Full Paper

Effects of OPC-51803, a Novel, Nonpeptide Vasopressin V₂-Receptor Agonist, on Micturition Frequency in Brattleboro and Aged Rats

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Abstract. We assessed the effects of OPC-51803 ((5*R*)-2-[1-(2-chloro-4-(1-pyrrolidinyl)benzoyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepin-5-yl]-*N*-isopropylacetamide), a nonpeptide vasopressin V₂-receptor agonist, on micturition frequency in female homozygous Brattleboro rats (strain carries hereditary diabetes insipidus) and aged male Sprague-Dawley rats with polyuria. Female homozygous Brattleboro rats exhibited more diuresis and a larger micturition frequency over a 24-h period than did the heterozygous controls. In Brattleboro rats, an oral administration of OPC-51803 at 0.03 and 0.3 mg/kg significantly decreased urinary frequency and was accompanied by decreased urine volume. However, little effect was seen in the mean and maximal micturition volume. Aged male Sprague-Dawley rats (25-month-old) showed a significant increase in urine volume throughout a 0- to 24-h period compared with mature (6-month-old) rats. Orally administered OPC-51803 at 0.3 mg/kg decreased not only urine volume but also urinary frequency in aged rats. Furthermore, OPC-51803 prolonged the time prior to the first micturition. Therefore, OPC-51803 decreased micturition frequency in both rat species by reducing urine outflow. This suggests that the compound will be useful for treating micturition disorders that result in frequent micturition, such as that from polyuria, nocturnal polyuria, and some kinds of urinary incontinence.

Keywords: OPC-51803, nonpeptide, vasopressin V₂ agonist, micturition frequency, antidiuresis

Introduction

Recently, we discovered a nonpeptide vasopressin (AVP) V₂-receptor agonist, OPC-51803, that has an affinity for AVP V₂-receptors and induced cyclic AMP production in HeLa cells that express human AVP V₂ receptors (1). When OPC-51803 was orally administered to female homozygous Brattleboro rats (harboring hereditary diabetes insipidus due to a congenital defect that prevents them from producing AVP and concentrating urine) and normally hydrated male Sprague-Dawley rats, urine volume decreased and urinary osmolality increased in a dose-dependent manner (2).

Frequent urination, commonly seen in people over 65, is a serious problem that disrupts social activities and sleep (3–6). Various etiologies mark these urinary disorders (7–10): One principal determinant is the reduction of the bladder's capacity to store urine, and another is polyuria (increased urine production). Patients with low bladder capacity given anticholinergic drugs (which relax bladder smooth muscles) have realized therapeutic effects (11–13), but few useful drugs exist to treat polyuria.

A lack of or lowering of endogenous AVP is generally cited as a cause of polyuria. In fact, an abnormal diurnal rhythm of plasma AVP has been observed in patients with primary nocturnal enuresis and nocturnal polyuria (14–18). We therefore chose to study the effects of OPC-51803 in two rat species with polyuria: homozygous Brattleboro rats and aged 25-month-old Sprague-Dawley rats. Although OPC-51803's ability to induce a decrease in urine volume has been previously confirmed

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in rats, the effects of OPC-51803 on micturition frequency and micturition volume per void have not yet been examined. We set up a system to measure micturition volume and frequency concomitantly and automatically, and then we estimated the micturition patterns of both rats with or without OPC-51803 treatment.

Materials and Methods

Materials

OPC-51803, (5*R*)-2-[1-(2-chloro-4-(1-pyrrolidinyl)benzoyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepin-5-yl]-*N*-isopropylacetamide, was synthesized at Otsuka Pharmaceutical Co., Ltd. (Tokyo). Gum arabic was purchased from Wako Pure Chemical Industries, Ltd. (Osaka). OPC-51803 was suspended in a 5% gum arabic solution and administered orally to each rat at 2 mL/kg.

Animals

Male Sprague-Dawley rats, aged 6 and 25 months, were purchased from Charles River Japan, Inc. (Yokohama), and female homozygous and heterozygous Brattleboro rats, aged 5 months, were bred at our facilities. Rats were housed in a temperature-, humidity-, and light-controlled room and given free access to food (MF; Oriental Yeast Co., Ltd, Tokyo) and water. The care and handling of these animals were in accordance with "The Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co., Ltd.; October 1, 1994."

Micturition pattern measured in Brattleboro rats

Each rat was placed in an individual metabolic cage (Sugiyama-Gen Iriki Co., Ltd., Tokyo) supplied with water and food. Water and food intakes were measured over a period of 0 to 8 h. Urine was collected in a plastic container, which was set on an electronic balance (Sartorius BP610; Sartorius K.K., Tokyo) that was connected to a printer (Sartorius YDP03-OCE), a personal computer (PC-VS16CS5CA1; NEC, Tokyo) through a T-type connector (Sartorius L99813), and a multiplexer (LMP-150N; Logitec, Tokyo). Spontaneously voided urine was weighed every 5 min for 8 h using a computer program designed to measure micturition frequency. If the difference in weight between two consecutive measurements was more than 0.1 g, it was classified as a micturition event. An aliquot of urine was collected, and urinary osmolality was determined by analyzing the freezing-point depression using a Fiske Osmometer (Model 3400; Fiske Associates, Norwood, MA, USA). The experiments were conducted in a triple-crossover-dosing design at approximately 3- or 4-day intervals, with each rat orally given the vehicle (5% gum arabic solution, p.o.) and both doses of OPC-51803

(0.03 and 0.3 mg/kg, p.o.).

Micturition pattern measured in aged Sprague-Dawley rats

The micturition pattern was monitored during a 0- to 24-h period as described above. Each rat was orally given both the vehicle (5% gum arabic solution, p.o.) and OPC-51803 (0.3 mg/kg, p.o.) at approximately 1-week intervals. Urine volume and urinary osmolality were determined as mentioned above, and urinary AVP concentrations were measured by radioimmunoassay using commercial kits from Mitsubishi Yuka Bio-Chemical Laboratories (Tokyo).

Statistical analyses

All values were expressed as the mean $\pm$ S.E.M. Differences were analyzed by a two-tailed paired *t*-test or an ANOVA with a crossover design followed by a two-tailed Dunnett's multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

Results

Micturition characteristics in Brattleboro rats

The micturition characteristics of female homozygous Brattleboro rats and age-matched heterozygous control rats are shown in Table 1. Homozygous Brattleboro rats demonstrated not only an increase in diluted urine but also an increase in micturition frequency. Micturition intervals were shorter, and mean and maximal micturition volumes were larger in homozygous Brattleboro rats than in the heterozygous controls.

The effects of OPC-51803 on urine volume and micturition frequency were examined in a three-period crossover design using nine female Brattleboro rats. Oral doses of OPC-51803 (0.03 and 0.3 mg/kg) resulted in a significant and dose-dependent decrease in urine volume during a 0- to 8-h period (Fig. 1), as reported in our previous study (2). In the 0.3 mg/kg group, urine osmolality showed a statistically significant increase.

Micturition frequency was also significantly suppressed at both 0.03 and 0.3 mg/kg, compared to the vehicle-treated group (Fig. 2). OPC-51803 treatment tended to increase mean micturition volume in a dose-dependent manner, but this increase was not statistically significant. Maximal micturition volume was not affected by drug treatment. Water intake decreased after OPC-51803 dosing, but no significant change in body weight or food intake was seen between the OPC-51803- and vehicle-treated groups.

Table 1. Characteristics of female homozygous and heterozygous Brattleboro rats

	Homozygous	Heterozygous	<i>P</i> value
Body weight (g)	210.5 ± 5.4	295.6 ± 4.6	<0.0001
Water intake (g/24 h)	140.4 ± 4.4	21.9 ± 0.4	0.0001
Food intake (g/24 h)	12.6 ± 0.6	14.2 ± 0.5	0.0657
Urine volume (g/24 h)	120.7 ± 4.1	10.3 ± 0.5	0.0001
Urine osmolality (mOsm/kg)	160 ± 5	2445 ± 126	0.0001
Micturition frequency (voids/24 h)	44.6 ± 6.4	15.4 ± 0.9	0.0004
Mean micturition intervals (min)	39.9 ± 4.0	96.4 ± 5.8	<0.0001
Mean micturition volume (g/void)	3.2 ± 0.3	0.7 ± 0.1	0.0001
Maximal micturition volume (g/void)	7.9 ± 0.3	1.6 ± 0.2	0.0001

Values are expressed as the mean ± S.E.M. of 16 homozygous and 10 heterozygous Brattleboro rats. The difference between homozygous and heterozygous Brattleboro rats was determined by a Student's *t*-test (two tailed). A value of *P*<0.05 was taken to be significant.

Fig. 1. Effects of OPC-51803 on urine volume and urinary osmolality during an 8-h period postdose in female homozygous Brattleboro rats. Values are expressed as the mean ± S.E.M. of nine rats from each dosing group in a triple-crossover design. Differences were analyzed by an ANOVA with a crossover design followed by a two-tailed Dunnett's multiple comparison test. ***P*<0.01 vs vehicle.

Micturition characteristics in aged Sprague-Dawley rats

Eleven aged Sprague-Dawley rats (25-month-old) and 14 mature rats (6-month-old) were used. In aged rats, spontaneously voided urine volume was significantly greater than that in mature rats over a 0- to 24-h period. Urinary osmolality at that time was lower in aged rats than in mature rats. Micturition frequency was almost the same in both mature and aged rats, but mean and maximal micturition volume was larger in aged rats than in mature rats (Table 2). Urinary AVP content was less in aged rats than in mature rats (Table 2).

Fig. 2. Effects of OPC-51803 on micturition frequency and mean and maximal micturition volume during an 8-h period postdose in female homozygous Brattleboro rats. Values are expressed as the mean ± S.E.M. of nine rats from each dosing group in a triple-crossover design. Differences were analyzed by an ANOVA with a crossover design followed by a two-tailed Dunnett's multiple comparison test. ***P*<0.01 vs vehicle.

The effects of oral OPC-51803 administration on urine volume and micturition frequency are seen in

Table 2. Urinary parameters during 0–24 h in 6-month-old and 25-month-old Sprague-Dawley rats

	6-months-old (n = 14)	25-months-old (n = 11)	P values
Body weight (g)	599.6 ± 9.8	869.9 ± 45.8	0.0001
Urine volume (g/24 h)	18.9 ± 0.8	28.1 ± 3.6	0.0282
Urinary osmolality (mOsm/kg)	1669 ± 72	864 ± 80	<0.0001
Micturition frequency (voids/24 h)	12.5 ± 1.0	13.5 ± 1.6	0.6007
Mean micturition volume (g/void)	1.6 ± 0.1	2.2 ± 0.2	0.0120
Maximal micturition volume (g/void)	2.7 ± 0.1	4.0 ± 0.4	0.0044
Urinary AVP excretion (pg/24 h)	2504 ± 217	1490 ± 183	0.0022

Values are expressed as the mean ± S.E.M. The difference between 6-month-old rats and 25-month-old rats was determined by a Student's *t*-test (two tailed). A value of $P < 0.05$ was considered significant.

Fig. 3. Effects of OPC-51803 on urine volume and micturition frequency in 25-month-old Sprague-Dawley rats. Values are expressed as the mean ± S.E.M. of 11 rats. The difference was determined by a paired *t*-test (two tailed) at each time period. ** $P < 0.01$.

Fig. 3. At 0.3 mg/kg, OPC-51803 significantly decreased urine volume and micturition frequency during 0 to 4 h. Furthermore, OPC-51803 delayed the time when rats excreted urine for the first time after being placed in individual metabolic cages (vehicle: 86 ± 11 min, OPC-51803: 248 ± 41 min).

Discussion

Female homozygous Brattleboro rats with hereditary diabetes insipidus showed not only an increase in diluted urine but also an increase in micturition frequency in accordance with previous reports (19, 20). The marked

increase in urine output induced changes in micturition patterns (e.g., shortening micturition intervals and large micturition volume per void). In particular, increases in mean and maximal micturition volume suggest that homozygous Brattleboro rats adapted themselves to the increased urine excretion by enlarging their bladder capacity.

In our previous report (2), we demonstrated that single and multiple oral dosing of OPC-51803 induced antidiuretic effects in homozygous Brattleboro rats. Those results were consistent with this study's observations: Over an 8-h period postdose with 0.03 and 0.3 mg/kg of OPC-51803, urine volume significantly decreased and urine osmolality increased, compared to the vehicle treatment. We further assessed the effects of OPC-51803 on increased micturition frequency: Over an 8-h period postdose, micturition frequency was 28.7 ± 5.9 voids in the vehicle-treated group and 16.4 ± 3.6 times and 6.8 ± 2.0 voids in the 0.03 mg/kg- and 0.3-mg/kg-treatment groups, respectively. On the other hand, mean and maximal micturition volumes were not significantly changed by OPC-51803 treatment, suggesting that OPC-51803 has no direct action on bladder capacity. By using cystometry in urethane-anesthetized rats, we confirmed that OPC-51803 did not influence bladder functions including bladder capacity (unpublished data). These results showed that OPC-51803 significantly suppressed micturition frequency in accordance with reduced urine excretion.

Since it is well known that normal micturition patterns are impaired in the elderly (3, 5), we compared the micturition patterns of 6- and 25-month-old Sprague-Dawley rats and assessed the differences based on age. Twenty-five-month-old rats showed higher urine volume and lower urinary osmolality than 6-month-old rats, suggesting that their ability to concentrate urine decreased with age. Geelen and Corman (21) reported that urinary osmolality in rats decreased with age, although plasma AVP levels or urinary AVP excretion

was comparable between 10-, 20-, and 30-month-old rats. Therefore, the phenomenon is likely related to an impaired responsiveness of the kidney to AVP. On the other hand, Zbuzek et al. (22) showed that plasma AVP concentrations decreased in aged rats because of the reduced release of AVP from the neurohypophysis. In our study, during 0 to 24 h, the urinary AVP content measured in 25-month-old rats was less than that seen in 6-month-old rats (Table 1). Our results were identical to those observed by Zbuzek et al. and suggest that with age, there is a decline in AVP secretion from the neurohypophysis. Furthermore, low plasma AVP concentrations may also lead to the excretion of diluted urine. OPC-51803 significantly decreased both urine volume and micturition frequency in aged rats. Hence, although the rats lacked endogenous AVP, OPC-51803 promoted water reabsorption and normalized micturition behavior.

In conclusion, OPC-51803 improved frequent urination by reducing urine outflow in Brattleboro rats and aged Sprague-Dawley rats. Cannon et al. (23) and Hilton and Stanton (24) reported that a peptide AVP V_2 agonist, DDAVP, reduced urinary frequency in the male and female subjects. We need to study further whether OPC-51803 shows the same improvement in patients with frequent urination as seen in the present study.

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