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In vivo and in vitro characterisation of a nonpeptide vasopressin V_{1A} and V_2 receptor antagonist (YM087) in the rat

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Abstract

This paper reports the in vitro and in vivo characterisation of a nonpeptide, orally active, vasopressin V_{1A} and V_2 receptor antagonist, YM087 (methyl-1,4,5,6-tetrahydroimidazo[4,5-d][1]benzoazepine-6-carbonyl)-2-phenylbenzanilide monohydrochloride) in the rat. YM087 dose dependently displaced the vasopressin V_{1A} receptor antagonist radioligand, ^{125}I-labelled [d(CH_2)$_5$,sarcosine^7]vasopressin at vasopressin V_{1A} receptors in liver and kidney medulla membranes and caused a concentration dependent displacement of the vasopressin V_2 receptor antagonist radioligand [^{3}H]desGly-NH$_2^9$[d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin at vasopressin V_2 receptors in kidney medulla membranes. In vitro binding kinetic studies showed YM087 acted as a competitive antagonist at liver V_{1A} and kidney V_{1A} and V_2 vasopressin receptors. Oral administration of YM087 (0.1–3 mg/kg) dose dependently inhibited vasopressin binding to liver V_{1A} and kidney V_{1A} and V_2 vasopressin receptors over 24 h. Oral YM087 (1–3 mg/kg/day) for 7 days in normotensive rats caused a dose dependent aquaresis with no effect on systolic blood pressure. These results show that YM087 is an orally effective vasopressin V_{1A} and V_2 receptor antagonist that may be useful in the treatment of conditions characterised by vasoconstriction and fluid retention such as congestive heart failure.

Keywords: YM087; Nonpeptide; Vasopressin; Vasopressin receptor; (Rat)

1. Introduction

Arginine vasopressin plays a pivotal role in salt and water homeostasis and blood pressure control through its effects at the vasopressin V_{1A} receptor to cause vasoconstriction and at the renal vasopressin V_2 receptor to mediate antidiuresis (Johnston, 1985; Mohr and Richter, 1994; Johnston et al., 1998). Vasopressin receptors have been classified according to their second messenger system and/or pharmacological profile of antagonist displacement (Michell et al., 1979; Jard et al., 1986) and at least three vasopressin receptor subtypes (V_{1A}, V_{1B}/V3 and V_2) have been identified and cloned (Morel et al., 1993; Sugimoto et al., 1994). Vasopressin V_{1A} receptors are found in many tissues including liver, vascular smooth muscle cells, kidney and brain, whilst the vasopressin V_{1B} receptor is found in the anterior pituitary, and vasopressin V_2 receptor has only been definitively identified in the kidney (Phillips et al., 1990).

Several nonpeptide vasopressin receptor antagonists have been developed. OPC-21268 and SR49059 are selective vasopressin V_{1A} receptor antagonists. OPC-21268 blocks the pressor effects of vasopressin and lowers blood pressure in mineralocorticoid hypertension in the rat (Burrell et al., 1993, 1994), whilst SR 49059 is a potent and selective vasopressin V_{1A} receptor antagonist in the human internal mammary artery (Liu et al., 1995). OPC-31260, OPC-41061 and SR 121463A antagonise the effect of vasopressin at the vasopressin V_2 receptor to cause an aquaresis (Yamamura et al., 1992; Serradeil-Le Gal et al., 1996; Burrell et al., 1998; Yamamura et al., 1998). YM087 is an orally effective nonpeptide antagonist of both the vasopressin V_{1A} and V_2 receptors (Tahara et al., 1997). In vitro YM087 antagonises the action of vasopressin in cultured rat vascular smooth muscle cells indicating vaso-

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pressin V_{1A} receptor antagonism, and blocks vasopressin induced cAMP production by cultured renal epithelial cells indicating vasopressin V_2 receptor antagonism (Tahara et al., 1997). In vivo, the vasopressin V_{1A} receptor antagonistic properties of YM087 are demonstrated by its ability to block the pressor response to vasopressin in rats and dogs (Tahara et al., 1997; Yatsu et al., 1997), whilst its aquaretic actions in the dog indicate vasopressin V_2 receptor blockade (Yatsu et al., 1997).

The high oral bioavailability of nonpeptide vasopressin antagonists allows long-term studies to be performed which will further elucidate the role of vasopressin in cardiovascular disease such as hypertension, heart failure and renal failure. The acute effects of an intervention do not necessarily predict its long-term efficacy, and to date there have been no studies assessing the long-term efficacy of YM087. This study further investigates the vasopressin receptor antagonist properties of YM087 in vitro and in vivo in the rat using selective vasopressin V_{1A} and V_2 receptor antagonist radioligands (Phillips et al., 1990), and also assesses the effect of long term oral administration of YM087 on blood pressure and renal parameters in the rat.

2. Materials and methods

Experimental procedures were approved by the Austin Hospital Animal Research Ethics Committee and performed according to the National Health and Medical Research Council of Australia guidelines for animal experimentation. YM087 (methyl-1,4,5,6-tetrahydroimidazo[4,5-d][1]benzoazepine-6-carbonyl)-2-phenylbenzanilide monohydrochloride) was a generous gift from Yamanouchi Pharmaceutical (Japan).

2.1. Animals

Male Sprague–Dawley rats (250–300 g) obtained from the Austin Hospital animal house were used in all studies. Animals were housed at 20°C in a cycle of 12 h light:12 h darkness, with access to water and food ad libitum. Systolic blood pressure was measured using the indirect tail-cuff method (IITC Instruments, CA, USA) in warmed, lightly restrained rats.

2.2. Membrane preparation

In all studies rats were killed by decapitation and livers and kidneys were removed and membranes prepared as previously described (Burrell et al., 1993).

2.3. Radioligands

A selective vasopressin V_{1A} receptor antagonist [1-(β-mercapto-β,β-cyclopentamethylene propionic acid), 7-sarcosine]vasopressin (abbreviated: [d(CH_2)$_5$, sarcosine^7]vasopressin) (Auspep, Melbourne, Australia) was radioiodinated (specific activity: 2394 Ci/mmol) and purified as previously described (Kelly et al., 1989; Trinder et al., 1991).

The selective vasopressin V_2 receptor antagonist radioligand, [Phe-3,4,5-^{3}H]$_9$-des-Gly-NH_2, [1-(β-mercapto-β,β-cyclopentamethylene propionic acid) 2-D-Ile,4-

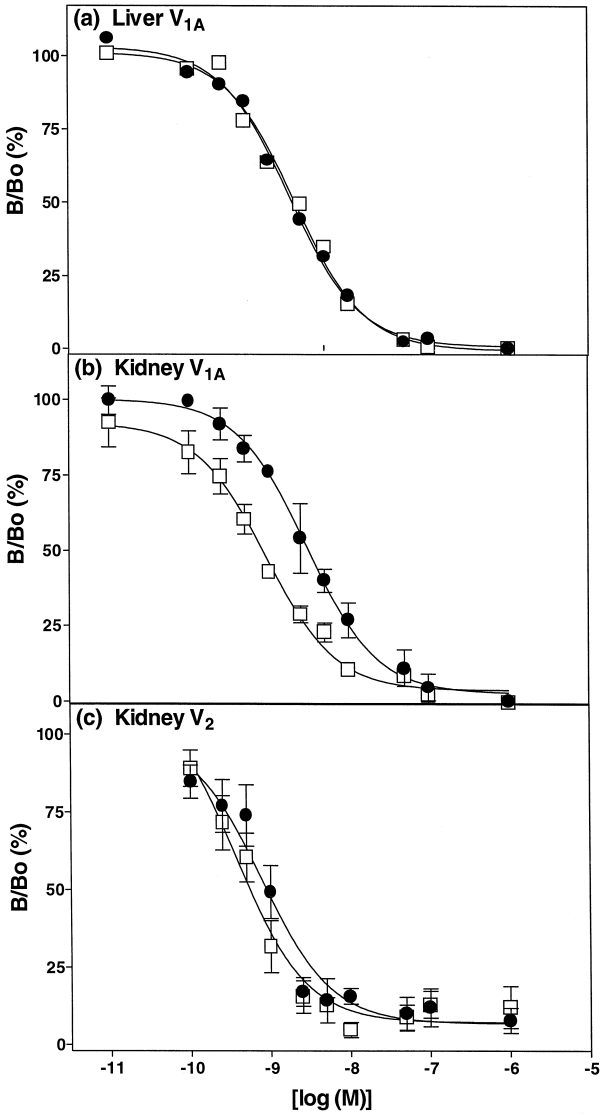

Fig. 1. (a) Displacement of the specific binding of the vasopressin V_{1A} receptor antagonist radioligand ^{125}I-labelled [d(CH_2)$_5$, sarcosine^7] vasopressin from liver membranes by increasing concentrations of unlabelled vasopressin (●) and YM087 (□). (b) Displacement of the specific binding of the vasopressin V_{1A} receptor antagonist radioligand ^{125}I-labelled [d(CH_2)$_5$, sarcosine^7]vasopressin from kidney medulla membranes by increasing concentrations of unlabelled vasopressin (●) and YM087 (□). (c) Displacement of the specific binding of the vasopressin V_2 receptor antagonist radioligand [^{3}H]desGly-NH_2^9[d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin from kidney medulla membranes by increasing concentrations of unlabelled vasopressin (●) and YM087 (□). B and Bo represent the amount of specific radioligand binding in the presence and absence of unlabelled compound respectively. Each point represents the mean of three separate determinations performed in triplicate.

Table 1
In vitro studies: Liver membranes were prepared from untreated rats, and incubated with ^{125}I-labelled [d(CH$_2$)$_5$, sarcosine^7]vasopressin in the absence or presence of YM087 (0.25 and 2.5 nM) to determine the in vitro effect of YM087 on the number and affinity of vasopressin V_{1A} receptors. Kidney medulla membranes were prepared from untreated rats, and incubated with [^{3}H]desGly-NH$_2^9$[d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin in the absence or presence of YM087 (0.01 and 0.1 nM) to determine the in vitro effect of YM087 on the number and affinity of vasopressin V_2 receptors. Values are mean ± S.E.M. ($n = 4$ per group)

Group	B_{max} (fmol/mg)	K_{dapp} (nM)
Liver V_{1A}		
Vehicle	294 ± 27	1.5 ± 0.2
YM087 (0.25 nM)	230 ± 20	1.7 ± 0.3
YM087 (2.5 nM)	284 ± 37	8.8 ± 1.1[a]
Kidney V_2		
Vehicle	8.7 ± 1.0	1.0 ± 0.1
YM087 (0.01 nM)	5.9 ± 0.8	1.4 ± 0.3
YM087 (0.10 nM)	10.1 ± 1.7	2.5 ± 0.3[a,b]

[a] $P < 0.05$ vs. vehicle.
[b] $P < 0.05$ vs. 0.01 nM YM087.

Ile]vasopressin (abbreviated: [^{3}H]desGly-NH$_2^9$[d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin) was obtained from Dupont, Boston, MA, USA (specific activity: 57.2 Ci/mmol).

2.4. In vitro inhibition by YM087 of vasopressin binding

These experiments were designed to investigate the inhibition by YM087 of selective V_{1A} and V_2 antagonist radioligand binding to hepatic V_{1A} and renal V_2 vasopressin receptors.

In vitro inhibition by YM087 of selective V_{1A} antagonist radioligand binding was determined by incubating liver (60 μg) or kidney (250 μg) membranes from untreated rats in a buffer containing 100 mM Tris–HCl, 10 mM $MgCl_2$, 0.1% bovine serum albumin (buffer A, pH 7.4; Sigma, St. Louis, MO, USA) and 0.5 mg/ml bacitracin, 100 IU/ml aprotinin (buffer B) with ^{125}I-labelled [d(CH$_2$)$_5$,sarcosine^7]vasopressin for 1 h at 20°C. The displacement of ^{125}I-labelled [d(CH$_2$)$_5$,sarcosine^7]vasopressin binding to liver or kidney membranes by YM087 was measured using 0.5 nM (liver) or 1 nM (kidney) of V_{1A} tracer in the presence of YM087 or vasopressin in the concentration range 10 pM to 1 μM. Specific binding was calculated as total counts minus nonspecific binding in the presence of 1 μM unlabelled vasopressin (Peninsula Laboratories, Belmont, CA, USA) and was approximately 80% of total binding for liver and 65% of total binding for kidney vasopressin V_{1A} receptors.

The in vitro inhibition by YM087 of selective V_2 antagonist radioligand binding was determined by incubating kidney (250 μg) membranes in buffer B with [^{3}H]desGly-NH$_2^9$[d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin for 2 h at 20 °C. The displacement of [^{3}H]desGly-NH$_2^9$[d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin binding to kidney membranes was measured using 1 nM V_2 tracer in the presence of YM087 or vasopressin in the concentration range 10 pM to 1 μM. Specific binding was calculated as total counts minus nonspecific binding in the presence of 1 μM unlabelled vasopressin and was approximately 50% of total binding for kidney vasopressin V_2 receptors.

2.5. In vitro vasopressin binding kinetics

The in vitro effects of YM087 on maximum binding site density (B_{max}) and the apparent affinity (K_d) of the liver V_{1A} and kidney V_2 vasopressin receptor were determined by analysis of the saturation binding of the selective vasopressin V_{1A} and V_2 receptor radioligands. Liver mem-

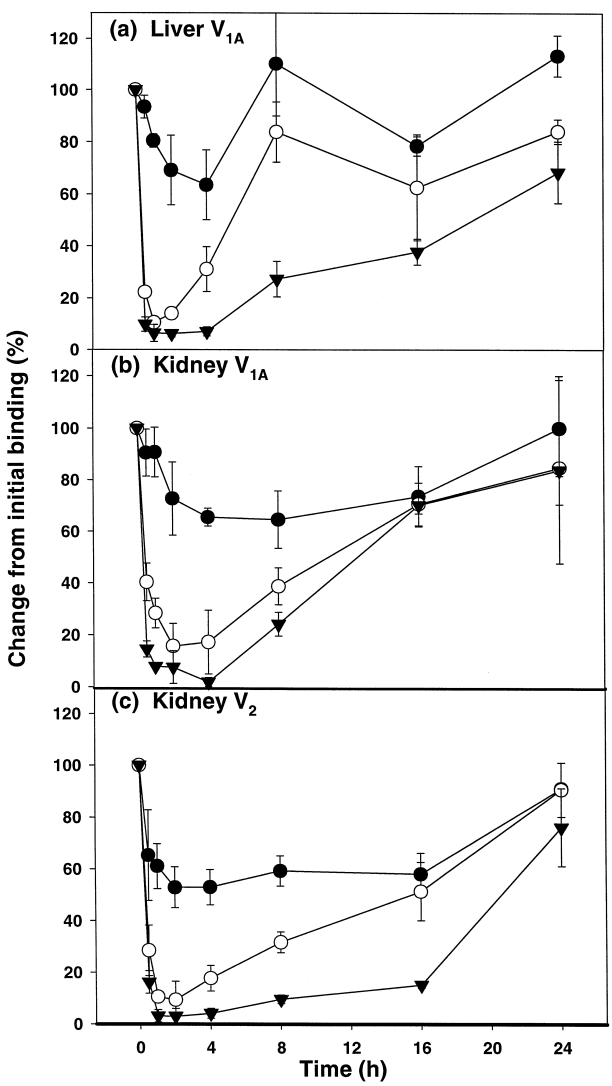

Fig. 2. Effect of orally administered YM087 at 0.1 (●), 1(○) and 3 (▼) mg/kg on the in vitro specific binding of ^{125}I-labelled [d(CH$_2$)$_5$, sarcosine^7]vasopressin to (a) liver and (b) kidney medulla membranes and [^{3}H]desGly-NH$_2^9$[d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin binding to (c) kidney medulla membranes. Results are expressed as percent change from initial binding at time zero. Each point represents the mean ± S.E.M. of three estimations.

branes prepared from untreated animals were incubated with ^{125}I-labelled [d(CH$_2$)$_5$,sarcosine^7]vasopressin as described above, in the absence ($n = 4$) or presence ($n = 4$) of YM087 (0.25 and 2.5 nM). Kidney membranes prepared from untreated animals were incubated with [^{3}H]desGly-NH$_2^9$[d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin as described above, in the absence ($n = 4$) or presence ($n = 4$) of YM087 (0.01 and 0.1 nM). The B_{max} and K_d values were determined by Scatchard analysis.

2.6. In vivo treatment with YM087 on in vitro vasopressin binding

To assess the in vivo inhibition of YM087 on vasopressin binding to liver and kidney V_{1A} and kidney vasopressin V_2 receptors, rats were gavaged with vehicle (0.5% methyl cellulose (BDH, Poole, UK)) or YM087 (0.1, 1 and 3 mg/kg suspended in methyl cellulose). Rats were killed by decapitation at 0, 0.5, 1, 2, 4, 8, 16 and 24 h after gavage. Liver and kidneys were collected and membranes prepared. Three animals were used for each concentration of YM087 and at each time point. Binding assays for liver and kidney V_{1A} and kidney V_2 vasopressin receptors were performed and specific binding was calculated as total counts minus nonspecific counts in the presence of 1 μM unlabelled vasopressin.

2.7. Effect of long term oral YM087 on blood pressure and renal parameters

Baseline systolic blood pressure and body weights were measured in male Sprague–Dawley rats (300 g, $n = 30$) which were then randomised to vehicle, YM087 (1 mg/kg) or YM087 (3 mg/kg). Systolic blood pressure was determined on days 1, 3 and 7 at 90 min post gavage. On day 7 of treatment rats were placed into metabolic cages and water intake and urinary volume, and urine sodium and osmolality measured for 24 h. Rats were then killed by decapitation and trunk blood collected into chilled lithium heparin tubes for the measurement of plasma sodium, osmolality and vasopressin. Kidneys were collected ($n = 4$ rats/group) and rapidly frozen in dry-ice chilled isopentane and stored at −80°C until used for vasopressin V_{1A} receptor in vitro autoradiography (Phillips et al., 1990). Plasma sodium was measured using an Instrumentation Laboratory Ilyte ion specific electrode analyser (Milan, Italy) and plasma osmolality was determined using an Advanced Instruments Micro-Osmometer model 3300 (MA, USA). Plasma vasopressin was extracted using acetone and petroleum ether and measured as previously described (Pullan et al., 1978). The inter-assay and intra-assay coefficients of variation were less than 8% and the limit of detection was approximately 1 pM.

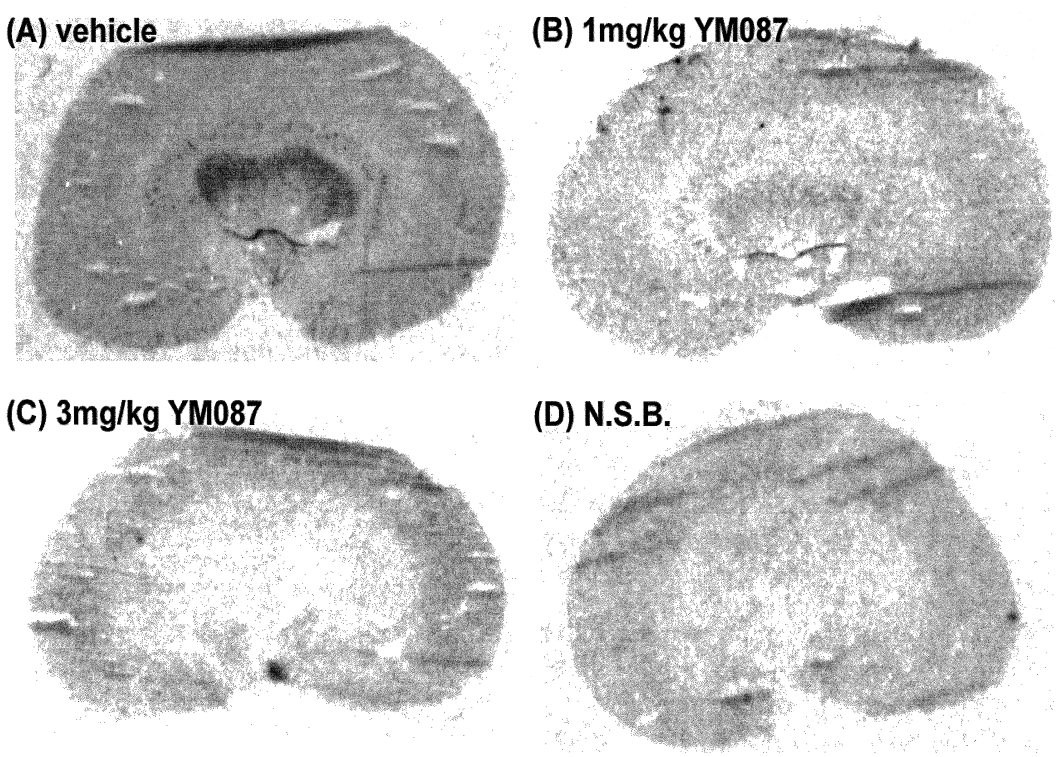

Fig. 3. Computer generated images of ^{125}I-labelled [d(CH$_2$)$_5$, sarcosine^7]vasopressin binding to rat kidney. Dark grey represents high level of binding and light grey low to undetectable levels of binding. The autoradiographs represents binding of ^{125}I-labelled [d(CH$_2$)$_5$, sarcosine^7]vasopressin to kidney V_{1A} receptors following 7 days treatment with vehicle (A) 1 mg/kg YM087 (B) and 3 mg/kg YM087 (C). Non-specific binding is depicted in panel (D).

2.8. Analysis of data

Results are expressed as mean ± S.E.M. and were analysed by one way analysis of variance using the Fisher post hoc comparison or Student–Newman–Keuls tests where appropriate.

3. Results

3.1. In vitro inhibition by YM087 of vasopressin binding

YM087 dose dependently displaced the selective vasopressin V_{1A} receptor antagonist radioligand ^{125}I-labelled [d(CH_2)$_5$,sarcosine^7]vasopressin at vasopressin V_{1A} receptors in both rat liver and kidney medulla membranes. The concentration of YM087 that displaced 50% of specific vasopressin binding (IC_{50}) was 2.2 ± 0.1 nM for liver V_{1A} and 0.9 ± 0.1 nM for kidney vasopressin V_{1A} receptors ($n = 3$). The IC_{50} for vasopressin was 1.9 ± 0.1 nM for liver V_{1A} and 3.0 ± 0.1 nM for kidney V_{1A} ($n = 3$) (Fig. 1a, b). YM087 also caused a concentration dependent displacement of the vasopressin V_2 receptor antagonist radioligand [^{3}H]desGly-NH$^9_2$[d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin to vasopressin V_2 receptors in kidney medulla membranes. The IC_{50} for YM087 was 0.4 ± 0.1 nM (n = 3) and that of vasopressin, 0.8 ± 0.2 nM for kidney vasopressin V_2 receptors ($n = 3$) (Fig. 1c).

3.2. In vitro vasopressin binding kinetics

YM087 caused a concentration dependent increase in the apparent K_d of liver vasopressin V_{1A} receptor binding sites ($P < 0.05$) with no significant effect on B_{max} indicating competitive inhibition at the vasopressin V_{1A} receptor (Table 1). Similarly, YM087 caused a concentration dependent increase in the apparent K_d of kidney vasopressin V_2 receptor binding sites ($P < 0.05$) with no effect on B_{max} indicating competitive inhibition at the vasopressin V_2 receptor (Table 1).

3.3. In vivo treatment with YM087 on in vitro vasopressin binding

Orally administered YM087 (3 mg/kg) caused a significant reduction in binding of ^{125}I-labelled [d(CH_2)$_5$,sarcosine^7]vasopressin to liver vasopressin V_{1A} receptors for up to 24 h ($P < 0.001$) and to kidney vasopressin V_{1A} receptors for up to 8 h ($P < 0.001$) (Fig. 2a, b). At 1 mg/kg YM087 reduced binding to liver ($P < 0.01$) and kidney ($P < 0.05$) vasopressin V_{1A} receptors for up to 16 h. Low dose YM087 (0.1 mg/kg) reduced binding in liver vasopressin V_{1A} receptors at 0.5 h ($P < 0.01$).

Fig. 2c shows the effect of orally administered YM087 on the in vitro binding of [^{3}H]desGly-NH$^9_2$[d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin to vasopressin V_2 receptors in kidney membranes. YM087 at 3 mg/kg caused a significant reduction in binding of [^{3}H]desGly-NH$^9_2$[d(CH_2)$_5$, D-Ile^2, Ile^4]vasopressin to vasopressin V_2 receptors for up to 24 h ($P < 0.001$). At 1 and 0.1 mg/kg, YM087 reduced binding to vasopressin V_2 receptors for up to 16 h ($P < 0.001$).

3.4. Effect of long term oral YM087 on blood pressure and renal parameters

Oral YM087 had no effect on systolic blood pressure (Fig. 4a) despite significant blockade of the vasopressin V_{1A} receptor ($P < 0.01$) (Figs. 3 and 4b) and caused a dose dependent increase in urine volume and fluid intake, and decreased urine osmolality with no natriuresis (Fig. 5). Biochemical and hormonal parameters are shown in Fig. 6.

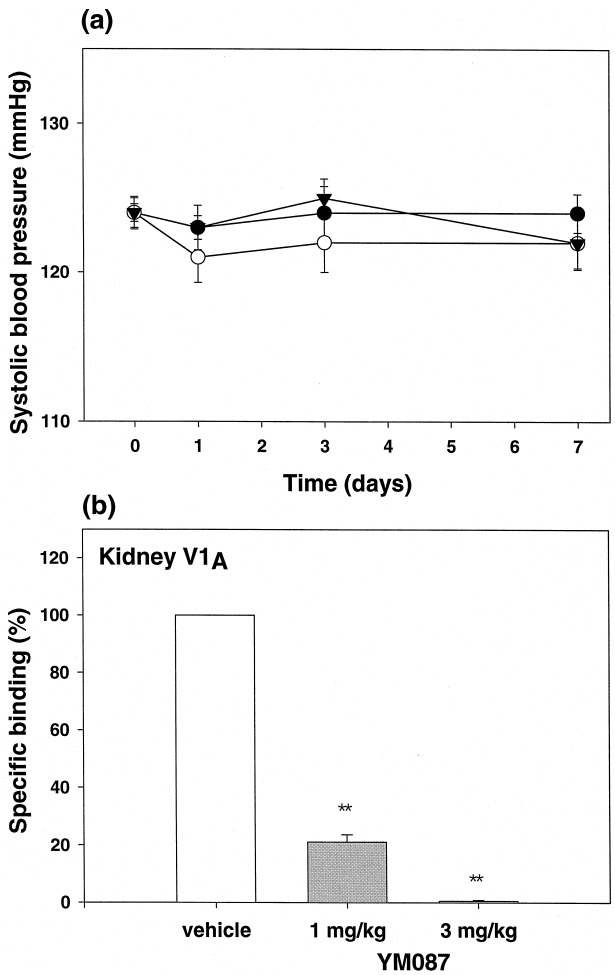

Fig. 4. (a) Systolic blood pressure of male Sprague–Dawley rats measured 90 min after gavage with 1 mg/kg YM087 (○), 3 mg/kg YM087 (▼), or vehicle (0.5% methyl cellulose) (●) on day 1, 3 and 7 of treatment. Each value represents mean ± S.E.M. ($n = 10$ rats/group). (b) Quantitative values obtained from computer analysis of the autoradiographs shown in Fig. 3. Results are expressed as percentage change from binding with vehicle. Kidney vasopressin V_{1A} receptors were significantly blocked after 7 days treatment with 1 and 3 mg/kg of oral YM087 (closed bars) compared to vehicle treated rats (open bar). Each bar represents mean ± S.E.M. ($n = 4$ rats/group).

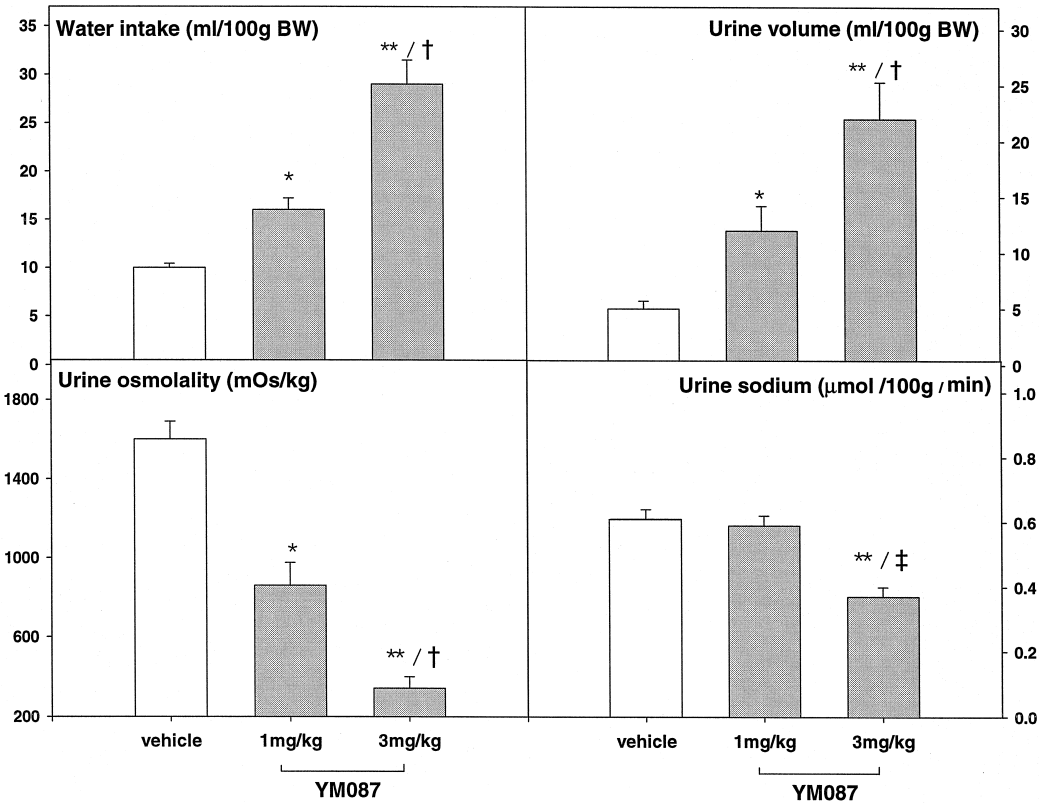

Fig. 5. Results of metabolic studies to assess vasopressin V_2 receptor blockade. Male Sprague–Dawley rats were placed into metabolic cages on day 7 of treatment and fluid intake, urine volume, osmolality and sodium were assessed for 24 h. Data are presented as mean ± S.E.M. ($n = 10$ rats/group). Open bars, vehicle; closed bars, YM087 (1 and 3 mg/kg). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle; $^{\dagger}P < 0.05$ vs. 1 mg/kg YM087, $^{\ddagger}P < 0.01$ vs. 1 mg/kg YM087.

YM087 (3 mg/kg) increased plasma sodium and osmolality ($P < 0.05$), and both doses of YM087 increased plasma vasopressin ($P < 0.05$–$P < 0.01$).

4. Discussion

The results of the present study indicate that in vitro, YM087 acts as a competitive inhibitor of vasopressin at liver and kidney vasopressin V_{1A} receptors, and kidney vasopressin V_2 receptors. Previous studies have used the agonist radioligand [^{3}H]vasopressin that binds to both V_{1A} and V_2 vasopressin receptors to assess the pharmacological profile of YM087 (Tahara et al., 1997, 1998; Yatsu et al., 1997). The use of the selective V_{1A} antagonist radioligand ^{125}I-labelled [d(CH$_2$)$_5$,sarcosine^7]vasopressin and the vasopressin V_2 receptor antagonist radioligand [^{3}H]desGly-NH$_2^9$[d(CH$_2$)$_5$, D-Ile^2, Ile^4]vasopressin allows the effects of YM087 to be discriminated at V_{1A} and V_2 vasopressin receptors which is of importance in tissues such as the kidney where both receptor subtypes exist. The IC_{50} values calculated from the radioligand displacement curves indicate that YM087 binds to the liver and kidney V_{1A} and kidney vasopressin V_2 receptor with similar affinity to vasopressin.

The in vivo time course and dose response studies demonstrated that oral YM087 is an effective V_{1A} and V_2 vasopressin receptor antagonist. The inhibitory effect of YM087 on kidney and liver vasopressin V_{1A} receptors and kidney vasopressin V_2 receptors was dose and time dependent. Maximal responses were seen at the highest dose of YMO87 (3 mg/kg), but at 1 mg/kg YM087 also caused significant inhibition of binding of vasopressin at both V_{1A} and V_2 vasopressin receptors for up to 16 h. These results confirm that YM087 exerts potent antagonistic effects at both V_{1A} and V_2 vasopressin receptors in vivo and suggest that its long lasting effects will enable once daily dosing.

To date, there have been no reports on the effect of long-term treatment with oral YM087 on physiological parameters. Certainly in the dog (Yatsu et al., 1997), a single intravenous injection of YM087 dose dependently inhibited the pressor response to exogenous vasopressin and also increased urine flow. In the present study, 7 days of oral YM087 had no effect on systolic blood pressure in normotensive hydrated rats although in vitro autoradiography confirmed blockade of vasopressin V_{1A} receptors by YM087. It is known, however, that blockade of the vasopressin V_{1A} receptor does not reduce blood pressure in normal animals due to the buffering effects of an intact sympathetic nervous system and activation of the renin angiotensin system (Johnston, 1985).

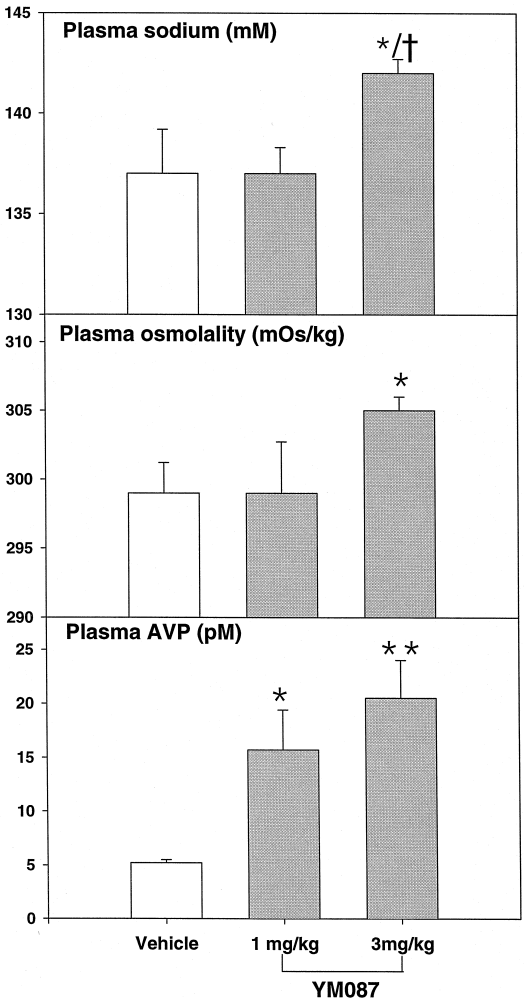

Fig. 6. Effect of oral administration of YM087 (1, 3 mg/kg) or vehicle on (a) plasma sodium, (b) plasma osmolality and (c) plasma vasopressin measured after 7 days of treatment. Each bar represents mean ± S.E.M. ($n = 5–10$ rats/group). Open bars, vehicle; closed bars, YM087 (1 and 3 mg/kg). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle; $^{\dagger}P < 0.05$ vs. 1 mg/kg YM087.

YM087 was also effective as a vasopressin V_2 receptor antagonist to cause significant aquaretic effects after 7 days of treatment. Although aquaresis on day 1 of treatment was not assessed in this study, previous work has shown YM087 to be effective acutely to cause an aquaresis (Yatsu et al., 1997). These data suggest that no tachyphylaxis with 7 days treatment had occurred. Furthermore, we have unpublished data indicating that the degree of aquaresis is similar after 5 months of treatment to that seen after 7 days. Previous studies have shown that the nonpeptide vasopressin V_2 receptor antagonist OPC-31260 also has sustained aquaretic effects in a coronary artery ligated rat model of heart failure (Burrell et al., 1998). This is an important point as chronic blockade of the vasopressin V_2 receptor using peptide receptor antagonists was not associated with persistent aquaresis (Wang et al., 1991). At the higher dose YM087 was associated with increased plasma sodium and osmolality, and reduced body weight (data not shown) suggesting rats were dehydrated despite having free access to water. These results indicate that care will be necessary in choosing the correct dose of nonpeptide vasopressin V_2 receptor antagonist to avoid excess water loss.

As noted in other studies of nonpeptide vasopressin V_2 receptor blockers (Burrell et al., 1998), YM087 was also associated with increased plasma vasopressin concentrations. The consequence of such an increase in vasopressin in terms of renal or haemodynamic effects remains unclear. Certainly in this study, increased plasma vasopressin did not appear to overcome vasopressin V_2 receptor blockade with YM087, and any contribution of elevated vasopressin to systemic vasoconstriction would be prevented by the vasopressin V_{1A} receptor blocking properties of YM087.

In conclusion, these studies confirm that YM087 is a potent nonpeptide orally effective V_{1A} and V_2 vasopressin receptor antagonist that has long lasting inhibitory effects at both V_{1A} and V_2 vasopressin receptors following oral dosing. These data suggests that YM087 may be useful in the management of heart failure where increased vasopressin contributes to increased vascular resistance through its vasopressin V_{1A} receptor effects and to hyponatraemia via its effects at the vasopressin V_2 receptor to cause fluid retention.

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