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Pharmacological profile of YM087, a novel nonpeptide dual vasopressin V_{1A} and V_2 receptor antagonist, in dogs

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Abstract

The pharmacological profile of YM087 (4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzazepin-6-yl)carbonyl]-2-phenylbenzanilide monohydrochloride) was investigated in dogs. YM087 showed high affinity for vasopressin V_{1A} and V_2 receptors in radioligand receptor binding studies with dog platelets (V_{1A}) and kidney (V_2). Intravenously injected YM087 (3–100 μg/kg) dose dependently inhibited the pressor response to exogenous vasopressin in anesthetized dogs. Intravenous (10–100 μg/kg) and oral (30–300 μg/kg) administration of YM087 dose dependently increased urine flow with little effect on urinary sodium and potassium excretion in normally hydrated conscious dogs. Concomitantly, the urine osmolality dropped below the plasma osmolality (300 mOsm/kg H_2O). In contrast, intravenously injected furosemide (300 μg/kg) increased urine flow with marked increases in urinary sodium and potassium excretion. These results indicate that YM087 is the first orally effective dual vasopressin V_{1A} and V_2 receptor antagonist and that it will be a new tool in the investigation of the physiological and pathophysiological role of vasopressin in the cardiovascular system and kidney. YM087 may be useful for the treatment of patients with congestive heart failure, renal diseases and water-retaining diseases.

Keywords: YM087; Vasopressin; Vasopressin V_{1A} receptor; Vasopressin V_2 receptor; Non-peptide antagonist

1. Introduction

Arginine vasopressin is produced and secreted by the hypothalamo-neurohypophysial system. The release of vasopressin from the posterior pituitary gland is stimulated by an increase in plasma osmolality and by volume depletion. Vasopressin has a wide variety of biological actions in the body. In the periphery, two classes of vasopressin receptor have been identified (Michel et al., 1979). The vasopressin V_{1A} receptor, which has been identified in vascular smooth muscle, liver and platelets (Thibonnier and Roberts, 1985), is coupled to phosphoinositide hydrolysis and elevation of intracellular Ca^{2+} (Chen et al., 1978). The vasopressin V_2 receptor, which has been identified in the kidney, stimulates the coupled adenylate cyclase and increases cyclic AMP. Vasopressin has dual actions, i.e., vasoconstriction and water retention via vasopressin V_{1A} and V_2 receptors, respectively, in the cardiovascular system and kidney. Vasopressin may play a role in a number of diseases, including congestive heart failure, hypertension, renal disease, edema, hyponatremia and the syndrome of inappropriate antidiuretic hormone secretion (Laszlo et al., 1991; Bouby et al., 1990). The development of vasopressin antagonists appears essential for assessing the pathophysiological roles of vasopressin and could lead to new therapeutic agents. Although a number of potent and selective peptide antagonists have been developed (Manning and Sawyer, 1989), the therapeutic usefulness of such peptide analogs is limited by their lack of oral activity. Recently, an orally effective, selective, nonpeptide vasopressin V_2 receptor antagonist was reported (Yamamura et al., 1992). The rapid water loss induced by acute vasopressin V_2 receptor blockade might lead to an increase in plasma osmolality and thereby stimulate vasopressin release. Consequent vasopressin V_{1A} receptor stim-

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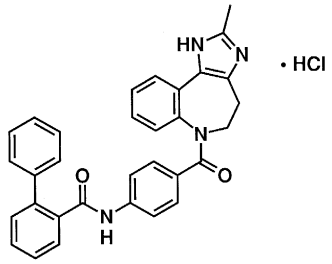

Fig. 1. Chemical structure of YM087, 4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzazepin-6-yl)carbonyl]-2-phenylbenzanilide monohydrochloride.

ulation by elevated plasma vasopressin might in turn induce unfavorable effects in the cardiovascular system and kidney. These undesirable effects could be prevented by vasopressin V_{1A} receptor blockade. We synthesized a series of azolbenzazepin derivatives to develop a novel nonpeptide dual vasopressin V_{1A} and V_2 receptor antagonist. Among these compounds, YM087 (4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzazepin-6-yl)carbonyl]-2-phenylbenzanilide monohydrochloride) was selected. In the present study, we report the pharmacological profile of YM087 (Fig. 1) in dogs.

2. Materials and methods

2.1. Vasopressin radioligand receptor binding assays

Platelets and kidney preparations were prepared from mongrel dogs of either sex weighing 8–15 kg. Canine platelets were prepared by modification of a previously described method (Vittet et al., 1986). Briefly, a citrated dog blood sample was centrifuged at $200 \times g$ for 10 min at room temperature, and the supernatant (platelet-rich plasma) was then centrifuged at $2500 \times g$ for 15 min at room temperature. The obtained pellet was washed and resuspended in iso-osmotic buffer (50 mM Tris[hydroxymethyl]aminomethane hydrochloride (pH 7.4), 100 mM sodium chloride, 5 mM disodium ethylenediamine tetracetate, 15 mM magnesium chloride). Canine kidney medulla plasma membranes were prepared by a modification of a previously described method (Pettibone et al., 1990). A kidney was isolated and homogenized by using a Polytron homogenizer (setting 7, 10 s × 3) in 10 mM Tris[hydroxymethyl]aminomethane hydrochloride (pH 7.4), containing 1 mM disodium ethylenediamine tetracetate and 0.5 mM dithiothreitol. The homogenate was filtered through a single layer of cheesecloth and centrifuged at $48\,000 \times g$ for 20 min at 4°C. The resulting pellet was washed twice and resuspended in hypo-osmotic buffer (50 mM Tris[hydroxymethyl]aminomethane hydrochloride (pH 7.4), 5 mM magnesium chloride). Competition binding experiments were conducted at equilibrium (platelets, 30 min, 30°C; kidney, 60 min, 25°C), using 0.5 nM [^{3}H]vasopressin in iso/hypo-osmotic buffer containing 0.1% bovine serum albumin in the presence of various concentrations of agonists or antagonists. The reactions were stopped with cold buffer containing 50 mM Tris[hydroxymethyl]aminomethane hydrochloride (pH 7.4), and 5 mM magnesium chloride. Membrane-bound radioactivity was separated from free ligand by filtration through a Whatman GF/C filter, using a Brandel cell harvester. The filters were washed twice and counted in a liquid scintillation counter. Non-specific binding was determined by using 1 μM unlabeled vasopressin. The concentration of test compound that caused 50% inhibition (IC_{50}) of the specific binding of [^{3}H]vasopressin was determined by regression analysis of displacement curves. The inhibitory dissociation constant (K_i) was calculated from the following formula: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of radioligand present in the tubes and K_d is the dissociation constant of radioligand obtained from the Scatchard plot. Data were analyzed by using the GraphPad PRISM program (GraphPAD Software, San Diego, CA, USA).

2.2. Vasopressin V_{1A} receptor antagonistic activity

Mongrel dogs of either sex (8–16 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.). A constant level of anesthesia was then maintained by i.v. infusion of sodium pentobarbital at a rate of 3–5 mg/kg/h with an infusion pump. After endotracheal intubation, the animals were artificially ventilated using a respiration pump (SN-480-4, Shinano Seisakusho, Tokyo, Japan) with room air administered at 18 strokes/min (20 ml/kg tidal volume). Body temperature was maintained at 37–38°C on a thermostatically controlled heating table (SN-662, Shinano Seisakusho, Tokyo, Japan). The right femoral artery was catheterized for measurement of systemic blood pressure with a pressure transducer (AP-600G, Nihon Kohden, Tokyo, Japan) and heart rate with a tachometer (AT-600G, Nihon Kohden, Tokyo, Japan) triggered by the arterial pulse wave. Changes in all parameters were recorded on a polygraph (RM-6000, Nihon Kohden, Tokyo, Japan). The right femoral vein was cannulated for intravenous administration of drugs. The vagus nerve was bilaterally resected at the neck to exclude reflex and/or indirect bradycardia due to the systemic vasoconstriction and/or the cardiac parasympathetic nerve activation induced by vasopressin.

Table 1
Effects of YM087 and vasopressin on specific binding of [^{3}H]vasopressin to canine platelets and kidney preparations

Compound	K_i (nM)	
	V_{1A} (platelets)	V_2 (kidney)
YM087	0.61 ± 0.16	0.66 ± 0.30
Vasopressin	1.88 ± 1.16	0.55 ± 0.14

Values are the means ± S.E.M. obtained from 4–5 independent experiments performed in duplicate.

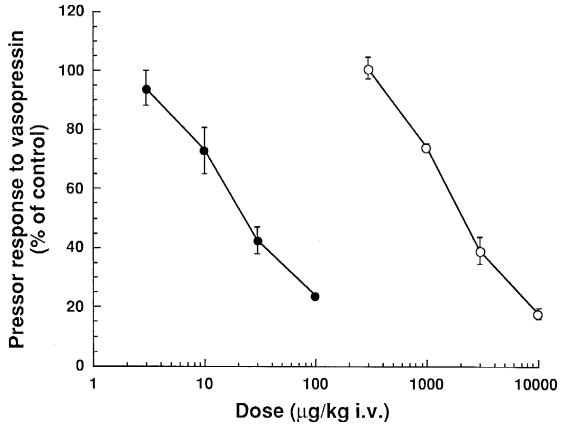

Fig. 2. Inhibitory effects of YM087 (●) and OPC-21268 (○) on the pressor response to vasopressin in anesthetized dogs. Data are expressed as the means ± S.E.M. for 3 dogs per group.

After an appropriate equilibration period, vasopressin (30 mU/kg i.v.) was injected at 60 and 30 min before drug administration. The mean blood pressure response to the second injection of vasopressin was defined as a control (100%) response. Animals were treated with increasing doses of drugs at approximately 40-min intervals. Ten minutes after the administration of each dose of a drug, the pressor response to vasopressin was determined. The data were expressed as percent inhibition of the control pressor response produced by vasopressin. The ID_{50} was the dose of drug inhibiting the control response by 50%.

2.3. Vasopressin V_2 receptor antagonistic activity

Experiments were performed on conscious female Beagle dogs (8–10 kg). Before but not during the experiment the dogs received water ad libitum. A urinary bladder catheter (Foley Pediatric 8 Fr) was inserted to collect urine under local anesthesia. After consecutive 10-min urine samples were collected for 30 min, the animals were i.v. or p.o. dosed with the test drug and urine collection was continued for a further 360 min. Urine osmolality was measured by the freezing point depression method, using

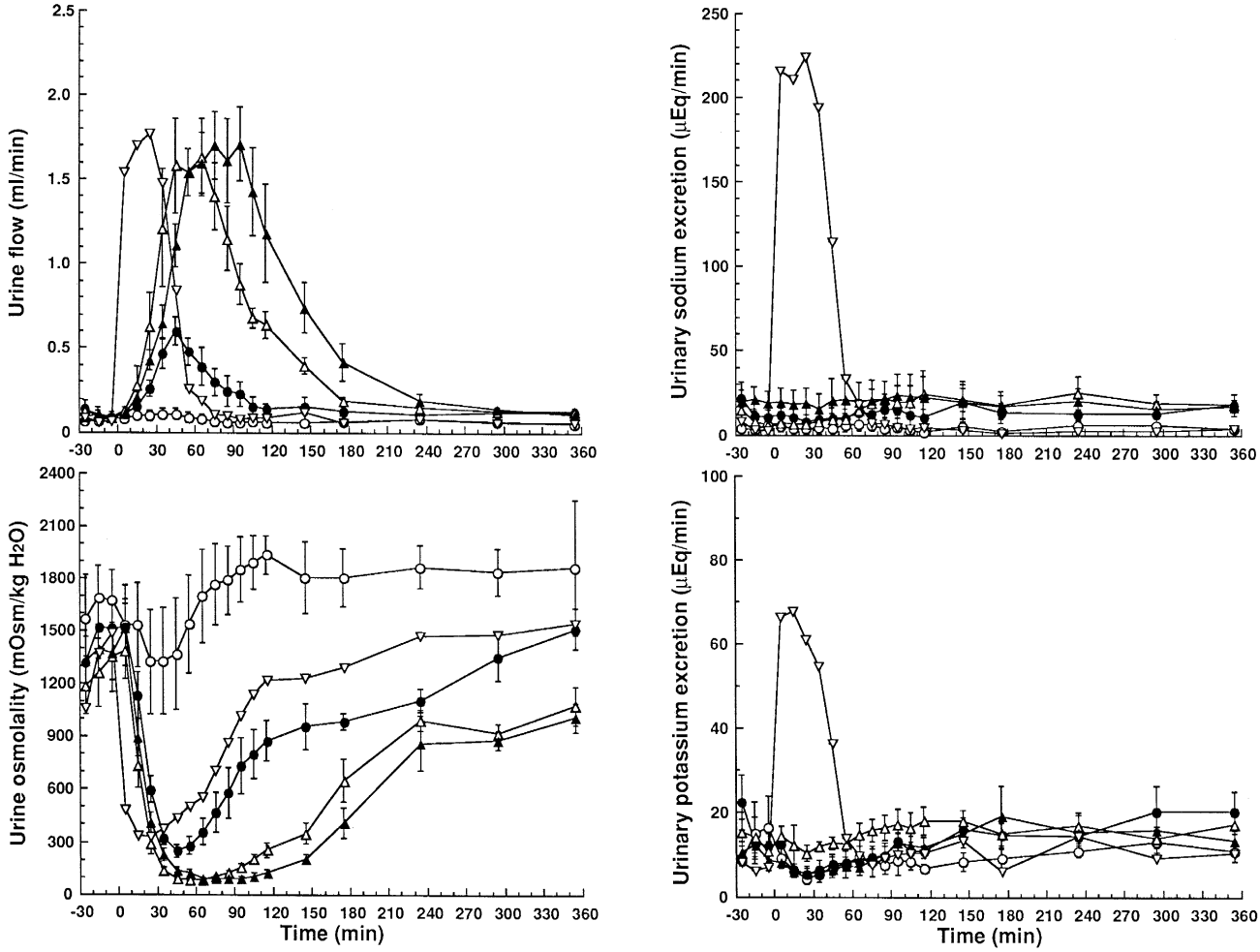

Fig. 3. Effects of intravenous administration of YM087 (●, 10; △, 30; ▲, 100 μg/kg,), furosemide (▽, 300 μg/kg) and vehicle (○, 0.25 ml/kg, 50% ethanol) on urine flow, urine osmolality, urinary sodium excretion and urinary potassium excretion in conscious dogs. Data are expressed as the means ± S.E.M. from 5 dogs per group except for the furosemide group, in which data are expressed as the means for 2 dogs.

an osmometer (Model 3C2, Advanced Instruments). Urinary sodium and potassium concentrations were measured by using a flame photometer (Model 710, Hitachi, Tokyo, Japan).

2.4. Drugs and data analysis

YM087 (4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzazepin-6-yl)carbonyl]-2-phenylbenzanilide monohydrochloride) and OPC-21268 (1-[1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl]-3,4-dihydro-2(1*H*)-quinolinone) were synthesized at Yamanouchi Pharmaceutical Co. (Ibaraki, Japan). Arginine vasopressin was obtained from the Peptide Institute (Osaka, Japan). The radioligand [^{3}H]vasopressin was obtained from DuPont-New England Nuclear (Boston, MA, USA) with a specific activity of 80 Ci/mmol. All other reagents were the best grade commercially available. For in vitro binding studies, YM087 was dissolved in dimethyl sulfoxide at 10^{-2} M and diluted to the desired concentration with assay buffer. The final concentration of dimethyl sulfoxide in the binding assay was 1% and had no effect on specific [^{3}H]vasopressin binding. For vasopressin V_{1A} antagonistic activity studies, YM087 was dissolved in dimethylformamide and diluted with distilled water to yield a final concentration of dimethylformamide $< 5\%$. The drug solution was administered i.v. at a volume of 0.2 ml/kg. For vasopressin V_2 antagonistic activity studies, YM087 was dissolved in ethanol and diluted with distilled water to yield a final concentration of ethanol $< 50\%$. The drug solution was administered i.v. at a volume of 0.25 ml/kg. For oral administration, YM087 was suspended in 0.5% methylcellulose in water and administered in a volume of 0.5 ml/kg. All data are expressed as the means ± S.E.M.

3. Results

3.1. Vasopressin radioligand receptor binding assays

YM087 and vasopressin competed with almost equal potency for [^{3}H]vasopressin binding to canine platelets

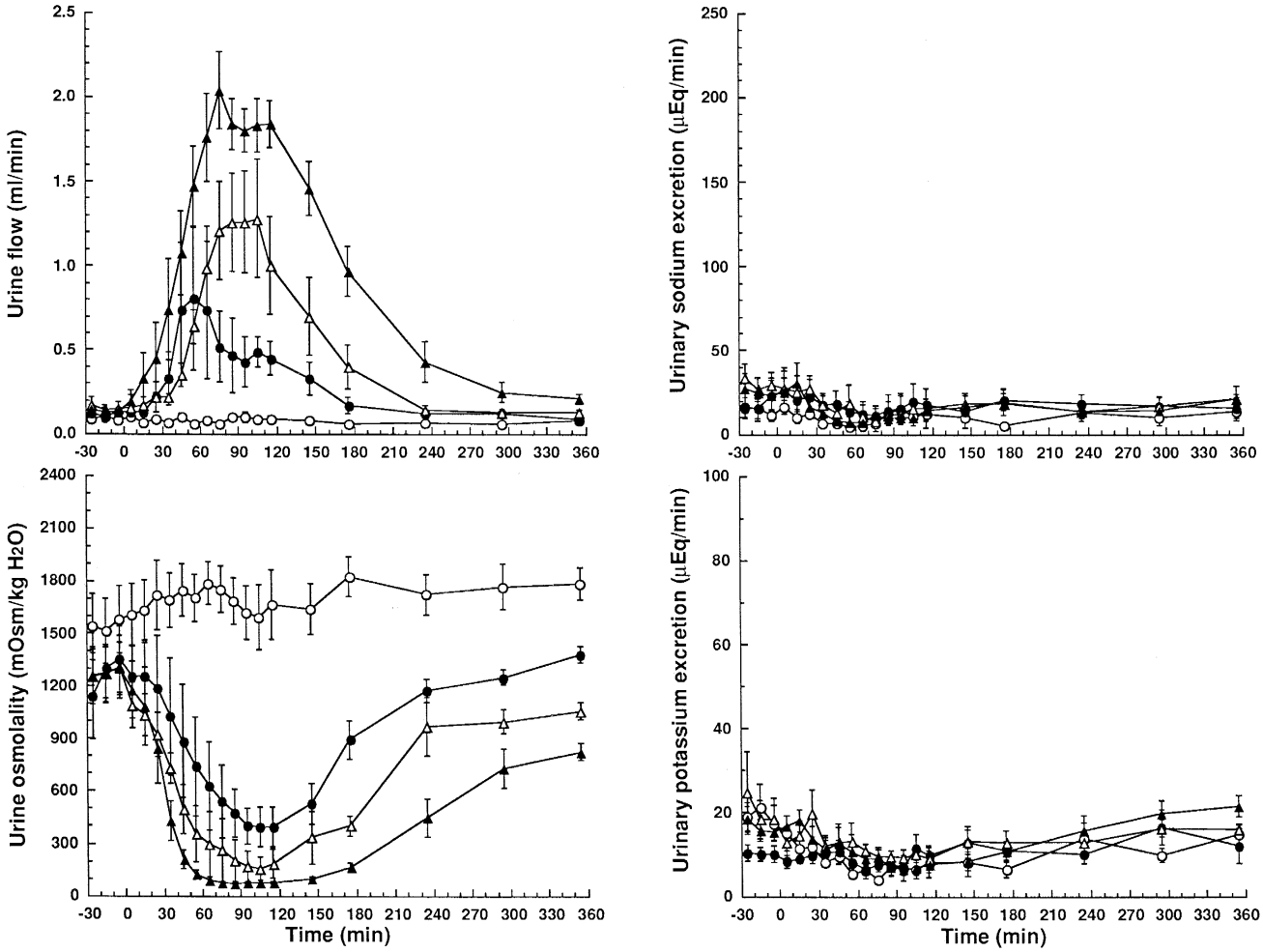

Fig. 4. Effect of oral administration of YM087 (●, 30; △, 100; ▲, 300 μg/kg) and vehicle (○, 0.5 ml/kg, 0.5% methylcellulose) on urine flow, urine osmolality, urinary sodium excretion and urinary potassium excretion in conscious dogs. Data are expressed as the means ± S.E.M. for 5–7 dogs per group.

(V_{1A}) and kidney (V_2) preparations. The calculated K_i values of YM087 were 0.61 ± 0.16 nM and 0.66 ± 0.30 nM for vasopressin V_{1A} and V_2 receptors, respectively (Table 1). The K_i values of vasopressin were 1.88 ± 1.16 nM and 0.55 ± 0.14 nM for vasopressin V_{1A} and V_2 receptors, respectively. YM087 therefore possesses high and equal binding affinity for the vasopressin V_{1A} and V_2 receptor subtype binding sites, with K_i values comparable to those of vasopressin in the subnanomolar range.

3.2. Vasopressin V_{1A} receptor antagonistic activity

In anesthetized dogs, intravenous administration of YM087 (3–100 μg/kg) dose dependently inhibited vasopressin-induced pressor responses without changing basal blood pressure (Fig. 2). ID_{50} values of YM087 and the selective vasopressin V_{1A} antagonist OPC-21268 were 26.0 μg/kg i.v. and 2550 μg/kg i.v., respectively, making the vasopressin V_{1A} antagonistic activity of YM087 approximately 100 times more potent than that of OPC-21268. The pressor responses to repeated challenge with vasopressin in animals treated with vehicle were consistent (data not shown).

3.3. Vasopressin V_2 receptor antagonistic activity

In conscious dogs, intravenous administration of YM087 (10–100 μg/kg) dose dependently increased urine flow and decreased urine osmolality with little effect on urinary sodium and potassium excretion (Fig. 3). In contrast, intravenous administration of furosemide at a dose of 300 μg/kg markedly increased urinary sodium and potassium excretion. Oral administration of YM087 (30–300 μg/kg) also produced a dose-dependent aquaresis (Fig. 4).

4. Discussion

The present study demonstrates that YM087 is the first orally effective dual vasopressin V_{1A} and V_2 receptor antagonist. Vasopressin receptors in platelets have been identified as vasopressin V_{1A} receptors (DiTullio et al., 1985; Vanderwel et al., 1983), as have vascular (Fox, 1988; Thibonnier and Roberts, 1985) and hepatic (Vittet et al., 1986) vasopressin receptors. We therefore used platelets and kidney from dogs to evaluate the affinity of the nonpeptide antagonist YM087 for vasopressin V_{1A} and V_2 receptors. In the present binding studies, YM087 exhibited high and equal binding affinity for the vasopressin V_{1A} and V_2 receptor subtype binding sites with K_i values comparable to those of vasopressin in the nanomolar range.

To investigate whether YM087 acts as a dual vasopressin V_{1A} and V_2 receptor antagonist in vivo, we examined the effects of YM087 in anesthetized and conscious dogs. The vasopressin V_{1A} receptor antagonistic property of YM087 was demonstrated in anesthetized dogs by the dose-dependent inhibition of the pressor responses induced by vasopressin bolus injection. YM087 was approximately 100-fold more potent than the selective vasopressin V_{1A} receptor antagonist OPC-21268 (Yamamura et al., 1991), as measured by the ID_{50} value.

In conscious dogs, YM087 dose dependently increased urine flow and reduced urine osmolality with little effect on urinary sodium and potassium excretion. The two doses (30 and 100 μg/kg i.v.) markedly decreased urine osmolality to less than 100 mOsm/kg H_2O, strongly suggesting that YM087 antagonizes the antiaquaretic effect of endogenous vasopressin mediated by vasopressin V_2 receptors in the kidney. Furosemide induced marked increases in urinary sodium and potassium excretion because of its effect as a potent loop diuretic. Thus the mechanisms of action of YM087 and conventional diuretics are fundamentally different. Furthermore, oral administration of YM087 induced a dose-dependent aquaresis in conscious dogs. YM087 was very well absorbed, since the aquaretic effect obtained at 30 μg/kg i.v. was nearly the same as that at 100 μg/kg p.o. Under the same experimental conditions, we confirmed that the selective vasopressin V_2 antagonist OPC-31260 (Yamamura et al., 1992) also induced an aquaretic effect (data not shown).

It has been reported that intravenous and oral administration of OPC-31260 induces an increase in plasma osmolality and a rapid elevation of plasma vasopressin in humans and dogs, and that these effects are due to rapid water loss as a result of vasopressin V_2 receptor blockade (Ohnishi et al., 1993, 1995; Shimizu, 1995; Naitoh et al., 1994). It is therefore conceivable that in the presence of vasopressin V_2 receptor blockade the increased circulating levels of vasopressin could interact with the vasopressin V_{1A} receptor subtype to produce an undesirable biological response in some diseases, including congestive heart failure and renal disease. This consideration is supported by several studies which have shown that combined administration of vasopressin V_{1A} and V_2 receptor antagonists exerts a more beneficial effect than that produced by each antagonist alone in dogs with congestive heart failure (Naitoh et al., 1994), and that a combination of vasopressin V_{1A} and V_2 receptor antagonists produces renal protective effects in rats with progressive renal failure (Okada et al., 1994). In the present study, we demonstrated that YM087 could block vasopressin V_{1A} receptor agonism by exogenously administered vasopressin in the dose range in which YM087 induces aquaresis. YM087 therefore offers advantages over selective vasopressin V_{1A} and V_2 receptor antagonists in the treatment of some diseases, including congestive heart failure and renal disease.

The present study reveals that YM087 is the first orally active, potent, nonpeptide dual vasopressin V_{1A} and V_2 receptor antagonist. YM087 may therefore be a new tool for the investigation of the physiological and pathophysiological role of AVP in the cardiovascular system and

kidney. The therapeutic potential of the drug will be evaluated in clinical studies.

References

Bouby, N., S. Bachmann, D. Bichet and L. Bankir, 1990, Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat, Am. J. Physiol. 258, F973.

Chen, J.-L.J., D.F. Babcock and H.A. Dardy, 1978, Norepinephrine, vasopressin, glucagon, and A23187 induce efflux of calcium from an exchangeable pool in isolated rat hepatocytes, Proc. Natl. Acad. Sci. USA 75, 2234.

DiTullio, N.W., L.B. Kinter and B.L. Storer, 1985, Pharmacological characterization of vasopressin aggregation response of human platelets, Thromb. Res. 40, 425.

Fox, A.W., 1988, Vascular vasopressin receptors, Gen. Pharmacol. 19, 639.

Laszlo, F.A., F. Laszlo and D. De Wied, 1991, Pharmacology and clinical perspectives of vasopressin antagonist, Pharmacol. Rev. 43, 73.

Manning, M. and W.H. Sawyer, 1989, Discovery, development, and some uses of vasopressin and oxytocin antagonists, J. Lab. Clin. Med. 114, 617.

Michel, R.H., C.J. Kirk and M.M. Billah, 1979, Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin, Biochem. Soc. Trans. 7, 861.

Naitoh, M., H. Suzuki, M. Murakami, A. Matsumoto, K. Arakawa, A. Ichihara, H. Nakamoto, K. Oka, Y. Yamamura and T. Saruta, 1994, Effects of oral AVP receptor antagonists OPC-21268 and OPC-31260 on congestive heart failure in conscious dogs, Am. J. Physiol. 267, H2245.

Ohnishi, A., Y. Orita, R. Okahara, H. Fujihara, T. Inoue, Y. Yamamura, Y. Yabuuchi and T. Tanaka, 1993, Potent aquaretic agent, a novel nonpepitide selective vasopressin 2 antagonist (OPC-31260) in men, J. Clin. Invest. 92, 2653.

Ohnishi, A., Y. Orita, N. Takagi, T. Fujita, T. Toyoki, Y. Ihara, Y. Yamamura, T. Inoue and T. Tanaka, 1995, Aquaretic effect of a potent, orally active, nonpeptide V_2 antagonist in men, J. Pharmacol. Exp. Ther. 272, 546.

Okada, H., H. Suzuki, Y. Kanno, Y. Yamamura and T. Saruta, 1994, Effects of vasopressin V_1 and V_2 receptor antagonists on progressive renal failure in rats, Clin. Sci. 86, 399.

Pettibone, D.J., C.J. Woyden and J.A. Totaro, 1990, Identification of functional oxytocin receptors in lactating rat mammary gland in vitro, Eur. J. Pharmacol. 188, 235.

Shimizu, K., 1995, Aquaretic effects of the nonpeptide V_2 antagonist OPC-31260 in hydropenic humans, Kidney Int. 48, 220.

Thibonnier, M. and J.M. Roberts, 1985, Characterization of human platelet vasopressin receptors, J. Clin. Invest. 76, 1857.

Vanderwel, M., D.S. Lum and R.J. Haslam, 1983, Vasopressin inhibits the adenylate cyclase activity of human platelet particular fraction through V1-receptors, FEBS Lett. 164, 340.

Vittet, D., A. Rondot, B. Cantau, J.M. Launay and C. Chevillard, 1986, Nature and properties of human platelet vasopressin receptors, Biochem. J. 223, 631.

Yamamura, Y., H. Ogawa, T. Chihara, K. Kondo, T. Onogawa, S. Nakamura, T. Mori, M. Tominaga and Y. Yabuuchi, 1991, OPC-21268, an orally effective, nonpeptide vasopressin V_1 receptor antagonist, Science 252, 572.

Yamamura, Y., H. Ogawa, H. Yamashita, T. Chihara, H. Miyamato, S. Nakamura, T. Onogawa, T. Yamashita, T. Hosokawa, T. Mori, M. Tomonaga and Y. Yabuuchi, 1992, Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin V_2 receptor antagonist, Br. J. Pharmacol. 105, 787.