Blood Pressure-Lowering Effect of an Orally Active Vasopressin V1 Receptor Antagonist in Mineralocorticoid Hypertension in the Rat


Abstract

We studied the contribution of vasopressin to the maintenance of high blood pressure in deoxycorticosterone acetate (DOCA)-salt hypertension in the rat using the non-peptide orally effective vasopressin V1 receptor antagonist OPC-21268. Binding kinetic studies demonstrated that oral OPC-21268 (30 mg/kg) acted as a competitive antagonist at the vasopressin V1 receptor in DOCA-salt and salt control rats. Basal mean intra-arterial blood pressure was 140±4 mm Hg (n=12) in DOCA-salt rats compared with 111±2 mm Hg in salt control rats (n=18). Acute oral OPC-21268 (30 mg/kg) significantly (P<.01) reduced mean intra-arterial pressure in DOCA-salt hypertension, with an average maximal decrease of 24±3 mm Hg occurring at 2.5±0.7 hours after dosing. Systolic blood pressure (tail-cuff) in DOCA-salt rats was 178±2 mm Hg. Chronic oral OPC-21268 (30 mg/kg) twice daily for 7 days significantly (P<.01) reduced mean intra-arterial pressure in DOCA-salt hypertensive rats. Basal mean intra-arterial blood pressure was 140±4 mm Hg in DOCA-salt rats (n=20) was unchanged by chronic oral OPC-21268 (30 mg/kg twice daily for 7 days), and this was confirmed by direct measurement of mean intra-arterial pressure. After chronic oral OPC-21268 (30 mg/kg twice daily for 7 days) hepatic V1 receptor binding was significantly reduced for up to 10 hours (P<.05). The results of this study suggest that vasopressin does not play a major role in the regulation of normal blood pressure in the rat but support a role for vasopressin in the maintenance of mineralocorticoid hypertension in the rat. OPC-21268 may be of use in the treatment of hypertensive conditions associated with elevated vasopressin concentrations.

Key Words: hypertension, experimental; blood pressure; vasopressins; mineralocorticoids; OPC-21268

Although most studies of DOCA-salt hypertension and AVP receptor blockade have been acute because of the peptide nature of available AVP antagonists, such initial responses to blockade of homeostatic systems may not predict long-term responses. The development of the nonpeptide AVP V1 receptor antagonist OPC-21268 provides a new tool that can be used to assess the effects of both acute and chronic AVP V1 receptor blockade in DOCA-salt hypertension. Characterization of OPC-21268 has shown it to be an orally effective, selective, competitive V1 receptor antagonist with little effect at the V2 receptor.

The aim of this study was to examine the role of AVP in the maintenance of high BP in DOCA-salt hypertension using the nonpeptide AVP V1 receptor antagonist OPC-21268. First, the effects of OPC-21268 on hepatic and renal AVP V1 receptor binding kinetics were studied to determine the accessibility of orally administered OPC-21268 to its putative site of action. Next, the effects of acute and chronic AVP V1 receptor blockade on BP were studied in DOCA-salt hypertensive rats. Finally, the effects of chronic AVP V1 blockade on BP regulation and AVP V1 binding in normotensive rats were investigated.

Methods

Experimental procedures were approved by the Austin Hospital Animal Research Ethics Committee and performed according to the National Health and Medical Research Council of Australia guidelines for animal experimentation. OPC-21268 (1-[4-[(3-acetylaminopropoxy)benzoyl]-4-piperidyl]-3,4-dihydro-2(1H)-quinolinone) was a gift from the Otsuka Pharmaceutical Co Ltd. The selective AVP V1 recep-
tor antagonist [1-(β-mercapto-β,β-cyclopentamethylene propionic acid),7-sarcosine]AVP ([d(CH2)5, sarcosine]AVP) was obtained from Auspep. Ethrane was obtained from Abbott Australasia Pty Ltd and pentobarbital from Arnoinds Pty Ltd. AVP was purchased from Peninsula Laboratories Inc. Aprotinin, bacitracin, Tris-HCl, and DOCA were obtained from Sigma Chemical Co. Bovine serum albumin was obtained from CSL Ltd. All other reagents were obtained from either BDH or Ajax Chemicals.

Animals

Female adult Sprague-Dawley rats (150 to 250 g) obtained from the Austin Hospital animal house were used in all experiments. Animals were housed at 20°C with a 12-hour light/dark cycle and ad libitum food containing 0.4% to 0.6% NaCl (Norco). For experiments 1, 2, and 3, rats were divided into two treatment groups: DOCA-salt and salt controls. The DOCA-salt group received twice weekly subcutaneous injections of DOCA in peanut oil, 70 mg/kg body wt, for 10 weeks with 1% saline to drink ad libitum. Salt controls received twice-weekly subcutaneous injections of peanut oil for 10 weeks with 1% saline to drink ad libitum. For experiments 4, 5, and 6, rats were housed as above and received similar food but had tap water to drink and no subcutaneous injections (water control group).

Blood Pressure Measurements

Systolic blood pressure (SBP) was measured by the indirect tail-cuff technique (W&K recorder, model 800S). Animals were preheated in a 37°C warming chamber and lightly restrained during measurement. For direct intra-arterial mean arterial pressure (MAP) recording, rats underwent brief ether anesthesia before intubation and ventilation with a mixture of ethrane and oxygen. A polyethylene cannula (PE-50) was inserted into the left carotid artery and exteriorized in the interscapular area. Rats were allowed to recover for 24 hours with free access to food and 1% saline (experiment 2) or tap water (experiment 6). On the study day conscious rats were weighed, and a BP transducer (model DPT 3003-S, Peter von Berg) was calibrated and attached to the intra-arterial catheter. Transducer signals were preamplified (model 7C, Grass Instrument Co) before analog-digital conversion (MacLab/8TM, Analog Digital Instruments Pty, Ltd) for data recording and storage.

Membrane Preparation

Rats were killed by decapitation, and kidneys and liver were removed. Kidney medulla13 and liver14 membranes were prepared by previously described methods and stored in liquid nitrogen before use within 48 to 72 hours.

Radioligands

The selective AVP V1 receptor antagonist [d(CH2)5, sarcosine]AVP was iodinated and purified as previously described15: its specific activity was 2394 Ci/nmol.

Characterization of OPC-21268

Characterization of OPC-21268 has shown it to be an orally effective, selective, competitive V1 receptor antagonist with little effect at the V2 receptor. OPC-21268 caused a concentration-dependent displacement of the selective V1 receptor antagonist radioligand 125I-[d(CH2)5, sarcosine]AVP to V1 receptors in both rat liver and kidney membranes (IC50, 40±0.3 nmol/L liver; 150±0.2 nmol/L kidney) but had little effect on the selective V2 antagonist radioligand [H]des-Gly-NH2-d(CH2)3[β-Ile8,Lle9]AVP binding to V2 receptors in renal membranes (IC50, >100 μmol/L). In vivo time-course and dose-response studies demonstrated that oral OPC-21268 was an effective hepatic and renal V1 receptor antagonist.

Analytic Methods

Plasma AVP was extracted with acetone and ether and measured by radioimmunoassay (interassay and intra-assay coefficients of variation both <8%) using a specific rabbit AVP antiserum. Plasma osmolality was measured with a Wescor Vapor Pressure Osmometer 5100C. Membrane protein was measured by the method of Bradford with γ-globulin as standard.

Experiments

Experiment 1: Effect of Acute Oral OPC-21268 on In Vivo Binding Kinetics in DOCA-Salt Hypertension

The in vivo effects of OPC-21268 on binding site density (Bmax) and the apparent affinity (Kd) of the V1 receptor were determined in rat liver and kidney medulla membranes by analysis of the saturation binding of the selective V1 receptor antagonist radioligand 125I-[d(CH2)5, sarcosine]AVP.

DOCA-salt hypertensive rats (n=26) and salt control rats (n=26) were gavaged daily with vehicle (5% arabic gum) for 3 days before the study to accustom them to the procedure. On the study day rats were weighed, gavaged with vehicle (DOCA, n=13; salt control, n=13) or OPC-21268 (30 mg/kg) (DOCA, n=13; salt control, n=13), and killed 1 hour later. Trunk blood was collected for the measurement of plasma osmolality and AVP concentrations, and liver and kidney tissue was collected for membrane preparation.

The Bmax and apparent Kd values of the V1 receptor were determined by incubating liver (60 μg) or kidney (250 μg) membranes from DOCA-salt+OPC-21268, DOCA-salt+vehicle, salt control+OPC-21268, and salt control+vehicle rats in a buffer containing 100 mmol/L Tris-HCl, 10 mmol/L MgCl2, and 0.1% bovine serum albumin (buffer A, pH 7.4) with 0.5 mg/mL bacitracin and 100 IU/mL aprotinin (buffer B) with 125I-[d(CH2)5, sarcosine]AVP (liver, 0.1 nmol/L; kidney, 2 nmol/L) and increasing concentrations of cold peptide for 1 hour at 20°C. Bound and free ligands were separated by rapid filtration through a glass microfiber filter (Whatman GF/B) using a Brandel automatic filtration apparatus. The tubes and filters were washed three times with 2.5 mL ice-cold buffer A. Gamma radiation was measured in an LKB 1260 Multigamma 11 counter. Specific binding was calculated as total counts minus nonspecific binding in the presence of 1 μmol/L unlabeled AVP (Peninsula Laboratories). Specific binding was approximately 70% of total binding. Bmax and Kd values were calculated by Scatchard analysis.

Experiment 2: Effect of Acute Oral OPC-21268 on Direct MAP in DOCA-Salt Hypertension

DOCA-salt hypertensive rats (n=12) and salt control rats (n=18) were gavaged daily with vehicle for 3 days before the study to accustom them to the procedure. An intra-arterial cannula was inserted as previously described, and rats were allowed to recover for 24 hours. Rats were fully conscious and freely moving throughout the study, with access to food and 1% saline drinking water. Baseline measurements of MAP were taken once the rats were settled and BP was stable. The rats were randomly allocated to receive either OPC-21268 (30 mg/kg) (DOCA-salt, n=6; salt control, n=9) or vehicle (DOCA-salt, n=6; salt control, n=9) by oral gavage. MAP recordings were made continuously for 7 hours after gavage and the rats were killed with a lethal intra-arterial injection of pentobarbital (50 mg/kg).

Experiment 3: Effect of Chronic Oral OPC-21268 on Indirect SBP in DOCA-Salt Hypertension

DOCA-salt hypertensive rats (n=17) were gavaged daily with vehicle for 3 days before the study to accustom them to the procedure. Baseline measurements of indirect tail-cuff SBP were taken on three separate occasions before the start of
the study. For statistical analysis the average of the three readings was taken to give a single pressure determination.

The DOCA-salt hypertensive rats were weighed and randomly allocated to receive twice-daily gavage with OPC-21268 (30 mg/kg) (n=9) or vehicle (n=8). On days 1, 4, and 7, SBP was measured 1 hour after gavage by an independent observer unaware of which treatment had been administered. After 7 days gavage was stopped, but SBP was measured daily for the next 5 days. Rats continued to receive subcutaneous injections of DOCA during the period of the experiment. On day 12 animals were killed, and trunk blood was collected for measurement of plasma osmolality and AVP concentration.

**Experiment 4: In Vitro Effect of OPC-21268 on Mesenteric Vascular Resistance in Water Control Rats**

Water control rats were killed by decapitation, and the small intestine and mesenteric vasculature were removed. Resistance vessels of the first- or second-order branch of the superior mesenteric artery were dissected, and vessels of approximately 150 to 300 μm in external diameter were mounted in a microvascular myograph. The chamber was filled with physiological salt solution (PSS) containing (mmol/L) NaCl 119, NaHCO3 14.9, KCl 4.7, KH2PO4 1.18, MgSO4 1.17, CaCl2 2.5, glucose 5.5, and EDTA 0.026 at 37°C aerated with 5% CO2 and 95% O2. The potassium solution (KPSS) was made by equimolar substitution of KCl for NaCl in PSS. Two mesenteric resistance arteries were tested in each experiment. After the medial cross-sectional area was determined, the vessel was set to 90% of the tension for a transmural pressure of 100 mm Hg. After the normalization procedure the vessels equilibrated for 1 hour, and the viability of the vessels was assessed by exposure to KPSS. Vessels with a contraction of less than 3 mN/mm were rejected. Contraction to KPSS was used as the standard, and responses to AVP and OPC-21268 were expressed as a percentage of the KPSS response. Vessels were exposed to graded concentrations of either AVP (10 pmol/L to 1 μmol/L) or OPC-21268 (10 pmol/L to 1 μmol/L) in a random order. Mean curves were obtained from five vessel segments from different rats.

**Experiment 5: Effect of Chronic Oral OPC-21268 on Indirect SBP in Water Control Rats**

Water control rats were gavaged daily with vehicle for 3 days to accustom them to the procedure, and baseline measurements of indirect tail-cuff SBP were taken on three separate occasions before the start of the study. Rats were then weighed and randomly allocated to receive twice-daily gavage with OPC-21268 (30 mg/kg) (n=10) or vehicle (n=10). On days 1, 3, 5, and 7, SBP was measured 1 hour after gavage by an independent observer. After 7 days of treatment rats were killed, and blood was collected for the measurement of plasma osmolality and AVP concentration.

**Experiment 6: Effect of Chronic Oral OPC-21268 on Direct MAP in Water Control Rats**

Water control rats were weighed daily and gavaged twice daily with OPC-21268 (30 mg/kg) for 7 days. On day 6 an intra-arterial cannula was inserted as previously described, and rats were allowed to recover for 24 hours. The next day rats were fully conscious and freely moving with access to food and normal drinking water and continued to receive OPC-21268. After weighing and baseline measurements of direct MAP, rats were randomly allocated to receive either OPC-21268 (30 mg/kg) (n=5) or vehicle (n=5) by oral gavage. MAP recordings were made for the next 7 hours, and the rats were killed with a lethal intra-arterial injection of pentobarbital (50 mg/kg).

<table>
<thead>
<tr>
<th>Group</th>
<th>Osmolality, mOsm/kg</th>
<th>AVP, pg/mL</th>
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<tr>
<td>Salt control</td>
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<td></td>
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<tr>
<td>Vehicle</td>
<td>285±2</td>
<td>13±1</td>
</tr>
<tr>
<td>OPC-21268</td>
<td>287±2</td>
<td>13±1</td>
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<tr>
<td>DOCA-salt*</td>
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<td>Vehicle</td>
<td>292±2</td>
<td>21±2</td>
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<tr>
<td>OPC-21268</td>
<td>294±4</td>
<td>16±2</td>
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</table>

AVP indicates arginine vasopressin; DOCA, deoxycorticosterone acetate. Rats received 30 mg/kg OPC-21268 and were killed at 1 hour. Values are mean±SEM; n=13 per study.

*P<.05 DOCA-salt rats compared with salt control rats. OPC-21268 had no significant effect on plasma AVP or osmolality compared with vehicle in either DOCA-salt or salt control groups.

**Experiment 7: Effect of Chronic Oral OPC-21268 on In Vivo Inhibition of OPC-21268 at V1 Receptor**

Water control rats were weighed daily and gavaged twice daily with OPC-21268 (30 mg/kg) (n=24) or vehicle (n=4) for 7 days. Vehicle-treated rats were killed after the final gavage, and OPC-21268-treated rats were killed at 1, 4, 8, 10, 16, and 24 hours after final gavage (n=4 per time point). Trunk blood was collected for the measurement of plasma osmolality concentrations and liver collected for membrane preparation.

The number of hepatic V1 binding sites was determined by incubating membranes (60 μg) with 125I-[d(CH2)5], sarcosine]AVP (0.5 nmol/L) in the presence or absence of unlabeled AVP (1 μmol/L) for 1 hour at 20°C. The results are expressed as the change from binding in vehicle-treated rats.

**Statistics**

Results are presented as mean±SEM. Statistical analyses were performed using Student's unpaired t test or ANOVA followed by analysis of simple main effects and the Newman-Keuls test as appropriate. Significant differences were obtained at a value of P<.05.

**Results**

BP increased in all rats treated with subcutaneous DOCA and 1% saline drinking solution over the 10-week treatment period. By week 8 SBP reached 179 to 193 mm Hg and remained at this level over the next 2 weeks. The general condition of DOCA-salt rats was good throughout the study and was no different from that of salt-control animals. The DOCA-salt hypertensive rats continued to gain weight during the study. These observations indicate that the rats had established hypertension but were not in the malignant phase.

**Experiment 1: Effect of Acute Oral OPC-21268 on In Vivo Binding Kinetics in DOCA-Salt Hypertension**

There was no significant difference in the body weight of DOCA-salt rats (26±8 g, n=26) compared with salt control rats (26±7 g, n=26). Mean SBP in DOCA-salt rats (170±5 mm Hg, n=26) was significantly higher than in salt control rats (128±1 mm Hg, n=26) (P<.01). Both plasma AVP concentrations and plasma osmolality were significantly elevated in DOCA-salt compared with salt control rats (P<.05) (Table 1). There was no effect of OPC-21268 compared with vehicle on plasma osmolality.
TABLE 2. Vasopressin V1 Receptor Binding Kinetics After Acute Oral Gavage With OPC-21268 or Vehicle in DOCA-Salt and Salt Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Kᵦ, nmol/L</th>
<th>Bₘₐₓ, fmol/mg</th>
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<td>Liver</td>
<td></td>
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<tr>
<td>Salt control</td>
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<tr>
<td>Vehicle</td>
<td>1.0±0.2</td>
<td>251±19</td>
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<tr>
<td>OPC-21268</td>
<td>4.2±0.5*</td>
<td>276±32</td>
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<tr>
<td>DOCA-salt</td>
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<tr>
<td>Vehicle</td>
<td>1.0±0.1</td>
<td>224±12</td>
</tr>
<tr>
<td>OPC-21268</td>
<td>4.2±0.3*</td>
<td>276±20</td>
</tr>
<tr>
<td>Kidney</td>
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<tr>
<td>Salt control</td>
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<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.2±0.1</td>
<td>46±6</td>
</tr>
<tr>
<td>OPC-21268</td>
<td>1.7±0.3</td>
<td>52±7</td>
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<tr>
<td>DOCA-salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.1±0.1</td>
<td>51±6</td>
</tr>
<tr>
<td>OPC-21268</td>
<td>1.4±0.2</td>
<td>47±6</td>
</tr>
</tbody>
</table>

DOCA indicates deoxycorticosterone acetate. Rats received 30 mg/kg OPC-21268 and were killed at 1 hour. Values are mean±SEM; n=13 per study.

*P<.05, OPC-21268 compared with vehicle in salt control and DOCA-salt rats.

AVP concentrations and plasma osmolality in either group.

Table 2 shows the effect of orally administered OPC-21268 (30 mg/kg) on apparent Kᵦ and Bₘₐₓ of V₁ binding sites in rat liver and kidney membranes in DOCA-salt and salt control rats. Oral OPC-21268 led to a significant increase in the apparent Kᵦ (P<.05) of the liver V₁ receptor in both DOCA-salt and salt control rats. There was no change in liver Bₘₐₓ. Neither the Bₘₐₓ nor Kᵦ of kidney V₁ binding sites in DOCA-salt or salt control rats was significantly altered by treatment with OPC-21268.

**Experiment 2: Effect of Acute Oral OPC-21268 on Direct MAP in DOCA-Salt Hypertension**

The baseline direct MAP of DOCA-salt rats (140±4 mm Hg, n=12) was significantly higher than in salt control rats (111±2 mm Hg, n=18) (P<.01). Oral OPC-21268 (30 mg/kg) had a significant antihypertensive effect in DOCA-salt hypertensive rats (P<.01). The results are shown in Fig 1a and are expressed as the change from basal MAP. The average maximal fall in MAP in the DOCA-salt group treated with OPC-21268 was 24±3 mm Hg, and the average time to the maximal effect was 2.5±0.7 hours. The slight increase in MAP in the salt control rats treated with OPC-21268 did not reach statistical significance.

**Experiment 3: Effect of Chronic Oral OPC-21268 on Indirect SBP in DOCA-Salt Hypertension**

The baseline indirect SBP in DOCA-salt-treated rats was 178±2 mm Hg (n=17). The effect of oral OPC-21268 (30 mg/kg) on SBP is shown in Fig 1b, with the results expressed as the change from basal SBP. Treatment with OPC-21268 significantly lowered SBP compared with vehicle (P<.01) over the 7-day treatment period. The average maximal fall in SBP was 27±5 mm Hg. When treatment with oral OPC-21268 was stopped, SBP slowly rose back to basal SBP.

Chronic treatment with OPC-21268 (30 mg/kg twice daily for 7 days) had no significant effects on body weight compared with vehicle over the period of the experiment (day 1: OPC-21268, 252±7 g; vehicle, 247±5 g; day 7: OPC-21268, 262±6 g; vehicle, 268±5 g). There were no significant differences in plasma osmolality or AVP concentration in the OPC-21268-treated versus vehicle-treated groups of DOCA-salt hypertensive rats (data not shown). The similar body weights and biochemical parameters in both groups indicate similar sodium intake and do not suggest a differential effect of OPC-21268 on sodium balance.

**Experiment 4: In Vitro Effect of OPC-21268 on Mesenteric Vascular Resistance**

The mean internal diameter of the rat mesenteric resistance arteries was 280±13 μm at an equivalent transmural pressure of 100 mm Hg. AVP caused a
Hypotensive Effect of OPC-21268 in DOCA-Salt Hypertension

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**Experiment 5: Effect of Chronic Oral OPC-21268 on Indirect SBP in Water Control Rats**

The baseline SBP in water control rats was 120±1 mmHg (n=20). Chronic oral OPC-21268 (30 mg/kg twice daily for 7 days) had no effect compared with vehicle on SBP as shown in Fig 3a. The results are expressed as the change from basal SBP. Chronic treatment with OPC-21268 had no significant effects on body weight compared with vehicle (day 1: OPC-21268, 138±3 g; vehicle, 138±4 g; day 7: OPC-21268, 163±3 g; vehicle, 166±4 g) nor any effect on biochemical parameters (plasma osmolality: OPC-21268, 281±1 mOsm/kg; vehicle, 281±1 mOsm/kg; plasma AVP: OPC-21268, 6.7±0.4 pg/mL; vehicle, 6.8±0.7 pg/mL). The higher plasma AVP and plasma osmolality measured in the DOCA-salt hypertensive and salt control rats (Table 1) reflect the consumption of 1% saline drinking solution.

**Experiment 6: Effect of Chronic Oral OPC-21268 on Direct MAP in Water Control Rats**

Neither oral OPC-21268 (30 mg/kg) nor vehicle had significant direct MAP-lowering effects in water control rats treated chronically with OPC-21268 (30 mg/kg twice daily for 7 days). Fig 3b shows the results expressed as the change from baseline intra-arterial MAP of 99±4 mm Hg (n=10).

**Experiment 7: Effect of In Vivo Chronic Oral OPC-21268 Treatment on In Vitro AVP Binding**

Fig 3c shows the effect of chronic OPC-21268 (30 mg/kg twice daily for 7 days) on the in vitro binding of $^{125}$I-[d(CH$_2$)$_3$]sarcosine$^1$AVP to hepatic V1 receptors in water control rats. OPC-21268 caused a significant reduction in the V1 binding at 1, 4 ($P<.01$), and 8 ($P<.05$) hours after the final dose of OPC-21268 but had no effect on plasma sodium or osmolality at any time compared with vehicle-treated rats.

**Discussion**

This report demonstrates the antihypertensive effect of the orally active, nonpeptide, selective vasopressin V1 antagonist OPC-21268 and indicates a pressor role for AVP in mineralocorticoid hypertension in the rat. In addition, chronic AVP V1 blockade did not alter BP in water control rats, suggesting that AVP does not play a major role in normal BP regulation in the rat.
These results are consistent with some but not all previous acute studies of intravenous AVP V1 blockade in DOCA-salt hypertension. In general, DOCA-salt-treated rats have been studied in the malignant phase of hypertension, which is associated with the complicating influences of escalating BP, weight loss, markedly elevated AVP levels, and increased mortality. The results of the present study are more easily interpretable because the DOCA-salt-treated rats had established hypertension but were not in the malignant phase. This is the first report of the antihypertensive efficacy of chronic oral AVP V1 receptor blockade in established DOCA-salt hypertension. Such chronic studies are important because the short-term effects of receptor blockade do not necessarily predict long-term responses.

The nonpeptide OPC-21268 is known to be an orally effective, competitive V1 receptor AVP analogue, with little effect at the V2 receptor. The V1 antagonist properties of OPC-21268 have been confirmed in studies in conscious rats, dogs, and humans. In the present study acute oral OPC-21268 (30 mg/kg) caused a significant reduction in MAP in conscious DOCA-salt hypertensive rats. The response, which was maximal at approximately 2 hours and persisted for up to 4 hours, agrees with our previous findings of the inhibitory effect of oral OPC-21268 (30 mg/kg) on liver V1 receptor binding. Although OPC-21268 caused a nonsignificant rise in MAP in the salt control group, suggesting it may act as a partial agonist, this was not confirmed by in vitro rat resistance vessel myographic studies. Also, preincubation of rat resistance arteries with OPC-21268 caused significant antagonism of AVP-induced vasoconstriction (unpublished data).

The antihypertensive effect of oral OPC-21268 was confirmed after chronic treatment with OPC-21268 (30 mg/kg twice daily for 7 days). On day 8, when indirect SBP was measured 16 hours after the last gavage with OPC-21268, SBP was still significantly lower than baseline values (13±3 mm Hg below baseline). The antihypertensive effect of OPC-21268 on SBP was gradually reversed 5 days after OPC-21268 was stopped, supporting the conclusion that elevated endogenous AVP levels were contributing to hypertension and that OPC-21268 treatment was responsible for lowering the BP.

It is likely that OPC-21268 lowers BP by antagonism at the vascular V1 receptor site, although the specific mechanism remains to be determined. Certainly, the results from the binding kinetic study in both DOCA-salt hypertensive and salt control rats demonstrate that oral OPC-21268 is absorbed and acts competitively at V1 receptors. Although a similar effect on kidney V1 receptor binding was not seen, these results are consistent with previous findings in the rat which demonstrated that the maximum effect of OPC-21268 on kidney V1 receptor binding in vivo was in fact at 30 minutes rather than 1 hour. In vitro binding kinetic studies confirm that OPC-21268 acts as a competitive antagonist at the kidney V1 receptor as effectively as in the liver. The differences in time courses of inhibition of binding sites may reflect differences in tissue distribution or partial agonism of the drug. Alternatively, differences in the structure-activity relation between the V1 receptor in the liver and kidney.

It is of interest that the antihypertensive effect of OPC-21268 appeared to outlast its antagonism at AVP V1 receptors in the DOCA-salt hypertensive rats. This suggests that OPC-21268 is acting through additional mechanisms to its effects at the vascular V1 receptor, particularly as chronic oral OPC-21268 in water control rats inhibited V1 receptor binding for only 10 hours. AVP-like immunoreactivity has been demonstrated in blood vessels, so it is possible that OPC-21268 acts at the tissue level to inhibit the effect of locally produced AVP. Alternatively, peripherally administered OPC-21268 may have effects within the central nervous system to modulate BP. The permeability of the blood-brain barrier to oral OPC-21268 is not known.

Several other explanations for the antihypertensive effect of OPC-21268 exist. Antidiuretic effects of AVP mediated by the renal V2 receptor with consequent volume expansion may be involved in DOCA-salt hypertension. It has been shown that DOCA-salt hypertension can be prevented using a V1/V2 receptor antagonist (albeit at the expense of increased mortality), although in other studies dDAVP had less effect compared with AVP in increasing BP in Brattleboro rats treated with DOCA and salt. It is unlikely that the antihypertensive effect of OPC-21268 is due to renal V2 receptor antagonism, given the lack of effect of OPC-21268 on the V2 receptor in vitro. OPC-21268 was 100 times less potent than AVP for the liver and kidney V1 receptor but approximately 10 000 times more selective for V1 receptors than kidney V2 receptors. Furthermore, we have treated a group of DOCA-salt hypertensive rats with oral OPC-31260 (30 mg/kg), an AVP V2 receptor antagonist. Selective AVP V2 receptor blockade caused significant diuresis, weight loss, hypernatremia, and mortality. The consequent increase in plasma AVP levels may have led to further vasoconstriction and accounted for the finding that indirect SBP did not change (unpublished observations). Thus, the contribution of the tubular effects of AVP to DOCA-salt hypertension remains unclear.

In some species AVP acts not only as a powerful vasoconstrictor via V1 receptors but also as a vasodilator via extrarenal V2 receptors. In the dog the selective V2 agonist dDAVP causes vasodilation independently of the kidneys. After V1 receptor blockade in the normotensive dog, exogenous AVP exhibits vasodilator hemodynamic effects probably mediated by extrarenal V2 receptors. These findings are relevant in the interpretation of the antihypertensive effect of OPC-21268, in that blocking the vasoconstrictor V1 receptor may unmask the effect of endogenous AVP on a vasodilator V2 receptor. As the site of the vasodilator V2-like receptor is unknown and extrarenal V2 receptors have yet to be convincingly demonstrated, any role of such receptors in the antihypertensive response to OPC-21268 is unclear.

In conclusion, the results indicate that AVP does not play a major role in BP regulation in normotensive rats and provide evidence for a pressor role for AVP in the maintenance of DOCA-salt hypertension in the rat. These findings suggest that OPC-21268 has potential as a therapeutic agent in conditions associated with elevated plasma AVP concentrations that may be causing vasoconstriction via V1 receptors.
Acknowledgments

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References

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