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Pharmacological profile of YM358, a novel nonpeptide angiotensin AT_1 receptor antagonist

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Abstract

The pharmacological profile of YM358, 2,7-diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole potassium salt monohydrate, a novel non-peptide angiotensin AT_1 receptor antagonist, was studied in vitro and in vivo. YM358 competed with [^{125}I][Sar^1, Ile^8]angiotensin II for angiotensin AT_1 receptors in rat liver membranes. YM358 displayed competitive kinetics and the pK_i value was calculated as 8.79. In contrast, YM358 had little effect on the binding of [^{125}I][Sar^1, Ile^8]angiotensin II to the angiotensin AT_2 receptor in bovine cerebellum. In isolated rabbit aorta, YM358 produced a parallel rightward shift in the concentration–response curve for angiotensin II with a pA_2 value of 8.82. YM358 had no effect on the contraction induced by KCl, norepinephrine, serotonin, histamine, prostaglandin $F_{2\alpha}$ or endothelin-1 even at 10^{-5} M. On the basis of pK_i values in the binding assay and pA_2 values in the isolated tissues, YM358 was approximately 3–10 times more potent than losartan in antagonizing angiotensin AT_1 receptors. In pithed rats, intravenous administration of YM358 inhibited an increase in mean blood pressure induced by intravenous infusion of angiotensin II in a dose-dependent manner. In conscious normotensive rats, YM358 at 3–30 mg/kg p.o. inhibited the angiotensin II-induced pressor response in a dose-dependent manner. YM358 at 30 mg/kg caused maximum and complete inhibition 30 min after dosing, and inhibition lasted more than 24 h. These results demonstrate that YM358 is a potent, AT_1-selective and competitive nonpeptide angiotensin receptor antagonist. Moreover, YM358 is both orally active and long-lasting. This pharmacological profile suggests that YM358 would be suitable for the treatment of cardiovascular disorders such as hypertension and chronic heart failure. © 1997 Elsevier Science B.V.

Keywords: Angiotensin II; Angiotensin AT_1 receptor antagonist; YM358; Losartan

1. Introduction

The renin–angiotensin system is of principal importance in the regulation of blood pressure and fluid and electrolyte homeostasis (Cody, 1986). Angiotensin II, the primary biologically active peptide hormone of the renin–angiotensin system, produces multiple physiological effects, most of which seem to be mediated by the angiotensin AT_1 receptor subtype. In contrast, the functions of the AT_2 receptor subtype are not yet well understood (Philipps, 1987; Herblin et al., 1991; Timmermans et al., 1991). Prevention of the formation of angiotensin II via inhibition of the angiotensin I converting enzyme has proved a powerful strategy for the treatment of hypertension and congestive heart failure (Gavras et al., 1978; Turini et al., 1979; Cody, 1986). However, there is evidence to suggest that side effects of angiotensin I converting enzyme inhibitors such as dry cough and angioedema result from the lack of specificity of angiotensin I converting enzyme for angiotensin I (Skidgel and Erdos, 1987; Williams, 1988). A possible approach to overcome these side effects is the specific blockade of angiotensin II receptors.

Recent reports on the discovery of losartan and analogues, a new class of specific nonpeptide angiotensin II receptor antagonists (Timmermans et al., 1991; Smith et al., 1992), indicate a promising future for the development of orally active nonpeptide angiotensin II receptor antagonists. In our own efforts toward the development of a nonpeptide angiotensin II receptor antagonist, we discov-

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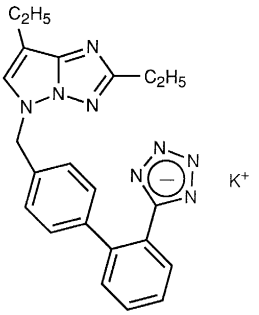

YM358

Fig. 1. Chemical structure of YM358.

ered 2,7-diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-b][1,2,4]triazole potassium salt monohydrate (YM358; Fig. 1), a novel, highly potent and selective angiotensin AT_1 receptor antagonist from a series of pyrazolotriazole derivatives. In the present study, we report the in vitro and in vivo pharmacology of YM358, compared with those of the well-characterized angiotensin AT_1 receptor antagonists losartan and its active metabolite, EXP3174.

2. Materials and methods

2.1. In vitro angiotensin II receptor antagonistic activities

2.1.1. Angiotensin II receptor binding

Binding assay for the angiotensin AT_1 receptor in rat liver membranes or for angiotensin AT_2 receptor in bovine cerebellar membranes was performed with a commercial assay kit (DuPont NEN, Boston, MA, USA). [^{125}I][Sar^1, Ile^8]angiotensin II was used as radioligand for the angiotensin AT_1 and AT_2 receptor binding assay (17 pM for angiotensin AT_1 receptor and 74 pM for angiotensin AT_2 receptor). Test compounds (25 μl) and radioligands (25 μl) were added to test tubes. Membrane fractions (200 μl) for each receptor binding assay were then added to initiate binding. Incubation was performed for 3 h at room temperature in the angiotensin AT_1 receptor binding assay or for 1 h at 37°C in the angiotensin AT_2 receptor binding assay. After incubation, the reaction was terminated by addition of 3 ml ice-cold 0.9% saline. To separate bound and free radioactivity, the reaction mixtures were filtered immediately under pressure through a glassfiber GF/C filter for the angiotensin AT_1 receptor or a GF/B filter for the angiotensin AT_2 receptor (Whatman, Tokyo, Japan), with each tube and filter rinsed twice with ice-cold 0.9% saline. The radioactivity trapped on the filters was counted in a γ-counter (ARC-300, Aloka, Tokyo, Japan). Nonspecific binding of [^{125}I][Sar^1, Ile^8]angiotensin II to the receptor was estimated in the presence of 10^{-6} M unlabeled DuP753 for angiotensin AT_1 receptor or 10^{-6} M unlabeled angiotensin II for angiotensin AT_2 receptor. The inhibitory concentration of test compound that caused 50% inhibition of the specific binding of angiotensin II (IC_{50}) was determined by regression analysis of displacement curves. Inhibition dissociation constants (K_i) were calculated from the formula $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of radioligand present in the tubes (Cheng and Prusoff, 1973) and K_d is the dissociation constant of radioligand obtained from the Scatchard plot (Scatchard, 1949).

In separate experiments, rat liver membranes were incubated with increasing concentrations of [^{3}H]angiotensin II for saturation analysis. The saturation binding of [^{3}H]angiotensin II was measured with and without the drug. Data on the saturation curve were plotted by the method of Scatchard (1949).

2.1.2. Functional antagonism, specificity, and potency in isolated rabbit aorta

New Zealand White male rabbits weighing 2.0–4.5 kg were killed by cervical dislocation and exsanguinated. The thoracic aorta was removed and cleaned of adherent fat and connective tissue. The vascular endothelium was removed by gently rubbing the intimal surface of the vessel. Aortic strips (2–3 mm wide and 30 mm long) were then prepared according to Furchgott and Bhadrakom (1953) and mounted in 30 ml organ baths containing Krebs–Henseleit solution (NaCl 118.4, KCl 4.7, $MgSO_4$–$7H_2O$ 1.2, KH_2PO_4 1.2, $CaCl_2$–$2H_2O$ 2.5, $NaHCO_3$ 25.0, and glucose 11.1 mM) maintained at 37°C and bubbled with a 95% O_2, 5% CO_2 gas. Under a resting tension of 1.5 g, isometric tension changes were recorded on a polygraph (Rikadenki Kogyo, Tokyo) through a force displacement transducer (Nihon Kohden, Tokyo). After equilibration for 1 h, a single contractile–response curve to the cumulative addition of angiotensin II was constructed. The strips were then washed 2 times and allowed to relax to the baseline tension. Each strip was then incubated for 30 min with the vehicle or with a single concentration of compound before a second contractile–response curve to angiotensin II was obtained. The results are expressed as a percentage of the maximal angiotensin II response obtained with the first curve, which served as control. pA_2 and slope values were determined from Schild plots using the method of least squares (Arunlakshana and Schild, 1959). As for the compound which was found to exert noncompetitive (insurmountable) angiotensin II antagonism, the pD'_2 value, the negative logarithm value of the concentration of the compound which inhibits the maximum response by 50%, was calculated.

In a second series of experiments, agonists used were KCl, norepinephrine, serotonin, histamine, prostaglandin $F_{2\alpha}$ and endothelin-1. A control contractile–response curve for each agonist was obtained for each tissue, then the concentration of each agent required to obtain a submaximum response was determined. Except for endothelin-1, a

control contractile response at the submaximum concentration of each agent was obtained. After washing, the tissue was then incubated with YM358 at 10^{-5} M for 30 min, and the contractile response was estimated again in the presence of YM358. For endothelin-1, control contractile response and contractile response after treatment of YM358 at 10^{-5} M were obtained in separate tissues, because tissue contracted with endothelin-1 required rinsing several times to relax to baseline tension.

2.2. In vivo angiotensin II receptor antagonistic activities

2.2.1. Inhibition of pressor response to angiotensin II in pithed rats

Male Wistar rats weighing 250–400 g (12–22 weeks old) were anesthetized with ether and pithed by inserting a steel rod through the orbit and foramen magnum down into the spinal canal. Immediately after pitching, the rats were vagotomized bilaterally at the neck and artificialy ventilated with room air with a tidal volume of 1 ml/100 g body weight at a rate of 50 breaths/min using a rodent respirator (SN-480-7, Shinano, Japan). Arterial blood pressure was measured at the left carotid artery via a pressure transducer (TP-400T, Nihon Kohden, Japan) and recorded on a polygraph recorder (RM-6000, Nihon Kohden, Japan). The left and right femoral veins were cannulated for intravenous infusion of angiotensin II and intravenous administration of compound, respectively. Angiotensin II was infused to elicit an approximately 50–80 mm Hg increase in diastolic blood pressure. After diastolic blood pressure had reached a steady level, the compound was administered in an ascending-dose manner, during which diastolic blood pressure responses were recorded.

2.2.2. Inhibition of pressor response to angiotensin II in conscious normotensive rats

Male Wistar rats aged 16–24 weeks were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and the left carotid artery and vein were cannulated with polyethylene tubes for the measurement of blood pressure and the intravenous administration of angiotensin II, respectively. The catheters were passed subcutaneously, exteriorized at the neck, and filled with saline containing heparin. The animals were allowed to recover from surgery for at least 3–4 days before beginning the experiment. The arterial catheter was connected to a pressure transducer (TP-400T, Nihon Kohden, Japan) and diastolic blood pressure was recorded with a polygraph (RM-6000, Nihon Kohden, Japan). First, in order to determine the intravenous dose of angiotensin II which produces a submaximal increase in diastolic blood pressure, the dose–pressor response curve to angiotensin II was obtained. Second, after establishing control pressor responses to the submaximal dose of angiotensin II, YM358 or losartan was administered orally. Thereafter, angiotensin II was injected repeatedly at given times. The pressor responses to angiotensin II after the administration of agents were compared with the responses before the treatment with the agents.

All experiments were performed under the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical.

2.3. Drugs

YM358, losartan, EXP3174 and PD123319 were synthesized in Institute for Drug Discovery Research, Yamanouchi Pharmaceutical, Japan. In in vivo experiments, the drugs were suspended in a 0.5% methylcellulose solution and administered by oral gavage at a volume of 5 ml/kg for rats.

2.4. Statistical analysis

Results are expressed as the mean ± S.E.M. Data were analyzed by one-way analysis of variance. When overall statistical significance was achieved ($P < 0.05$), Dunnett multiple range test was used to compare each of the doses to the vehicle control. Probability values less than 0.05 were considered to be significant.

3. Results

3.1. Angiotensin II receptor binding

YM358 displaced the specific binding of [^{125}I][Sar^1, Ile^8]angiotensin II to a single population of binding site (angiotensin AT_1 receptor) in rat liver membranes (Fig. 2), with a pK_i value calculated as 8.79 ± 0.04 (Table 1). In the case of bovine cerebellar membranes (angiotensin AT_2 receptor), PD123319 displaced the specific binding of [^{125}I][Sar^1, Ile^8]angiotensin II with pK_i value of 7.79 ± 0.38. In contrast, YM358 revealed low affinity to the

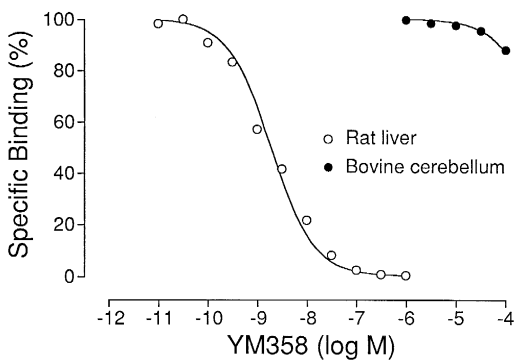

Fig. 2. Inhibitory effects of YM358 on specific binding of [^{125}I][Sar^1, Ile^8]angiotensin II to rat liver membranes (angiotensin AT_1 receptor) or bovine cerebellar membranes (angiotensin AT_2 receptor). Each point represents the mean value of three separate experiments, performed in duplicate.

Table 1
Effects of YM358, losartan, EXP3174 and PD123319 on specific binding of [^{125}I][Sar^1, Ile^8]angiotensin II to rat liver membranes (angiotensin AT_1 receptor) and [^{125}I][Sar^1, Ile^8]angiotensin II to bovine cerebellar membranes (angiotensin AT_2 receptor)

Compound	pK_i value	
	angiotensin AT_1 receptor	angiotensin AT_2 receptor
YM358	8.79 ± 0.04	3.48 ± 0.04
losartan	7.81 ± 0.05	3.56 ± 0.04
EXP3174	11.08 ± 0.32	3.84 ± 0.03
PD123319	4.36 ± 0.05	7.79 ± 0.38

Each value represents the mean ± S.E.M. of 3 separate experiments, performed in duplicate.

angiotensin AT_2 site with a pK_i value of 3.48 ± 0.04 (Table 1).

To establish the nature of the antagonism exerted by YM358, we determined its effect on saturation curves of [^{3}H]angiotensin II binding in rat liver membranes in comparison with those of losartan and EXP3174. Scatchard plot analysis (Fig. 3) showed that YM358 (5 nM) and losartan (30 nM) increased the K_d of [^{3}H]angiotensin II binding from 2.58 ± 0.23 to 5.17 ± 0.47 nM and from 2.01 ± 0.21 to 6.13 ± 1.59 nM, respectively, without affecting B_{max}. In contrast, EXP3174 (1 nM) decreased B_{max} from 1602 ± 49 to 928 ± 59 fmol/mg protein without affecting K_d.

3.2. Functional antagonism, specificity, and potency in isolated rabbit aorta

Pretreatment with YM358 produced a rightward parallel shift in the concentration–response curve for angiotensin II without affecting the maximal response to angiotensin II (Fig. 4). Schild analysis of the data gave a pA_2 value of 8.82 (8.74–8.93, 95% confidence limits) and a slope of 0.98 (0.88–1.08, 95% confidence limits), not significantly different from unity. The antagonism produced by losartan was also characterized by a rightward shift of the angiotensin II concentration–contractile response curve, and the pA_2 value and slope were 8.37 (7.87–13.76, 95% confidence limits) and 1.00 (0.15–1.85, 95% confidence limits), respectively. In contrast, EXP3174 caused nonparallel shifts to the right of the dose–response curve to angiotensin II and decreased the maximal response with a pD'_2 value of 9.13 (8.79–9.34, 95% confidence limits).

YM358 had no effect on the contraction induced by KCl, norepinephrine, serotonin, histamine, prostaglandin $F_{2\alpha}$ or endothelin-1 even at 10^{-5} M (Table 2).

3.3. Inhibition of pressor response to angiotensin II in pithed rats

Intravenous infusion of angiotensin II at 0.1 μg/kg per min increased diastolic blood pressure from 51.2 ± 1.2 mmHg to 114.3 ± 2.2 mmHg ($n = 12$). YM358 and losartan inhibited this increase in diastolic blood pressure in a dose-dependent manner (Fig. 5). The intravenous doses of YM358 and losartan required to induce a 50% decrease in the maximum pressor response to angiotensin II infusion were 0.02 ± 0.003 mg/kg and 0.11 ± 0.029 mg/kg, respectively.

3.4. Inhibition of pressor response to angiotensin II in conscious normotensive rats

Bolus injection of angiotensin II at 3–1000 ng/kg i.v. produced a transient and dose-dependent increase in diastolic blood pressure. A submaximal pressor dose of angiotensin II was 100 ng/kg i.v., and the increase in diastolic blood pressure was 48.0 ± 1.8 mm Hg ($n = 4$) YM358 and losartan by oral administration (3–30 mg/kg)

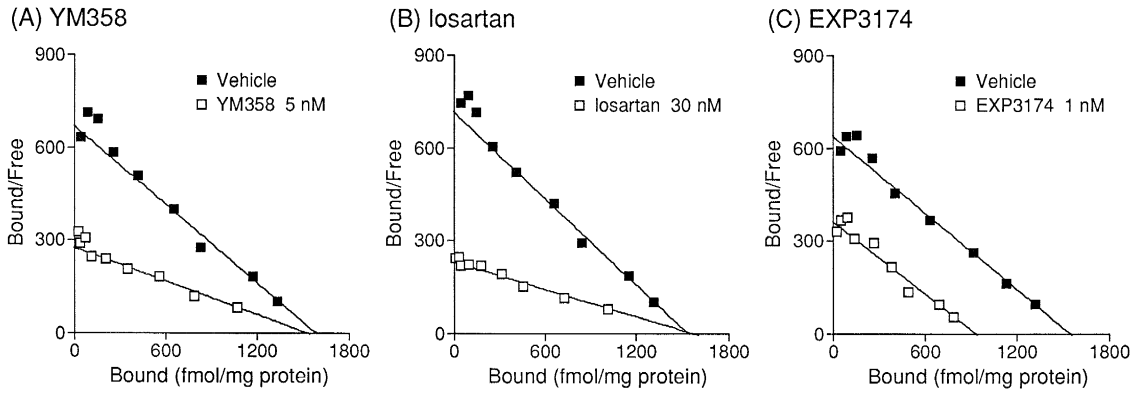

Fig. 3. Scatchard analysis of YM358 (A), losartan (B) and EXP3174 (C) on the binding of [^{3}H]angiotensin II in rat liver membrane. Data shown are representative of three separate experiments, performed in duplicate.

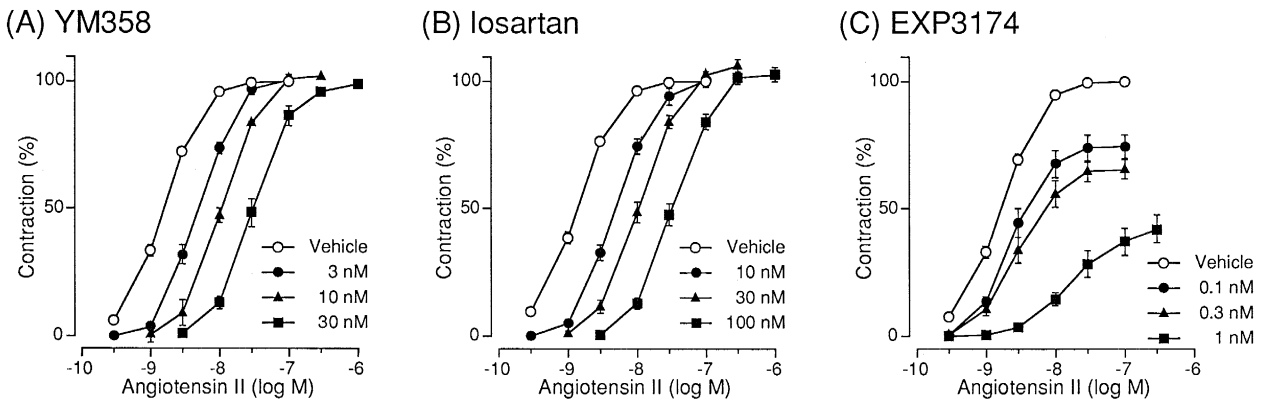

Fig. 4. Effects of YM358 (A), losartan (B) and EXP3174 (C) on the log concentration–contractile response curve of angiotensin II in isolated rabbit aortic strips. The results are expressed as a percentage of the maximal angiotensin II response obtained with the first curve, which served as control. Each point represents the mean ± S.E.M. of 2–21 experiments.

inhibited the angiotensin II (100 ng/kg i.v.)-induced pressor response in a dose-dependent manner (Fig. 6). Maximal inhibitory effect of YM358 at each dose was produced at 1–2 h after oral administration. Maximal inhibitory effect of YM358 at 3 and 10 mg/kg p.o. were approximately 70.6 ± 3.3% and 93.7 ± 1.6%, respectively. YM358 at 30 mg/kg p.o. caused complete inhibition of the angiotensin II-induced pressor response and its inhibition was still evident 24 h after dosing. The maximal inhibitory effect of losartan at 3, 10 and 30 mg/kg was approximately 49.7 ± 3.3%, 85.4 ± 4.0% and 98.5 ± 0.9%, respectively, with maximum inhibition reached at 6, 5 and 2 h after administration, respectively. On the basis of ED_{50} values, YM358 (ED_{50} = 1.0 mg/kg p.o.) was approximately 3 times more potent than losartan (ED_{50} = 3.0 mg/kg p.o.).

Table 2
Effect of YM358 on the contractile response of rabbit aortic strips induced by various agonists

Agonist	Concentration of agonist	n [a]	Absolute contraction (g)	
			pretreatment	YM358 [b] treatment
KCl	30 mM	6	2.77 ± 0.34	3.12 ± 0.21
Norepinephrine	3 μM	6	3.24 ± 0.14	3.46 ± 0.06
Serotonin	1 μM	8	2.67 ± 0.24	2.44 ± 0.15
Histamine	30 μM	5	3.37 ± 0.17	2.81 ± 0.40
Prostaglandin$F_{2\alpha}$	3 μM	7	3.12 ± 0.34	3.39 ± 0.31
Endothelin-1	10 nM	6	1.78 ± 0.18	1.63 ± 0.08

[a] Number of experiments.
[b] Concentration of YM358 is 10^{-5} M.

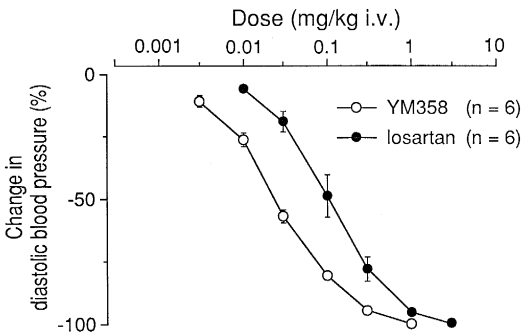

Fig. 5. Effects of intravenous administration of YM358 and losartan on the increase in diastolic blood pressure induced by infusion of angiotensin II in pithed rats. Each point represents the mean ± S.E.M. of 4–6 animals.

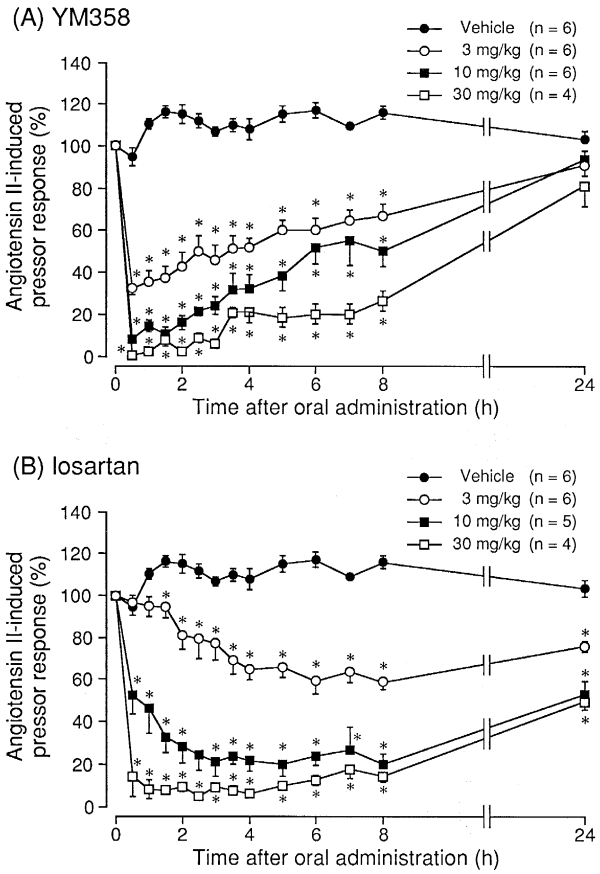

Fig. 6. Effects of oral administration of YM358 (A) and losartan (B) on the pressor response to angiotensin II (100 ng/kg i.v.) in conscious normotensive rats. Each point represents the mean ± S.E.M. of 4–6 animals. * $P < 0.05$, significantly different from the vehicle treated group.

4. Discussion

The present studies show that YM358, a novel pyrazolotriazole derivative, is a potent, selective and specific angiotensin AT_1 receptor antagonist in vitro and in vivo.

Recent binding studies using several nonpeptide angiotensin II receptor antagonists have confirmed the existence of at least two angiotensin II receptor subtypes. Angiotensin AT_1 receptor subtype was found in tissues such as human uterus, rat vascular smooth muscle and rat adrenal cortex (Whitebread et al., 1989; Chiu et al., 1990; Wong et al., 1990a). Angiotensin AT_2 receptor subtype was found in tissues such as bovine cerebellum (Wiest et al., 1991) and rat adrenal medulla (Herblin et al., 1991). In binding assays using rat liver membrane (angiotensin AT_1 receptor), YM358 increased the K_d of the radioligand for its receptor without a change in the maximum number of receptors, suggesting that YM358 interacts in a reversible and competitive manner with angiotensin AT_1 receptors. YM358 did not affect angiotensin AT_2 receptors in bovine cerebellar membrane. These results suggest that YM358 specifically and competitively antagonizes angiotensin II at angiotensin AT_1 receptors.

In rabbit aorta, YM358 produced a rightward parallel shift in the concentration–response curve for angiotensin II without a change in the maximal contractile response. The slope calculated from the Schild plots was 0.98, close to the theoretical value of unity. These results indicate that YM358 showed competitive angiotensin II receptor antagonism in functional as well as in binding studies. Moreover, YM358 is a specific antagonist for angiotensin II, as it did not inhibit the contraction induced by KCl, norepinephrine, serotonin, histamine, prostaglandin $F_{2\alpha}$ or endothelin-1.

Angiotensin AT_1 receptor antagonists have been categorized as either surmountable (competitive) or insurmountable on the basis of their ability to shift angiotensin II concentration–response curves to the right without or with a reduction in maximal angiotensin II response, respectively (Gaddum et al., 1955). In the present functional assay, YM358 and losartan were classified as surmountable angiotensin AT_1 receptor antagonists. In contrast, EXP3174 showed insurmountable antagonism. Consistent with the result in the vasoconstriction study, Scatchard analysis in the saturation binding experiments demonstrated that YM358 and losartan behaved as competitive (surmountable) antagonists, whereas EXP3174 was noncompetitive (insurmountable). The relative in vivo efficacy of competitive and noncompetitive angiotensin AT_1 receptor antagonists is currently under discussion, and no conclusion has been reached. With regard to antihypertensive effects, YM358 and losartan showed comparable effects in several hypertensive models in rats by single or repeated administration (Yamaguchi et al., 1997; Shibasaki et al., 1997). Thus, there appears to be no difference in antihypertensive efficacy between competitive and noncompetitive AT_1-receptor antagonisms.

In the pithed rat, the blood pressure lowering effect of YM358 after intravenous dosing was approximately 6 times more potent than that of losartan. In the oral administration study, in contrast, the angiotensin II inhibitory effect of YM358 in the conscious rats was only two-fold greater than that of losartan. This difference in potency ratio between intravenous and oral dosing may be due to the formation of a major metabolite of losartan, EXP3174, in rats. That is, EXP3174 generated by the oral administration of losartan may be responsible for much of the inhibitory effect of the pressor response to angiotensin II (Wong et al., 1990b). This explanation is supported by the observation that the time required to achieve the maximum effect of losartan after oral administration was longer than that of YM358.

The present study demonstrates that YM358 is a potent, AT_1-selective and competitive nonpeptide angiotensin II receptor antagonist. YM358 is both orally active and long-lasting. This pharmacological profile suggests that YM358 may be valuable in the treatment of cardiovascular disorders such as hypertension and chronic heart failure.

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