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Pharmacological profile of ME3221, a novel angiotensin II receptor antagonist

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Abstract

The pharmacological profile of a new surmountable angiotensin AT_1 receptor antagonist, ME3221, 3-methoxy-2,6-dimethyl-4-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methoxy]pyridine, was studied in several animal models, and was compared with that of losartan. EF2831, 3-hydroxy-2,6-dimethyl-4-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methoxy]pyridine, a metabolite of ME3221, is also a surmountable angiotensin AT_1 receptor antagonist, whose potency was 1/30 that of ME3221 in vitro, but equal to or 1/3 of that of ME3221 in in vivo experiments. In rats and marmosets, ME3221 antagonized angiotensin II-induced pressor responses, but did not affect bradykinin-induced depressor responses. ME3221 lowered the blood pressure in renal hypertensive rats and spontaneously hypertensive rats (SHR), and its ED_{25} value was 3 times that of losartan. Repeated administration of ME3221 to SHR had a stable and long-lasting antihypertensive effect without influencing heart rate. Thus ME3221, like losartan, may be useful in the treatment of renal and essential hypertension.

Keywords: ME3221; Angiotensin AT_1 receptor antagonist, surmountable; Antihypertensive drug; Losartan; Telemetry

1. Introduction

The renin-angiotensin system plays an important role in controlling cardiovascular function. Angiotensin converting enzyme inhibitors are widely used clinically for the treatment of hypertension and congestive heart failure (Williams, 1988; Cody, 1986). Non-peptide angiotensin II receptor (AT_1) antagonists have now been developed as a new type of therapeutic drug; their therapeutic potency exceeds that of the angiotensin converting enzyme inhibitors in clinical use (Smith et al., 1992; Timmermans et al., 1993). The angiotensin AT_1 receptor antagonists, losartan (DuP753, 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl)methyl]imidazole, potassium salt) (Wong et al., 1990a,b) and TCV-116 (Shibouta et al., 1993), are now being used in clinical trials as antihypertensives. These compounds are pro-drug type agents. Although the mother compounds show considerable potency of angiotensin II antagonistic activity, the main metabolites are more potent. Further, the mother compounds, losartan and TCV-116, show surmountable (competitive) angiotensin AT_1 receptor antagonism, while the metabolites, EXP3174, (2-*n*-butyl-4-chloro-1-[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid and CV11974, respectively, show insurmountable (non-competitive) angiotensin AT_1 receptor antagonism.

We have previously reported on a novel angiotensin II receptor (AT_1 specific) antagonist, ME3221 (3-methoxy-2,6-dimethyl-4-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methoxy]pyridine) (Fig. 1) (Katano et al., 1991). It has been reported that ME3221 bound strongly to angiotensin AT_1 receptor sites without binding to angiotensin AT_2 receptor sites and that it showed surmountable angiotensin II receptor antagonism, but had no effect on other receptors (e.g., adrenoceptors, acetylcholine receptors, serotonin 2 receptors), on angiotensin converting enzyme, or on Ca^{2+} channels (Nagura et al., 1994; Kawano et al., 1994). The present study, using several animal models, was carried out to characterize the pharmacological profile of ME3221

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and its major metabolite, EF2831 (3-hydroxy-2,6-dimethyl-4-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methoxy]pyridine) (Fig. 1), and to compare these profiles with those of the angiotensin AT_1 receptor antagonist, losartan, and that of enalapril, an angiotensin converting enzyme inhibitor. The comparison with losartan is particularly interesting, since the major metabolite of losartan, EXP3174, shows insurmountable angiotensin AT_1 receptor antagonism (Wong et al., 1990c,1991).

2. Materials and methods

2.1. In vitro angiotensin II antagonism in rabbit aorta preparation

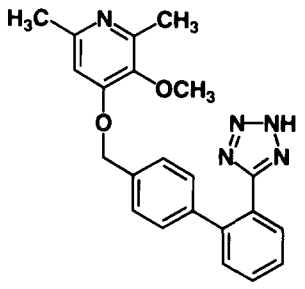
The thoracic aorta was removed from Japanese white male rabbits (2.5–3.0 kg) (Japan Laboratory Animals, Tokyo, Japan). Helical strips were prepared and mounted in tissue baths containing oxygenated (95% O_2-5% CO_2) Krebs-Henseleit solution at 37°C, as described previously (Kawano et al., 1994). The contraction signal was recorded with an isometric transducer (Nihon Kohden, T-512), under a tension of 1.5–2.0 g. The sensitivity was adjusted with norepinephrine (10^{-5} M) at the end of the equilibration period, and the cumulative concentration-contractile response curve for angiotensin II was obtained for each tissue before and after 30-min incubation with ME3221 or EF2831. The response was expressed as a percentage of the maximal angiotensin II contractile response. To measure the potency of the antagonist, the pA_2 values of ME3221 and EF2831 were determined by means of the Schild equation (Arunlakshana and Schild, 1959).

2.2. Experimental procedures in rats

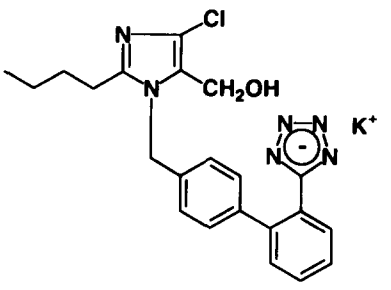
Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and a vascular catheter was inserted into the femoral artery. The catheter was passed subcutaneously to the dorsal side of the neck and exteriorized. After the animals had completely recovered from anesthesia and surgical trauma (16–24 h), the arterial catheter was connected to a pressure transducer (Nihon Kohden, DX-300) and a blood pressure amplifier (Nihon Kohden, AP-641G) to measure the mean blood pressure. The heart rate was obtained with a cardiotachometer (Nihon Kohden, AT-601G) triggered by the arterial pulse. These rats remained conscious and moved freely.

2.3. Measurement of blood pressure responses induced by angiotensin II and other vasoactive agents in conscious rats

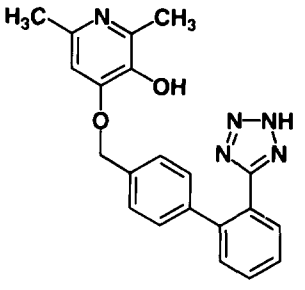
Group 1. In the first series of experiments, male Sprague-Dawley rats (weighing 250–350 g, obtained from Nihon Clea) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and two vascular catheters were implanted, one in the femoral artery



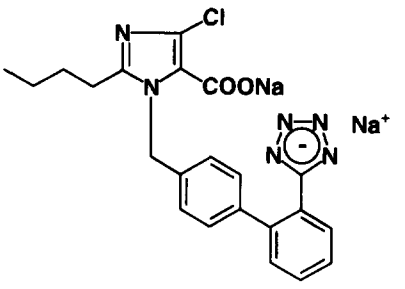
ME3221



Losartan



EF2831



EXP3174

Fig. 1. Chemical structures of ME3221, losartan, and their major metabolites.

and one in the femoral vein. The arterial catheter was used for the measurement of blood pressure, and the venous catheter was used for the i.v. administration of angiotensin II. The rats were allowed to recover overnight from anesthesia and were allowed free access to water. Food was withheld when the animals received the oral drug treatment. Mean blood pressure was measured by the method described above under Experimental Procedures. After the blood pressure was stabilized, the animals were challenged several times with submaximal, bolus i.v. doses of 0.1 μg/kg of angiotensin II until the blood pressure response was reproducible. Drugs were given and angiotensin II was then administered i.v. at 15- to 60-min intervals for 3 or 6 h after dosing.

Group 2. In the second series of experiments, the acute effects of ME3221 and enalapril on blood pressure responses to several vasoactive agents were evaluated. Male Sprague-Dawley rats (250–350 g) were used for this test. The procedures for the operation and the measurement of mean blood pressure were the same as those employed in the group 1 experiment. Angiotensin I (0.3 μg/kg), angiotensin II (0.1 μg/kg), angiotensin III (1 μg/kg), norepinephrine (1 μg/kg), and bradykinin (3 μg/kg) were given as bolus injections into the femoral vein at appropriate intervals to obtain the control response. One to three hours after the p.o. administration of ME3221 or enalapril, the same doses of angiotensin I, angiotensin II, angiotensin III, norepinephrine, and bradykinin were administered i.v. again and the responses were compared to the control responses.

2.4. Measurement of angiotensin II-induced blood pressure response in conscious common marmosets

Conscious common marmosets (male and female, 250–500 g, bred in the Laboratories of Meiji Seika Kaisha) were used for these experiments. The marmosets were anesthetized with ketamine hydrochloride (20 mg/kg i.m.) and placed in a prepared holding device, and two vascular catheters, containing heparin (500 units/ml), were inserted, one into the femoral artery and one into the femoral vein. Lidocaine was then applied to the wound. About 2 h after the operation, the marmosets had recovered completely from the systemic anesthesia. After the blood pressure and heart rate were stabilized, the animals were challenged with i.v. bolus doses of angiotensin II (1 μg/kg) and the pressor responses were recorded. A single dose of the test drug was administered intravenously or orally, and changes in blood pressure and heart rate were monitored. The mean blood pressure and heart rate were measured according to the same procedures as employed for the rats, with the same monitoring schedule. For oral administration, a nasogastric feeding tube was used and the drugs were delivered directly into the stomach.

2.5. Preparation of renal hypertensive rats and measurement of blood pressure

Male Sprague-Dawley rats (250–350 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the left renal artery was completely ligated with a 4-O silk suture (Cangiano et al., 1979). Six days after the ligation, the animals were anesthetized with sodium pentobarbital, and the femoral artery was cannulated with a vascular catheter for the measurement of mean blood pressure. On the next day, after mean blood pressure was confirmed to be stabilized, drugs were given p.o. The parameters were measured continuously for more than 10 h, and again at 24 h. The dose-response curves at the maximum response during the observation period were analyzed by the least-squares method, and doses that decreased mean blood pressure to 25 mm Hg from the pretreatment level (ED_{25}) were used to compare potency.

2.6. Measurement of blood pressure in spontaneously hypertensive rats (SHR)

The procedures for cannula insertion to SHR (male, 28 weeks old, obtained from Charles River Japan), and the measurement of mean blood pressure and drug treatment were the same as those described for the renal hypertensive rat study. Efficacy was expressed in terms of ED_{25} values.

2.7. Measurement of mean blood pressure and heart rate in SHR with radio telemetry system

The surgical procedure and measurement of blood pressure and heart rate have been described previously (Brockway et al., 1991). After SHR (male, 24 weeks old, Charles River Japan) were anesthetized with sodium pentobarbital (50 mg/kg i.p.), a midline abdominal incision was made, and a sensor catheter was inserted into the descending aorta below the renal artery, pointing upstream. A sensor (TA-11PA; Data Sciences, MN, USA) was stitched to the inside musculus rectus abdominis. The rats received an i.m. injection of 5000 units of penicillin (Meiji Seika, Tokyo, Japan), and were kept in isolation cages and allowed to recover for at least 7 days. A plastic cage was placed above the telemetry receiver (RA1310, Data Sciences) for the measurement of mean blood pressure and heart rate. The data were collected for 10 s at 5-min intervals, using a computer system (Dataquest III, Data Sciences) and the mean values for each 30-min interval were calculated. ME3221 (10 mg/kg), or vehicle, was given p.o. to the rats every day at 11:00 a.m. for 14

days. The mean blood pressure and heart rate parameters were obtained from a cross-over trial performed after 7 days without the drug.

2.8. Compounds

ME3221, EF2831, losartan, and EXP3174 were synthesized at the Laboratory of Meiji Seika Kaisha. Enalapril was purchased from Sigma Chemicals (St. Louis, MO, USA). In the in vitro studies, ME3221 and EF2831 were dissolved in 0.25 N NaOH at 10^{-1} M and diluted with saline. For oral dosing, ME3221 was suspended in 0.5% carboxymethylcellulose. Losartan and enalapril were dissolved in distilled water; all drugs were administered in a volume of 2 ml/kg body weight. For intravenous dosing, ME3221 and EF2831 were dissolved in a small volume of 1 N NaOH and diluted with saline. EXP3174 was dissolved in saline.

Angiotensin I (human), angiotensin II (human), angiotensin III (human), and bradykinin were purchased from the Peptide Institute (Osaka, Japan); norepinephrine was from obtained Sigma Chemicals. These agents were dissolved in saline for i.v. injection (100 μl/kg).

2.9. Statistics

All values were expressed as means ± S.E.M. Statistical analyses used were analysis of variance and Duncan's multiple range test, Dunnetts 2-sided test, or the paired Student's *t*-test.

3. Results

3.1. In vitro angiotensin II antagonism in rabbit aorta preparation

Fig. 2 shows the angiotensin II-induced concentration-contractile response curves of ME3221 (3–30 nM) and its metabolite, EF2831 (0.1–1 μM), in the rabbit aorta. Each of the response curves of the two compounds was shifted in parallel to the right when the concentration was reduced, and the maximal response to angiotensin II was not altered in either case, indicating surmountable antagonism (Fig. 2). The pA_2 values for ME3221 and EF2831 were 8.91 ± 0.13 ($n = 6$) and 7.47 ± 0.14 ($n = 6$), and the slopes of the Schild plot were 1.11 ± 0.11 and 1.02 ± 0.37, respectively (neither value differed significantly from 1).

3.2. Effects of ME3221 on blood pressure responses induced by angiotensin II and other vasoactive agents in conscious rats

Experiment 1. When 0.1 μg/kg angiotensin II was injected into the rats, the blood pressure rose transiently to about 40 mm Hg, and the pressor response did not change during the 3- or 6-h observation period in the vehicle-treated group. Intravenous treatment with ME3221 at 0.03 mg/kg, 0.1 mg/kg, and 0.3 mg/kg dose dependently and significantly ($P < 0.05$) inhibited the angiotensin II-induced pressor response. The effect lasted for at least 3 h at doses of 0.1 and 0.3 mg/kg (Fig. 3). EF2831, a metabolite of ME3221, dose dependently and significantly ($P < 0.05$ and $P < 0.01$) inhibited the response for at least 3 h at 0.1–1 mg/kg i.v. (Fig. 3). EXP3174, a metabolite of losartan, also dose dependently and significantly ($P < 0.05$ and $P < 0.01$) inhibited the response for at least 3 h, at 0.03–0.3 mg/kg i.v. (Fig. 3). Orally administered ME3221, at 0.1, 0.3, and 1 mg/kg, dose dependently and significantly ($P < 0.05$) inhibited the angiotensin II-induced pressor response for at least 5 h (Fig. 3). ME3221, at the oral doses noted above, did not alter basal blood pressure or heart rate (data not shown). Similarly, losartan, at doses of 1, 3, and 10 mg/kg p.o., caused a significant ($P < 0.05$) dose-dependent inhibition of the pressor response to angiotensin II (Fig. 3). Losartan

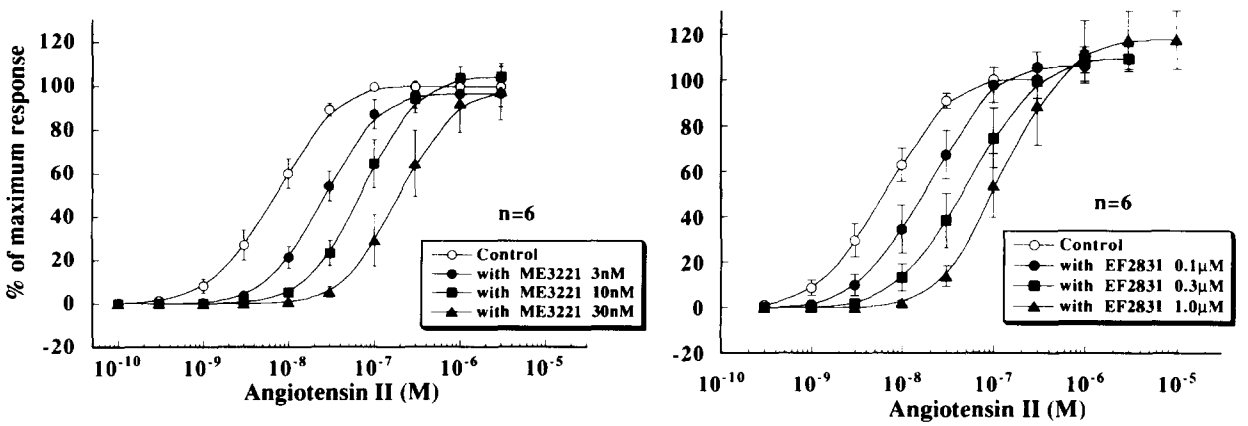


Fig. 2. Effects of ME3221 and EF2831 on concentration-contractile response curves for angiotensin II in the isolated rabbit aorta.

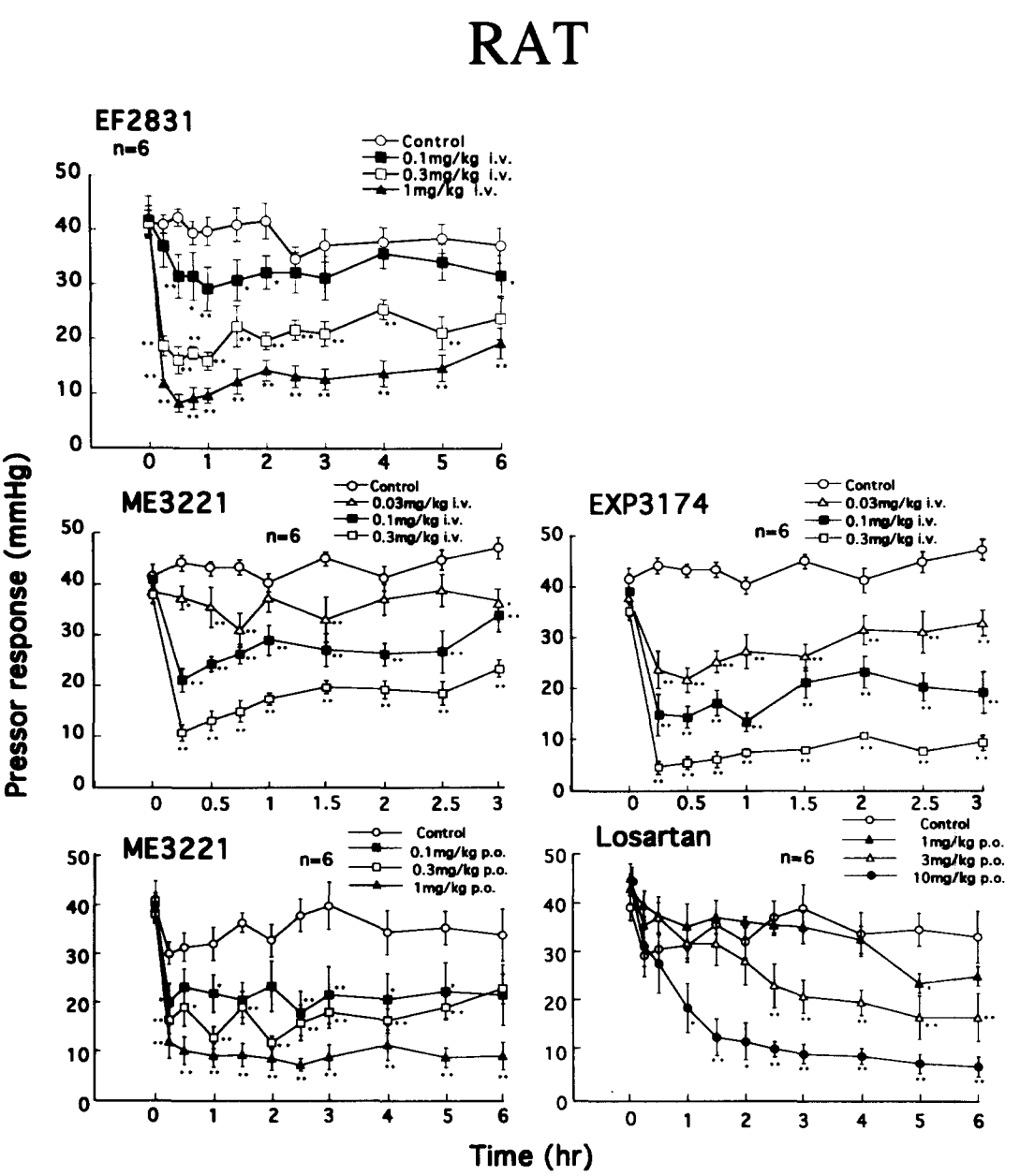


Fig. 3. Effects of ME3221, losartan, and their major metabolites on pressure response to angiotensin II (0.1 μg/kg i.v.) in conscious normotensive rats. *,** Significant difference from the control, $P < 0.05$ and $P < 0.01$, respectively.

gradually inhibited the angiotensin II pressor response; the inhibitory effect reached its highest level 5–6 h after administration. The time course of the inhibition of the pressor response by losartan was different from that for ME3221. Oral treatment with losartan did not alter the basal blood pressure or the heart rate (data not shown), similarly to the effect of ME3221.

Experiment 2. When 0.3 μg/kg angiotensin I, 0.1 μg/kg angiotensin II, or 1 μg/kg angiotensin III was injected into the rats, the blood pressure rose to 32 ± 6.8 mm Hg, 29 ± 3.8 mm Hg, and 39 ± 7.4 mm Hg, respectively. Similarly, 1 μg/kg norepinephrine elevated the blood pressure to 36 ± 1.7 mm Hg, while 3 μg/kg bradykinin lowered the blood pressure to 15 ± 3.6 mm Hg. ME3221 (10 mg/kg) significantly ($P < 0.01$) inhibited the pressor responses induced by angiotensin I, angiotensin II, and angiotensin III, whereas the responses induced by norepinephrine and bradykinin were unaffected. Enalapril, however, inhibited only the pressor response to angiotensin I ($P < 0.01$) and did not inhibit the responses to angiotensin II, angiotensin III, and norepinephrine. Furthermore, enalapril signifi-

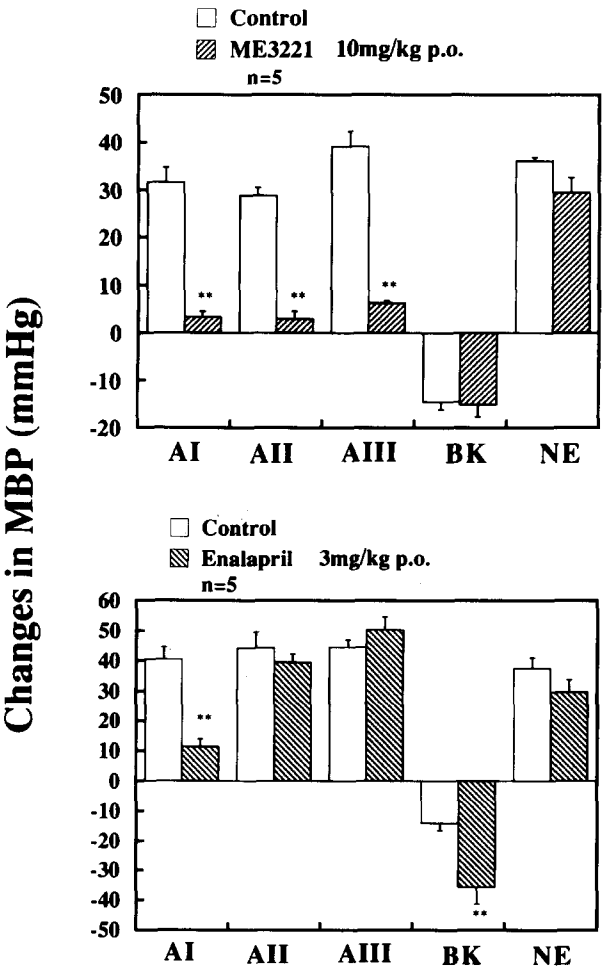


Fig. 4. Effects of ME3221 and enalapril on blood pressure responses induced by angiotensin I (AI), angiotensin II (AII), angiotensin III (AIII), bradykinin (BK), and norepinephrine (NE) in conscious normotensive rats. MBP: mean blood pressure, ** $P < 0.01$, compared with control.

cantly ($P < 0.01$) potentiated the bradykinin-induced vasodepressor response, from 13 ± 5.0 to 35 ± 12.5 mm Hg (Fig. 4).

3.3. Effects of ME3221 on angiotensin II-induced blood pressure response in conscious common marmosets

When 1 μg/kg angiotensin II was injected into common marmosets, the blood pressure rose transiently to about 60 mm Hg, and the signal pattern of the pressor response was not changed for 3 h (i.v.) or 6 h (p.o.) by repeated injection of angiotensin II to the vehicle-treated control. The intravenous injection of ME3221, at 0.3, 1, and 3 mg/kg, dose dependently and significantly ($P < 0.05$ or $P < 0.01$) inhibited the angiotensin II-induced pressor response, and the effect lasted for at least 3 h at 3 mg/kg (Fig. 5). EXP3174, at doses of 0.1–1 mg/kg i.v., also dose dependently and significantly ($P < 0.05$ and $P < 0.01$) inhibited the pressor response for at least 3 h at 1 mg/kg (Fig. 5). Similarly, the oral administration of ME3221, at 1, 3, and 10 mg/kg and losartan at 30 mg/kg significantly ($P < 0.05$ and $P < 0.01$) inhibited the angiotensin II-induced pressor response for at least 6 h (Fig. 5). With 30 mg/kg losartan, inhibition of the angiotensin II-induced pressor response was observed for 2 h (Fig. 5). Neither i.v. nor p.o. administration of ME3221 and losartan altered basal blood pressure or heart rate (data not shown).

3.4. Antihypertensive effects of ME3221 in renal hypertensive rats

The initial mean blood pressure values in conscious renal hypertensive rats were 134–144 mm Hg. When ME3221 at 0.3, 1, and 3 mg/kg was administered orally, the blood pressure was lowered to 21–46 mm Hg at the maximal response. The response was dose-dependent (Fig. 6). The maximal response was obtained 4–9 h after the administration of ME3221, and the significant antihypertensive effect lasted for at least 10 h. With 3 mg/kg ME3221, the blood pressure was significantly ($P < 0.05$) lowered even 24 h after drug administration. Similarly, losartan, at 1, 3, and 10 mg/kg p.o., significantly ($P < 0.05$) lowered blood pressure in a dose-dependent manner (Fig. 6). The antihypertensive effect of losartan lasted almost as long as that of ME3221. The ED_{25} values (95% confidence limits) of ME3221 and losartan were 0.48 (0.23–0.99) and 1.49 (0.85–2.64) mg/kg, respectively, indicating that ME3221 was approximately 3 times more potent than losartan in renal hypertensive rats.

3.5. Antihypertensive effects of ME3221 in SHR

The initial mean blood pressure values in conscious SHR were 141–154 mm Hg. When ME3221, at doses of 3, 10, and 30 mg/kg, was given orally, the mean blood pressure was lowered to 25–45 mm Hg, in a dose-dependent manner (Fig. 7). The maximal response was obtained 2–6 h after dosing. The significant ($P < 0.05$) antihypertensive effect of ME3221, at doses of 3, 10, and 30 mg/kg, was sustained for at least 10 h. With 30 mg/kg ME3221, the effect was observed even 24 h after drug administration. Losartan, at doses of 3, 10, and 30 mg/kg p.o., also caused a dose-dependent decrease in mean blood pressure (Fig. 7), with a long-lasting effect similar to that of ME3221 at 30 mg/kg. Neither of these two drugs significantly changed the heart rate throughout the experimental period. The ED_{25} values (95% confidence limits) for ME3221 and losartan were 2.49 (0.55–11.19) and 7.15 (2.75–18.60) mg/kg, respectively, indicating that ME3221 was approximately 3 times more potent than losartan in SHR.

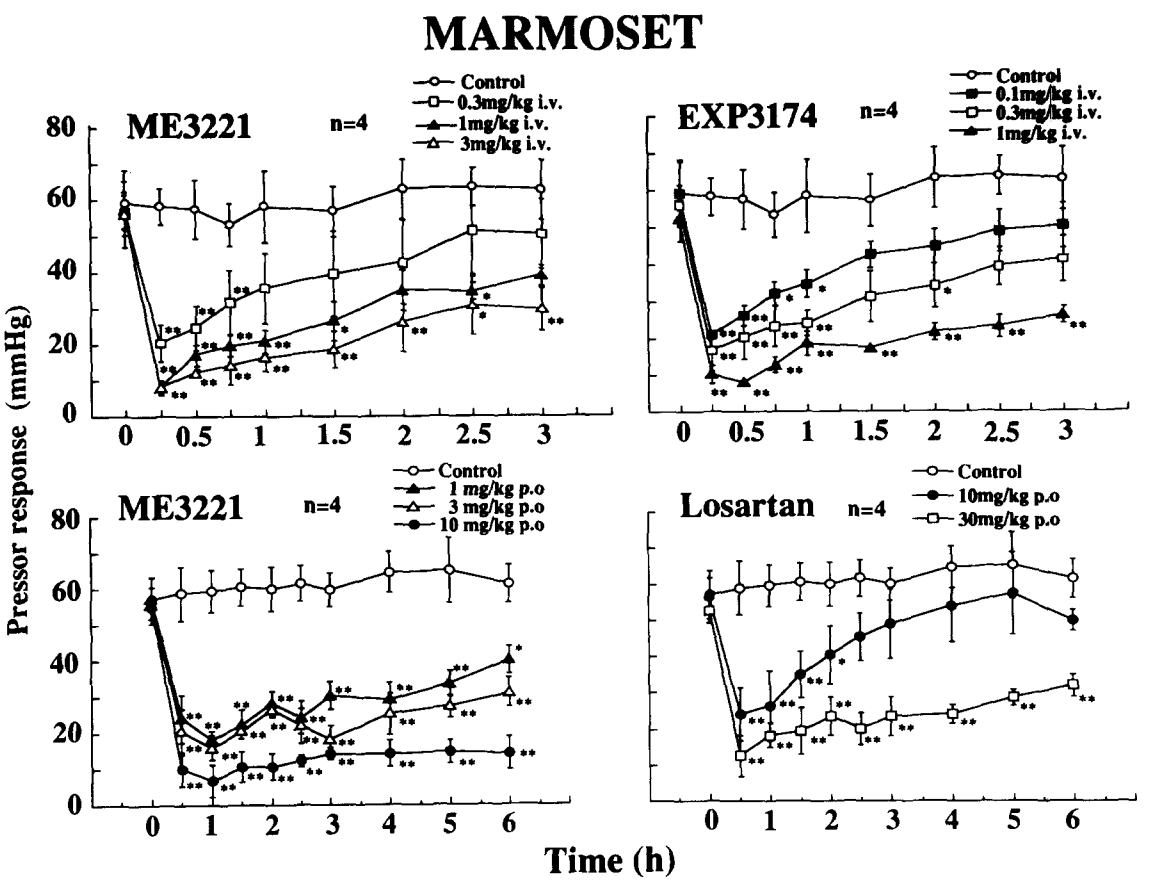


Fig. 5. Effects of ME3221, losartan, and EXP3174 on pressure response to angiotensin II (1 μg/kg i.v.) in conscious common marmosets. *,** Significant difference from the control, $P < 0.05$ and $P < 0.01$, respectively.

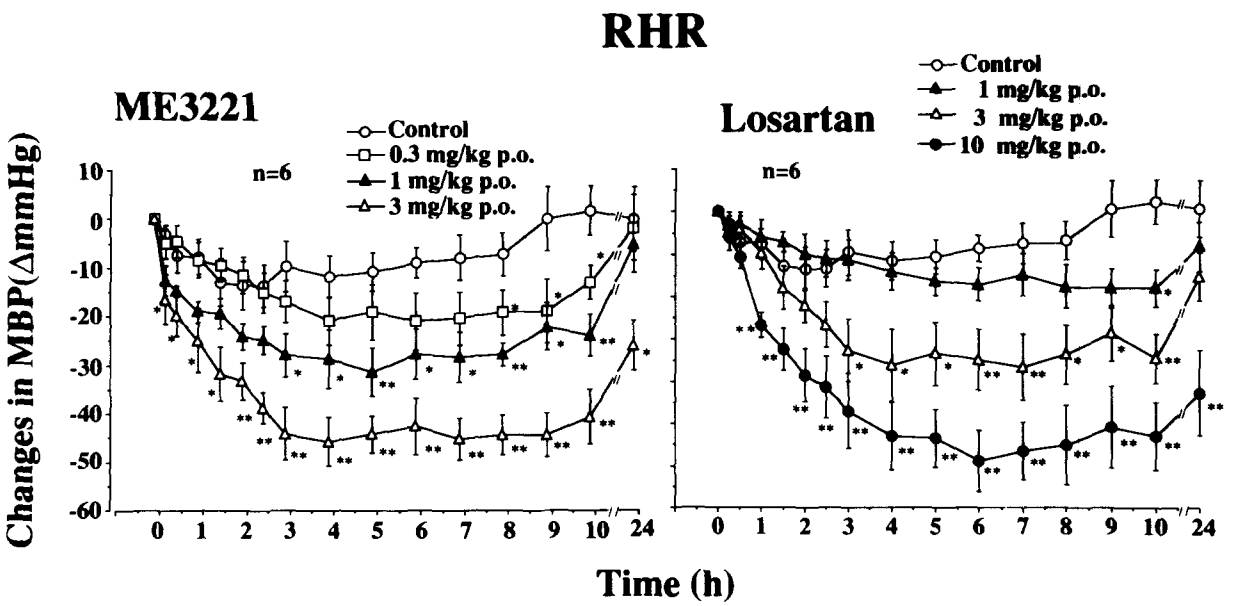


Fig. 6. Effects of ME3221 and losartan on mean blood pressure (MBP) in renal hypertensive rats (RHR). *,** Significant difference from the control, $P < 0.05$ and $P < 0.01$, respectively.

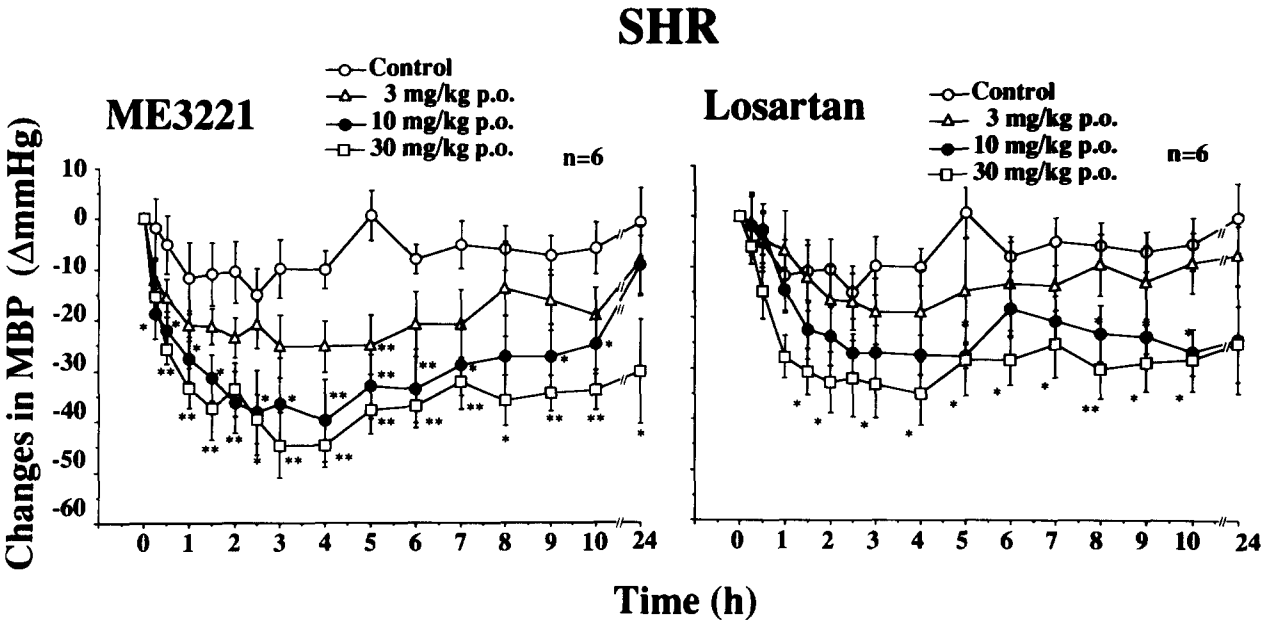


Fig. 7. Effects of ME3221 and losartan on mean blood pressure (MBP) in spontaneously hypertensive rats (SHR). *,** Significant difference from the control, $P < 0.05$ and $P < 0.01$, respectively.

3.6. Antihypertensive effect of repeated administration of ME3221 in SHR

Fig. 8 illustrates the effects of multiple administration of ME3221 on mean blood pressure and heart rate in SHR.

The mean blood pressure and heart rate in the vehicle-treated control group changed from 115 to 140 mm Hg and from 200 to 275 beats/min, respectively. The maximal blood pressure response to ME3221 at 10 mg/kg/day was reached about 2–6 h after the daily administration. The maximal depressive response was increased significantly ($P < 0.05$ or $P < 0.01$) from days 1 to 3. Although the maximal response was comparable on the 3rd and 7th days, depression of the mean blood pressure 24 h after each dosing was significantly longer for the 7-day treatment than for the 3-day treatment. On the 7th and the 14th days, mean blood pressure at 0, 6, and 24 h was significantly reduced ($P < 0.05$ and $P < 0.01$) from the control mean blood pressure. On the 21st day, that is, 7 days after the cessation of ME3221 administration, the antihypertensive effect was not observed, and no subsequent increase in blood pressure occurred. There was no significant difference in heart rate between control and ME3221-treated groups throughout the experimental period.

4. Discussion

EF2831, the demethylated product of ME3221, is the major metabolite of ME3221 in humans (unpublished data). The in vitro potency of EF2831 is about 1/30 that of ME3221, but its in vivo potency is equal to or 1/3 that of ME3221. That is, the potency of EF2831 is close to that of ME3221 in vivo. The increase of the in vivo activity of EF2831 relative to the in vitro activity of ME3221 may be due, at least in part, to differences in serum protein binding affinity between the two drugs. The rat serum binding rates of ME3221 and EF2831 were found to be 98.4–97.8% and 94.7–93.1%, respectively, and the concentration of free EF2831 was about 3 times higher than that of free ME3221 (unpublished observations). If it is assumed that the plasma free compound exerts the antihypertensive effect, the 3-fold higher plasma free concentration of EF2831 compensates for the weaker in vitro activity relative to ME3221. Therefore, the antihypertensive effect of ME3221 in humans may be explained as follows: ME3221 absorbed orally leads to a faster onset of the antihypertensive effect, and the metabolite, EF2831, which has a long-lasting antihypertensive effect, is responsible for the duration of the effect. Thus, due to the combination of the effects of surmountable ME3221 itself, and its surmountable metabolite, EF2831, ME3221 could exert potent and long-lasting effects in hypertensive patients.

In the present study, we found that both the novel angiotensin AT_1 receptor antagonist, ME3221, and its metabolite, EF2831, showed surmountable angiotensin AT_1 receptor antagonism. Losartan is reported to be converted in the body to a more active metabolite, EXP3174, and shows a slow onset of action and potent antihypertensive effect. Although losartan shows surmountable angiotensin AT_1 receptor antagonism, the metabolite, EXP3174, shows insurmountable an-

giotensin AT_1 receptor antagonism (Wong et al., 1990c, 1991). Similarly, as reported by Shibouta et al. (1993), TCV-116 is also converted to an active metabolite, CV-11974, which also shows insurmountable angiotensin AT_1 receptor antagonism. The relative efficacy of these antagonists is a current topic of discussion. The present study showed that the surmountable antagonists, ME3221 and EF2831, showed an antihypertensive activity comparable to that of the surmountable losartan and the insurmountable EXP3174 in vivo.

With regard to the inhibition of the angiotensin II pressor response in rats, ME3221 showed a fast onset of action and steady inhibition for the observation period, while losartan showed a slow onset and gradually increasing inhibition for up to 6 h of the observation period. In marmosets, ME3221 showed an inhibition pattern similar to that obtained in rats, while losartan showed a faster onset and faster recovery than in rats. The recovery rate with losartan was somewhat faster than that with ME3221.

ME3221 thus showed less species difference in inhibition pattern than losartan, suggesting that losartan metabolism differs considerably in rats and marmosets. Indeed, Stearns et al. (1992), in an experiment with liver slices, showed that metabolism of losartan differs in rats, monkeys, and humans.

The antihypertensive potency of ME3221 was about 3 times that of losartan in both renal hypertensive rats and SHR. Our findings regarding the effect of losartan in inhibiting the angiotensin II pressor response in rats and in inducing hypotension in renal hypertensive rats coincide with those reported by Wong et al. (1990a,b).

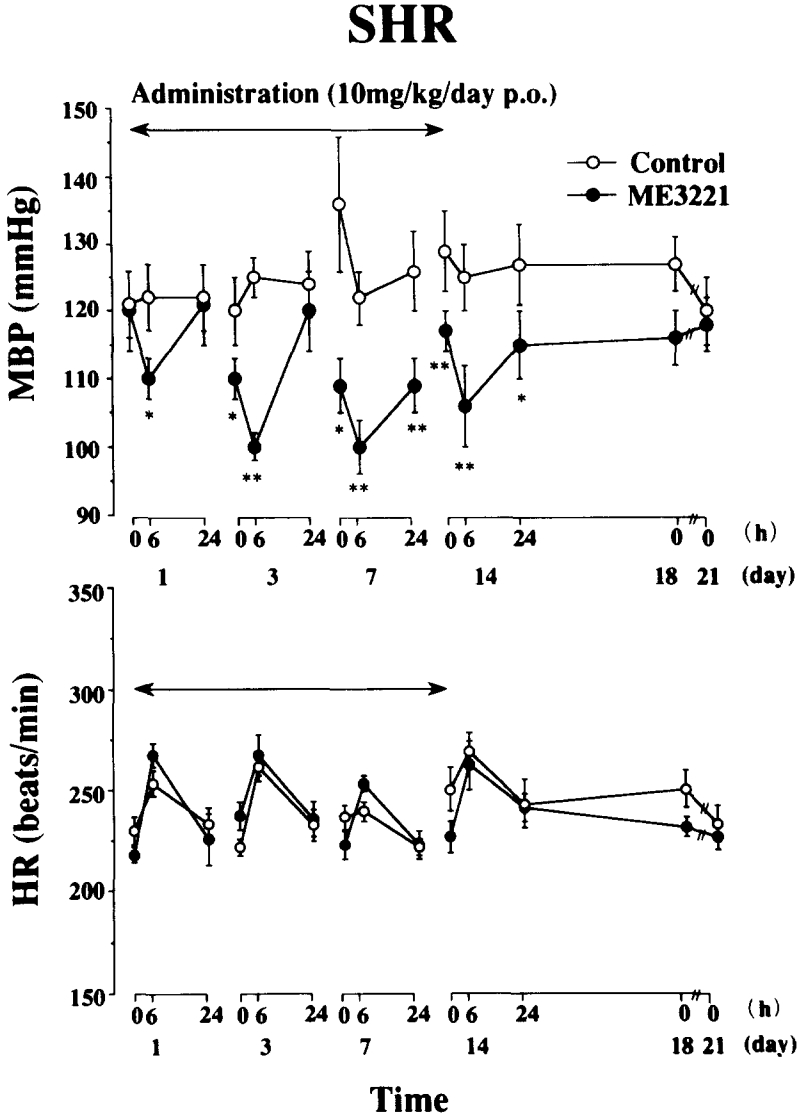


Fig. 8. Effects of repeated daily administration of ME3221 on mean blood pressure (MBP) and heart rate (HR) in spontaneously hypertensive rats (SHR). These parameters were measured with a radio-telemetry system. Values were obtained from a cross-over trial. *,** Significant difference from the control, $P < 0.05$ and $P < 0.01$, respectively.

The difference in the effective dose range of ME3221 in the two animal models is associated with differences in plasma renin activity. The higher plasma renin activity in renal hypertensive rats compared with SHR coincides with the more potent antihypertensive effect of ME3221 in the renal hypertensive rats. The fast onset and potent action of ME3221 both i.v. and p.o. on the angiotensin II pressor response may reflect the high bioavailability of ME3221 in rats; these characteristics also indicate that the ME3221 molecule itself is the bioactive molecule (Nagura et al., 1994).

The radio-telemetry systems can measure blood pressure and heart rate in freely moving animals without inducing any stress (Brockway et al., 1991). Using this system, we found that ME3221, on repeated administration for 14 days, lowered mean blood pressure significantly in SHR, without inducing tachycardia or tolerance. The antihypertensive effect gradually increased on repeated administration from days 1 to 7, and on the 7th day the effect was saturated. The antihypertensive effect of ME3221 gradually decreased after cessation of administration, and no rebound phenomenon was observed. At least two explanations may be considered for the increased antihypertensive effect caused by repeated administration. One is possible drug accumulation in the animals. However, no differences were observed in the absorption, distribution, metabolism, or elimination of ME3221 between single and repeated administration (our unpublished data). The other explanation is related to the dissociation rate of ME3221 from receptor sites. Since the binding of ME3221 to the angiotensin AT_1 receptor is strong in vitro (Kawano et al., 1994), it is conceivable that the ME3221 molecules bound to the receptors, especially to those of the resistant vessels, were incompletely dissociated, leading to accumulation on repeated administration. However, further experiments are necessary to confirm that this type of accumulation does occur.

Some advantages over angiotensin converting enzyme inhibitors are anticipated with angiotensin II receptor antagonists. The most important advantage is the absence of such side-effects as coughing and angioedema, which arise from the accumulation of bradykinin with angiotensin converting enzyme inhibition (Morice et al., 1989; McEwan and Fuller, 1989). ME3221 inhibited the pressor response to angiotensin I and angiotensin III, as well as that to angiotensin II, and did not enhance the bradykinin-induced vasodepressor response even when used at a high dose, whereas enalapril inhibited only the pressor response to angiotensin I and potentiated the depressor response to bradykinin.

In conclusion, the present results indicated that the antihypertensive and angiotensin II-induced pressor response blocking effects of ME3221 were 3 times more potent than those of losartan in several models. With regard to antihypertensive efficacy, there was no remarkable difference between surmountable and insurmountable angiotensin AT_1 receptor antagonists. Repeated administration of ME3221 in SHR had a stable and long-lasting antihypertensive effect without influencing heart rate. Thus, ME3221, like losartan, may be of therapeutic value for the treatment of both renal and essential hypertension.

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References

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative use of drug antagonism, Br. J. Pharmacol. 14, 48.

Brockway, B.P., P.A. Mills and S.H. Azar, 1991, A new method for continuous chronic measurement and recording of blood pressure, heart rate and activity in the rat via radio-telemetry, Clin. Exp. Hypertens. 13, 885.

Cangiano, J.L., C. Rodriguez-Sargent and M. Martinez-Malonado, 1979, Effects of antihypertensive treatment on systolic blood pressure and renin in experimental hypertension in rats, J. Pharmacol. Exp. Ther. 208, 310.

Cody, R.J., 1986, Conceptual and therapeutic approaches to inhibition of the renin-angiotensin system in chronic heart failure, J. Cardiovasc. Pharmacol. 8 (Suppl. 1), S58.

Katano, K., H. Ogino, E. Shitara, H. Watanabe, J. Nagura, N. Osada, Y. Ichimaru, F. Konno and T. Tsuruoka, 1991, Pyridine derivative with angiotensin II antagonism, International Patent WO 91/19697.

Kawano, K., S. Yasuda, K. Fujishima, J. Nagura, M. Hachisu and F. Konno, 1994, In vitro profile of ME3221, a novel nonpeptide angiotensin II (AII) receptor antagonist, Jpn. J. Pharmacol. 64 (Suppl. 1), 331P.

McEwan, J.R. and R.W. Fuller, 1989, Angiotensin converting enzyme inhibitors and cough, J. Cardiovasc. Pharmacol. 13 (Suppl. 3), S67.

Morice, A.H., M.J. Brown and T. Higenbottam, 1989, Cough associated with angiotensin converting enzyme inhibition, J. Cardiovasc. Pharmacol. 13 (Suppl. 3), S59.

Nagura, J., K. Fujishima, K. Kawano, S. Yasuda, M. Hachisu and F. Konno, 1994, Pharmacological profile of ME3221, a novel angiotensin II (AII) receptor antagonist, J. Hypertens. 12 (Suppl. 3), S99.

Shibouta, Y., Y. Inada, M. Ojima, T. Wada, M. Noda, T. Sanada, K. Kubo, Y. Kohara, T. Naka and K. Nishikawa, 1993, Pharmacological profile of a highly potent and long-acting angiotensin II receptor antagonist, 2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-1*H*-benzimidazole-7-carboxylic acid (CV-11974), and its prodrug, (±)-1-(cyclohexyloxycarbonyloxy)-ethyl 2-ethoxy-1-[[2′-(1*H*-terazol-5-yl) biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylate (TCV-116), J. Pharmacol. Exp. Ther. 266, 114.

Smith, R.D., A.T. Chiu, P.C. Wong, W.F. Herblin and P.B.M.W.M. Timmermans, 1992, Pharmacology of nonpeptide angiotensin II receptor antagonists, Annu. Rev. Pharmacol. Toxicol. 32, 135.

Stearns, R.A., R.R. Miller, G.A. Doss, P.K. Chakravarty, A. Rosegay, G.J. Gatto and S.-H.L. Chiu, 1992, The metabolism of DuP753, a nonpeptide angiotensin II receptor antagonist, by rat, monkey, and human liver slices, Drug Metab. Dispos. 20, 281.

Timmermans, P.B.M.W.M., P.C. Wong, A.T. Chiu, H.P. Benfield, D.J. Carini, R.J. Lee, R.R. Wexler, J.A.M. Saye and R.D. Smith, 1993, Angiotensin II receptors and angiotensin II receptor antagonists, Pharmacol. Rev. 45, 205.

Williams, G.H., 1988, Converting enzyme inhibitors in the treatment of hypertension, New Engl. J. Med. 319, 1517.

Wong, P.C., W.A. Price, A.T. Chiu, J.V. Duncia, D.J. Carini, A.L. Johnson, R.R. Wexler and P.B.M.W.M. Timmermans, 1990a, Nonpeptide angiotensin II receptor antagonists. VIII. Characterization of functional antagonism displayed by DuP753, an orally active antihypertensive agent, J. Pharmacol. Exp. Ther. 252, 719.

Wong, P.C., W.A. Price, A.T. Chiu, J.V. Duncia, D.J. Carini, A.L. Johnson, R.R. Wexler and P.B.M.W.M. Timmermans, 1990b, Nonpeptide angiotensin II receptor antagonists. IX. Antihypertensive activity in rats of DuP753, an orally active antihypertensive agent, J. Pharmacol. Exp. Ther. 252, 726.

Wong, P.C., W.A. Price, A.T. Chiu, J.V. Duncia, D.J. Carini, A.L. Johnson, R.R. Wexler and P.B.M.W.M. Timmermans, 1990c, Nonpeptide angiotensin II receptor antagonists. XI. Pharmacology of EXP3174: an active metabolite of DuP753, an orally active antihypertensive agent, J. Pharmacol. Exp. Ther. 255, 211.

Wong, P.C., S.D. Hart, J.V. Duncia and P.B.M.W.M. Timmermans, 1991, Nonpeptide angiotensin II receptor antagonists. XIII. Studies with DuP753 and EXP3174 in dogs, Eur. J. Pharmacol. 202, 323.