Receptor Occupancy in Myocardium, Adrenal Cortex, and Brain by TH-142177, a Novel AT_1 Receptor Antagonist in Rats, in Relation to Its Plasma Concentration and Hypotensive Effect

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Received January 14, 1998; accepted March 2, 1998

Purpose. To study the relationship between angiotensin II (AII) receptor occupancy *ex vivo* in tissues plasma concentration and hypotensive effect of a novel AII receptor antagonist, TH-142177 and losartan in rats.

Methods. At 2, 8 and 24 hr after oral administration of TH-142177 and losartan in rats, AII receptors in myocardium, adrenal cortex and cerebral cortex were determined by radioligand binding assay using [^{125}I]Sar^1,Ile^8-AII. Plasma concentrations of both drugs and metabolite in rats were also measured using validated HPLC assays. Further, systolic blood pressure (SBP) in conscious renal hypertensive rats treated orally with TH-142177 and losartan were measured by using a tail cuff plethysmographic method.

Results. Oral administration of TH-142177 (1.8 and 5.5 μmol/kg) and losartan (6.5 and 21.7 μmol/kg) in rats brought about dose-dependent decreases in [^{125}I]Sar^1,Ile^8-AII binding sites (Bmax) in myocardium and adrenal cortex. The extent of receptor occupancy by both drugs in adrenal cortex was maximal at 2 hr later but that in myocardium at 8 hr later. Further, the receptor occupancy was more sustained in myocardium than adrenal cortex. The *ex vivo* binding affinity of TH-142177 for AII receptors in these tissues was roughly three times higher than that of losartan. Also, cerebral cortical [^{125}I]Sar^1,Ile^8-AII binding was significantly reduced by oral administration of losartan but not by TH-142177. The time course of AII receptor occupancy by both drugs in adrenal cortex appeared to be in parallel with that of their plasma concentrations, while the time course in myocardium correlated with that of their hypotensive effects rather than plasma concentrations.

Conclusions. TH-142177 produced a relatively selective and sustained occupancy *ex vivo* of AII receptors in myocardium and adrenal cortex of rats with approximately three times greater potency than losartan. Its time course of myocardial receptor occupancy was in parallel with that of hypotensive effect rather than plasma concentration.

KEY WORDS: angiotensin II receptor antagonist; TH-142177; rat tissues; *ex vivo* receptor occupancy; pharmacokinetics; pharmacodynamics.

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ABBREVIATIONS: AII, angiotensin II; AT_1, angiotensin II type 1 receptor; Kd, apparent dissociation constant; Bmax, maximal number of binding sites; SBP, systolic blood pressure.

INTRODUCTION

Renin-angiotensin system (RAS) plays pivotal roles in the control of cardiovascular function. Angiotensin II (AII), the biologically active peptide of RAS, has been suggested to modulate blood pressure mostly through the stimulation of AII receptors in the vascular smooth muscle (1). The clinical use of angiotensin-converting enzyme (ACE) inhibitors have revealed an implicated role of RAS in controlling blood pressure under physiological and pathological conditions (2). The nonpeptide AT_1 receptor antagonists such as losartan were shown to have antihypertensive effects in clinical trials (3) without adverse effects such as dry cough. TH-142177 (N-n-butyl-N-[2′-(1-H-tetrazole-5-yl) biphenyl-4-yl]-methyl-(N-carboxymethyl-benzyl- amino)-acetamide) (Fig. 1) is a novel orally active AT_1 receptor antagonist (4). Oral administration of TH-142177 exerted a potent and long-lasting antihypertensive action in rats. Furthermore, this compound inhibited the AII-induced contractions in rat isolated aorta and inhibited specific [^{125}I]AII binding in rat aortic membranes. It has been shown that *in vitro* receptor binding affinities of AII receptor antagonists do not necessarily correlate with their *in vivo* pharmacological potencies (4,5). There are many explanations for this discrepancy, including differences between pharmacokinetics or pharmacodynamics. We have previously demonstrated that the extent and duration of *ex vivo* and *in vivo* receptor occupancy by 1,4-dihydropyridine calcium channel antagonists differed markedly among tissues in spite of little difference in their *in vitro* affinity and that the occupancy of cardiovascular receptors correlated closely with the pharmacological effects (6–8). Thus, characterization of receptor binding of AII antagonists under physiological conditions in relation to the pharmacokinetics must be important for the analysis of their pharmacodynamics as shown previously in CNS drugs (9,10). The present study was performed to determine the time relationship between *ex vivo* AII receptor occupancy in tissues (myocardium, adrenal cortex and cerebral cortex), plasma concentration, and pharmacological response of TH-142177 and losartan in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (290–380 g, body weight) were obtained from Charles River Japan Inc. (Atsugi, Japan) and housed three or four per cage in the laboratory with free access to food (normal rat chow) and water, and maintained on a 12-hr dark/light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). All procedures were performed according to protocols approved by the Animal Ethics Committee of Taiho Pharmaceutical Co. for use and care of laboratory animals.

Tissue Preparation

For the *in vitro* binding assay, the myocardial tissues from rats were minced with scissors and homogenized by a Kinematica Polytron homogenize in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The myocardial homogenate was centrifuged at 500× g for 10 min, and the supernatant fraction

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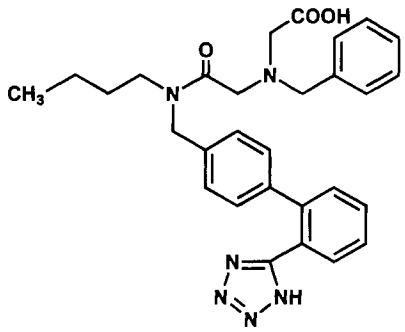

Fig. 1. Chemical structure of TH-142177.

was centrifuged at 40,000× g for 15 min. The pellet was resuspended in the ice-cold buffer, and the suspension was centrifuged again at 40,000× g for 15 min. The adrenal cortex and cerebral cortex were homogenized in 20 volumes of 50 mM Tris-HCl buffer with a Polytron homogenizer, and each homogenate was centrifuged at 40,000× g for 15 min. The pellet was washed twice by centrifugation. The resulting pellet was finally resuspended in the buffer for use in the binding assay.

For the *ex vivo* receptor occupancy, rats were fasted for 16 hr before the administration of drugs, and administrated orally with TH-142177 (1.8, 5.5 μmol/kg) or losartan (6.5, 21.7 μmol/kg) through a gastric tube to rats. Control animals were administrated with the vehicle. At 2, 8, and 24 hr after the drug administration, rats were sacrificed by taking the blood from descending aorta under light anesthesia with ethyl ether, and the myocardium, brain, and adrenal cortex were perfused with 0.9% saline from the aorta. Then, these tissues were removed. The methods of membrane preparation were essentially the same as those described above for the *in vitro* binding assay. All steps for the tissue preparation were performed at 4°C. Yamada *et al.* (14) have previously showed that the dissociation of antagonists from receptor sites at 4°C was much slower than at 25 or 37°C. Thus, in the *ex vivo* experiment with TH-142177 and losartan, the dissociation of these drugs from receptor sites during tissue preparation (homogenization and suspension) appears to be minimized. Protein concentration was measured according to the method of Bradford *et al.* (11) with bovine serum albumin as standard.

Receptor Binding Assay

The binding assay of [^{125}I]Sar^1,Ile^8-AII (AII receptor antagonist radioligand) was performed according to the methods of Sechi *et al.* (12) and Nozawa *et al.* (13). Briefly, the membranes (200–600 μg of protein) prepared from rat myocardium, adrenal cortex, and cerebral cortex incubated with different concentrations (0.05–1 nM) of [^{125}I]Sar^1,Ile^8-AII for saturation experiment (0.2 nM [^{125}I]Sar^1,Ile^8-AII for the competition experiment) in the assay buffer contained 50 mM Tris, 100 mM NaCl, 1 mM sodium EGTA and 10 mM $MgCl_2$ (pH 7.2) for 90 min at 25°C. The reaction was terminated by rapid filtration through Whatman GF/C glass fiber filters, and filters were rinsed three times with 4 ml of ice-cold buffer. Tissue-bound radioactivity was determined by a γ-counter (Model 1282; Pharmacia LKB, Uppsala, Sweden) at 80% efficiency. Specific [^{125}I]Sar^1,Ile^8-AII binding was determined experimentally from the difference between counts in the absence and presence of 3 μM unlabelled AII. All assays were conducted in duplicate.

Determination of TH-142177, Losartan, and EXP3174 in Plasma

For the determination of plasma concentrations, rats were administered TH-142177 (5.5 μmol/kg) and losartan (6.5 μmol/kg) orally, and, at 1, 4, 12, and 24 hr later, the blood was taken from the aorta. The plasma from rat blood was isolated by centrifugation, and stored at −80°C until plasma concentrations were determined. Plasma concentrations of TH-142177, losartan, and EXP3174 (active metabolite of losartan) were determined by high-performance liquid chromatography (HPLC). Following the addition of acetonitrile, the plasma sample was centrifuged for 5 min at 2000× g. After adding of ethyl acetate, the supernatant fraction was shaken mechanically. The organic layer was transferred to a test-tube and evaporated dry at 40°C under a nitrogen flow. The residue was dissolved in the ethanol, and aliquots were injected into the HPLC column. The HPLC system consisted of a Shimadzu LC-6AD pump, a SIL-6B autoinjector, and a SPD-6A UV- spectrophotometric detector (Shimadzu, Kyoto, Japan). An Inertsil ODS-3 column (150 mm × 4.6 mm I.D., 5 μm, GL Sciences, Tokyo, Japan) was used for the HPLC separations. The efferent was monitored at 250 nm. The mobile phase, degassed by bubbling with helium, was prepared by 23% acetonitrile and 77% phosphate buffer. The detection limits of TH-142177, losartan, and EXP3174 with a coefficient of variation of <10% were approximately 18, 22, and 23 pmol/assay, respectively.

Measurement of Blood Pressure

Renal hypertension was induced in 6-week-old male Wistar rats (Charles River, Atsugi, Japan) by means of a silver clip placed on the right renal artery. Approximately 4 weeks later, systolic blood pressure (SBP) was measured by using a tail cuff plethysmographic method (PE-300, Narco, TX, USA), and rats exhibiting SBP over 160 mmHg were used. Under light ether anesthesia, a catheter connected to a battery-operated biotelemetry device (TA11PA-CA, Data Sciences Inc., USA) was introduced into the abdominal aorta via the femoral artery. The biotelemetry devices were placed intraperitoneally. A week after the operation, SBP was continuously measured using an appropriate data processor (Physiotel-DATAQuest III, Data Sciences Inc., U.S.A.) in a quiet room while the animals were conscious and unrestrained. TH-142177 (5.5 μmol/kg) and losartan (6.5 μmol/kg) were orally administered between 9 and 11 a.m.

Data Analysis

The analysis of binding data was performed as described previously (14). The apparent dissociation constant (Kd) and maximal number of binding sites (Bmax) for [^{125}I]Sar^1,Ile^8-AII binding were estimated by Rosenthal analysis of the saturation data. The ability of TH-142177 to inhibit specific [^{125}I]Sar^1,Ile^8-AII (0.2 nM) binding *in vitro* was estimated by IC_{50} values, which are the molar concentrations of unlabeled drug necessary for displacing 50% of the specific binding (estimated by log probit analysis). A value for the inhibition constant, Ki, was

calculated from the equation, Ki = IC_{50} / (1 + L / Kd), where L equals the concentration of [^{125}I]Sar^1,Ile^8-AII. The Hill coefficients for inhibition by TH-142177 and losartan were obtained by the Hill plot analysis. The data from the TH142177 and losartan-induced inhibition of adrenal cortical [^{125}I]Sar^1,Ile^8-AII binding were analyzed with a computer program (NONLIN) that performs iterative nonlinear least-squares regression analysis (15). The occupancy (%) of AII receptors by TH-142177 was calculated by the equation: {[Bmax(control)-Bmax(TH-142177 or losartan)]/Bmax(control)} × 100. Statistical analysis was conducted by a one-way analysis of variance (ANOVA) followed by a Dunnett's test for multiple comparisons. Statistical significance was accepted at $P < 0.05$.

Drugs

[^{125}I]Sar^1,Ile^8-AII (81.4 TBq / mmol) was purchased from DuPont-NEN Co. Ltd. (Boston, MA, USA). TH-142177 (N-n-butyl-N-[2′-(1-H-tetrazole-5-yl)biphenyl-4-yl]-methyl-(N-carboxymethyl-benzylamino)-acetamide), losartan, and EXP3174 were synthesized by Taiho pharmaceutical Co., Ltd. (Tokyo, Japan). AII and saralasin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Drugs were dissolved in dimethyl sulfoxide and diluted in reaction buffer. For oral administration, drugs were suspended in 0.5% hydroxypropylmethyl cellulose solution, and given in a volume of 5 ml/kg.

RESULTS

In Vitro Inhibition of AII Receptor Binding

Specific binding of [^{125}I]Sar^1,Ile^8-AII (0.05–1.0 nM) in myocardial, adrenal cortical, and cerebral cortical membranes of rats appeared to be saturable and Rosenthal analysis revealed a linear plot, suggesting the existence of a single population of binding sites with Kd values of 0.43 ± 0.03 (myocardium), 0.95 ± 0.08 (adrenal cortex), and 0.72 ± 0.08 (cerebral cortex) nM, respectively (mean ±S.E., n = 4). The Bmax values for [^{125}I]Sar^1,Ile^8-AII in these tissues were 198 ± 16 (myocardium), 2023 ± 91 (adrenal cortex), and 161 ± 20 (cerebral cortex) fmol/mg protein, respectively. TH-142177 and losartan at 1 nM to 10 μM competed with [^{125}I]Sar^1,Ile^8-AII for myocardial and cerebral cortical binding sites in a concentration-dependent manner (Fig. 2). The inhibitory effect of TH-142177 in myocardium and cerebral cortex was two and three times more potent than that of losartan, respectively (Table I). The Hill coefficients for both drugs in these tissues were close to unity. Both TH-142177 (0.1 nM–10 μM) and losartan (0.1 nM–100 μM) also inhibited specific [^{125}I]Sar^1,Ile^8-AII-AII binding in rat adrenal cortex, and their inhibition curves were biphasic as shown by the Hill coefficients of 0.4 to 0.5 (Fig. 2, Table I). The nonlinear least squares regression analysis showed the existence of high (Ki values for TH-142177 and losartan = 4.4 ± 0.7 and 18.9 ± 0.8 nM, respectively) and low (Ki values for TH-142177 and losartan = 82.6 ± 7.4 and 99.1 ± 4.8 μM, respectively) affinity [^{125}I]Sar^1,Ile^8-AII binding sites for both drugs. The relative concentrations of high and low affinity sites (62.3 and 37.7%, respectively) on the basis of inhibition by TH-14277 in rat adrenal cortex agreed well with those (61.2 and 38.8%, respectively) by losartan.

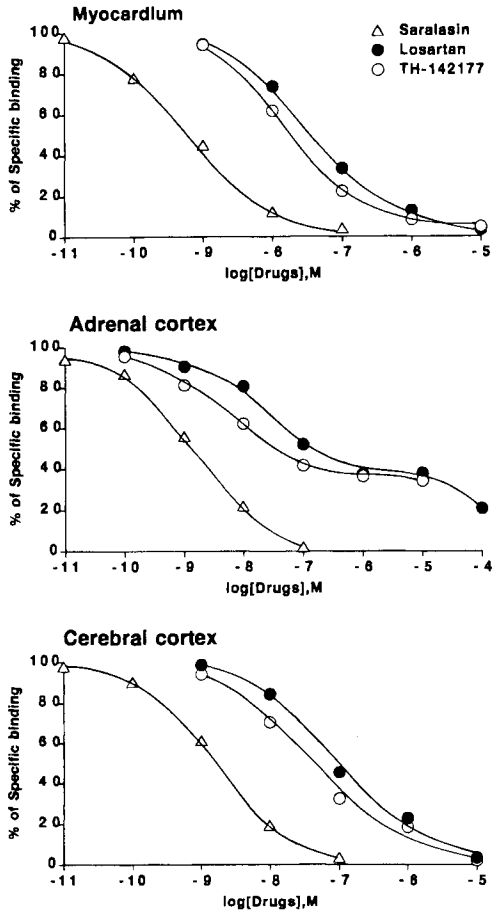

Fig. 2. Inhibition of specific [^{125}I]Sar^1,Ile^8-AII binding to myocardium, adrenal cortex and cerebral cortex by saralasin (△), TH-142177 (○) and losartan (●). The inhibition of specific [^{125}I]Sar^1,Ile^8-AII binding by these drugs was determined by incubating the ligand with four to seven concentrations of each drug. Ordinate, percentage of specific [^{125}I]Sar^1,Ile^8-AII binding in the absence of any drugs. Each point represents the average of four to five determinations.

Effects of Oral Administration of TH-142177 and Losartan on AII Receptor Binding

The effect of oral administration of TH-142177 and losartan on AII receptors in myocardial, adrenal cortical, and cerebral cortical tissues of rats was investigated. Following oral administration of TH-142177 at doses of 1.8 and 5.5 μmol/kg, there were significant decreases in Bmax values for specific [^{125}I]Sar^1,Ile^8-AII binding to myocardial and adrenal cortical membranes compared to the control values, as shown in Table II. In myocardial tissue, the decreases in Bmax values at 2 and 8 hr at the dose of 1.8 μmol/kg were 36.4 and 52.0%, respectively, and the decreases at 2, 8, and 24 hr at the dose of 5.5 μmol/kg were 67.2, 75.0 and 53.4%, respectively. Thus, the inhibitory effect by TH-142177 was dose-dependent and it was maximal at 8 hr. Similar reductions in Bmax values by TH-14277 (1.8 and 5.5 μmol/kg) at 2 and 8 hr were observed also in rat adrenal cortex, but the effect was maximal at 2 hr. The Bmax value in adrenal cortex at 24 hr was almost similar to that in control tissue. The Kd values for [^{125}I]Sar^1,Ile^8-AII binding in both myocardium and adrenal cortex were unaltered by oral administration of TH-142177 at doses of 1.8 and 5.5 μmol/kg

Table I. Inhibition of Specific [^{125}I]Sar^1,Ile^8-AII Binding to Myocardium, Adrenal Cortex, and Cerebral Cortex by Saralasin, TH-142177, and Losartan

	Saralasin[a]		TH-142177[a]		Losartan[a]	
	Ki (nM)	Hill coefficient	Ki (nM)	Hill coefficient	Ki (nM)	Hill coefficient
Myocardium	0.51 ± 0.02	0.88 ± 0.12	8.03 ± 0.7	0.87 ± 0.099	15.6 ± 0.7	0.85 ± 0.18
Adrenal Cortex	1.61 ± 0.08	0.92 ± 0.07	89.3 ± 5.4	0.43 ± 0.04	313.2 ± 14.8	0.50 ± 0.11
Cerebral Cortex	1.39 ± 0.10	0.95 ± 0.05	16.2 ± 1.1	0.88 ± 0.07	48.6 ± 3.4	0.90 ± 0.10

[a] Mean ± SE of duplicate determination from four to five rats.

except a slight increase at 24 hr in myocardium at the dose of 5.5 μmol/kg (0.48 ± 0.11 *vs* 0.65 ± 0.11 nM, $P < 0.05$). In contrast to the marked decrease in [^{125}I]Sar^1,Ile^8-AII binding sites in rat myocardial and adrenal cortical membranes, oral administration of TH-142177 (1.8 and 5.5 μmol/kg) had little significant effect on Bmax values for the radioligand in cerebral cortical membranes. The *ex vivo* occupancy by TH-142177 (5.5 μmol/kg) of AII receptors in rat tissues was illustrated in Fig. 3A.

As shown in Table III, oral administration of losartan at doses of 6.5 and 21.7 μmol/kg brought about a significant reduction in Bmax value of specific [^{125}I]Sar^1,Ile^8-AII binding to rat myocardial and adrenal cortical membranes without a change in Kd values (data not shown). The decreases in myocardium and adrenal cortex at 6.5 μmol/kg were 35.8 and 53.8%, respectively, at 2 hr, and they were 58.3 and 39.8%, respectively, at 8 hr. Further reduction in Bmax values was seen at 21.7 μmol/kg of losartan, and the decrease in myocardium was sustained for more than 24 hr. The receptor occupancy by losartan in myocardium and adrenal cortex was maximal at 8 and 2 hr, respectively. There was a significant (36–57%) reduction of Bmax values for [^{125}I]Sar^1,Ile^8-AII binding in rat cerebral cortex at 2 and/or 8 hr after oral administration of losartan at doses of 6.5 and 21.7 μmol/kg (Table III). The *ex vivo* occupancy by losartan (6.5 μmol/kg) of AII receptors in rat tissue was illustrated in Fig. 4A.

Table II. Effects of Oral Administration of TH-142177 on B_{max} Values of Specific [^{125}I]Sar^1,Ile^8-AII Binding to Myocardium, Adrenal Cortex, and Cerebral Cortex of Rats

	B_{max} values[a], fmol/mg protein (Average receptor occupancy)		
	Myocardium	Adrenal Cortex	Cerebral Cortex
Control	208 ± 21	2179 ± 86	190 ± 23
TH-142177			
1.8 μmol/kg			
2 h	132 ± 5* (36.4)	965 ± 185* (55.7)	178 ± 10 (6.0)
8 h	99.5 ± 3.3* (52.0)	1282 ± 28* (41.2)	195 ± 23 (−3.0)
24 h	176 ± 5 (15.4)	1972 ± 116 (9.5)	N.D.[b]
5.5 μmol/kg			
2 h	68.0 ± 9.4* (67.2)	622 ± 20* (71.4)	149 ± 14 (21.6)
8 h	51.8 ± 12.2* (75.0)	942 ± 38* (56.8)	198 ± 26 (−4.4)
24 h	96.7 ± 10.8* (53.4)	2053 ± 47 (5.8)	198 ± 12 (−4.5)

[a] Mean ± SE of duplicate determinations from four to five rats.
[b] Not determined.
* Significantly difference from control values, $P < 0.01$.

Plasma Concentrations of TH-142177, Losartan, and EXP3174

Plasma concentrations of TH-142177 (5.5 μmol/kg) at 1 to 24 hr after oral administration in rats were determined. They

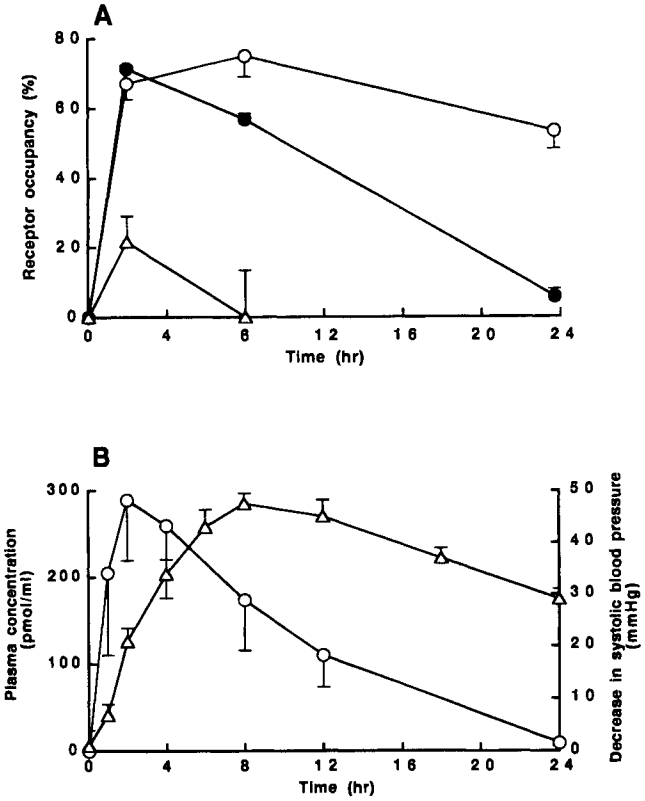

Fig. 3. Time relationship of AII receptor occupation in myocardium (○), adrenal cortex (●) and cerebral cortex (△) (panel A), plasma concentration (○) and hypotensive effect (△) (panel B) after oral administration of TH-142177 (5.5 μmol/kg) in rats. The receptor occupancy (%) in rat tissues was determined from the decrease by the TH-142177 administration (Table II), and the plasma concentration of TH-142177 in these rats was measured. The decrease by TH-142177 in the systolic blood pressure in conscious renal hypertensive rats was measured. Each point represents the mean ± SE of four to seven rats.

Table III. Effects of Oral Administration of Losartan on B_{max} Values of Specific [^{125}I]Sar^1,Ile^8-All Binding to Myocardium, Adrenal Cortex, and Cerebral Cortex of Rats

	B_{max} values[a], fmol/mg protein (Average receptor occupancy)		
	Myocardium	Adrena Cortex	Cerebral Cortex
Control	208 ± 21	2179 ± 86	190 ± 23
Losartan			
6.5 μmol/kg			
2 h	133 ± 5** (35.8)	1007 ± 294** (53.8)	121 ± 5* (36.1)
8 h	86.5 ± 4.3** (58.3)	1312 ± 69** (39.8)	147 ± 5 (22.4)
24 h	185 ± 11 (11.1)	2025 ± 63 (7.1)	163 ± 7 (14.2)
21.7 μmol/kg			
2 h	63.5 ± 8.0** (69.3)	746 ± 59** (65.7)	87.1 ± 24.0* (54.1)
8 h	49.0 ± 2.9** (76.4)	1026 ± 73** (52.9)	82.3 ± 15.2** (56.6)
24 h	60.8 ± 2.5** (70.7)	1976 ± 76 (9.3)	212 ± 7 (−11.9)

[a] Mean ± SE of duplicate determinations from four to five rats.
* Significantly difference from control values, $P < 0.05$.
** Significantly difference from control values, $P < 0.01$.

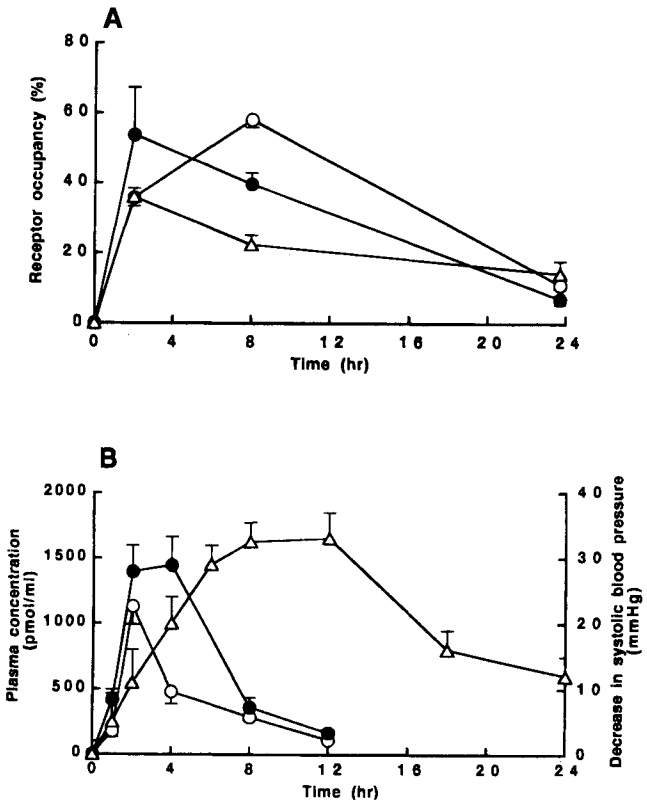

Fig. 4. Time relationship of AII receptor occupation in myocardium (○), adrenal cortex (●) and cerebral cortex (△) (panel A), plasma concentration (○: losartan, ●: EXP3174) and hypotensive effect (△) (panel B) after oral administration of losartan (6.5 μmol/kg) in rats. The receptor occupancy (%) in rat tissues was determined from the decrease by the losartan administration (Table III), and the plasma concentrations of losartan and EXP3174 in these rats were measured. The decrease by losartan in the systolic blood pressure in conscious renal hypertensive rats was measured. Each point represents the mean ± SE of three to seven rats.

were 205 ± 95 (1 hr), 289±70 (2 hr), 260 ± 84 (4 hr), 174 ± 59 (8 hr), 110 ± 37 (12 hr), and 19.2 ± 2.7 (24 hr), respectively, pmol/ml (mean ± S.E., n = 4-5) (Fig. 3B). Plasma concentrations of losartan (6.5 μmol/kg) at 1 to 12 hr after oral administration in rats were 178 ± 48 (1 hr), 1130 ± 137 (2 hr), 480 ± 94 (4 hr), 287 ± 24 (8 hr), and 111 ± 14 (12 hr), respectively, and those of EXP3174 were 419 ± 48 (1 hr), 1396 ± 204 (2 hr), 1446 ± 215 (4 hr), 362 ± 73 (8 hr), and 167 ± 25 (12 hr), respectively, pmol/ml (mean ± S.E., n = 4–5) (Fig. 4B).

Hypotensive Effects

At 4 weeks after a silver clip had been placed on the rat right renal artery, the SBP in conscious rats was 172 ± 11 mmHg (n = 11). Oral administration of TH-142177 at the dose of 5.5 μmol/kg reduced SBP in conscious renal hypertensive rats and its maximal decrease (48 ± 2 mmHg, n = 6) was appeared at approximately 8 hr (Fig. 3B). The hypotensive effect was still sustained 24 hr after the administration of this dose of TH-142177. Losartan at the dose of 6.5 μmol/kg also produced a significant antihypertensive effect, and the maximal decrease in SBP (33 ± 4 mmHg, n = 5) was seen at 12 hr (Fig. 4B). The extent and duration of decreases in SBP by TH-142177 at the dose of 5.5 μmol/kg were greater than those by 6.5 μmol/kg of losartan.

DISCUSSION

Binding characteristics of TH-142177 to AII receptors in rat tissues have been investigated in comparison with those of losartan. TH-142177 competed with [^{125}I]Sar^1,Ile^8-AII for the binding sites in myocardium, adrenal cortex, and cerebral cortex of rats *in vitro*, and its inhibitory effect was two to four times more potent than that of losartan. Thus, specific [^{125}I]Sar^1,Ile^8-AII binding in myocardium and cerebral cortex of rats was markedly inhibited by these AT_1 receptor antagonists with higher (nanomolar) affinity, thereby demonstrating the predominant existence of AT_1 receptor in these tissues. On the other hand, in rat adrenal cortex, TH-142177 and losartan inhibited specific [^{125}I]Sar^1,Ile^8-AII binding with biphasic fashion and their Hill coefficients were less than unity. Consequently, non-linear least-squares regression analysis showed the presence of two classes of AII binding sites (AT_1 and AT_2 receptors) in rat adrenal cortex, and their relative densities were calculated to be about 60 and 40%, respectively. Such a distribution of AII receptor subtypes in rat tissue has confirmed previous observations (16).

The technique of *ex vivo* receptor binding is useful in predicting the dose and duration of action of drugs in relation to the pharmacokinetics and pharmacodynamics (6–9). This technique has been used to investigate *in vivo* occupancy of AII receptors in rat tissues by TH-142177 and losartan. At 2 and 8 hr after oral administration of TH-142177 at the dose of 1.8 μmol/kg, there was a significant (36~56%) reduction in [^{125}I]Sar^1,Ile^8-AII binding sites (Bmax) in myocardium and

adrenal cortex of rats. The administration of higher dose (5.5 μmol/kg) brought about more sustained and greater (53~75%) decrease in Bmax values for myocardial [^{125}I]Sar^1,Ile^8-AII binding at 2, 8, and 24 hr later. The extent of receptor occupancy by TH-142177 at these doses in myocardium was maximal at 8 hr later but that in adrenal cortex at 2 hr later. The AII receptor density in myocardium returned to the control level at 24 hr after oral administration of TH-142177 at the dose of 1.8 μmol/kg, and it was still considerably (53%) lower than the control value, at that time of 5.5 μmol/kg. In adrenal cortex, there was little significant decrease in AII receptor density at 24 hr after the oral administration of TH-142177 at both doses. Thus, TH-142177 caused more sustained occupancy of AII receptors in myocardium than in adrenal cortex. Similarly, oral administration of losartan at the dose of 6.5 μmol/kg in rats brought about a significant (36–58%) reduction in Bmax value for [^{125}I]Sar^1,-Ile^8-AII binding in myocardium and adrenal cortex of rats at 2 and 8 hr later. This inhibitory effect by losartan was almost identical to the effect by TH-142177 at the dose of 1.8 μmol/kg in terms of the extent and duration, but it was considerably weaker than that at the dose of 5.5 μmol/kg. The extent and duration of AII receptor occupancy by 21.7 μmol/kg losartan in these tissues was rather similar to those by 5.5 μmol/kg TH-142177. Thus, *ex vivo* binding affinity of TH-142177 for AII receptors in myocardium and adrenal cortex of rats was roughly at least three times higher than that of losartan, consistent with the *in vitro* binding affinity in these tissues as described above. Also, the receptor occupancy by oral administration of losartan, as seen in the case of TH-142177, appeared to be maximal at 8 hr later in myocardium and at 2 hr later in adrenal cortex. In the *ex vivo* experiment, both TH-142177 and losartan brought about mainly a change in the Bmax of AII receptor sites with little change in the Kd. This may mean that in the present experimental condition, the dissociation from receptor sites during tissue preparation at low temperature (4°C) is minimized, as previously suggested (14). The drugs such as TH-142177 and losartan which cause predominant decrease in the receptor density in the *ex vivo* receptor binding experiment are generally considered to exert considerable slow kinetics in the association with and dissociation from receptor sites, as reported in dihydropyridine calcium channel antagonists (6,8).

From the *ex vivo* receptor binding experiment, Marchall *et al.* (17) have shown that losartan and GR117289, (a novel AII receptor antagonist) are able to cross the blood brain barrier and occupy central AII receptors. In agreement with previous observation (17), oral administration of losartan at 6.5 and 21.7 μmol/kg reduced significantly cerebral cortical [^{125}I]Sar^1,Ile^8-AII binding (Bmax), and the decreases in receptor density at 2 hr were 36 and 54%, respectively. Similar reduction (57%) at the higher dose was seen also at 8 hr. The doses of losartan described here correlated well with doses that have been shown to antagonize central actions of AII (18). In the present study, oral administration of TH-142177 at doses of 1.8 and 5.5 μmol/kg had little significant effe ct on specific [^{125}I]Sar^1,Ile^8-AII binding in cerebral cortex of rats. Inasmuch as TH-142177 competed with [^{125}I]Sar^1,Ile^8-AII for binding sites in rat cerebral cortex with three times higher affinity than losartan *in vitro,* such a difference in the *ex vivo* occupancy of brain AII receptors between these drugs may be ascribable to the permeability through the blood-brain barrier. In other words, this suggests that TH-142177 does not penetrate the blood-brain barrier in rats. This compound will therefore be useful to investigate further the role of renin-angiotensin system in peripheral tissues. Consequently, *ex vivo* receptor binding data have indicated that, unlike losartan and GR117289, TH-142177 caused a relatively selective and sustained occupancy of myocardial AII receptors in rats.

The time relationship of AII receptor occupancy of TH-142177 and losartan to the plasma concentration and to the hypotensive effect in rats has been investigated. The plasma concentration of TH-142177 and losartan in rats was greatest at 2 hr after the oral administration, and then it decreased periodically. Wong *et al.* (19) have reported that losartan is metabolized *in vivo* to a related compound EXP3174, which has a high affinity for AT_1 receptors. The plasma concentration of EXP3174 after oral administration of losartan in rats was also maximal at 2 and 4 hr. Thus, it is possible that the action of losartan observed in the present study is partly a result of receptor blockade by EXP3174. As illustrated in Figs. 3 and 4, such a time course of plasma concentration of both TH-142177 and losartan (also EXP3174) in rats appeared to correlate closely with that of their *ex vivo* occupancies of AII receptors in adrenal cortex. However, the time course of occupancy of myocardial AII receptors by both drugs correlated with that of their hypotensive effects in conscious renal hypertensive rats rather than the plasma concentration. Such a difference in the time course of receptor occupancy by AT_1 antagonists between myocardium and adrenal cortex might be due to the difference in *in vivo* receptor binding kinetics (rates of association and dissociation) in addition to pharmacokinetic factors such as organ blood flow, distribution, and metabolism. The mechanism whereby TH-142177 and losartan exhibit the different time course in the receptor occupation between myocardium and adrenal cortex remains to be elucidated.

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