Pharmacological Profile of VP-343, a Novel Selective Vasopressin V2 Receptor Antagonist, in Rats

Akira Naito, a Yasuhiro Ohtake, a Hisashi Hasegawa, a Yasuhiro Fukaya, a Takashi Kurasa, a Kenji Naito, a Hidehiko Matsukawa, a Touro Oguma, a Yohji Ezure, a Yoshihiro Tsuriya, a Hikaru Tanaka, b Katsuko Koike, b and Koki Shigenobu b

Sagami Research Laboratory, Wakamoto Pharmaceutical Co., Ltd, 378 Kanade, Ohi-machi, Ashigarakamigun, Kanagawa 258-0018, Japan and Toho University School of Pharmaceutical Sciences, 1 Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan. Received May 12, 1999; accepted October 18, 1999

The pharmacological profile of a novel selective vasopressin V2 receptor antagonist, VP-343(N-[4-[(2S,3aR)-2-hydroxy-2,3,3a,4-tetrahydropyrrolo[1,2-a]quinazolin-5(1H)-yl]carbonyl][phenyl]-4'-methyl[1,1'-biphenyl]-2-carboxamide) was characterized in several in vitro and in vivo rat models. The IC50 values of VP-343 for vasopressin V1a and V2 receptors were 110 and 0.77 nm, respectively, VP-343 inhibited dose-dependently the pressor response to exogenous arginine vasopressin (AVP; 30 mU/kg, i.v.) in pithed rats, with an ID50 value of 0.57 mg/kg (i.v.). VP-343 induced strong aquaregia in normal saline-loaded conscious rats. Antidiuretic activities of VP-343 have not yet been detected in AVP deficient Brattleboro rats, showing its lack AVP V2 agonistic activity. During repeated administration for 21 d (3 mg/kg, p.o.) and after recovery, the aquaretic action of VP-343 still remained. In the aged (17 month) saline-loaded conscious rats study, VP-343 (3 mg/kg, p.o.) exhibited remarkable diuretic action. In a single dose oral toxicity study in mice, VP-343 did not produce any clinical signs and mortality at any of the tested doses.

The results indicate that VP-343 is a potent, orally active, selective V2 receptor antagonist, suggesting that it can be expected to be useful as an aquaretic drug.

Key words VP-343; vasopressin; vasopressin V1 receptor; nonpeptide antagonist; aquaregia; diuretic

Arginine vasopressin (AVP) has a variety of physiological actions, including vasconstriction, stimulation of platelet aggregation, hepatocyte glycogenolysis, corticotropin release stimulation and antidiuresis caused by in the binding of AVP to its specific receptors. The AVP receptors have been classified into at least three subtypes (V1A, V1B, and V2). V1A receptors have been found in several tissues such as liver, platelets, vascular smooth muscle cells and mesangial cells. V1B receptor has been found in kidney and V1B mainly in anterior pituitary. V2 receptors are coupled to phosphoinositol hydrolysis followed by the elevation of intracellular Ca2+ and V2 activation stimulates the coupled adenylyl cyclase, leading to increased cyclic AMP levels.

AVP may play roles in several disease conditions, including congestive heart failure, hypertension, edema, hyponatremia and the syndrome of inappropriate antidiuretic hormone secretion. Therefore, AVP antagonists may be useful for the treatment of these diseases. Although several peptide vasopressin antagonists have been developed as therapeutic drugs, their partial agonistic activity and lack of oral bioavailability have restricted their clinical and therapeutic usefulness. Orally effective nonpeptide AVP antagonists (OPC-31260, a selective V2 receptor antagonist; YM087, a dual V1A and V2 receptor antagonist; OPC-21268, a selective V1A receptor antagonist) were recently developed. Oral administration of OPC-31260 induced marked aquarexia, which inevitably led to significant increases in serum osmolality and plasma AVP. YM087 inhibited V1A receptor-mediated pressor response and V2 receptor-mediated antidiuretic response.

We previously reported novel and potent vasopressin receptor antagonists, including VP-343(N-[4-[(2S,3aR)-2-hydroxy-2,3,3a,4-tetrahydropyrrolo[1,2-a]quinazolin-5(1H)-yl]carbonyl][phenyl]-4'-methyl[1,1'-biphenyl]-2-carboxamide). In the present study, to elucidate the pharmacological and toxicological properties of VP-343, we performed receptor binding and in vivo studies, in comparison with YM087, OPC-31260 and furosemide.

MATERIALS AND METHODS

Materials The radioligands of d(CH2)3Tyr(Me)-[3H]-arginine vasopressin ([3H]V1 antagonist; NET-945; specific activity, 1813 GBq/m mol) and d(CH2)3-D-Ile5, Des-Gly-NH2-[3H]arginine vasopressin ([3H]V2 antagonist; NET-1010; specific activity, 2116.4 GBq/m mol) were obtained from Du pont. [125I]Ornithine vasotocin analog ([125I]OVTA; specific activity, 81.4 TBq/m mol) was obtained from NEN Life Science Products, Inc. AVP and oxytocin (OT) and angiotensin II were obtained from Sigma Chemical Co., Ltd. YM087 and OPC-31260 were synthesized at Yamamoto Pharmaceutical Co., Ltd. (Kanagawa, Japan). Furosemide and arabic gum were from Wako Pure Chemicals. Pitressin® injection and noradrenaline were obtained from Sankyo Co., Ltd. d(CH2)3Tyr(Et)VAP (dAVP) was obtained from Funakoshi Co., Ltd. VP-343 was synthesized as described previously.

Fig. 1. Chemical Structure of VP-343

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Animals Male ICR mice, male/female Sprague-Dawley rats (Charles River Japan, Inc.) and Brattleboro rats (Harlan) were used. Animals were housed in communal cages and maintained on a 12 h light/dark cycle with food and water available ad libitum.

Receptor Binding Studies $V_{IA}$, $V_2$ and OT receptor binding studies were performed according to a previously reported method \(^{4}\) with slight modification. Briefly, female Sprague-Dawley rats (7 week) were sacrificed by decapitation. Liver, kidney and uterus were quickly removed and were washed with an ice-cold medium containing 50 mM Tris–HCl (pH 7.4 at 4 °C). The isolated tissues were homogenized with a Polytron homogenizer in 20 vol. of 50 mM Tris–HCl (pH 7.4 at 4 °C). The homogenate was centrifuged at 50000×g for 20 min (liver and kidney) or 30 min (uterus). The pellets thus obtained were stored at −80 °C and used as plasma membrane.

For $V_{IA}$ receptor binding assay, various concentrations of the test compounds (3 pM—1 μM) and 2.12 nM $[^3H]V_2$, antagonist were incubated with 5 mg (protein equivalent) of liver membrane, in 1 ml of 50 mM Tris–HCl (pH 7.4) containing 10 mM KCl, 10 mM MgCl₂, and 0.1% bovine serum albumin (BSA). For $V_2$ receptor binding assay, various concentrations of the test compounds (10 nM—10 μM) and 1.8 nM $[^3H]V_2$, antagonist were incubated with 5 mg (protein equivalent) of kidney membrane, in 1 ml of 50 mM Tris–HCl (pH 7.4) containing 2 mM KCl, 1 mM MgCl₂, and 0.1% BSA. For OT receptor binding assay, various concentrations of the test compounds (3 pM—1 μM) and 0.8 nM $[^25]$OTVA were incubated with 10 mg (protein equivalent) of uterus membrane, in 1 ml of 50 mM Tris–HCl (pH 7.4) containing 2 mM CaCl₂ and 1 mM MgCl₂.

After incubation for 60 min at 25 °C, the incubation mixture was rapidly filtered by vacuum through Whatman GF/C glass fiber filters using a Cell Harvester, and the filters were washed three times with 3 ml of 50 mM Tris–HCl (pH 7.4). The filters were then dried and the radioactivity trapped on the filters was counted in a toluene-based scintillator with a liquid scintillation counter. Nonspecific binding was determined with 10 μM unlabeled AVP or 100 μM unlabeled OT. Specific binding was calculated as the difference between the total binding and the nonspecific binding. The IC₅₀ values of test compounds (nM, the concentration required to cause 50% inhibition of the specific binding of $[^3H]V_1$, antagonist, $[^3H]V_2$, antagonist and $[^25]OTVA$, were determined by regression analysis of displacement curves.

Blood Pressure Measurements in Pithed Rats Male Sprague–Dawley rats (9—12 week) were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). After tracheal intubation, systemic blood pressure (SBP) was measured through a catheter in the right common carotid artery with a pressure transducer (MPU-0.5, NIH, Japan) and amplifier (AP-601G, NIH, Japan). Heart rate (HR) was measured with a cardiometer (AF-601G, NIH, Japan) triggered by the arterial pressure pulse. All measurement parameters were recorded on an ink writing polygraph (RM-6000, NIH, Japan). The rats were bilaterally vagotomized. The left femoral vein was cannulated for intravenous administration of drugs. The central nervous system was destroyed by means of a steel rod inserted in the brain via the orbit and then pushed down into the whole length of the spinal canal (pithing). Artificial ventilation with room air was started immediately with a rodent respirator (SN-480-7, Shinano Seisakusho, Japan) at a frequency of 60 stroke/min and a tidal volume of 1—1.2 ml/100 g body weight.

After the blood pressure became stable, VP-343, YM087 and OPC-31260 (all dissolved in ethanol) or 100% ethanol were given (0.1 ml/kg, i.v.) about 3 min before injecting AVP (30 μU/kg, i.v.). In addition, VP-343 or 100% ethanol were given (0.1 ml/kg, i.v.) about 3 min before the injection of angiotensin II (0.1 μg/kg, i.v.) and noradrenaline (2 μg/kg, i.v.). ID₅₀ (μg/kg, i.v.), the dose required to cause 50% inhibition of the pressor response to AVP was calculated.

Aquatic Effects in Normal Conscious Saline-Loaded Rats Studies were performed with conscious male Sprague-Dawley rats (8 week). Rats were fasted for 16—20 h, giving water ad libitum. Immediately after the test compounds or vehicle (5% arabic gum (AG)) were orally administered to the rats, saline (25 ml/kg) was given orally. During the experiments, the rats were placed individually in metabolic cages and deprived of food and water for 4 h. Spontaneous voided urines were collected during 0—2 h and 2—4 h. Urine collected during 0—2 h was used for the evaluation of aquatic effect by measuring the urine volume and urine osmolality which was determined by freezing point depression with an osmometer (Auto & STAT OM-6030, Kyoto Da II chi Kagaku, Japan). Total urine volume collected from 0 to 4 h was measured. The dose of test compounds required to increase the total urine volume to three times that of in vehicle group was determined (ED₅₀, mg/kg, p.o.), and was used for the evaluation of diuretic activity.

Urinary Electrolyte Analysis The same urine samples as those described above for the evaluation of aquatic effect were used. Urinary sodium, potassium and chloride concentrations were determined with a flame photometer (PVA-α II, Analytical Inc., Japan).

Studies with Brattleboro Rats Male Brattleboro rats (homozygous for hypothalamic diabetes insipidus) were used. They were placed in metabolic cages and given food and water ad libitum throughout the experiment. VP-343 (30 mg/kg) and 5% AG were orally administered in a volume of 2 ml/kg, and (CH₃)₂Syr-d-(Et)VAVP (10 μg/kg) was subcutaneously administered in a volume of 1 ml/kg. Urine was collected for 6 h with metabolic cages. Urine volumes were measured, and urine osmolalities measured with a freezing point osmometer.

Aquatic Activity of VP-343 during Repeated Oral Dose and Recovery Two series of experiments were performed with conscious male Sprague-Dawley rats (8—13 week). The scheme of the experimental protocol is shown in Fig. 2. In the 1st series of experiments, 5% AG (group 1) or VP-343 (3 mg/kg, p.o.; group 2) were given once a day repeatedly for 21 d, and then a single dose of 5% AG or VP-343 (3 mg/kg, p.o.) were given after 14 d of recovery. The diuretic activities of VP-343 were estimated on the 1st, 7th, 14th and 21st day, and on the 14th day of recovery by the same method as described above.

The second series of experiments were the control studies for the 1st series of experiments. On the next day after the repeated oral dose of 5% AG once a day for 6 d (group 3, 13 (group 4), and 20 d (group 5), VP-343 (3 mg/kg, p.o.) was
administered and the diuretic activity was estimated as described above. In addition, in group 5, animals were allowed to recover for 14 d after the administration of VP-343. On the next day after the recovery, another administration of VP-343 (3 mg/kg, p.o.) was administered, and its diuretic activity was estimated.

**Studies with Aged (17 month) Saline-Loaded Conscious Rats** The same type of diuretic experiments were performed using aged conscious male Sprague-Dawley rats (17 month). The diuretic activity of VP-343 (3 mg/kg, p.o.), YM087 (3 mg/kg, p.o.), OPC-31260 (3 mg/kg, p.o.) and furosemide (10 mg/kg, p.o.) were estimated by the method described above.

**Single Dose Oral Toxicity Study** General toxicity studies were performed by giving orally a single dose of VP-343, YM087 and OPC-31260 to male ICR mice (6 week). The general symptoms and body weights were checked every day during 7 d after the oral administration of test compounds. On the last day, necropsy was performed on all surviving mice.

**Statistical Analysis** Experimental results are expressed as the mean±S.E. One way analysis of variance (ANOVA) followed by Williams, Williams–Shirley or Dunnett’s test was used. The level of significance was taken as p<0.05.

**RESULTS**

**Receptor Binding Studies** VP-343 and YM087 displaced [3H]V₁-receptor antagonist and [3H]V₂-receptor binding and OPC-31260 displaced [3H]V₂-receptor antagonist binding in a concentration-dependent manner. The IC₅₀ values of VP-343 were 0.77 nm for the V₂ receptor, 110 nm for the V₁A receptor and >1000 nm for the OT receptor, respectively. The IC₅₀ value of VP-343 for the V₂ receptor was 142.8 times higher than that for the V₁A receptor (Table 1). On the other hand, the IC₅₀ values of YM087 were 0.18 nm for the V₂ receptor, 3.38 nm for the V₁A receptor and 1000 nm for the OT receptor. The IC₅₀ value of YM087 for the V₂ receptor was 18.8 times higher than that for the V₁A receptor (Table 1). The IC₅₀ values of OPC-31260 were 7.71 nm for the V₂ receptor, >1000 nm for the V₁A receptor and >1000 nm for the OT receptor. The inhibitory potency of YM087 for V₁A and V₂ receptors was greater than those of VP-343. However, VP-343 was 10 times more selective to the V₂ receptor than OPC-31260. VP-343 had no affinity for oxytocin receptors (Table 1).

**Studies on Pithed Rats** In pithed rats, intravenous administration of VP-343 and YM087 inhibited the AVP-induced pressor response in a dose-dependent manner. The ID₅₀ values of VP-343, YM087 and OPC-31260 were 0.57, 0.01 and >1 mg/kg, respectively. The inhibitory effect of YM087 on AVP-induced pressor response in pithed rats was 57 times higher than that of VP-343 (Table 2, Fig. 3). In addition, VP-343 had no effect on the pressor response to angiotensin II or norepinephrine (Fig. 4).

**Aqueietic Effects on Normal Conscious Saline-Loaded Rats** VP-343, YM087, OPC-31260 and furosemide increased urine volume dose-dependently (Fig. 5). The ED₅₀ values of VP-343, YM087, OPC-31260 and furosemide were 0.22, 0.23, 3.2 and 11.7 mg/kg, respectively (Table 2). The diuretic effect of VP-343 was similar to that of YM087, and was 14.5 and 53.2 times more potent than that of OPC-31260 and furosemide, respectively. VP-343, YM087 and OPC-31260 decreased the urine osmolality level depending on the urine volume, thus indicating the clear aqueietic activity of these compounds. On the contrary, furosemide did not show the aqueietic effect as shown in Fig. 6a. The urinary elec-

<table>
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<th>Exp.</th>
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<th>Period B</th>
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<tr>
<td></td>
<td>Period A</td>
<td></td>
<td>Period A</td>
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</table>

Fig. 2. Scheme of the Experimental Protocol

<p>| Table 1. Binding Affinity of VP-343, YM087 and OPC-31260 for AVP and OT Receptor Subtypes |
|---------------------------------|-----------------|-----------------|</p>
<table>
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<tr>
<th>IC₅₀ (nm)</th>
<th>V₁A (Liver)</th>
<th>V₂ (Kidney)</th>
<th>V₁A/V₂</th>
<th>OT (Uterus)</th>
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<td>110</td>
<td>0.77</td>
<td>142.8</td>
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<td>YM087</td>
<td>3.38</td>
<td>0.18</td>
<td>18.8</td>
<td>≈1000</td>
</tr>
<tr>
<td>OPC-31260</td>
<td>&gt;1000</td>
<td>7.71</td>
<td>129.7</td>
<td>&gt;1000</td>
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</tbody>
</table>

Each value represents the mean.
Table 2. Antagonistic Activities of VP-343, YM087, OPC-31260 and Furosemide to V₁ and V₂ Receptors

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<th>V₂</th>
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<td>Binding assay</td>
<td>Pithed rat</td>
<td>Binding assay</td>
</tr>
<tr>
<td></td>
<td>IC₅₀ (μM)</td>
<td>ID₅₀ (mg/kg, i.v.)</td>
<td>ED₅₀ (mg/kg, p.o.)</td>
</tr>
<tr>
<td>VP-343</td>
<td>110</td>
<td>0.57</td>
<td>0.77</td>
</tr>
<tr>
<td>YM087</td>
<td>3.38</td>
<td>0.01</td>
<td>0.18</td>
</tr>
<tr>
<td>OPC-31260</td>
<td>&gt;1000</td>
<td>&gt;1.0</td>
<td>7.71</td>
</tr>
<tr>
<td>Furosemide</td>
<td>N.T.</td>
<td>N.T.</td>
<td>11.7</td>
</tr>
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</table>

In addition to the values from binding assay, values obtained in pithed rats and saline-loaded rats are shown for V₁ and V₂ antagonistic activity, respectively (see Text). N.T.: Not tested.

Fig. 3. Effects of VP-343, YM087 and OPC-31260 on the Pressor-Response to AVP of Pithed Rats

Ordinate: inhibition (%) of increase in blood pressure produced by AVP (30 mU/kg, i.v.). Abscissa: dose of test compounds. Symbols and vertical bars represent the mean±S.E. of 4–8 animals.

Fig. 4. Inhibitory Effects of VP-343 (3 mg/kg, i.v.) on the Pressor-Response to AVP (30 mU/kg, i.v.), Norepinephrine (2 μg/kg, i.v.) and Angiotensin II (0.1 μg/kg, i.v.) of Pithed Rats

Each column and vertical bars represent the mean±S.E. of 8 animals. NE, norepinephrine; Ang II, Angiotensin II.

trolyte (Na⁺, K⁺ and Cl⁻) excretions of VP-343, YM087 and OPC-31260 were lower than that by furosemide. In other words, VP-343, YM087 and OPC-31260 excreted water more selectively than furosemide (Fig. 6b, c, d).

**Studies on Brattleboro Rats** As shown in Fig. 7, VP-343 significantly increased the urine volume accompanied by the decrease in urine osmolality, indicating the clear aquaretic effect of VP-343 in Brattleboro rats. In contrast, d(CH₂)₄Tyr(εT)VAVP, which exhibited diuresis in normal rats, showed antidiuretic effects in Brattleboro rats as reported previously.²⁵

**Aquaretic Effect of VP-343 on Repeated Dose with Recovery** As shown in Fig. 8, VP-343 exhibited potent aquaretic activity throughout the period of repeated administration. The diuretic effect of VP-343 during repeated administration was about the same as that produced by single administration.

**Studies with the Aged (17 Month) Saline-Loaded Conscious Rats** As shown in Fig. 9, VP-343 exhibited a remarkable diuretic action in the aged (17 month) saline-loaded rats.

**Single Dose Oral Toxicity Study** 1) **VP-343**: As shown in Table 3, six mice were used in each group. No abnormalities were observed in general symptoms during the experimental periods. The body weight gain of mice administered 500 and 1500 mg/kg of VP-343 was similar to that of control mice except that the body weight gain was significantly lower on the second day.

2) **YM087**: As shown in Table 4, five mice were used in each group. General symptomatic changes observed were hypolocomotion, sedation, hypothermia, prone position, ptosis and closing eyelids. At the doses of 500 and 1500 mg/kg, two out of five mice died; at doses 50 and 150 mg/kg, all mice survived.

3) **OPC-31260**: As shown in Table 5, five mice were used in each dose group. Hypolocomotion, sedation, abnormal gait, tremor, clonic convulsion, hypothermia, prone position, ptosis and closing eyelids were observed as the general symptoms. At 1500 mg/kg, four out of five mice died; at the
Fig. 6. Effects of VP-343, YM087, OPC-31260 and Furosemide on Urinary Electrolyte (Na⁺ (b), K⁺ (c), Cl⁻ (d)) and Urine Osmolality (a) in Normal Conscious Saline-Loaded Rats

Each parameter is plotted as a function of urine volume. Symbols and vertical bars represent the mean±S.E. of 4—8 animals.

Fig. 7. Urine Volume, Water Intake and Urinary Osmolality in Brattleboro Rat over a 6h Collection Period after Oral Administration of VP-343 (30 mg/kg, Closed Column, n=6) and Vehicle (5% AG, Open Column, n=7)

Effects of subcutaneously administered d(CH₂)₅Tyr(ε)-NVP (10 μg/kg, hatched column, n=6) are also shown for comparison. Each column and vertical bars represent the mean±S.E. of 6—7 animals. * p<0.05, ** p<0.01: significantly different from the value of vehicle group (5% AG).

Fig. 8. Effect of VP-343 on Urine Volume (A) and Urine Osmolality (B) in Rats during the Period of Repeated Oral Administration and Recovery

Open column: vehicle (5% AG). Dotted and closed column: VP-343 given during the period of repeated administration of VP-343 or vehicle, respectively. Each column and vertical bars represent the mean±S.E. of 6 animals. * p<0.01: significantly different from the corresponding values of the vehicle group (5% AG).
lower doses, all mice survived.

**DISCUSSION**

In the present study, we investigated the pharmacological properties of VP-343 in comparison with YM087, OPC-31260 and furosemide. For this purpose, we performed radioligand binding studies and examined the effects of these compounds in several animal models.

The IC$_{50}$ values of VP-343 for V$_{1A}$ and V$_{2}$ receptors were 110 and 0.77 nm, respectively, thus this compound is 142.8 times more potent as an antagonist of the V$_{2}$ receptor than the V$_{1A}$ receptor. The results indicated that VP-343 possesses relatively selective binding affinity for V$_{2}$ receptors. In contrast, YM087 showed potent binding affinity for both V$_{1A}$ and V$_{2}$ receptors and OPC-31260 exclusively for the V$_{2}$ receptor, which was in good agreement with the results reported previously.$^{25,26}$ VP-343 and OPC-31260 did not display substantial affinity for the OT receptor, whereas YM087 exhibited a low affinity, as reported previously.$^{26}$

In general, antagonism to vasopressin-induced pressor response in pithed rats has been established as an animal model for the evaluation of a V$_{1A}$ antagonistic effect.$^{23,24,26}$ and aquaretic effects in normal conscious rats for V$_{2}$ antagonistic effects.$^{23,26,30,31}$ Therefore, we studied the effects of VP-343 on vasopressin-induced pressor response in pithed rats and its aquaretic effects in conscious saline-loaded rats. In pithed rats, the inhibitory effect of VP-343 was smaller than that of YM087; the ID$_{50}$ values of VP-343 and YM087 to antagonize AVP-induced pressor response being 0.57 and 0.01 mg/kg (i.v.), respectively. VP-343 did not affect the pressor responses to angiotensin II and norepinephrine, suggesting its selective antagonism to vasopressin. Yatsu et al. reported the lack of antagonism by YM087 for pressor response to angiotensin II and norepinephrine.$^{28}$ OPC-31260 had no in-

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**Table 3. Single Dose Toxicity Study of VP-343**

<table>
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<tr>
<th>Species, strain, age, sex</th>
<th>Mouse, C57-CDI (ICR), six week, male</th>
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<td>Route</td>
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<td>Dose (mg/kg)</td>
<td>Vehicle</td>
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<tr>
<td>Number of mice</td>
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<td>Necropsy</td>
<td>— Necropsy</td>
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---: No remarkable findings. *: Significantly lower than that in vehicle group. N.T.: Not tested.

**Table 4. Single Dose Toxicity Study of YM087**

<table>
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<th>Species, strain, age, sex</th>
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<td>Dose (mg/kg)</td>
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<td>Necropsy</td>
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</table>

---: No remarkable findings. *: Significantly lower than that in vehicle group. N.T.: Not tested.
hibitory effect on the pressor response to AVP in pithed rats.

In normal saline-loaded rats, orally administered VP-343, YM087 and OPC-31260 showed diuretic effects with ED_{50} values of 0.22, 0.23 and 3.2 mg/kg, respectively. Urine osmolality values after administration of these compounds were significantly lower than that after furosemide. From these results showing the potent aquaretic effect and weak inhibitory effect on AVP-induced pressor response in pithed rats, VP-343 was considered to possess pharmacological properties different from YM087 and OPC-31260.

Since it is important from a clinical point of view to show lack of tachyphylaxis after repeated oral administration, we performed repeated oral administration of VP-343 to rats, followed by recovery. We found that VP-343 exhibited about the same diuretic activity with low urine osmolality throughout the period of repeated administration.

To confirm the diuretic activity in aged patients is important from the clinical standpoint. Thus, we performed studies with aged rats (17 month), and found that oral administration (3 mg/kg) of VP-343 showed a potent diuretic activity.

It has been reported that peptide V_2 receptor antagonists such as SKE-101926 did not show diuretic activity in clinical trials, partly due to its partial AVP V_2 agonistic activity, indicating that the partial AVP V_2 agonistic activity of a drug can restrict its clinical applications. Therefore, we investigated whether VP-343 has any AVP V_2 agonistic activity using Brattleboro rats. As a result, VP-343 increased urine volume and decreased urine osmolality in Brattleboro rats, which clearly indicates that VP-343 does not substantially possess V_2 agonistic activity.

The increase in urine volume and decrease in urine osmolality can be explained as follows. In the Brattleboro rat, oxytocin could interact with the V_2 receptor and increase water permeability in the rat collecting duct. Furthermore, the possible secretion of small amounts of vasopressin by peripheral organs such as the adrenal glands and testis where immunoreactive AVP has been detected.

VP-343 may inhibit these antidiuretic effects.

To investigate the safety of VP-343, a single dose oral toxicity study was conducted. YM087 and OPC-31260 produced a decrease in body weight gain and death at high doses; changes in general symptoms seemed to be related to the suppression of the central nervous system, including hypothermia, ptosis, prone position and hypolocomotion. In contrast, VP-343 did not show any toxicological signs, except for a slight decrease in the body weight gain observed on the second day. Therefore, VP-343 was considered to be safer than YM087 and OPC-31260, as judged from the single dose oral toxicity studies.

In conclusion, VP-343, a nonpeptide AVP V_2 receptor antagonist, had potent and selective V_2 ligand activity in rat preparations. VP-343 showed powerful aquaretic effects in some animal models without substantial V_2 agonistic properties. VP-343 displayed good safety in the single dose oral toxicity study. Therefore, VP-343 is a suitable tool for studying the pathophysiological role of V_2 receptors and may be a useful drug for water-retaining diseases.

REFERENCES AND NOTES

1) A part of this work was presented at the 118th annual meeting of the Pharmaceutical Society of Japan.


