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Pharmacological characterization of YM471, a novel potent vasopressin V_{1A} and V_2 receptor antagonist

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Abstract

The pharmacologic profile of YM471 ((*Z*)-4′-{4,4-difluoro-5-[2-(4-dimethylaminopiperidino)-2-oxoethylidene]-2,3,4,5-tetrahydro-1*H*-1-benzoazepine-1-carbonyl}-2-phenylbenzanilide monohydrochloride), a novel potent vasopressin V_{1A} and V_2 receptor antagonist, was investigated using several in vitro and in vivo techniques. YM471 showed high affinity for rat vasopressin V_{1A} and V_2 receptors, exhibiting K_i values of 0.16 and 0.77 nM, respectively. In contrast, YM471 exhibited much lower affinity for rat vasopressin V_{1B} and oxytocin receptors, with K_i values of 10.5 μM and 31.0 nM, respectively. In conscious rats, oral administration of YM471 (0.1–3.0 mg/kg) produced dose-dependent inhibition of the pressor response caused by exogenous vasopressin and increased urine excretion and decreased urine osmolality; this effect lasted more than 8 h. In all biological assays used, YM471 exhibited no agonistic activity. These results demonstrate that YM471 exerts potent and long-lasting antagonistic activity on both vasopressin V_{1A} and V_2 receptors, and that this compound may be a useful tool for clarifying the physiologic and pathophysiologic roles of vasopressin and the therapeutic usefulness of the vasopressin receptor antagonist.

Keywords: YM471; Vasopressin V_{1A} receptor; Vasopressin V_2 receptor; Nonpeptide antagonist

1. Introduction

The peptide hormone arginine vasopressin plays important roles in the regulation of blood pressure and fluid volume homeostasis. These effects are mediated by membrane-bound receptors located in a variety of tissues and organs. So far, three vasopressin receptor subtypes (V_{1A}, V_{1B} and V_2) have been identified based on their primary structure, their coupling mechanisms, their tissue distributions and their pharmacologic properties (Birnbaumer et al., 1992; Sugimoto et al., 1994; Thibonnier et al., 1994; Morel et al., 1992; De Keyzer et al., 1994; Lolait et al., 1992). Vasopressin V_{1A} receptors have been shown to be present in vascular smooth muscle cells, hepatocytes, platelets, mesangial cells, cardiomyocytes, brain, testis, adrenal glands, spinal cord and sympathetic ganglia by radioligand binding techniques. These receptors serve to mediate the contraction, proliferation and hypertrophy of cells, platelet aggregation, hepatocyte glycogenolysis, enhancement of learning and memory, and steroid secretion (Weingartner et al., 1981; Thibonnier and Roberts, 1985; Jard et al., 1986; Phillips et al., 1990; Howl et al., 1991; Guillon et al., 1982; Serradeil-Le Gal et al., 1995; Tahara et al., 1997a). Vasopressin V_{1B} receptors are located in the anterior pituitary, β-cells of pancreas and adrenal medulla, where they stimulate corticotropin, insulin and catecholamine release, respectively (Jard et al., 1986; Lee et al., 1995; Grazzini et al., 1996). In contrast, vasopressin V_2 receptors are present in a renal epithelial cell line (LLC-PK_1), as well as in the medullary portion of the kidney, where they control water and urea reabsorption (Butlen et al., 1978; Jans et al., 1989).

Vasopressin causes potent vasoconstriction via vasopressin V_{1A} receptors and induces water retention via vasopressin V_2 receptors, respectively; consequently, vasopressin plays an important role in regulating blood pressure as well as fluid and electrolyte homeostasis in normal physiologic and various pathophysiologic states, such as congestive heart failure, liver cirrhosis, renal disease, hyponatremia,

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the syndrome of inappropriate antidiuretic hormone secretion, nephrotic syndrome, and dysmenorrhea (Fujisawa et al., 1993b; Naitoh et al., 1994; Laszlo et al., 1991; Mah and Hofbauer, 1987; Sorensen et al., 1995). Therefore, the development of vasopressin receptor antagonists is essential for assessing the pathophysiologic roles of vasopressin and could lead to new therapeutic agents. Recently, several orally effective nonpeptide vasopressin receptor antagonists have been discovered, namely the vasopressin V_{1A} receptor-selective antagonists OPC-21268 (1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone; Yamamura et al., 1991) and SR 49059 ((2*S*) 1-[(2*R* 3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl)]-pyrrolidine-2-carboxamide; Serradeil-Le Gal et al., 1993), the vasopressin V_2 receptor-selective antagonists OPC-31260 (5-dimethylamino-1-{4-(2-methylbenzoylamino)benzoyl}-2,3,4,5-tetrahydro-1*H*-benzazepine; Yamamura et al., 1992), OPC-41061 (7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoyl-amino)benzoyl]-2,3,4,5-tetrahydro-1*H*-1-benzazepine; Yamamura et al., 1998) and SR 121463A (1-[4-(*N*-tert-butyl-carbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy) cyclohexane]indol-2-one; equatorial isomer; Serradeil-Le Gal et al., 1996) and the vasopressin V_{1A}/V_2 receptor antagonist conivaptan (YM087, 4′-(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-d][1]benzoazepine-6-carbonyl)-2-phenylbenzanilide monohydrochloride; Burnier et al., 1999; Yatsu et al., 1999; Matsuhisa et al., 2000).

We have previously reported on the discovery and characterization of a high-affinity mixed vasopressin V_{1A}/V_2 receptor antagonist, conivaptan (Tahara et al., 1997b). Although the recent identification of nonpeptide vasopressin receptor antagonists represents an important milestone in vasopressin research, it is likely that elucidation of the role of vasopressin in the pathophysiology of diseases in various systems will require potent compounds for both animals and humans. In this study, we report on the characterization of YM471 ((*Z*)-4′-{4,4-difluoro-5-[2-(4-dimethylaminopiperidino)-2-oxoethylidene]-2,3,4,5-tetrahydro-1*H*-1-benzoazepine-1-carbonyl}-2-phenylbenzanilide monohydrochloride; Fig. 1) in rats. YM471 is the lead compound from a new chemical series of potent nonpeptide vasopressin receptor antagonists.

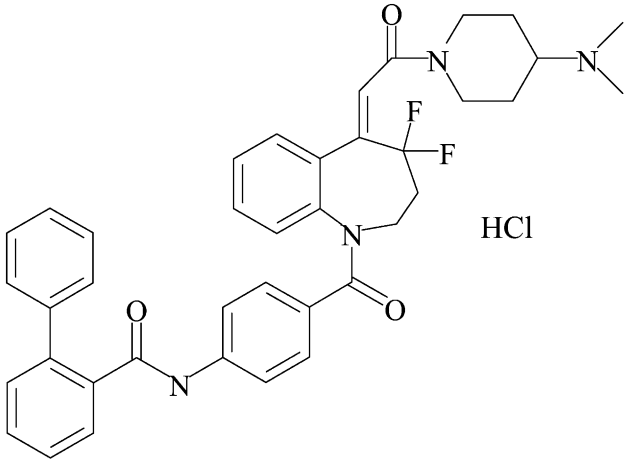

Fig. 1. Chemical structure of YM471, (*Z*)-4′-{4,4-difluoro-5-[2-(4-dimethylaminopiperidino)-2-oxoethylidene]-2,3,4,5-tetrahydro-1*H*-1-benzoazepine-1-carbonyl}-2-phenylbenzanilide monohydrochloride.

2. Materials and methods

2.1. Materials

The radioligands [^{3}H]vasopressin and [^{3}H]oxytocin with a specific activity of 80 and 50 Ci/mmol, respectively, were obtained from DuPont, New England Nuclear (Boston, MA, USA). Vasopressin and oxytocin were obtained from the Peptide Institute (Osaka, Japan). Furosemide was obtained from Sigma (St. Louis, MO, USA). YM471, SR 49059 and SR 121463A were synthesized at the Yamanouchi Pharmaceutical (Ibaraki, Japan). The structure of these compounds was determined by ^{1}H-nuclear magnetic resonance, mass spectrometry and elemental analysis. Their purity was measured by high-pressure liquid chromatography and was > 98%. For in vitro studies, these nonpeptide antagonists were initially dissolved in dimethylsulfoxide at 10^{-2} M and diluted to the desired concentration with assay buffer. The final concentration of dimethylsulfoxide in the assay buffer did not exceed 1%, a concentration at which specific [^{3}H]vasopressin or [^{3}H]oxytocin binding was not affected. For in vivo studies, drugs were dissolved in dimethylformamide for intravenous administration and in 0.5% methylcellulose solution for oral administration. Diethylstilbestrol dipropionate was obtained from Sigma. Bovine serum albumin was purchased from Nacalai Tesque (Kyoto, Japan). Reagents for protein assay were purchased from Bio-Rad Laboratories (Richmond, CA, USA). All other chemicals were of the highest reagent grade available.

2.2. Animals

Male and female Wistar rats (250–300 g) were used as indicated. All animals were housed in communal cages and maintained on a 12-h light/dark cycle with food and water available ad libitum. All experimental procedures involving animals or animal tissues conformed to the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical and "The Guide for the Care and Use of Laboratory Animals" (U.S. Department of Health and Human Services, 1985 NIH Publication No. 86-23).

2.3. Binding assays

Rats were anesthetized with ether and killed by decapitation; the liver, kidneys, pituitary gland and uterus were

quickly removed. All subsequent preparative steps were carried out at 4 °C. Membrane preparations from rat liver (Nakamura et al., 1983), kidney (Campbell et al., 1972) and pituitary (Lutz-Bucher and Koch, 1983) were prepared as previously described. Uterine plasma membranes were prepared by the method of Pettibone et al. (1990) from uterine tissue isolated from female Wistar rats treated with diethylstilbestrol dipropionate at 0.3 mg/kg i.p. 18–24 h before isolation. For saturation binding studies, membrane preparations were incubated with various concentrations of [^{3}H]vasopressin or [^{3}H]oxytocin (0.1–3.0 nM). For competition studies, radioligand (0.5–1.0 nM) was added to each membrane preparation, which was incubated with various concentrations of test compounds in 250 μl of assay buffer containing 50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$ and 0.1% bovine serum albumin. The binding reactions were initiated by the addition of the plasma membrane preparations and incubations were for 60 min at 25 °C, which allowed equilibrium to be established. After incubation, the reaction was terminated by the addition of 3 ml of ice-cold Tris buffer (50 mM Tris–HCl, pH 7.4, and 10 mM $MgCl_2$) followed immediately by rapid filtration through 96-well GF/B UniFilter Plates using a MicroMate Cell Harvester (Packard Instrument, Meriden, CT, USA). The filters were rinsed twice and the radioactivity retained on the filters was counted with a TopCount Microplate Scintillation Counter (Packard Instrument). Nonspecific binding was determined using 1 μM unlabeled vasopressin or oxytocin. Specific binding was calculated as the total binding minus nonspecific binding. The concentration of test compound that caused 50% inhibition (IC_{50}) of the specific binding of [^{3}H]vasopressin or [^{3}H]oxytocin was determined by regression analysis of displacement curves. The inhibitory dissociation constant (K_i) was calculated from the following formula (Cheng and Prusoff, 1973): $K_i = IC_{50}/(1+[L]/K_D)$, where $[L]$ is the concentration of radioligand present in the tubes and K_D is the dissociation constant of radioligand obtained from the saturation studies. Data were analyzed using the GraphPAD PRISM (GraphPAD Software, San Diego, CA, USA).

2.4. Inhibition of pressor response to vasopressin in pithed rats

Male Wistar rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and the left carotid artery was cannulated with a polyethylene tube (PE-50) to measure blood pressure with a pressure transducer (AP-200T; Nihon Kohden; Tokyo, Japan), and heart rate with a cardiotachometer (AT-200T; Nihon Kohden) triggered by the blood pressure pulse wave. The left femoral vein was cannulated for intravenous administration of vasopressin and YM471. The vagus nerve was bilaterally resected at the neck to prevent reflex actions, indirect bradycardia due to the systemic vasoconstriction and cardiac parasympathetic nerve activation induced by vasopressin. Rats were pithed by inserting a steel rod through the orbit and foramen magnum down the whole length of the spinal canal. Immediately after pithing, the rats were artificially ventilated with a tidal volume of 0.01 ml/g body weight at a frequency of 50 cycles/min using a rodent ventilator (SN-480-4; Shinano Seisakusho; Tokyo, Japan). Rats were kept warm at 37 °C by means of a thermostatically controlled heating board. After arterial blood pressure and heart rate had stabilized, YM471 or the vehicle was administered intravenously (0.1 ml/kg) 5 min before the injection of vasopressin (30 mU/kg i.v.). The dose of antagonist causing a 50% inhibition of the pressor response induced by vasopressin (ID_{50}) was calculated from peak inhibition percentage with several doses of antagonist.

2.5. Inhibition of pressor response to vasopressin in conscious normotensive rats

Male Wistar rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). The left carotid artery was cannulated with a polyethylene tube (PE-50) to measure blood pressure. The catheter passed subcutaneously to the back, where it exited at the neck, and was filled with saline containing heparin. For 1–2 days, rats recovered from the operation; during this time, they were allowed free access to rat food and water. Measurement of blood pressure and heart rate was as previously described. This configuration permitted direct recording of arterial blood pressure and heart rate in individually housed, conscious, and unrestrained animals. The left femoral vein was cannulated for intravenous administration of vasopressin. After calibration of pressure transducers and an appropriate equilibration period, exogenous vasopressin (30 mU/kg) sufficient to induce a rise in diastolic blood pressure of 40–60 mm Hg was injected intravenously (50 μl/100 g in saline) two or three times at 15-min intervals to establish a reproducible control pressor response and subsequently at 30, 60, 90, 120, 180, 240, 360 and 480 min after a single p.o. dose of YM471 or the vehicle. Percentage of inhibition of the pressor response to exogenous vasopressin challenges during the subsequent 8-h period was used to measure vasopressin inhibition.

2.6. Aquaretic effect in dehydrated conscious rats

Male Wistar rats were deprived of drinking water for 16–20 h to stimulate endogenous vasopressin secretion. After YM471 or the vehicle was administered intravenously or orally, spontaneously voided urine was collected for 2 h using a metabolic cage.

2.7. Aquaretic effect in normally hydrated conscious rats

Male Wistar rats were housed individually in metabolic cages with water and food ad libitum for 2–3 days before the experiments. YM471, furosemide, or the vehicle was administered orally to hydrated conscious rats by gavage (5

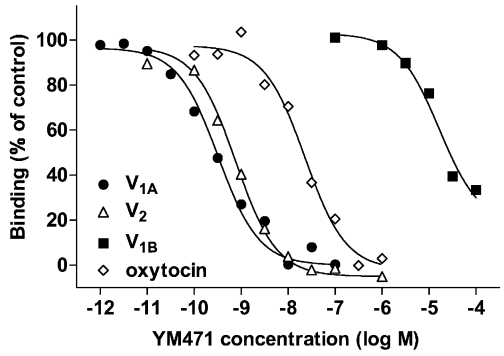

Fig. 2. Displacement of the specific binding of [^{3}H]vasopressin or [^{3}H]oxytocin to rat liver vasopressin V_{1A}, kidney V_2, pituitary V_{1B}, and uterus oxytocin receptors by YM471. Specific binding of [^{3}H]vasopressin or [^{3}H]oxytocin is expressed as a percentage of control binding. Results are representative data from three to seven independent experiments performed in duplicate. The combined results of all experiments are summarized in Table 1.

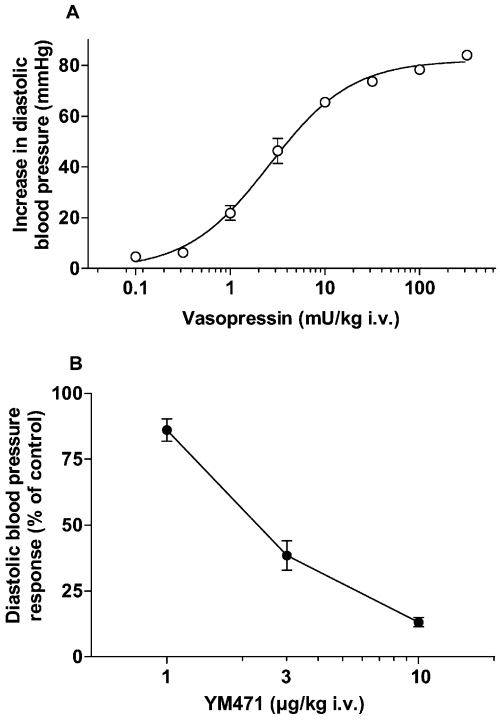

Fig. 3. (A) Vasopressin-induced dose–pressor response curve in pithed rats. (B) Inhibitory effect of intravenous administration of YM471 on the pressor response induced by vasopressin in pithed rats. YM471 was given 5 min before the injection of vasopressin (30 mU/kg i.v.). Values are means± S.E.M. for six animals in each group.

ml/kg). After treatment, each rat was placed in its own metabolic cage and provided with food and water ad libitum and spontaneously voided urine was collected at 2-h intervals for 10 h or for 4 h. After the volume of collected urine was measured, a portion was centrifuged at 2000 × *g* for 10 min. The supernatant was used for measurement of urinary variables. After urine sampling, the rats were decapitated, and trunk blood was collected into a tube to obtain plasma by centrifugation at 2000 × *g* for 15 min. Plasma and urine osmolality were measured by the freezing point depression method, using an osmometer (Model 3C2; Advanced Instruments; Needham Heights, MA, USA). Free water clearance (C_{H_2O}) was calculated as the urine flow rate minus osmolal clearance (C_{osm} = urine flow rate × urine osmolality/plasma osmolality). Plasma and urinary Na^+ and K^+ concentrations were measured using a flame photometer (Model 710; Hitachi, Tokyo, Japan). Plasma vasopressin concentration was measured with a vasopressin RIA kit (Mitsubishi Yuka Bio-chemical Laboratories, Tokyo, Japan) after Sep-Pak C18 extraction.

Table 1
Affinity of nonpeptide vasopressin receptor antagonists for rat vasopressin and oxytocin receptor subtypes

Compound	K_i (nM)			
	Vasopressin			Oxytocin[a] receptors
	V_{1A} receptors	V_2 receptors	V_{1B} receptors	
YM471	0.16 ± 0.04	0.77 ± 0.16	10,500 ± 500	31.0 ± 10.2
Conivaptan[b]	0.48 ± 0.07	3.04 ± 1.51	>100,000	44.4 ± 13.1
SR 49059	1.43 ± 0.17	285 ± 36	206 ± 46	83.9 ± 20.3
SR 121463A	5480 ± 520	2.83 ± 0.94	3840 ± 1600	984 ± 258

Values represent means±S.E.M. obtained from three to seven independent experiments performed in duplicate.

[a] Competition experiments with [^{3}H]oxytocin.

[b] Corresponding values of conivaptan were taken from previously published data (Tahara et al., 1997b).

2.8. Statistical procedures

Experimental results are expressed as the means±S.E.M. For in vivo studies, data were analyzed by one-way analysis

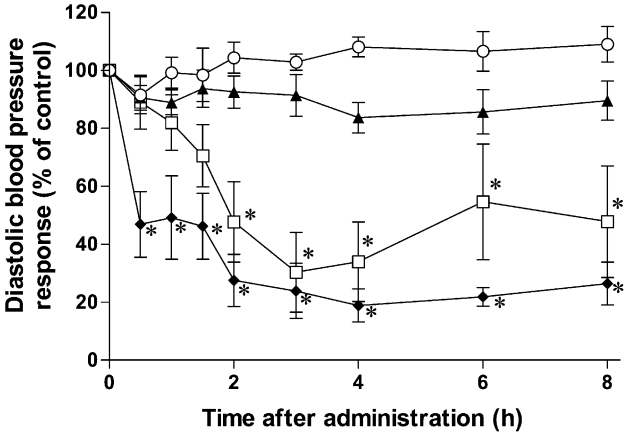

Fig. 4. Time course of the inhibitory effect of oral administration of YM471 (▲, 0.1; □, 0.3; ♦, 1.0 mg/kg) or the vehicle (○) on exogenous vasopressin (30 mU/kg i.v.)-induced hypertension in conscious rats. Each rat was treated with a single p.o. dose of the vehicle or YM471. Values are means±S.E.M. for six animals in each group. *P < 0.05 compared with the vehicle group.

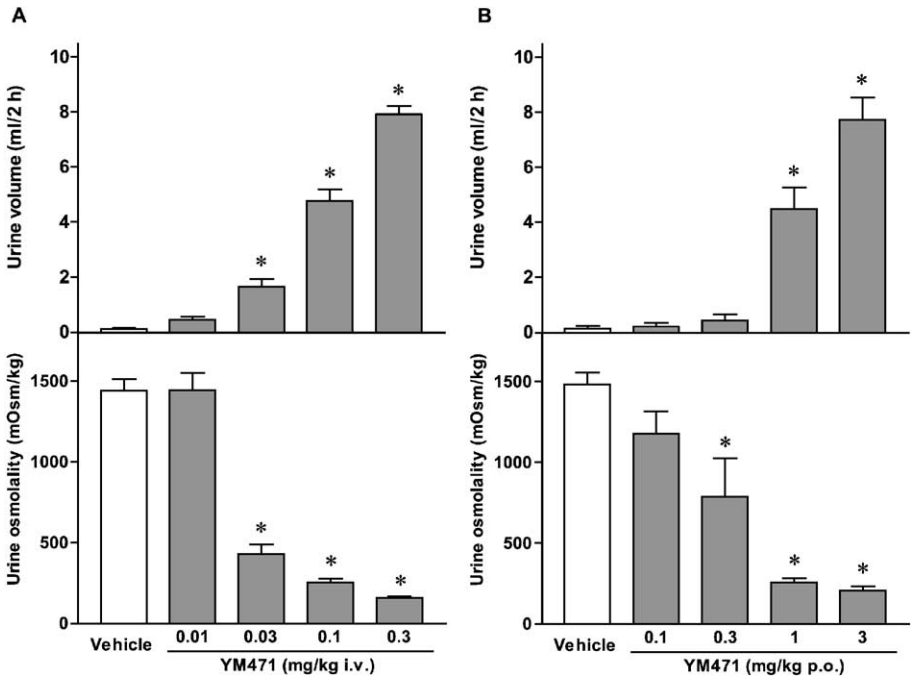

Fig. 5. Urine volume and osmolality in dehydrated conscious rats over a 2-h collection period after (A) intravenous or (B) oral administration of YM471 or the vehicle. Values are means±S.E.M. for five animals in each group. *$P<0.05$ compared with the vehicle group.

of variance. When overall statistically significant differences were detected ($P<0.05$), Dunnett's multiple range test was used to compare each of the doses to the vehicle control.

3. Results

3.1. Radioligand binding studies

YM471 showed high affinity for rat liver vasopressin V_{1A} and kidney V_2 receptors (Fig. 2, Table 1); measured K_i values were 0.16 ± 0.04 nM for V_{1A} and 0.77 ± 0.16 nM for V_2 receptors. The affinity of YM471 for vasopressin V_{1A} receptors was nine and three times higher than that of SR 49059 ($K_i = 1.43 \pm 0.17$ nM) and conivaptan ($K_i = 0.48 \pm 0.07$ nM), whereas that for vasopressin V_2 receptors was four times higher than that of SR 121463A ($K_i = 2.83 \pm 0.94$ nM) and conivaptan ($K_i = 3.04 \pm 1.51$ nM). In contrast, YM471 exhibited low affinity for rat pituitary vasopressin V_{1B} and uterus oxytocin receptors with K_i values of 10.5 ± 0.5 μM and 31.0 ± 10.2 nM, respectively.

3.2. Inhibition of pressor response to vasopressin in pithed rats

In pithed rats, intravenous administration of vasopressin dose-dependently induced a transient rise in diastolic blood pressure (Fig. 3A). Intravenous administration of

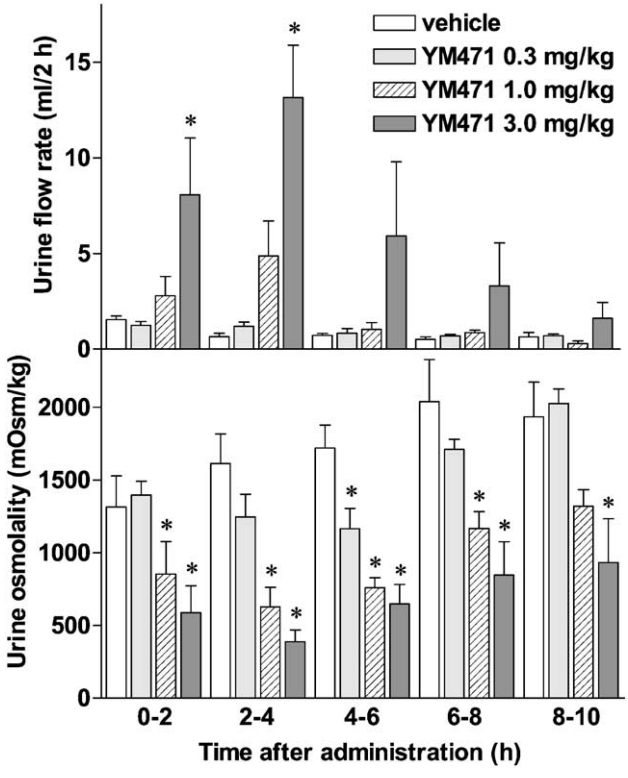

Fig. 6. Time course of YM471 action on urine flow rate and osmolality after oral administration to hydrated conscious rats. The aquaretic effect was measured by collecting urine at 2-h intervals for 10 h after oral administration of YM471 or the vehicle. Values are means±S.E.M. for five animals in each group. *$P<0.05$ compared with the vehicle group.

Table 2
Effect of oral administration of YM471 on urine volume, osmolality, and urinary electrolyte excretion in hydrated conscious rats

	Compound	Time after administration (h)					
		0–2	2–4	4–6	6–8	8–10	0–10
Urine volume (ml)	vehicle	1.55 ± 0.18	0.66 ± 0.17	0.72 ± 0.10	0.50 ± 0.14	0.64 ± 0.23	4.07 ± 0.28
	YM471	8.08 ± 2.96*	13.2 ± 2.7*	5.92 ± 3.86	3.30 ± 2.25	1.61 ± 0.84	32.1 ± 10.2*
Urine osmolality (mOsm/kg)	vehicle	1310 ± 210	1610 ± 200	1720 ± 160	2040 ± 290	1940 ± 240	1630 ± 170
	YM471	587 ± 185*	387 ± 82*	649 ± 131*	847 ± 229*	932 ± 302*	477 ± 112*
Urinary Na^+ excretion (μEq)	vehicle	204 ± 32.8	89.5 ± 41.9	144 ± 31	58.5 ± 21.1	45.1 ± 21.6	542 ± 98
	YM471	200 ± 13	318 ± 28*	139 ± 25	129 ± 26	92.2 ± 34.3	878 ± 69*
Urinary K^+ excretion (μEq)	vehicle	291 ± 40	191 ± 60	181 ± 31	158 ± 45	241 ± 80	1060 ± 90
	YM471	548 ± 29*	637 ± 36*	245 ± 105	166 ± 45	107 ± 30	1700 ± 190*

The aquaretic effect was measured by collecting urine at 2-h intervals for 10 h after oral administration of YM471 (3 mg/kg) or the vehicle in hydrated conscious rats. Values represent means±S.E.M. for five animals in each group.
* $P<0.05$ compared with the vehicle group.

YM471 (1.0–10 μg/kg) inhibited this exogenous vasopressin (30 mU/kg)-induced pressor response dose-dependently, exhibiting an ID_{50} value of 2.7 μg/kg (Fig. 3B). YM471 exhibited no agonistic property in this model.

3.3. Inhibition of pressor response to vasopressin in conscious normotensive rats

In conscious normotensive rats, bolus injection of vasopressin (30 mU/kg) produced a transient increase in dias-

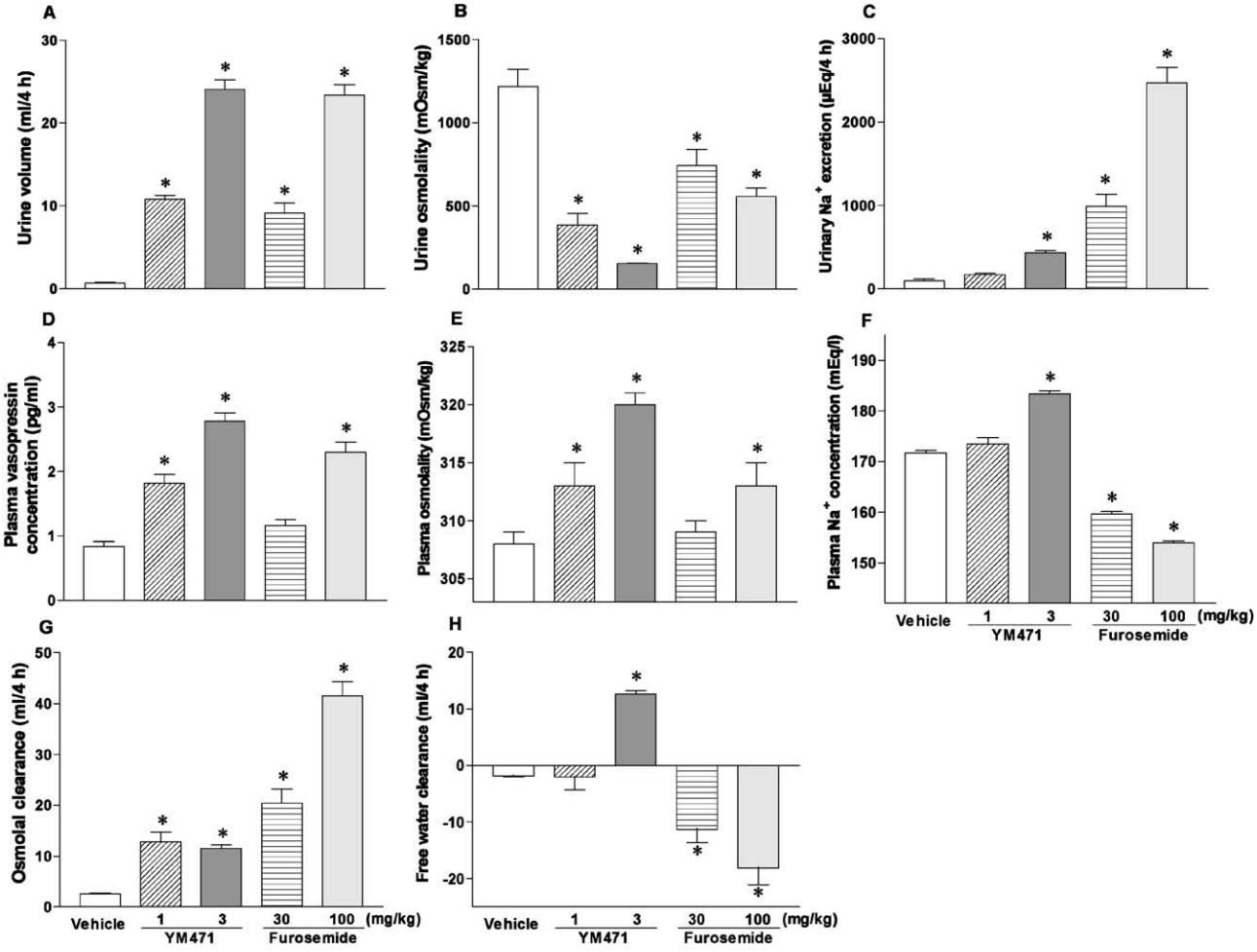

Fig. 7. Effects of oral administration of YM471 (1 and 3 mg/kg) or furosemide (30 and 100 mg/kg) on urinary and plasma variables in hydrated conscious rats. Values are means±S.E.M. for five animals in each group. * $P<0.05$ compared with the vehicle group.

tolic blood pressure (40–60 mm Hg). Oral administration of YM471 (0.1–1.0 mg/kg) produced a dose-dependent inhibition of the pressor response to exogenous vasopressin without changing basal blood pressure and heart rate (Fig. 4). Vasopressin receptor agonistic activity was not observed in that there was no significant increase in blood pressure with doses of YM471 that strongly inhibited the pressor response to vasopressin. The maximal inhibitory effect of YM471 at each dose occurred 3–4 h after oral administration. Maximal inhibitory effects of YM471 at 0.1, 0.3 and 1.0 mg/kg were approximately 16%, 69% and 81%, respectively, and the ID_{50} value was 0.27 mg/kg. YM471 at 1.0 mg/kg caused potent inhibition of the vasopressin-induced pressor response and this inhibition was still evident 8 h after dosing, demonstrating the long-lasting oral effectiveness of YM471 in counteracting the hypertensive response to vasopressin in vivo.

3.4. Aquaretic effect in dehydrated conscious rats

In dehydrated conscious rats, intravenous administration (0.01–0.3 mg/kg) and oral administration (0.1–3.0 mg/kg) of YM471 dose-dependently caused a significant increase in urine volume and a concomitant decrease in urine osmolality (Fig. 5).

3.5. Aquaretic effect in normally hydrated conscious rats

In hydrated conscious rats, oral administration of YM471 (0.3–3.0 mg/kg) dose-dependently increased urine excretion and decreased urine osmolality (Fig. 6). This effect was significant from 1.0 mg/kg on urine osmolality and at a dose of 3.0 mg/kg on urine excretion and reached its maximal effect 2–4 h after administration of YM471. The effects of the highest dose (3.0 mg/kg) lasted more than 8 h. Table 2 summarizes the effects of YM471 on urinary Na^+ and K^+ excretion. Oral administration of YM471 (0.3 and 1.0 mg/kg) had no measurable effect on urinary Na^+ and K^+ excretion (data not shown); however, the 3.0 mg/kg dose caused a significant increase. YM471 exhibited no antidiuretic property in this model.

3.6. Comparison of YM471 and furosemide

The doses of YM471 and furosemide that had a similar effect on the extracellular volume were determined. Oral administration of YM471 (1 and 3 mg/kg) and furosemide (30 and 100 mg/kg) increased urine volume to about 15 and 35 times that of the control at 4 h postdosing, respectively (Fig. 7A). There were no differences in urine volume between the YM471 and furosemide groups at both the low doses and high doses. Both YM471 and furosemide dose-dependently decreased urine osmolality (Fig. 7B). Furosemide caused a dose-dependent increase in urinary electrolyte (Na^+ and K^+) excretion (Fig. 7C, Table 3). Although YM471 also increased urinary electrolyte excretion, the extent of the increase was smaller than that seen in furosemide-treated groups. YM471 and furosemide significantly elevated the plasma vasopressin concentration and plasma osmolality to the same extent (Fig. 7D,E). YM471 significantly elevated the plasma electrolyte (Na^+ and K^+) concentration, but furosemide significantly decreased it (Fig. 7F, Table 3). YM471 markedly elevated free water clearance to a positive value. In contrast, furosemide elevated only osmolal clearance but not free water clearance (Fig. 7G,H).

4. Discussion

In receptor binding studies, YM471 potently inhibited [^{3}H]vasopressin binding to rat vasopressin V_{1A} and V_2 receptors at subnanomolar concentrations. In contrast, YM471 showed much lower affinity for rat vasopressin V_{1B} and oxytocin receptors. These results indicate that YM471 possesses potent affinity and selectivity for vasopressin V_{1A} and V_2 receptors. In in vivo studies, YM471 potently and dose-dependently antagonized the pressor response to exogenous vasopressin and exerted an aquaretic effect. Additionally, these effects lasted for more than 8 h if YM471 was given at a dose of 1.0 mg/kg or higher, demonstrating that YM471 exerted a long-lasting oral effect. The systemic bioavailability, estimated by the ratio of the dose-normalized area under the plasma concentration–time curve of YM471 (1 mg/kg i.v. and 10 mg/kg p.o.), amounted to 23.3% in rats

Table 3
Effect of oral administration of YM471 and furosemide on urinary electrolyte excretion and plasma electrolyte concentration in hydrated conscious rats

Compound	Dose (mg/kg)	Urinary electrolyte excretion (μEq/4 h)		Plasma electrolyte concentration (mEq/l)	
		Na^+	K^+	Na^+	K^+
Vehicle		98.8 ± 20.3	145 ± 19	172 ± 0.5	4.10 ± 0.22
YM471	1	169 ± 15	183 ± 46	173 ± 1.2	4.74 ± 0.08*
	3	430 ± 30*	350 ± 140	183 ± 0.6*	5.39 ± 0.05*
Furosemide	30	986 ± 147*	267 ± 44	160 ± 0.5*	3.50 ± 0.10*
	100	2470 ± 180*	701 ± 94*	154 ± 0.3*	3.19 ± 0.09*

The diuretic effect was measured by collecting urine for 4 h after oral administration of YM471 (1 and 3 mg/kg), furosemide (30 and 100 mg/kg) or the vehicle in hydrated conscious rats. Values represent means ± S.E.M. for five animals in each group.

* $P < 0.05$ compared with the vehicle group.

and 28.1% in dogs, respectively, after oral administration (unpublished data). In contrast, YM471 alone exhibited no pressor activity or antidiuretic properties at these intravenous and oral doses in vivo. Furthermore, in the absence of vasopressin, YM471 did not increase the intracellular Ca^{2+} concentration in Chinese hamster ovary (CHO) cells expressing human vasopressin V_{1A} receptors and cAMP production in CHO cells expressing human V_2 receptors (Tsukada et al., 2001), indicating that YM471 possesses no agonistic activity at vasopressin V_{1A} and V_2 receptors. These results show that YM471 exerts potent and long-lasting antagonistic activity without agonistic properties at both vasopressin V_{1A} and V_2 receptors in vivo.

Vasopressin is a neuroendocrine factor which regulates potent systemic vasoconstriction through vasopressin V_{1A} receptors as well as water retention through V_2 receptors. Elevation of plasma vasopressin increases peripheral vascular resistance and body fluid retention, leading to a deterioration of cardiac function and water-retaining states. Indeed, several experimental and clinical studies have demonstrated elevated plasma levels of vasopressin in various water-retaining conditions including congestive heart failure (Szatalowicz et al., 1981; Goldsmith et al., 1986; Gines and Jimenez, 1996; Burmeister et al., 1986; Pyo et al., 1995; Manoogian et al., 1988; Cowley et al., 1981). Moreover, the aquaporin-2 water channel, which controls the water permeability of the collecting duct under the regulation of vasopressin, is markedly up-regulated in these diseases (Xu et al., 1997; Fernandez-Llama et al., 1999; Schrier et al., 1998; Fujita et al., 1995). These observations suggest that vasopressin is one of the most important neurohormones implicated in the pathophysiology of various water-retaining conditions such as congestive heart failure, hyponatremia, cirrhosis, nephrotic syndrome, the syndrome of inappropriate antidiuretic hormone secretion and hypertension (Fujisawa et al., 1993b; Naitoh et al., 1994; Bichet et al., 1982). Thus, a vasopressin receptor antagonist may be a valuable therapeutic agent in the treatment of these chronic disorders.

In the pathological condition of congestive heart failure, vasopressin promotes the renal reabsorption of water, and thus blockade of vasopressin action through vasopressin V_2 receptors, to correct the abnormal water retention, might be useful in the treatment of heart failure (Nishikimi et al., 1996; Fujisawa et al., 1993a). In the present study, YM471 increased urine volume, decreased urine osmolality and markedly elevated free water clearance to a positive value. In contrast, furosemide also increased urine volume, but elevated only osmolal clearance but not free water clearance. These results suggest that YM471 and furosemide exert an aquaretic and a natriuretic effect, respectively. These differences in the mode of diuretic action reflected the changes in plasma electrolyte levels. YM471 elevated plasma electrolyte levels, but furosemide decreased them and caused hyponatremia and hypokalemia. This aquaretic effect of YM471 is clinically important for the treatment of water-retaining diseases such as congestive heart failure and liver cirrhosis, because dilutional hyponatremia and hypokalemia frequently develop secondarily to these diseases. Especially, the plasma Na^+ level is one of the most powerful predictors of cardiovascular mortality, with hyponatremic patients showing a substantially shorter survival than patients with a normal plasma Na^+ level (Lee and Packer, 1986; Martin and Schrier, 1997). These results indicate that YM471 may be beneficial in the treatment of water-retaining diseases without the well-known side effects of conventional saliuretics, such as hyponatremia or hypokalemia.

In the present study, YM471 significantly increased urinary Na^+ and K^+ excretion, but the extent of the increase was lower than that seen in furosemide-treated rats. It was previously demonstrated that vasopressin V_2 receptor antagonists increased urinary electrolyte excretion in rats (Tomura et al., 1999; Yamamura et al., 1998) but not in dogs (Yamashita et al., 1993) or humans (Shimizu, 1995). These findings are consistent with some reports that rats and mice possess vasopressin-sensitive adenylate cyclase activity in the thick ascending limb of Henle's loop, whereas dogs and humans do not (Chabardes et al., 1977; Morel, 1981; Ruggles et al., 1985). Therefore, the present results suggest that YM471 inhibits electrolyte absorption at the vasopressin-sensitive segment in the thick ascending limb of Henle's loop.

YM471 possesses high affinity for, and exerts potent antagonistic activity at, vasopressin V_{1A} receptors. Several experimental and clinical studies have demonstrated that vasopressin V_{1A} receptor antagonists cause significant hemodynamic improvement with decreased peripheral vascular resistance in congestive heart failure and hypertension (Raya et al., 1990; Naitoh et al., 1994, 1997; Nicod et al., 1985; Yamada et al., 1994; Burrell et al., 1994, 1995). These results indicate that vasopressin contributes to the raised peripheral vascular resistance in congestive heart failure and hypertension through vasopressin V_{1A} receptors (Wang et al., 1991), and that YM471 may be therapeutically useful in the treatment of these circulatory diseases.

In summary, the present in vitro and in vivo assay results indicate that YM471 is an orally active, nonpeptide dual vasopressin V_{1A} and V_2 receptor antagonist with high affinity and potency. Furthermore, this compound is devoid of vasopressin-like agonist activity. Therefore, YM471 will not only be useful for elucidating the physiologic and pathophysiologic roles of vasopressin, but also for studying the etiology and possible treatment of diseases such as heart failure, hyponatremia and the syndrome of inappropriate antidiuretic hormone secretion.

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