-salt treatment with no change to the K<sub>D</sub> (Table 2).

If the B<sub>max</sub> values obtained using the selective V1 and V2 receptor antagonists are used to estimate the total number of renal AVP receptors, there was a significant decrease (p<0.05) in the number of AVP receptors in the DOCA-salt–treated hypertensive group when compared with the salt-treated group (Table 2).

In all experiments the production of cAMP by the renal membranes from the DOCA-salt–, salt–, and water-treated rats was increased in the presence of AVP (1 μM). However, the difference in cAMP production in the presence and absence of AVP was significantly reduced by the DOCA-salt treatment compared with the salt- and water-treated controls (p<0.01; Figure 2A), which is consistent with the downregulation of V2 binding sites. The percentage increase in AVP-stimulated cAMP production by the DOCA-salt–treated hypertensive animals (130±6%) was also reduced when compared with salt- (191±20%) and water- (185±19%) treated control animals, although the NaF-activated cAMP production (control) was similar in the three groups of animals, indicating that adenylyl cyclase was not non-specifically inactivated by DOCA-salt (Figure 2B).

Inositol phosphate levels in both the liver and kidney from the DOCA-salt–, salt–, and water-treated animals were increased by AVP (1 μM) in all experiments. However, the difference and the percentage increase in the inositol phosphate levels in the presence and absence of AVP by the DOCA-salt–, salt–, and water-
Discussion

In the present study rats developed hypertension during the 8-week period of treatment with DOCA-salt. This was associated with renal but not hepatic hypertrophy. These effects appear to be specific to mineralocorticoid treatment since these changes were not observed with animals treated with water or salt only. Similar changes to renal structure have been reported by others.21

The DOCA-salt–treated animals had elevated plasma sodium and osmolality levels, which is consistent with the observations that this model of hypertension is associated with increased renal sodium and water reabsorption22 and with expansion of extracellular fluid volume.23 The hypertensive animals also had elevated plasma AVP levels, a finding that has been reported previously by others1,2 and is probably due to an increased synthesis of AVP in the supraoptic and paraventricular nuclei of the hypothalamus.24

There was a decrease in the number of both AVP V1 receptors in the liver and V2 and total V1/V2 receptors in the kidney without any changes in the receptor affinity after chronic treatment with DOCA-salt. This is consistent with other studies, where DOCA-salt treatment also reduced the number of vascular AVP V1 receptors.25 The downregulation of the V1 and V2 receptors in these tissues appears to be directly controlled by the plasma AVP levels. In other studies using smooth muscle and kidney cell lines, which express V1 and V2 receptors, respectively, pretreatment with AVP induced a time- and concentration-dependent reduction in the number of AVP receptors.26,27 Similarly, in other physiological models where plasma AVP levels are raised such as dehydration, renal AVP receptors were also reduced.28

In contrast to hepatic V1 receptors, the number of renal V1 receptors was increased with no changes in receptor affinity in DOCA-salt–treated animals. Similarly, Swords et al.29 have observed recently that membranes from the brains of DOCA-salt–treated animals displayed an increased number of AVP receptors. These authors demonstrated that DOCA itself rather than AVP induced a direct increase in [3H]AVP binding
to cultured neuronal cells. Whether this control mechanism is also regulating renal \( V_1 \) receptors is unknown. However, the results obtained from the present study and that of Swords et al\(^{34} \) suggest that there are different cellular mechanisms regulating \( V_1 \) receptor levels in different tissues such as the liver, vasculature, kidney, and brain. Also, the number of \( V_1 \) and \( V_2 \) receptors found in the same tissue such as the kidney appear to be regulated by different mechanisms.

The downregulation of the number of renal medullary \( V_2 \) receptors in the DOCA-salt–treated hypertensive animals was associated with a reduction in the maximum level of the intracellular second messenger inositol phosphate turnover. This is probably also linked with the increase in plasma AVP concentration because Rajerison et al\(^{29} \) and Aiyar et al\(^{27} \) have reported that there was desensitization of cAMP responsiveness to AVP in renal medullary-papillary membranes and canine kidney cells pretreated with AVP. Yet other investigators were unable to detect any changes in AVP-stimulated cAMP levels in tubules (collecting ducts and loops of Henle) isolated from the renal medulla of DOCA-salt–treated animals.\(^{30} \) These findings are surprising since these structures contain a high density of \( V_2 \) receptors and are important sites of water reabsorption.\(^{31} \)

The maximum level of \( V_1 \) receptor–linked, inositol phosphate–stimulated responsiveness to AVP in the liver and kidney was not affected by DOCA-salt treatment. These findings would suggest that in these animals, the maximum level of inositol phosphate turnover was not regulated directly by changes in number of \( V_1 \) receptors. Although other workers have detected attenuated inositol phosphate responsiveness to AVP when smooth muscle cells were pretreated with AVP,\(^{26} \) our results indicate maximum inositol phosphate turnover in DOCA-salt–treated hypertensive animals was not controlled by plasma AVP levels. Therefore, the results obtained in the present study may be due to physiological effects of DOCA-salt hypertension and may involve changes to post-receptor regulation or alternatively indicate the presence of spare receptors that are not directly linked to inositol phosphate turnover.

The effects of DOCA-salt on \( V_1 \) and \( V_2 \) receptor density and maximum second messenger levels alone cannot explain the development of mineralocorticoid hypertension. Plasma AVP concentrations of the control and DOCA-salt–treated hypertensive animals were substantially below that required to saturate the AVP receptors. Therefore, in vivo the effect of increased plasma AVP levels to increase directly second messenger levels in the DOCA-salt–treated animals compared with the control animals might be greater than the effect of downregulation of \( V_2 \) receptors with subsequent reduction of adenylate cyclase activity. If this is the case, the increase in in vivo levels of AVP-stimulated adenylate cyclase in the DOCA-salt–treated animals would contribute to increased water and sodium reabsorption, which are characteristic of this model of hypertension and account for the essential role of AVP and \( V_2 \) receptors in the development of DOCA-salt hypertension.\(^{5,13} \) Although the effects of \( V_1 \) antagonists on blood pressure in DOCA-salt hypertension are small, it is also likely that an increase in vivo responsiveness of inositol phosphate turnover might contribute to increased AVP-mediated vasoconstriction and total peripheral resistance.

Another possible contribution to the hypertension of the DOCA-salt model is reduced vasodilatation mediated by \( V_2 \) receptors. Since AVP influences blood pressure by a direct \( V_1 \)-mediated vasoconstriction and a \( V_2 \)-mediated vasodilatation, the \( V_2 \) receptor downregulation observed here may be important in allowing a relatively greater \( V_1 \) vasopressor effect in DOCA-salt hypertension. Unfortunately, the site of \( V_2 \) vasodilator receptors is unknown,\(^{32} \) limiting specific studies to investigate whether the renal \( V_2 \) receptor downregulation seen here also occurs with the vasodilator \( V_2 \) receptors.

References


Regulation of vasopressin receptors in deoxycorticosterone acetate-salt hypertension.
D Trinder, P A Phillips, J Risvanis, J M Stephenson and C I Johnston

Hypertension. 1992;20:569-574
doi: 10.1161/01.HYP.20.4.569

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/20/4/569

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/