Antidiuretic Effects of a Nonpeptide Vasopressin V2-Receptor Agonist, OPC-51803, Administered Orally to Rats

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ABSTRACT
OPC-51803 is the first nonpeptide vasopressin (AVP) V2-receptor-selective agonist. Its pharmacological profile, including antidiuretic action and receptor binding, was characterized using conscious Brattleboro rats with hereditary diabetes insipidus and Sprague-Dawley rats. In membrane preparations from the liver and kidney, OPC-51803 displaced the [3H]AVP binding to V2-receptors (Kd = 49.8 ± 8.1 nM) more greatly than that to V1a-receptors (Kd = 1061 ± 60 nM), showing a 21 times higher affinity for V2-receptors. At single oral doses of 0.003 to 0.3 mg/kg in female Brattleboro rats, OPC-51803 decreased urine volume (from 10.8 ± 1.1 to 0.5 ± 0.2 ml during 0–2 h postdosing) and increased urinary osmolality (from 114 ± 9 to 432 ± 114 mOsm/kg) in a dose-dependent manner. During the period of 4-week treatment with OPC-51803, significant and constant antidiuresis was observed. In male Sprague-Dawley rats with normal plasma AVP levels, OPC-51803 at 0.03 to 0.3 mg/kg also produced a dose-dependent antidiuretic action (urine volume: from 2.6 ± 0.6 to 1.1 ± 0.2 ml at 0–4 h postdosing). Few changes in urinary parameters, serum parameters, or plasma hormone levels were observed. OPC-51803 did not change blood pressure or heart rate, or inhibit AVP-induced pressor response even at 30 mg/kg p.o. These results demonstrate that OPC-51803 is a V2-selective agonist that produces a significant antidiuretic action after single and multiple oral dosing in AVP-deficient and normal AVP states. The data suggest that OPC-51803 is a useful therapeutic drug in the treatment of hypothalamic diabetes insipidus, nocturnal enuresis, and some kinds of urinary incontinence.

Vasopressin (AVP) is a neurohypophysial peptide hormone that regulates water excretion. Its mechanism of action involves binding to V2-receptors coupled to Gs proteins, and activation of the adenylate cyclase system. It is thought that the ensuing increase in water permeability occurs through an action on water channels (aquaporin-2) in the kidney. In a dehydrated condition, AVP is secreted from the pituitary to prevent water loss. Conversely, hydration leads to a suppression of AVP secretion, resulting in increased water excretion. The same phenomenon occurs during diurnal rhythmic changes in plasma AVP, i.e., urine is concentrated during the night due to an increase in plasma AVP level, whereas in patients with hypothalamic diabetes insipidus or nocturnal enuresis (Newsome, 1979; Pullan et al., 1979; Norgaard et al., 1985; Rittig et al., 1989a,b; Steffens et al., 1993), insufficient secretion of endogenous AVP impairs water homeostasis.

In Brattleboro rats, which are hereditary AVP-deficient animals (Schmale and Richter, 1984; Valtin and Schroeder, 1997), a large volume of urine excretion at low urinary osmolality is typically displayed. Using this model, we undertook studies to develop a new nonpeptide AVP analog that would have a high specificity for V2-receptors. It is thought that activation of AVP receptor subtypes, V1a- or V1b-receptors, may result in an increase in blood pressure, release of adrenocorticotropic hormone, and other deleterious side effects. The advantage of a nonpeptide drug with a low molecular weight is that it is well absorbed by the oral route. In our research for developing two nonpeptide vasopressin V2-selective antagonists, OPC-31260 and OPC-41061 (Yamamura et al., 1992, 1998; Hirano et al., 2000), some compounds were shown to decrease urine output in alcohol-anesthetized water-loaded rats. Oral administration of these compounds showed significant antidiuretic effects in Brattleboro rats and in normal-hydrated rats. Further optimization of these compounds has recently yielded OPC-51803, which has been confirmed to be the first nonpeptide agonist for human AVP V2-receptors without agonistic activities for V1a- and V1b-receptors (Nakamura et al., 2000). In this study, we characterized a newly synthesized nonpeptide V2-agonist and assessed its antidiuretic and vasoconstrictive properties using Brattleboro rats and Sprague-Dawley rats.

Furthermore, chronic administration of a drug is a requirement in treating water metabolism disorders, such as hypo-

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ABBREVIATIONS: AVP, vasopressin; DDAVP, 1-desamino-8-D-arginine vasopressin; Kd, dissociation constant; Bmax, number of binding sites; Ki, inhibition constant; IC50, concentration required for 50% inhibition of specific binding; PAVP, plasma vasopressin.
thalamic diabetes insipidus, nocturnal enuresis, and some kinds of urinary incontinence (Mah and Hofbauer, 1988; Caltabiano and Kinter, 1991; Matthiesen et al., 1994; Asplund et al., 1999). We also examined repeated administration of OPC-51803 in Brattleboro rats to clarify the tolerance of antidiuretic action.

Experimental Procedures

Materials. OPC-51803, (5R)-2-[1-(2-chloro-4-(1-pyrrolidinyl)benzoyl)-2,3,4,5-tetrahydro-1H-1-benzazepin-5-yl]-N-isopropylacetamide, was synthesized in Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). AVP was purchased from Peptide Institute Inc. (Osaka, Japan) or Sigma (St. Louis, MO), and [3H]AVP from NEN LifeScience Products, Inc. (Boston, MA). 1-Desamino-8-D-arginine vasopressin (DDAVP) and BSA were purchased from Sigma. Phosphate-buffered salts were purchased from Tukara Shuzo Co., Ltd. (Kusatsu, Japan). EDTA was purchased from Dojindo Laboratories (Kumamoto, Japan) and Percoll and dextran T500 from Pharmacia Biotech AB (Uppsala, Sweden).

Animals. Male Sprague-Dawley rats, aged 7 to 8 weeks, were purchased from Charles River Japan, Inc. (Yokohama, Japan) and homozgyous Brattleboro rats (weighing 180–300 g) were bred in our animal house (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Rats were housed in a temperature-, humidity-, and light-controlled room and given free access to food (MF; Oriental Yeast, Osaka, Japan) and water. The care and handling of these animals were in accordance with The Guidelines for Animal Experimentation in Otsuka Pharmaceutical Co., Ltd., October 1, 1994.

Preparation of Crude Plasma Membranes. Liver and kidney plasma membranes were prepared from 90 male Sprague-Dawley rats weighing 280 to 380 g, as previously described (Yamamura et al., 1992). A sample of each preparation was dissolved in 0.1 N NaOH, and the protein quantitated by the dye method (Bradford, 1976) using BSA as a standard.

Radioligand Binding Assays. The saturation and competition binding experiments were also performed as described previously (Yamamura et al., 1992). For saturation binding studies, each membrane preparation was incubated with [3H]AVP at concentrations of 0.25 to 8 nM. For competition studies, increasing concentrations of OPC-51803, AVP, and DDAVP were incubated with approximately 1 or 2 nM [3H]AVP and liver or kidney membranes, respectively. Incubation was carried out for 10 min at 37°C (liver membrane) and 2 h at 4°C (kidney membrane).

Radioligand Binding Data Analysis. In saturation binding experiments, the dissociation constant (Kd) and the number of binding sites (Bmax) were determined by Scatchard (1949) analysis. The IC50 values of OPC-51803, AVP, and DDAVP were determined by a displacement procedure. The inhibition constants (Ki) were calculated from the IC50 values using equations in Cheng and Prusoff (1973).

Antidiuretic Experiment of a Single Oral Administration in Female Brattleboro Rats. OPC-51803 at doses of 0.003 to 0.3 mg/kg suspended in 5% arabic gum solution, and the vehicle (5% arabic gum) were orally administered to female homozgyous Brattleboro rats (n = 6 per each group, total n = 36) at a volume of 2 ml/kg using a stomach sonde. Spontaneously voided urine was collected every 2 h over a period of 8 h in metabolic cages (Sugiyama-Gen Medical Instruments Co., Ltd., Tokyo, Japan). Using an aliquot of urine collected, urinary osmolality was determined by analysis of the freezing-point depression using a Fiske osmometer (model 3400; Boston, MA).

Antidiuretic Experiment of 4-Week Repeated Oral Administrations in Female Brattleboro Rats. OPC-51803 at doses of 0.03 and 0.3 mg/kg and the vehicle (5% arabic gum) were orally administered to female Brattleboro rats once daily for 4 weeks (n = 6 per each group). The rats were placed in individual metabolic cages on days 0, 1, 7, 14, 21, 28, 29, 33, and 41. Urine was collected at 0 to 2, 2 to 4, 4 to 6, 6 to 8, and 8 to 24 h postdosing. Urine sampling on days 29, 33, and 41 was performed to obtain recovery responses. The antidiuretic effects of OPC-51803 were evaluated on the basis of the urine volume at 0 to 2, 0 to 8, and 0 to 24 h postdosing and maximal urinary osmolality in the four sampling periods until 8 h postdosing. As a reference, DDAVP dissolved in saline was administered subcutaneously to female Brattleboro rats (n = 6) at a dose of 3.0 ng/kg. This dose produced an antidiuretic action equivalent to 0.3 mg/kg p.o. of OPC-51803 when a single dose was done in female Brattleboro rats.

Antidiuretic Experiment of a Single Oral Administration in Male Sprague-Dawley Rats. OPC-51803 at doses of 0.03, 0.1, and 0.3 mg/kg and the vehicle (5% arabic gum) were orally administered to male Sprague-Dawley rats (n = 10 per group for urine collection, n = 6 per group for serum, and n = 6 per group for plasma). Spontaneously voided urine was collected during 0 to 4 h and 4 to 8 h in individual metabolic cages. Blood was drawn from the venal cava under ether anesthesia for measuring serum parameters or obtained postdecapitation for measuring plasma hormones at 4 h after administration. Urine and serum osmolality were determined by freezing-point depression using a Fiske osmometer. Electrolyte (sodium and potassium) concentrations were measured by the ion-electrode method (Synchron CX-3; Beckman Instruments, Brea, CA), and creatinine and urea nitrogen concentrations were measured using an AutoAnalyzer (Synchron CX-3; Beckman Instruments and COBAS FARAII; Roche, Basel, Switzerland). Hormone levels (AVP, renin activity, aldosterone, epinephrine, and norepinephrine) were measured by radioimmunoasay or by high-performance liquid chromatography.

Antipressor Experiment of a Single Oral Administration in Male Sprague-Dawley Rats. Male Sprague-Dawley rats (9 weeks old, n = 10) weighing 295 to 340 g were surgically operated to implant indwelling catheters as described previously (Yamamura et al., 1991) under anesthesia with sodium pentobarbital (40 mg/kg i.p., Nembutal solution; Abbott Laboratories, Abbott Park, IL). After a recovery period of 2 days, the rats were placed in individual boxes and arterial blood pressure and heart rate from pulse waves were recorded by a pressure transducer (MPU-0.5; NEC San-ei Instrument, Tokyo, Japan). After a reproducible pressor response to intravenous injection of AVP (30 mU/kg) was obtained, OPC-51803 at a dose of 30 mg/kg or the vehicle (5% arabic gum) was administered orally using a stomach sonde. Separately intravenous AVP injections were given at a dose of 30 mU/kg at 0.5, 1, 1.5, 2, 3, and 4 h after the administration of OPC-51803 or vehicle and their pressure responses were monitored. Blood pressure and heart rate in the steady state were recorded before each AVP injection.

Statistical Analysis. All values were expressed as the mean ± S.E.M. The differences in urine volume and urinary parameters between the OPC-51803-treated groups and the vehicle-treated group were determined by ANOVA based on repeated measurements, followed by a two-tailed Dunnett’s multiple comparison test at each time point. The differences in serum parameters and plasma hormone levels between the OPC-51803-treated groups and the vehicle-treated group were analyzed by one-way ANOVA followed by a two-tailed Dunnett’s multiple comparison test. The differences in the rise in blood pressure induced by AVP i.v. injections between the OPC-51803-treated groups and the vehicle-treated group were analyzed by ANOVA based on repeated measurements. The differences were considered significant at the P < .05 level. All analyses were performed with the Statistical Analysis System software (release 6.12, SAS Institute, Tokyo, Japan). The DDAVP-treated group was not included in the statistical analysis because this group was different from the other groups in terms of administration and solvent.
Results

[3H]AVP Binding to Rat Liver and Kidney Plasma Membranes. In plasma membranes from rat kidney and liver, the specific binding of [3H]AVP was saturated according to increasing concentrations. The $K_i$ and $B_{\text{max}}$ values calculated from Scatchard analysis were 1.63 ± 0.18 nM and 258 ± 23 fmol/mg of protein for rat V$_2$-receptors, and 1.06 ± 0.09 nM and 697 ± 67 fmol/mg of protein for rat V$_{1a}$-receptors, respectively. In the competition binding experiments, OPC-51803 displaced [3H]AVP binding to rat V$_2$- and V$_{1a}$-receptors in a concentration-dependent manner (Fig. 1); the $K_i$ value was about 21 times more selective for V$_2$-receptors than V$_{1a}$-receptors (Table 1). Unlabeled AVP displaced [3H]AVP binding for both receptor subtypes with similar $K_i$ values. DDAVP, which is well known as a selective V$_2$-agonist, displaced [3H]AVP binding to rat V$_2$- and V$_{1a}$-receptors; the affinity for V$_2$-receptors was 153 times higher than that for V$_{1a}$-receptors.

Antidiuretic Action of a Single Oral Dose of OPC-51803 in Female Brattleboro Rats. As shown in Fig. 2, OPC-51803 produced a dose-dependent decrease in urine volume with a concomitant increase in urine osmolality during a period of 0 to 2 h after administration. The decrease in urine volume was statistically significant at all doses compared with the vehicle-treated group. The antidiuretic action of OPC-51803 at 0.3 mg/kg lasted more than 6 h. From 0 to 8 h, the change in body weight and water intake in the vehicle-treated and OPC-51803-treated groups were monitored. A tendency for body weight to increase was seen at 0.3 mg/kg OPC-51803 (Table 2). Water intake significantly decreased only at the highest dose of OPC-51803. As a reference, subcutaneous DDAVP injection at 1 ng/kg also showed an antidiuretic action in female Brattleboro rats.

Antidiuretic Action of 4-Week Repeated Oral Doses of OPC-51803 in Female Brattleboro Rats. There were no significant differences in body weight between the vehicle-treated and OPC-51803-treated groups throughout the experimental period (vehicle, 241.8 ± 4.7 g; 0.03 mg/kg, 239.6 ± 6.2 g; 0.3 mg/kg, 235.9 ± 9.7 g on day 29). Figure 3 shows the progress of the values of urine volume and urinary osmolality during treatment and recovery. During the 0- to 2-h period

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### Table 1

<table>
<thead>
<tr>
<th>OPC-51803</th>
<th>AVP</th>
<th>DDAVP</th>
<th>V$<em>2$/V$</em>{1a}$ Ratio</th>
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</thead>
<tbody>
<tr>
<td>49.8 ± 8.1</td>
<td>1.73 ± 0.15</td>
<td>8.57 ± 1.50</td>
<td>1.40 ± 0.06</td>
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### Table 2

<table>
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<tr>
<th>Body Weight</th>
<th>Water Intake</th>
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<tr>
<td>Predosing</td>
<td>Changes</td>
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<tr>
<td>Vehicle</td>
<td>232.1 ± 9.8</td>
</tr>
<tr>
<td>OPC-51803 (mg/kg, p.o.)</td>
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</tr>
<tr>
<td>0.003</td>
<td>228.2 ± 9.6</td>
</tr>
<tr>
<td>0.01</td>
<td>229.4 ± 7.1</td>
</tr>
<tr>
<td>0.03</td>
<td>238.2 ± 8.3</td>
</tr>
<tr>
<td>0.1</td>
<td>231.6 ± 7.9</td>
</tr>
<tr>
<td>0.3</td>
<td>242.7 ± 8.4</td>
</tr>
<tr>
<td>DDAVP (ng/kg, s.c.)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>239.8 ± 10.2</td>
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</tbody>
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**$P < .01$ vs. vehicle.
after drug administration of OPC-51803 doses of 0.03 and 0.3 mg/kg, urine volume decreased significantly in a dose-dependent manner (Fig. 3a). The decrease in urine volume was shown constantly throughout the administration period. The significant and constant decreases in urine volume during 0 to 8 h postdosing were also seen in the 0.3 mg/kg group of OPC-51803, but there were little differences in urine volume from 0 to 24 h between the vehicle-treated and drug-treated groups (data not shown). As an index of the maximal capacity of urine concentration induced by OPC-51803, the maximal urinary osmolality was estimated. Throughout the administration period, 0.3 mg/kg OPC-51803 significantly and constantly increased urinary osmolality (Fig. 3b). After drug administration ceased, a transient but significant increase in urine volume was observed in the 0.3 mg/kg group of OPC-51803 compared with the vehicle-treated group. However, the increased urine volume returned to basal levels in a few days. The decrease in maximal urinary osmolality also returned to the basal level. DDAVP at 3 ng/kg was injected subcutaneously once a day for 4 weeks. A similar antidiuretic action was produced at the same level observed with an oral dose of 0.3 mg/kg OPC-51803 (data not shown).

Fig. 3. Antidiuretic action of 4-week repeated oral doses of OPC-51803 in female Brattleboro rats. a, urine volume during 0 to 2 h postdosing. b, maximal urinary osmolality defined under Experimental Procedures. Values are expressed as mean ± S.E.M. of six rats. Differences between each OPC-51803-treated group and the vehicle-treated group were analyzed using ANOVA based on repeated measurements in two periods (days 0–28 and days 28–41), followed by a two-tailed Dunnett’s multiple comparison test at each time point. *P < .05, **P < .01. □, vehicle (5% arabic gum); △, OPC 0.03 mg/kg/day; ◊, OPC 0.3 mg/kg/day.

Antidiuretic Action of OPC-51803 Orally Administered to Male Sprague-Dawley Rats. From 0 to 4 h after administration, OPC-51803 (0.03, 0.1, and 0.3 mg/kg) depressed urine volume to 81, 42, and 19% of the vehicle-treated group, respectively (Fig. 4a). Urinary osmolality was increased according to the decrease in urine volume (Fig. 4b). Both the decrease in urine volume and the increase in urinary osmolality reverted to basal levels at the 4- to 8-h period for all doses. OPC-51803 at 0.3 mg/kg tended to decrease urinary excretion of sodium, potassium, creatinine, and urea nitrogen from 0 to 4 h after administration (Fig. 4, c–f), but the values were not statistically different from one another. The effects of OPC-51803 on serum parameters and plasma hormone levels at 4 h after administration are shown in Tables 3 and 4. OPC-51803 slightly increased serum creatinine and urea nitrogen concentrations but only at the highest dose. The drug increased plasma norepinephrine concentra-

Fig. 4. Antidiuretic action of OPC-51803 administered orally to Sprague-Dawley rats. Urine volume (a); urinary osmolality (b); and urinary excretion of sodium (c), potassium (d), creatinine (e), and urea nitrogen (f) from 0 to 4 h and 4 to 8 h postdosing. Values are expressed as mean ± S.E.M. of 10 rats. Differences between each OPC-51803-treated group and the vehicle-treated group were statistically analyzed. *P < .05, **P < .01. □, vehicle; △, 0.03 mg/kg; ◊, 0.1 mg/kg; ◊, 0.3 mg/kg.
tion significantly but not in a dose-dependent manner. However, OPC-51803 did not affect serum osmolality, serum electrolyte concentrations, or other hormone levels.

**Effect of a Single Oral Dose of OPC-51803 on the Rise in Blood Pressure Induced by Intravenous AVP Injections in Male Sprague-Dawley Rats.** Blood pressure and heart rate at the steady state did not differ between the vehicle-treated and OPC-51803-treated groups (30 mg/kg, p.o.) throughout the experimental period (Fig. 5, a and b). There were no statistically significant differences in pressor responses induced by intravenous AVP injections at 30 mU/kg between the groups (Fig. 5c).

**Discussion**

The present study describes the pharmacological profile of OPC-51803, a newly synthesized nonpeptide AVP V2-agonist with potent oral antidiuretic properties. OPC-51803 inhibited [3H]AVP binding to rat kidney and liver plasma membranes with specificity 21 times higher for the rat V2-receptor subtype than for the rat V1a-receptor subtype. In our previous study using HeLa cells expressing human AVP receptor subtypes, OPC-51803 showed binding affinities for human V2- and V1a-receptors with \( K_i \) values of 91.9 ± 10.8 and 819 ± 39 nM, respectively, and the difference was about 9-fold.

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>Osmolality (mOsm/kg)</th>
<th>Na⁺ (mEq/l)</th>
<th>K⁺ (mEq/l)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea Nitrogen (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>309 ± 2</td>
<td>144.8 ± 0.7</td>
<td>5.26 ± 0.17</td>
<td>0.73 ± 0.01</td>
<td>20.2 ± 0.9</td>
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<tr>
<td>0.03 OPC-51803 (mg/kg, p.o.)</td>
<td>305 ± 1</td>
<td>144.7 ± 0.5</td>
<td>4.96 ± 0.19</td>
<td>0.71 ± 0.02</td>
<td>19.9 ± 0.7</td>
</tr>
<tr>
<td>0.1 OPC-51803 (mg/kg, p.o.)</td>
<td>306 ± 2</td>
<td>144.3 ± 0.4</td>
<td>4.97 ± 0.16</td>
<td>0.76 ± 0.02</td>
<td>21.9 ± 0.7</td>
</tr>
<tr>
<td>0.3 OPC-51803 (mg/kg, p.o.)</td>
<td>306 ± 1</td>
<td>145.3 ± 0.6</td>
<td>5.42 ± 0.33</td>
<td>0.79 ± 0.02*</td>
<td>23.4 ± 0.7*</td>
</tr>
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</table>

* \( P < .05 \) vs. vehicle.

**Table 4**

<table>
<thead>
<tr>
<th></th>
<th>AVP (pg/ml)</th>
<th>Renin Activity (ng/ml/h)</th>
<th>Aldosterone (ng/ml)</th>
<th>Norepinephrine (ng/ml)</th>
<th>Epinephrine (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.9 ± 0.1</td>
<td>7.51 ± 0.79</td>
<td>217 ± 36</td>
<td>6.78 ± 0.42</td>
<td>3.00 ± 0.35</td>
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<tr>
<td>0.03 OPC-51803 (mg/kg, p.o.)</td>
<td>0.9 ± 0.0</td>
<td>9.30 ± 3.72</td>
<td>200 ± 42</td>
<td>10.02 ± 0.70</td>
<td>4.04 ± 0.53</td>
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<tr>
<td>0.1 OPC-51803 (mg/kg, p.o.)</td>
<td>1.6 ± 0.9</td>
<td>8.03 ± 2.67</td>
<td>343 ± 86</td>
<td>10.59 ± 1.36*</td>
<td>4.18 ± 0.53</td>
</tr>
<tr>
<td>0.3 OPC-51803 (mg/kg, p.o.)</td>
<td>0.8 ± 0.1</td>
<td>6.08 ± 1.52</td>
<td>132 ± 35</td>
<td>9.54 ± 1.06</td>
<td>3.77 ± 0.43</td>
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</table>

* \( P < .05 \) vs. vehicle.
doses of 0.1 mg/kg and higher, OPC-51803 concentrated urine in a dose-dependent manner in Brattleboro rats. At 1997). Administered orally OPC-51803 decreased urine volume in normal-hydrated rats (Yamamura et al., 1992, 1998). These results suggest that OPC-51803 causes a concentration of urine to the same degree as circulating AVP in rats. In the multiple-dosing study, the decrease in urine volume induced by OPC-51803 in Brattleboro rats at doses of 0.03 and 0.3 mg/kg was almost constant during the 4-week period of repeated oral dosing, although the drug appeared to produce a slightly more potent antidiuretic action on the first dosing day. This result is similar to previous studies in Brattleboro rats using chronic AVP or AVP antagonists with intrinsic agonistic activity (Mah and Hofbauer, 1988). This suggests that OPC-51803 can sustain antidiuretic action by repeated administration. The results of our study suggest that OPC-51803 is a well tolerated antidiuretic and if the data can be extrapolated to humans, OPC-51803 may be useful in normalizing urine excretion in patients with central diabetes insipidus and in the treatment of nocturnal enuresis and polyuria.

Rittig et al. (1989b) reported that diurnal rhythm of plasma vasopressin levels (P_{AVP}) seemed to be related to urine excretion; for example, the increase in P_{AVP} during the night correlated to the decrease in urine production. In patients with nocturnal enuresis and some elderly subjects (Norgaard et al., 1985; Rittig et al., 1989b; Asplund and Aberg, 1991), however, the lack of the increase in P_{AVP} during the night may result in high nocturnal urine volume and low nocturnal urinary osmolality. As seen in normal Sprague-Dawley rats, OPC-51803 decreased urine volume in the presence of circulating AVP, suggesting that this drug can act as an antidiuretic in addition to the action of AVP and may thus restore nocturnal urine production impaired by the lack of the increase in P_{AVP}.

Occasionally, excess water retention leads to hyponatremia and hypokalemia. OPC-51803 did not significantly change serum sodium concentration in Sprague-Dawley rats even at those doses that significantly decreased urine volume. de Rouffignac et al. (1983) and Elalouf et al. (1984), using a micropuncture technique, reported that DDAVP slightly induced sodium and chloride reabsorption in the thick ascending limb of Henle's loop and/or distal tubules in rats. In our experiments, OPC-51803 showed a tendency to decrease urinary sodium excretion. Sodium reabsorption at Henle's loop or distal tubules may offset the decline in serum sodium concentration by water retention.

OPC-51803 slightly increased serum creatinine concentration and tended to decrease urinary excretion of creatinine, but only at the highest dose. The serum creatinine concentration and creatinine clearance have been used as a convenient index for evaluating the renal function. An increase in serum creatinine concentration predicts a deterioration of renal hemodynamics. However, Gellai et al. (1984) showed using Brattleboro rats that acute AVP infusion did not modify renal blood flow and glomerular filtration rate. Low urine output induced by OPC-51803 may lead to creatinine reabsorption in the kidney, causing an increase in serum creatinine (Bouby et al., 1996). Furthermore, OPC-51803 significantly increased serum urea nitrogen and tended to decrease urinary excretion of urea nitrogen but only at the highest dose. AVP reportedly activates the urea transporter via V_{2}-receptors and promotes urea reabsorption in the inner medullary collecting duct (Nielsen and Knepper, 1993; Inoue et al., 1999). OPC-51803 in the same way may induce urea reabsorption through the activation of the urea transporter via V_{2}-receptors, thereby increasing serum urea nitrogen concentration in Sprague-Dawley rats.

From the in vitro binding experiments, we showed that OPC-51803 has an affinity for the rat V_{2}- and V_{1a}-receptors. Even at a high dose of OPC-51803 (30 mg/kg) administered orally to Sprague-Dawley rats, no differences were seen in blood pressure and heart rate between the drug-treated and vehicle-treated groups. This suggests that perhaps OPC-51803 has no agonistic activity for rat V_{1a}-receptors. Consistent with this idea is that OPC-51803 did not increase intracellular Ca^{2+} concentration in HeLa cells expressing human V_{1a}-receptor (Nakamura et al., 2000). It is also noteworthy that OPC-51803 did not inhibit the pressor responses caused by injected AVP. At this point we are uncertain why V_{1a}-antagonism was not seen in vivo. It is possible that OPC-51803 may not inhibit endogenous AVP-induced effects on V_{1a}-receptors at least in the doses that produce significant antidiuresis.

In summary, oral administration of OPC-51803 produced an antidiuretic action via V_{2}-receptor activation in rats. OPC-51803 is a nonpeptide compound contrary to DDAVP (Hammer and Vilhardt, 1985; Janknegt et al., 1997; Asplund et al., 1999) and is absorbed well, so this drug may be a practical treatment for patients.

Acknowledgments

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References

Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K_{i}) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem Pharmacol 22:3199–3198.


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