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Nonpeptide angiotensin II receptor antagonist recognizes inter-species differences in angiotensin AT_1 receptors

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Abstract

Oral administration of the angiotensin AT_1 receptor antagonist 3-methyl-2,6-dimethyl-4-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methoxy] pyridine (ME3221) inhibited the pressor response to angiotensin II at doses of 0.3–1.0 mg/kg in rats. A higher dose of ME3221 (3–10 mg/kg) was required to obtain the same inhibitory potency in dogs. The antagonistic potency of ME3221 for angiotensin II-induced contraction in the rabbit aorta ($pA_2 = 8.82$) was about five times higher than that in the canine aorta ($pA_2 = 8.18$). The inhibition constant of ME3221 for displacing [^{125}I]angiotensin II binding to membrane fractions from the rabbit aorta ($K_i = 3.84$ nM) and rat liver ($K_i = 2.55$ nM) was significantly lower than that for the canine aorta ($K_i = 84.5$ nM), canine liver ($K_i = 122$ nM) and bovine adrenal cortex ($K_i = 21.5$ nM). In contrast, [Sar^1, Ala^8]angiotensin II had a similar inhibition constant ($K_i = 0.85–4.67$ nM) in the species investigated. Treatment with 5 mM dithiothreitol significantly ($P < 0.01$) reduced the angiotensin II-induced contractile response to 1.2% in the rabbit aorta, but it did not significantly reduce the response in the canine aorta (83.2%). Dithiothreitol reduced [^{125}I]angiotensin II binding to membrane fractions from the rabbit aorta and the rat liver but partially inhibited binding in preparations that had a low affinity for ME3221. These data indicate a species difference in the angiotensin AT_1 receptor: the canine and bovine angiotensin AT_1 receptor has a relatively low affinity for ME3221 and is slightly resistant to dithiothreitol. The species difference in the angiotensin AT_1 receptor reflects the in vivo efficacy of ME3221 in rats and dogs.

Keywords: Angiotensin AT_1 receptor; ME3221; [Sar^1, Ala^8]Angiotensin II; Species difference; Dithiothreitol

1. Introduction

Angiotensin II receptors are classified into two subtypes, namely AT_1 and AT_2 (Timmermans et al., 1991). The angiotensin AT_1 receptor is selectively antagonized by losartan, and the AT_2 receptor by PD 123,177. One of the sulfhydryl reagents, dithiothreitol, abolishes angiotensin II binding to the angiotensin AT_1 receptor, while it enhances binding to the AT_2 receptor (Catt and Abbott, 1991; Chappell et al., 1992). We have already reported the potent and selective angiotensin AT_1 antagonist, ME3221, 3-methyl-2,6-dimethyl-4-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methoxy] pyridine (Nagura et al., 1995). In the present study, we found a species difference in the ability of ME3221 to inhibit an angiotensin II-induced pressor response in conscious rats and dogs.

It has been reported that the canine aorta has an angiotensin AT_1-like receptor which has a lower affinity for losartan than that of typical angiotensin AT_1 receptors found in rats, rabbits and human (Burns et al., 1994). Despite the paucity of information about the angiotensin AT_1-like receptor, the different inhibitory efficacy of ME3221 in conscious rats and dogs might be due to differences in the affinity for ME3221 of the angiotensin AT_1 receptors in these species. Thus, we compared the angiotensin AT_1 receptor in dogs with the angiotensin AT_1 receptors found in other species. In addition, we evaluated the angiotensin AT_1 receptor of the bovine adrenal cortex, since previous reports showed that it has a low affinity for losartan (Sasaki et al., 1991; Ouali et al., 1992).

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2. Materials and methods

2.1. Recording of angiotensin II-induced pressor response in rats and dogs

All experiments were performed in accordance with institutional guidelines and extra care was taken to avoid animal suffering. Male Sprague–Dawley rats (250–500 g; Nihon clea, Japan) were anaesthetized with sodium pentobarbital 60 mg/kg i.p. and female Beagle dogs (8–10 kg; Japan Laboratory Animals, Japan) were anaesthetized with sodium pentobarbital 30 mg/kg i.v. A vascular catheter was inserted into the femoral artery under aseptic conditions. The other end of the catheter was subcutaneously advanced to the dorsal side of the neck and exteriorized for measurement of blood pressure. After the animals recovered from surgery, blood pressure was measured with a pressure amplifier (AP-641G, Nihon Kohden, Tokyo) through a pressure transducer (DX-300, Nihon Kohden) while the animals were conscious. After the blood pressure became stable, 0.1 μg/kg angiotensin II was administered i.v. until reproducible pressor responses were obtained. Thereafter, ME3221 was orally administered and angiotensin II-induced pressor responses were subsequently measured over 6 h at appropriate intervals.

2.2. Recording of angiotensin II-induced contraction in aortic strips from rabbits and dogs

The thoracic aortae were isolated from male Japanese white rabbits (2.5–3.0 kg; Japan Laboratory Animals) and female Beagle dogs under pentobarbital anaesthesia (30 mg/kg, i.v.). The connective tissues were carefully removed from the thoracic aorta and the aorta was cut into helical strips. The endothelia were removed by gently rubbing the lumen of the aorta with a cotton rod. These strips were suspended in 5-ml organ baths filled with Krebs–Henseleit solution of the following composition (in mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; $MgSO_4$, 1.2; and dextrose, 10.0. The solution was kept at 37°C and gassed with 95% O_2–5% CO_2. Active tension was continuously recorded with isometric transducers (T512, Nihon Kohden, Tokyo), under a passive tension of 1–2 g. After a 90-min equilibration periods, strips were contracted by addition of 0.1 μM noradrenaline for the rabbit aorta and 1 μM noradrenaline for the canine aorta. This process was repeated until successive contractions of equal size were obtained. Subsequently, the tissues were exposed to 10 μM noradrenaline for the rabbit aorta and 100 μM noradrenaline for the canine aorta. The contractile response induced by the concentration of noradrenaline was taken as 100% contraction and the magnitude of the angiotensin II responses is expressed in terms of this response in each preparation. Angiotensin II was cumulatively added to the organ baths to obtain a dose–response curve. After a 90 min-recovery period, rabbit aortic strips were exposed to ME3221 (3, 10 and 30 nM) for 30 min, and then angiotensin II was added cumulatively to the strips. For canine aortic strips, ME3221 (30, 100 and 300 nM) was added for 60 min and a dose–response curve for angiotensin II was obtained by a similar procedure.

The maximal responses and the EC_{50} values of concentration–response curves were estimated with the fitting equation (Leff et al., 1990):

$$E = \frac{\alpha[A]^m}{[A_{50}]^m + [A]^m}$$

where α, $[A_{50}]$ and m are the maximal response, EC_{50} and slope factor at half-maximal concentration, respectively. The Schild plot analysis gave the pA_2 value of ME3221 for angiotensin AT_1 receptors (Arunlakshana and Schild, 1959).

We tested the effect of dithiothreitol on the mechanical responses, using separate strips. After an equal size contractile response for noradrenaline was obtained, strips were contracted with 30 nM angiotensin II and 0.1 μM noradrenaline for the rabbit aorta and 100 nM angiotensin II and 1 μM noradrenaline for canine aorta, respectively. Dithiothreitol (5 mM) was added to the organ bath for 10 min, and then the bathing medium was washed out repeatedly. The contractile responses before treatment with dithiothreitol were taken as 100% and the magnitude of subsequent contractile responses was calculated.

2.3. Isolation of angiotensin II receptor membranes and radioligand binding assay

An angiotensin AT_1 receptor binding assay was carried out as follows: livers were isolated from male Sprague–Dawley rats or Beagle dogs, and aortae were isolated from Japanese white rabbits or Beagle dogs. The bovine adrenal gland and cerebellum were purchased from a slaughterhouse. The membrane fractions were prepared following the procedure described earlier (Chang and Lotti, 1990), except that Tris–HCl buffer was used instead of bicarbonate buffer. An aliquot of the membrane fraction was stored at −80°C until used for the receptor binding assay. The membrane fraction was incubated at 25°C with a reaction mixture composed of 50 mM Tris–HCl buffer (pH 7.4), 0.2% bovine serum albumin, 5 mM $MgCl_2$, 120 mM NaCl and resuspended pellet (0.2 mg/ml of protein) in a total volume of 150 μl. We did not use protease inhibitors to avoid the degradation of the ligand since combinations of these reagents did not enhance the specific binding of [^{125}I]angiotensin II under our experimental conditions (data not shown).

In the saturation study, the reaction mixture was incubated with 0.05–1.00 nM of [^{125}I]angiotensin II for 60 min at 25°C. Nonspecific binding was defined as [^{125}I]angiotensin II bound in the presence of unlabeled 1 μM [Sar^1,

Ile[8]]angiotensin II. To terminate the reaction, the reaction mixture was filtered through a glass fibre filter (Whatman GF/B, Maidstone, UK), using a cell-harvester (M-245, Brandel, MD, USA), and immediately washed five times with 1 ml of ice-cold washing buffer (50 mM Tris–HCl buffer, pH 7.4 at 4°C). The filter had been presoaked with the ice-cold washing buffer containing 0.3% polyethyleneimine to reduce the adsorption of radioligand to the filter. The radioactivity was counted with a γ-counter (Gamma 5500, Beckman, CA, USA). The dissociation constant (K_d value) and the receptor density (B_{max} value) were estimated by Scatchard analysis.

In the competition study, the inhibitory efficacy of the antagonists was determined under the same conditions as in the saturation study. The reaction mixture containing 0.2 nM [^{125}I]angiotensin II was incubated with various concentrations of competitors for 60 min at 25°C. The inhibition constants (K_i value) was calculated by using the Cheng and Prusoff equation (Cheng and Prusoff, 1973). The concentrations of protein in tissue samples were measured by using a protein assay kit (BCA protein assay reagent, Pierce, Rockford, IL, USA).

2.4. Influence of dithiothreitol on [^{125}I]angiotensin II binding

Membrane preparations from the rat liver, canine aorta, canine liver, rabbit aorta, bovine adrenal cortex and bovine cerebellum were incubated with 5 mM dithiothreitol at 37°C for 60 min. Subsequently, to terminate the reaction, the tubes were immediately placed at 4°C and centrifuged at 100 000 × g for 60 min. The pellets were suspended in 50 mM Tris–HCl buffer and incubated with [^{125}I]angiotensin II in the presence or absence of 1 μM [Sar[1], Ile[8]]angiotensin II. The specific binding of [^{125}I]angiotensin II to membrane fractions was compared with and without treatment with 5 mM dithiothreitol. All studies were performed in duplicate (n = 2–3).

2.5. Drugs

Angiotensin II, [Sar[1], Ile[8]]angiotensin II and [Sar[1], Ala[8]]angiotensin II were purchased from Peptide Research Laboratory (Osaka, Japan). Noradrenaline bitartarate and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). ME3221 and PD123,177 were synthesized by Meiji Seika Kaisha. [^{125}I]Angiotensin II (specific activity 2200 Ci/mmol) was obtained from New England Nuclear. Angiotensin II antagonists were dissolved in dimethylsulfoxide (DMSO), and the final concentration of solvent was up to 0.1% of DMSO in an organ bath.

2.6. Statistical analysis

The data are expressed as means ± S.E. Statistical analyses for the multiple comparisons of data were performed by analysis of variance (ANOVA) followed by a post hoc test. Two groups were compared by using a by paired-t test. A P-value of less than 0.05 was accepted as statistically significant. The linear and nonlinear least-squares curve fittings were analysed by means of a computer-assisted method, using Kaleida Graph software (Abelbeck software).

3. Results

3.1. Effects of ME3221 on angiotensin II-induced pressor responses in rats and dogs

In rats, intravenously injected angiotensin II temporarily raised the blood pressure by 40 mmHg. In the vehicle-

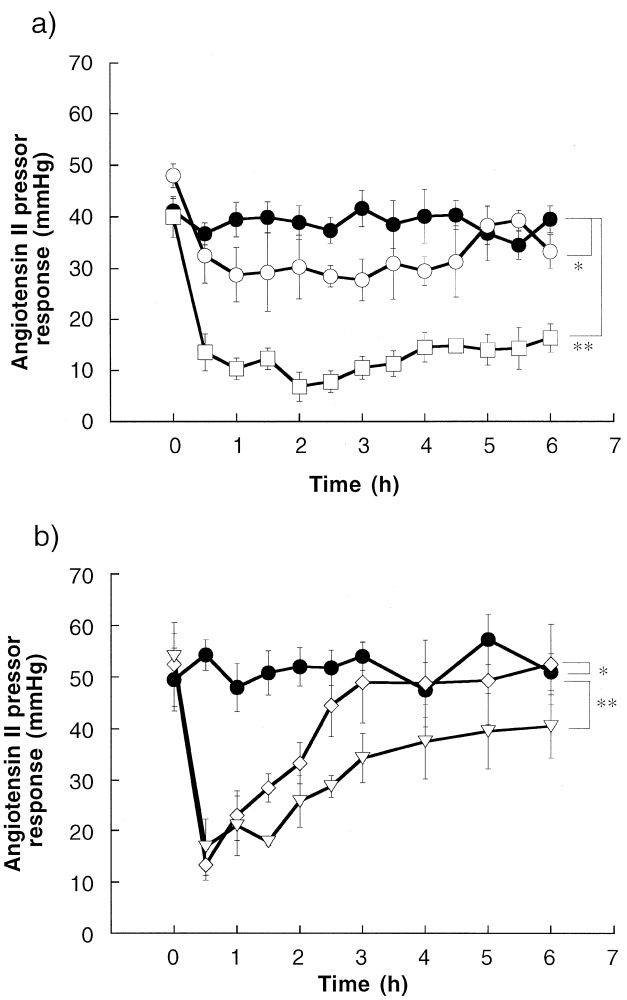

Fig. 1. Inhibitory effects of ME3221 on the pressor response to angiotensin II (0.1 μg/kg i.v.) with vehicle (●) or ME3221 in (a) rats and in (b) dogs. ME3221 was administered at zero time per os and the doses were 0.3 (○), 1.0 (□), 3 (◇) and 10 (△) mg/kg. The points and the bars represent the mean ± S.E. The number of animals used in the experiment was four for rats and three for dogs. The angiotensin II pressor responses in the ME3221-treated groups were significantly different from those in the vehicle-treated groups: * $P < 0.05$ or ** $P < 0.01$ (ANOVA).

treated groups, the pressor response did not change over the observation period up to 6 h. Oral administration of ME3221 at 0.3 and 1 mg/kg significantly ($P < 0.05$) reduced the angiotensin II-induced pressor response and the effect lasted for 6 h (Fig. 1a). In dogs, angiotensin II temporarily raised the blood pressure by 50 mmHg. In contrast to its effects in rats, ME3221 significantly ($P < 0.05$) inhibited the angiotensin II-induced pressor response at a relatively high dose (3 and 10 mg/kg) but for a shorter duration (Fig. 1b). ME3221 did not alter the heart rate in either species (data not shown).

3.2. Angiotensin II concentration–response relations in isolated aortas

In the rabbit aorta, ME3221 shifted the angiotensin II concentration–response curves to the right (Fig. 2a). The Schild analysis revealed a pA_2 value of 8.82 ± 0.14 and a slope factor of 1.12 ± 0.06 (Fig. 2a, inset). In the canine aorta, the maximal contractile responses to angiotensin II under ME3221 treatment tended to be augmented but not significantly. The pA_2 value and the slope factor of the Schild regression plot for the canine aorta were 8.18 ± 0.14 and 0.95 ± 0.09, respectively (Fig. 2b). The pA_2 value obtained for the canine aorta was significantly ($P < 0.05$) higher than that for the rabbit aorta.

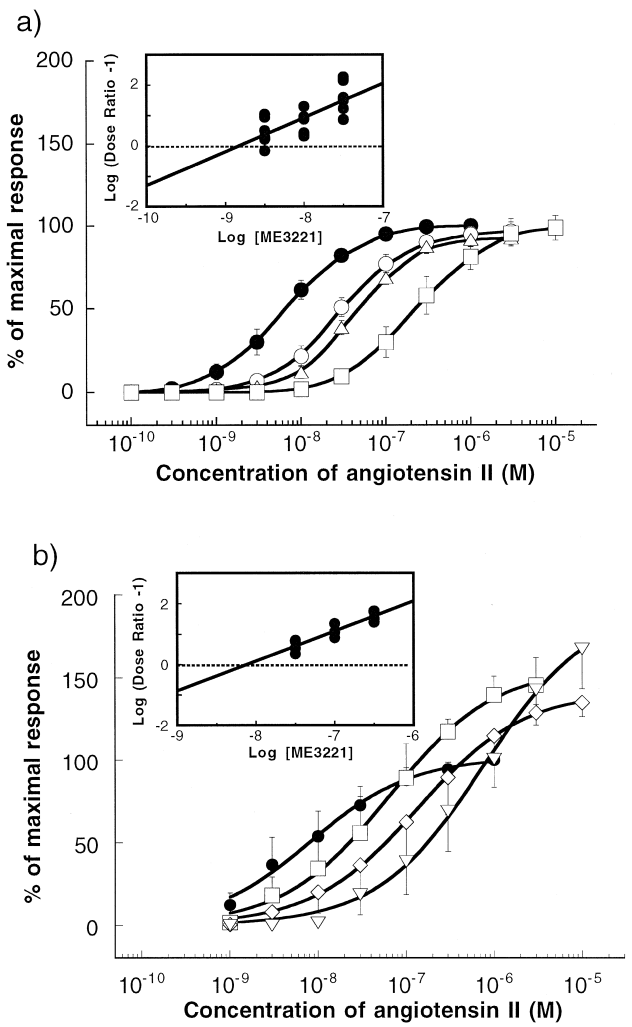

Fig. 2. Effects of ME3221 on contractile responses induced by cumulative concentrations of angiotensin II in (a) rabbit and (b) canine aortic strips in the absence (●) or presence of ME3221 (○ 3 nM, △ 10 nM, □ 30 nM, ◇ 100 nM and ▽ 300 nM). The points represent the mean ± S.E. of six replications for rabbits and four replications for dogs. Inset of the graph shows the Schild regression plot.

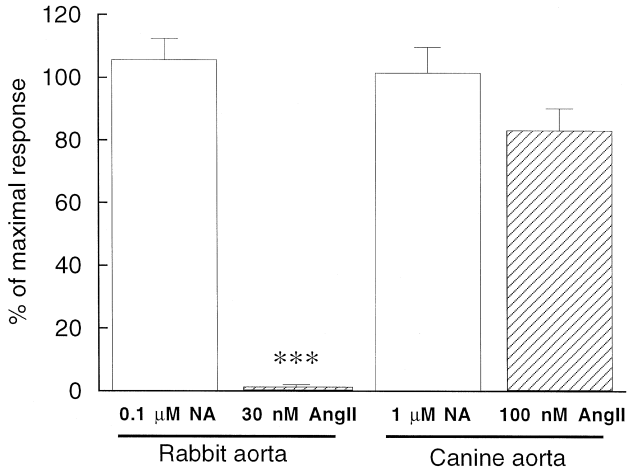

Fig. 3. Effects of dithiothreitol treatment on isolated preparations. Preparations were treated with dithiothreitol for 10 min. Then dithiothreitol was completely washed out with physiological saline and contractile responses to angiotensin II or noradrenaline were obtained. Each column indicates percentage of each response as mean ± S.E. for eight animals. Asterisk denotes statistical difference ($P < 0.001$, paired t) compared with the contractile response before treatment of dithiothreitol. NA, noradrenaline; AngII, angiotensin II.

3.3. Effect of dithiothreitol treatment on angiotensin II-induced contraction

In the rabbit aorta, treatment with dithiothreitol significantly ($P < 0.001$) reduced the angiotensin II-induced contractile response to $1.2 \pm 0.8\%$ (Fig. 3). Dithiothreitol partially reduced ($83.2 \pm 7.0\%$) the angiotensin II response in the canine aorta. Further incubation with dithiothreitol for up to 60 min failed to cause an additional reduction of the angiotensin II-induced contraction in canine aorta (data not shown). Dithiothreitol did not influence the contractile responses to noradrenaline in rabbit ($105.6 \pm 6.8\%$) and canine aortas ($101.6 \pm 8.2\%$).

3.4. Binding of [^{125}I]angiotensin II to membrane fractions from various tissues

[^{125}I]Angiotensin II binding to membrane fractions from rabbit and canine aortas was saturated at a ligand concentration of about 1 nM (Fig. 4a and b). Scatchard analysis revealed a single class of binding site with an apparent maximal receptor density (B_{max}) and dissociation constant (K_d) as shown in the inset of Fig. 4. Similarly, K_d values and B_{max} values were obtained for the rat liver, canine

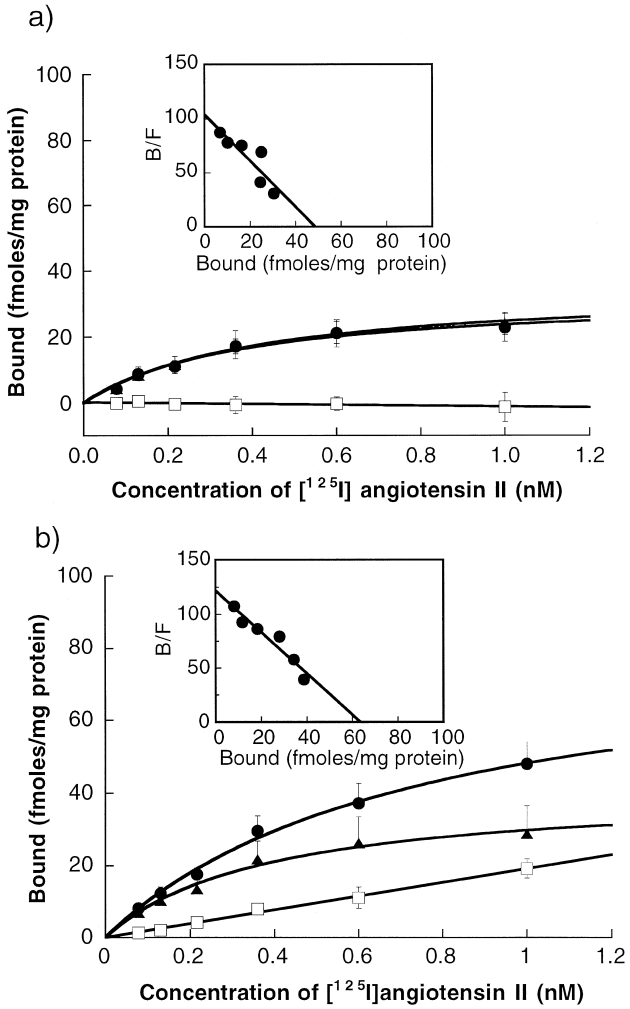

Fig. 4. The saturation curves for [^{125}I]angiotensin II binding to membrane preparations of (a) rabbit aorta and (b) canine aorta. Nonspecific binding is [^{125}I]angiotensin II binding in presence of 1 μM of [Sar^1, Ile^8]angiotensin II. Specific binding (▲) was obtained by subtracting the nonspecific binding (□) from the total binding (●). Each point represents mean ± S.E. of three replications. Inset of the graph shows a typical example of Scatchard plot.

liver and bovine adrenal cortex (Table 1). The K_d values for angiotensin II were not significantly different in the various tissues.

Table 1
Dissociation constant (K_d value) and receptor density (B_{max} value) for [^{125}I]angiotensin II binding in various tissues and species

Tissue	K_d value (nM)	B_{max} value (fmol/mg protein)	n
Rabbit aorta	0.72 ± 0.14	47.5 ± 7.92	3
Rat liver	0.27 ± 0.03	85.0 ± 12.0	4
Canine aorta	0.42 ± 0.15	47.6 ± 15.2	3
Canine liver	0.82 ± 0.16	101.9 ± 40.3	3
Bovine adrenal cortex	0.69 ± 0.06	267.24 ± 28.63	4

The K_d values and B_{max} values were obtained by Scathard analysis. The data are presented the means ± S.E. for 3–4 animals.

Fig. 5 shows the competition curves for the angiotensin II antagonists in rabbit and canine aortas. ME3221 and [Sar^1, Ala^8]angiotensin II completely displaced [^{125}I]angiotensin II binding from a single affinity site. The inhibition constants (K_i) of the tissues are shown in Table 2. The K_i values of [Sar^1, Ala^8]angiotensin II were not significantly different in the various tissues. In contrast, the K_i values for ME3221 in canine tissues were lower than those obtained in tissues from the rat and rabbit. The K_i value of ME3221 in the bovine adrenal cortex was four- to six-folds higher than in dogs but was lower than in rats and rabbits. PD 123,177 had a slightly higher affinity for the canine aorta than for the rabbit aorta.

3.5. Effect of dithiothreitol on [^{125}I]angiotensin II binding

Dithiothreitol reduced [^{125}I]angiotensin II binding to a membrane fraction from the rabbit aorta (15.3 ± 2.0% of vehicle group, $P < 0.01$, $n = 3$) and rat liver (6.5 ± 2.8%,

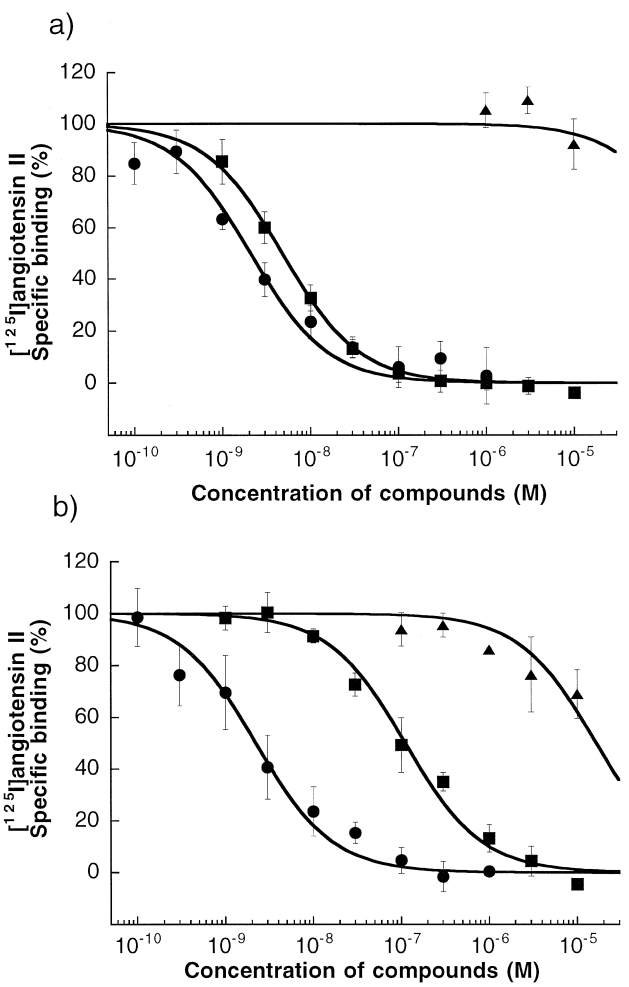

Fig. 5. Effects of [Sar^1, Ala^8]angiotensin II (●), ME3221 (■) and PD123,177 (▲) on specific binding of 0.2 nM [^{125}I]angiotensin II to membrane preparations of (a) rabbit and (b) canine aorta. The points represent the mean ± S.E. of four or three replications.

Table 2
Inhibition constant (K_i value) of angiotensin II receptor antagonists for displacement of [^{125}I]angiotensin II binding to membrane fractions from various tissues and species

Tissue	K_i value (nM)			n
	[Sar^1,Ala^8]angiotensin II	ME3221	PD123,177	
Rabbit aorta	1.86 ± 0.56	3.84 ± 0.86	10 000 <	3
Canine aorta	2.22 ± 1.18	84.5 ± 25.3[a,e]	10 000 <	3
Rat liver	0.85 ± 0.31	2.55 ± 0.60[c]	10 000 <	4
Canine liver	3.95 ± 1.15	122 ± 13.8[b,d,f]	10 000 <	3
Bovine adrenal cortex	4.67 ± 1.19	21.5 ± 7.85	10 000 <	4

The data are presented as means ± S.E. for 3–4 experiments.
[a] $P < 0.05$, [b] $P < 0.001$; significantly different from the rabbit aorta (Scheffé's test).
[c] $P < 0.01$, [d] $P < 0.001$; significantly different from rat liver (Scheffé's test).
[e] $P < 0.05$, [f] $P < 0.01$; significantly different from bovine adrenal cortex (Scheffé's test).

$P < 0.01$, $n = 3$). In contrast, dithiothreitol did not affect [^{125}I]angiotensin II binding in the bovine cerebellum (101.5%, $n = 2$). A decrease in [^{125}I]angiotensin II binding with dithiothreitol was found in the bovine adrenal cortex (41.7%, $n = 2$), canine aorta (33.5 ± 8.4%, $P < 0.05, n = 3$) and canine liver (42.7%, $n = 2$).

4. Discussion

We evaluated the inhibitory effect of ME3221 on the angiotensin II-induced pressor response in dogs and rats. In dogs, a considerably higher dose (10 times higher than that in rats) of ME3221 was needed to inhibit the angiotensin II pressor response, while the duration of the antagonistic action was even shorter than that in rats. The difference in the pharmacokinetics of ME3221 does not adequately explain the difference in potency (data not shown). Species differences in the in vivo potency of the angiotensin II receptor antagonist have previously been reported (Wong et al., 1991) but the mechanism responsible has not been evaluated. Thus, we compared the effect of ME3221 on the mechanical responses of the canine aorta with that on the responses of the rabbit aorta, which has angiotensin AT_1 receptors similar to those of rats and humans (Dudley et al., 1990; Mauzy et al., 1992).

The present results show that ME3221 bound with significantly higher affinity to the rabbit aorta than to the canine aorta. The maximum angiotensin II-induced contractile response in canine aorta tended to be augmented by treatment with ME3221. This is consistent with a previous report for losartan in canine aorta (Burns et al., 1994) and it can be explained by the two-state receptor model described by Robertson et al. (1994). The K_i values of ME3221 in the canine liver also support the species differences in angiotensin AT_1 receptors. In contrast, the peptide antagonist, [Sar^1, Ala^8]angiotensin II and angiotensin II had the same affinity for the angiotensin AT_1 receptor in canine and rat liver, as evaluated in the saturation and competition studies. These results suggest that ME3221 discriminates between different angiotensin AT_1 receptors while peptide ligands cannot because they are able to flexibly fit every receptor.

The alanine residue at position 163 in the fourth transmembrane domain of angiotensin AT_1 receptors is one of the possible binding sites of nonpeptide ligands, but not of peptide ligands (Ji et al., 1994). It is known that there are differences in the protein sequences of angiotensin AT_1 receptors in dogs, cattle and pigs (Sasaki et al., 1991; Itazaki et al., 1993; Burns et al., 1994), in which the alanine at position 163 is substituted by threonine. Our data show that the angiotensin AT_1 receptor in the bovine adrenal cortex has a low affinity for ME3221, but the K_i value was five times higher than that of the canine angiotensin AT_1 receptor. The porcine and bovine angiotensin AT_1 receptors are also reported to have a low affinity for losartan(Itazaki et al., 1993). Thus, the alanine residue at position 163 of the angiotensin AT_1 receptor could play a key role in determining the affinity of nonpeptide antagonists. Substitution of an amino acid in the angiotensin AT_1 receptor might determine the species difference in sensitivity to nonpeptide antagonist but not to peptide ligands. These results can be used to divide angiotensin AT_1 receptors into two groups: humans, rats and rabbits have a typical angiotensin AT_1 receptor and cattle, pigs and dogs have an atypical angiotensin AT_1 receptor. Unlike the angiotensin AT_2 receptor, which is highly conserved (Chappell et al., 1992; Tsutsumi et al., 1992), the angiotensin AT_1 receptor exists in different subtypes. Indeed, the angiotensin AT_1 receptor is divided into at least two subtypes, AT_{1A} and AT_{1B} receptors (Iwai et al., 1992; Llorens-Cortes et al., 1994), although the cloning of two subtypes has not been achieved in dogs and cattle.

In this report, we examined the effectiveness of sulfhydryl reagents on the atypical angiotensin AT_1 receptor. The cDNA cloning of the angiotensin II receptor revealed that the angiotensin II receptor has seven transmembrane domains and possesses several cysteine residues in the extracellular domain for forming disulfide bridges (Murphy et al., 1991; Mukoyama et al., 1993). A sulfhydryl reagent, dithiothreitol, abolished angiotensin II binding to the angiotensin AT_1 receptor, while it enhanced binding to

the angiotensin AT_2 receptor (Catt and Abbott, 1991; Chappell et al., 1992). In the present study, the angiotensin II-induced contractile response in the canine aorta was not reduced by dithiothreitol. In contrast, a great reduction in the angiotensin II-induced contractile response was observed in the rabbit aorta. In binding studies, dithiothreitol diminished specific binding to the membrane fraction from the rat liver and the rabbit aorta, both of which have typical angiotensin AT_1 receptors. Dithiothreitol did not affect [^{125}I]angiotensin II binding to the bovine cerebellum, which has an angiotensin AT_2 receptor. Intermediate sensitivity to dithiothreitol was found in the canine tissues and the bovine adrenal cortex. These findings also indicate that canine and bovine tissues have an atypical angiotensin AT_1 receptor that has partial resistance to dithiothreitol treatment.

Species differences in receptors has been reported, such as cholecystokinin-B/gastrin receptors and 5-HT_{1B} receptors (Murphy et al., 1991; Beinborn et al., 1993). In these cases, the endogenous ligand always showed the same affinity for each species whereas nonpeptide antagonists had a different affinity for the receptors. These and our results emphasize that information about drug-receptor interactions cannot always be simply extrapolated from animals to humans without verification.

In conclusion, species differences in the angiotensin AT_1 receptor were found: the canine and bovine angiotensin AT_1 receptor had a lower affinity for ME3221 than that of rats and rabbits. Such a difference in affinity explains why ME3221 shows a weak and short-lasting inhibition of the angiotensin II-induced pressor response in dogs.

References

Arunlakshana, O., Schild, H.O., 1959. Some quantitative use of drug antagonism. Br. J. Pharmacol. 14, 48–58.

Beinborn, M., Lee, Y.M., McBride, E.W., Quinn, S.M., Kopin, A.S., 1993. A single amino acid of the cholecystokinin-B/gastrin receptor determines specificity for nonpeptide antagonists. Nature 362, 348–350.

Burns, L., Clark, K.L., Bradley, J., Robertson, M.J., Clark, A.J., 1994. Molecular cloning of the canine angiotensin II receptor. An AT_1-like receptor with reduced affinity for DuP753. FEBS Lett. 343, 146–150.

Catt, K., Abbott, A., 1991. Molecular cloning of angiotensin II receptors may presage further receptor subtypes. Trends Pharmacol. Sci. 12, 279–281.

Chang, R.S., Lotti, V.J., 1990. Two distinct angiotensin II receptor binding sites in rat adrenal revealed by new selective nonpeptide ligands. Mol. Pharmacol. 37, 347–351.

Chappell, M.C., Diz, D.I., Jacobsen, D.W., 1992. Pharmacological characterization of angiotensin II binding sites in the canine pancreas. Peptides 13, 313–318.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Dudley, D.T., Panek, R.L., Major, T.C., Lu, G.H., Bruns, R.F., Klinkefus, B.A., Hodges, J.C., Weishaar, R.E., 1990. Subclasses of angiotensin II binding sites and their functional significance. Mol. Pharmacol. 38, 370–377.

Itazaki, K., Shigeri, Y., Fujimoto, M., 1993. Molecular cloning and characterization of the angiotensin receptor subtype in porcine aortic smooth muscle. Eur. J. Pharmacol. 245, 147–156.

Iwai, N., Inagami, T., Ohmichi, N., Nakamura, Y., Saeki, Y., Kinoshita, M., 1992. Differential regulation of rat AT_{1a} and AT_{1b} receptor mRNA. Biochem. Biophys. Res. Commun. 188, 298–303.

Ji, H., Leung, M., Zhang, Y., Catt, K.J., Sandberg, K., 1994. Differential structural requirements for specific binding of nonpeptide and peptide antagonists to the AT_1 angiotensin receptor. Identification of amino acid residues that determine binding of the antihypertensive drug losartan. J. Biol. Chem. 269, 16533–16536.

Leff, P., Harper, D., Dainty, I.A., Dougall, I.G., 1990. Pharmacological estimation of agonist affinity: detection of errors that may be caused by the operation of receptor isomerisation or ternary complex mechanisms. Br. J. Pharmacol. 101, 55–60.

Llorens-Cortes, C., Greenberg, B., Huang, H., Corvol, P., 1994. Tissular expression and regulation of type 1 angiotensin II receptor subtypes by quantitative reverse transcriptase-polymerase chain reaction analysis. Hypertension 24, 538–548.

Mauzy, C.A., Hwang, O., Egloff, A.M., Wu, L.H., Chung, F.Z., 1992. Cloning, expression, and characterization of a gene encoding the human angiotensin II type 1A receptor. Biochem. Biophys. Res. Commun. 186, 277–284.

Mukoyama, M., Nakajima, M., Horiuchi, M., Sasamura, H., Pratt, R.E., Dzau, V.J., 1993. Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven-transmembrane receptors. J. Biol. Chem. 268, 24539–24542.

Murphy, T.J., Alexander, R.W., Griendling, K.K., Runge, M.S., Bernstein, K.E., 1991. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. Nature 351, 233–236.

Nagura, J., Yasuda, S., Fujishima, K., Yamamoto, M., Chen, H., Kawano, K., Katano, K., Ogino, H., Hachisu, M., Konno, F., 1995. Pharmacological profile of ME3221, a novel angiotensin II receptor antagonist. Eur. J. Pharmacol. 1995, 201–211.

Ouali, R., Poulette, S., Penhoat, A., Saez, J.M., 1992. Characterization and coupling of angiotensin-II receptor subtypes in cultured bovine adrenal fasciculata cells. J. Steroid Biochem. Mol. Biol. 43, 271–280.

Robertson, M.J., Dougall, I.G., Harper, D., McKechnie, K.C., Leff, P., 1994. Agonist–antagonist interactions at angiotensin receptors: application of a two-state receptor model. Trends Pharmacol. Sci. 15, 364–369.

Sasaki, K., Yamano, Y., Bardhan, S., Iwai, N., Murray, J.J., Hasegawa, M., Matsuda, Y., Inagami, T., 1991. Cloning and expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor. Nature 351, 230–233.

Timmermans, P.B., Wong, P.C., Chiu, A.T., Herblin, W.F., 1991. Nonpeptide angiotensin II receptor antagonists. Trends Pharmacol. Sci. 12, 55–62.

Tsutsumi, K., Stromberg, C., Saavedra, J.M., 1992. Characterization of angiotensin II receptor subtypes in the rat spleen. Peptides 13, 291–296.

Wong, P.C., Hart, S.D., Duncia, J.V., Timmermans, P.B., 1991. Nonpeptide angiotensin II receptor antagonists. Studies with DuP 753 and EXP3174 in dogs. Eur. J. Pharmacol. 202, 323–330.