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Pharmacology of CS-866, a novel nonpeptide angiotensin II receptor antagonist

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Abstract

CS-866, (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl]phenyl}methylimidazol-5-carboxylate, a prodrug type angiotensin receptor antagonist, is deesterified to the active acid, RNH-6270. RNH-6270 inhibited [^{125}I]angiotensin II binding to bovine adrenal cortical membranes (angiotensin AT_1 receptors) with an IC_{50} value of 7.7 nM, but not [^{125}I]angiotensin II binding to bovine cerebellar membranes (angiotensin AT_2 receptors), indicating the selectivity of the compound for angiotensin AT_1 receptors. In guinea pig aortas, RNH-6270 reduced the maximal response of the concentration-contractile curve for angiotensin II ($pD'_2 = 9.9$), but had no effect on the contractile response induced by phenylephrine or KCl. In conscious rats, intravenously injected RNH-6270 inhibited angiotensin II-induced pressor responses in a dose-dependent manner, and orally administered CS-866 produced a long-lasting inhibition of angiotensin II pressor responses. SK&F-525A, a P-450 inhibitor, suppressed the angiotensin II inhibitory effect of losartan, but not that of CS-866. These results demonstrate that RNH-6270 is a potent and AT_1-selective angiotensin receptor antagonist and that, after oral administration, CS-866 has a long-lasting angiotensin II inhibitory action which is not affected by drug metabolizing enzymes in the liver.

Keywords: Angiotensin II; CS-866; Nonpeptide; Angiotensin receptor antagonist; Angiotensin AT_1 receptor, selective

1. Introduction

The renin-angiotensin system plays an important role in the regulation of blood pressure and fluid-electrolyte balance. The introduction of angiotensin converting enzyme inhibitors has demonstrated the benefit of blockade of the renin-angiotensin system in the treatment of hypertension and congestive heart failure (Cody, 1986; Williams, 1988). The treatment with angiotensin converting enzyme inhibitors is, however, associated with side effects, such as cough, angioedema, etc. (Chin and Buchan, 1990), probably because not only angiotensin I but also bradykinin, substance P and enkephalins serve as substrate for angiotensin converting enzyme (Erdös and Skidgel, 1986). Blockade of the renin-angiotensin system at the angiotensin receptor level is believed to be devoid of these side effects.

Although angiotensin II is mostly produced from angiotensin I by the action of angiotensin converting enzyme, a number of alternative pathways for angiotensin II production have been proposed from in vitro studies. These include tonin, cathepsin G, chymostatin-sensitive angiotensin generation enzyme, etc. (Dzau, 1989). Antagonism of the angiotensin receptor, therefore, may more effectively block the renin-angiotensin system than inhibition of angiotensin converting enzyme does.

Nonpeptidic and orally active angiotensin receptor antagonists such as losartan are under development as antihypertensive agents (Chiu et al., 1990). In this report, we describe the pharmacological profiles of a novel nonpeptide angiotensin receptor antagonist, CS-

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866, (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl]phenyl}methylimidazol-5-carboxylate, and its active form, RNH-6270 (Fig. 1).

2. Materials and methods

2.1. Angiotensin II binding assay

Bovine adrenal cortical membranes were prepared from fresh tissue by the method of Maeda et al. (1983) with a slight modification. Briefly, tissues were minced and homogenized, using a Polytron (KINETICA GmbH), in 10 mM sodium phosphate buffer (pH 7.4) containing 30 mM NaCl and 1 mM $MgCl_2$. The homogenate was centrifuged at $1500 \times g$ for 10 min. The supernatant was layered over 41% sucrose solution and centrifuged at $95\,000 \times g$ for 60 min. The white interfacial band between the supernatant and sucrose portions was collected and suspended in 50 mM Tris HCl buffer, and again centrifuged at $95\,000 \times g$ for 20 min. The pellet was re-suspended in buffer and stored at −80°C until use. The protein concentration was assayed using bovine serum albumin as a standard (Bio-Rad Protein Assay Kit).

Binding experiments were performed by incubating adrenal cortical membranes (10 μg/well), [^{125}I]angiotensin II (20 fmol/well, specific activity: 2200 Ci/mmol) and sample drugs in the assay buffer for 2 h at room temperature. The assay buffer had the following composition: 20 mM Tris HCl (pH 7.4), 120 mM NaCl, 5 mM $MgCl_2$, 0.05% bovine serum albumin, 1 μM (*p*-amidinophenyl)methanesulfonyl fluoride hydrochloride (Wako Pure Chem.), 0.5 mM EDTA and 0.1 mM dithiothreitol (Sigma). After incubation, the mixture was immediately filtered through a GF/C filter presoaked with 0.3% polyethyleneimine (Sigma) to separate membrane-bound radioligands from free ones, using a cell harvester. The filters were washed 3 times with cold 20 mM Tris buffer and the radioactivity was counted using a gamma counter. Specific binding was determined as the difference between binding in the absence and presence of 1 μM unlabeled angiotensin II. Binding of [^{125}I]angiotensin II to bovine cerebellar membranes was assayed using a radioreceptor assay kit (NED-001, du Pont). The reaction mixture was incubated for 3 h at room temperature.

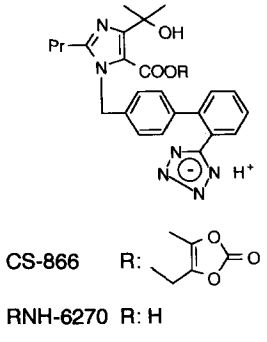

Fig. 1. Chemical structures of RNH-6270 and CS-866 (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl]phenyl}methylimidazol-5-carboxylate.

2.2. Angiotensin II-induced contraction in isolated guinea pig aortas

The experimental procedure for isolated aortas was similar to those described previously (Sada et al., 1989) with a slight modification. Briefly, male Hartley guinea pigs (300–450 g) were stunned by a blow on the head. Segments of the thoracic aorta were removed and dissected free of fat and connective tissue. The aorta was cut into ring segments approximately 2 mm wide, and its endothelium was removed by a cotton stick. Vascular rings were suspended in 30-ml organ baths containing Krebs-Henseleit solution. Krebs-Henseleit solution contained the following (mM): NaCl (119.8), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $NaHCO_3$ (25.0) and glucose (5.5). Medium in the tissue baths was kept at 36.5 ± 0.5°C and aerated with a 5% CO_2-95% O_2 mixture. The isometric tension of the aorta was measured with a force-displacement transducer (TB-612T, Nihon Kohden) connected to a carrier amplifier (AP-601G, Nihon Kohden), and recorded on a thermal pen-writing recorder (RJG-4128, Nihon Kohden). A force of 1.5 g was applied, and the strips were allowed to equilibrate for at least 60 min. Agonists (angiotensin II, phenylephrine or KCl) were added cumulatively to the baths and then washed out with the Krebs-Henseleit solution repeatedly for 60 min. Another two cumulative concentration-response curves for the agonist were obtained: the first response served as a control response, and the second one as response after drug treatment. The antagonist or vehicle was added 20 min before the third response.

In another series of experiments, the time course of angiotensin II inhibitory action during RNH-6270 exposure and after removal of RNH-6270 was observed. Contractile responses to angiotensin II (10 nM) were obtained every 30 min. When stable contractions were obtained, the next response was taken as a control. Aortas were then exposed to vehicle, RNH-6270 (0.3 nM), losartan (30 nM) or EXP3174 (1 nM) for 120 min. During drug exposure, angiotensin II contractile responses were obtained 4 times at 30-min intervals. Thereafter aortas were washed repeatedly with drug-free solution, and angiotensin II contractile responses were obtained at 30-min intervals.

2.3. Angiotensin II-induced pressor response in conscious normotensive rats

Male Wistar-Imamichi rats weighing 300–400 g were anesthetized with sodium pentobarbital, 50 mg/kg, i.p. The animal was surgically prepared with an arterial cannula inserted via the left femoral artery and a venous cannula inserted via the left femoral vein for measuring blood pressure and injecting drugs, respectively. The other ends of the cannulae were led under the skin and exteriorized at the back of the neck. The rat was placed in an individual cage after surgery and fasted for 24 h. On the next day, the arterial cannula was connected to a pressure transducer (TP-200T, Nihon Kohden), and mean blood pressure and heart rate were continuously recorded on a pen-writing recorder (WT-685G, Nihon Kohden). After blood pressure and heart rate stabilized, angiotensin II was administered at a dose of 50 ng/kg via the venous cannula. Intravenous administration of angiotensin II was repeated until a constant pressor response was obtained, and then test compound (CS-866, losartan, RNH-6270 or EXP3174) was administered p.o., or i.v. The pressor responses to angiotensin II were observed during the subsequent 8 h. The percentage inhibition was used as an index of angiotensin II antagonistic activity.

2.4. Angiotensin II-induced pressor response in anesthetized rats

Male Sprague-Dawley rats weighing 250–450 g were anesthetized with Inactin, 100 mg/kg, i.p. The animal was catheterized with two cannulae: an arterial cannula inserted via the left femoral artery and a caval cannula inserted via the left femoral vein. The aortic cannula was connected to a pressure transducer (TP-200T, Nihon Kohden), and mean blood pressure and heart rate were continuously recorded on a pen-writing recorder (WT-685G, Nihon Kohden). After blood pressure and heart rate stabilized, angiotensin II was administered at a dose of 50 ng/kg via the venous cannula. Intravenous administration of angiotensin II was repeated until constant pressor responses were obtained, and then SK&F-525A (50 mg/kg) or vehicle was administered intraperitoneally. Thereafter, the control angiotensin II responses were obtained two times, and then test compound (CS-866 or losartan) was administered i.v. The pressor responses to angiotensin II were observed during the subsequent 4 h. The percentage inhibition was used as an index of angiotensin II antagonistic activity.

2.5. Statistics

Data are presented as the means ± S.E.M. The statistical analysis was performed using Dunnett's test, and a value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Angiotensin II binding assay

Fig. 2 shows the concentration-dependent displacement of the curves for RNH-6270 and various standard agents in [^{125}I]angiotensin II binding studies. Specific [^{125}I]angiotensin II binding in bovine adrenal cortex was displaced by losartan, but not by PD-123,177. The binding in bovine cerebellum was displaced by PD-123,177, but not by losartan. The results demonstrate that angiotensin receptors in the bovine adrenal cortex are classified as AT_1 subtype, and those in the bovine cerebellum as AT_2 subtype. The concentration of RNH-6270 producing a 50% inhibition of [^{125}I]angiotensin II binding (IC_{50}) for the angiotensin AT_1 receptor was 7.7 ± 1.0 nM, approximately 12 and 2 times lower than those of losartan (92 ± 5 nM) and EXP3174 (16 ± 1 nM), respectively (Fig. 2A). RNH-6270 (100 μM) had no effect on the angiotensin II specific binding to bovine cerebellar membranes (angiotensin AT_2 receptor) (Fig. 2B). In the Scatchard analysis shown in

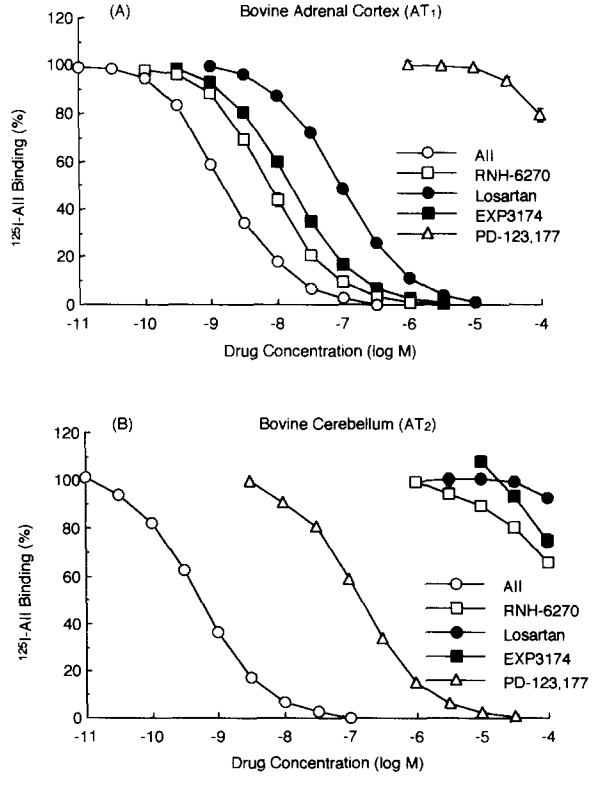

Fig. 2. Effects of various angiotensin II antagonists on specific binding of [^{125}I]angiotensin II to bovine adrenal cortical membranes (A) or bovine cerebellar membranes (B). Values are means ± S.E.M. Data from a typical experiment are shown.

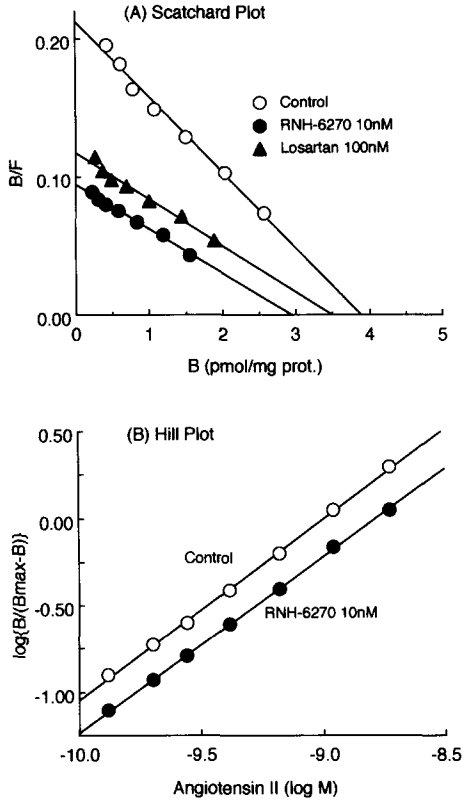

Fig. 3. (A) Scatchard analysis of RNH-6270 and losartan on the binding characteristics of [^{125}I]angiotensin II in bovine adrenal cortical membranes. (B) Hill analysis of the effect of RNH-6270 on the binding characteristics of [^{125}I]angiotensin II in bovine adrenal cortical membranes.

Fig. 3A, RNH-6270, like losartan, reduced the slope while having little effect on B_{max}. Hill analysis showed that the control Hill coefficient was not different from unity, and that RNH-6270 caused a shift to the right of the Hill plot without changing the slope (Fig. 3B). These results from two analyses suggest that RNH-6270 competitively interacts with angiotensin receptors.

3.2. *Angiotensin II-induced contraction in isolated guinea pig aortas*

RNH-6270, losartan and EXP3174 antagonized the angiotensin II-induced contraction in isolated guinea pig aorta in a dose-dependent manner (Fig. 4). RNH-6270 (0.1, 0.3 nM) reduced the maximal contraction induced by angiotensin II with a pD'_2 value of 9.9 (Fig. 4A). EXP3174 (0.1–1 nM) also caused a nonparallel shift to the right of the concentration-response curve for angiotensin II with a pA_2 value of 9.6 and reduced the maximal response to angiotensin II (Fig. 4B). As shown in Fig. 4C, losartan (10, 30 nM) produced a parallel rightward shift of the angiotensin II concentration-response curve with a pA_2 value of 8.2 without affecting the maximal contractile response. RNH-6270 at 1 μM did not affect the contractile response to phenylephrine or KCl (Fig. 5).

The kinetics of the inhibitory effect of RNH-6270, losartan and EXP3174 on angiotensin II-induced contraction are shown in Fig. 6. In vehicle-treated aortas, the contractile responses to angiotensin II (10 nM) were unchanged throughout the experimental period (Fig. 6A). The inhibitory effect of RNH-6270 (0.3 nM) on the angiotensin II contractile response became greater when the exposure time was increased from 30 to 90 min (Fig. 6B). The inhibition induced by losartan (30 nM) reached a maximum 30 min after drug exposure (Fig. 6C), and that induced by EXP3174 (1 nM) 60 min after drug exposure (Fig. 6D). In the washout period, the angiotensin II contractile response returned to pre-treatment levels 30 min after washout of losartan. The inhibitory effect of RNH-6270 persisted more than 90 min after drug washout. EXP3174 had some inhibitory effects 30 min after drug removal, but the full contraction recovered 60 min after drug removal.

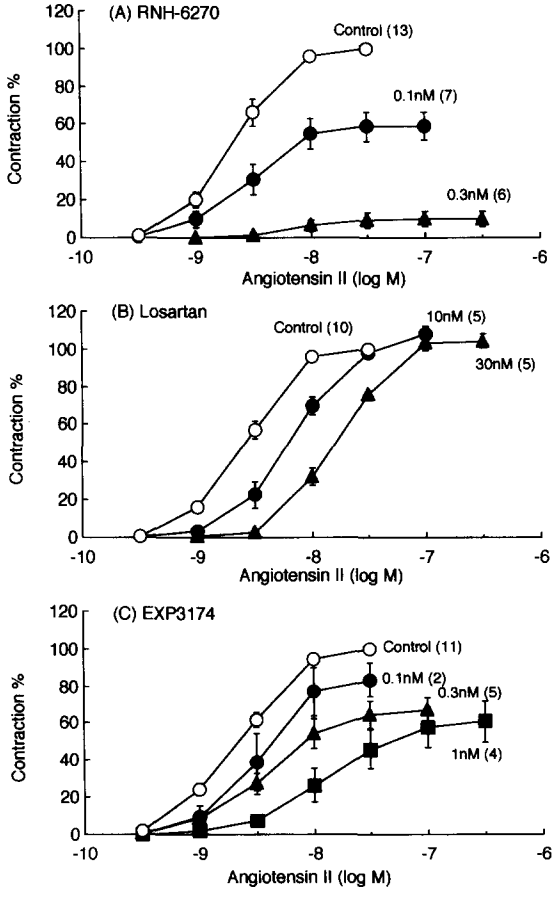

Fig. 4. Effect of RNH-6270 (A), losartan (B) or EXP3174 (C) on the concentration-contractile response curves for angiotensin II in isolated guinea pig aorta. Values are means ± S.E.M. The number of experiments is shown in parentheses.

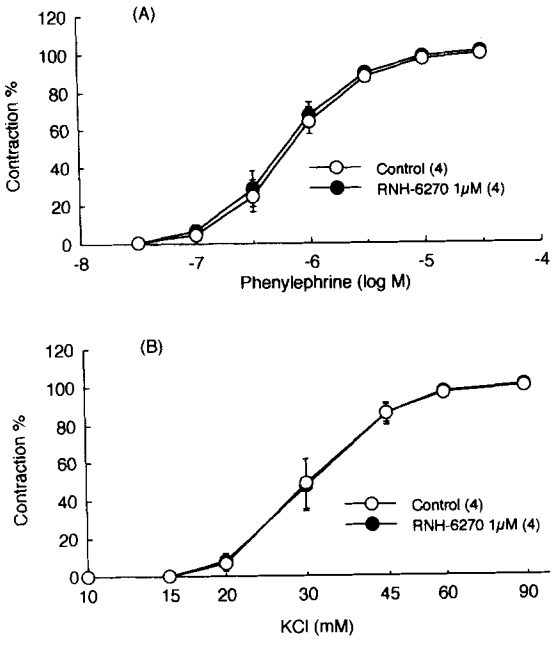

Fig. 5. Effect of RNH-6270 on the concentration-contractile response curves for phenylephrine (A) and KCl (B) in isolated guinea pig aorta. Values are means ± S.E.M. The number of experiments is shown in parentheses.

3.3. Angiotensin II-induced pressor response in conscious normotensive rats

In normotensive conscious rats, RNH-6270 (0.01,0.03 mg/kg), unlike saralasin, did not raise blood pressure when administered intravenously. This indicates that

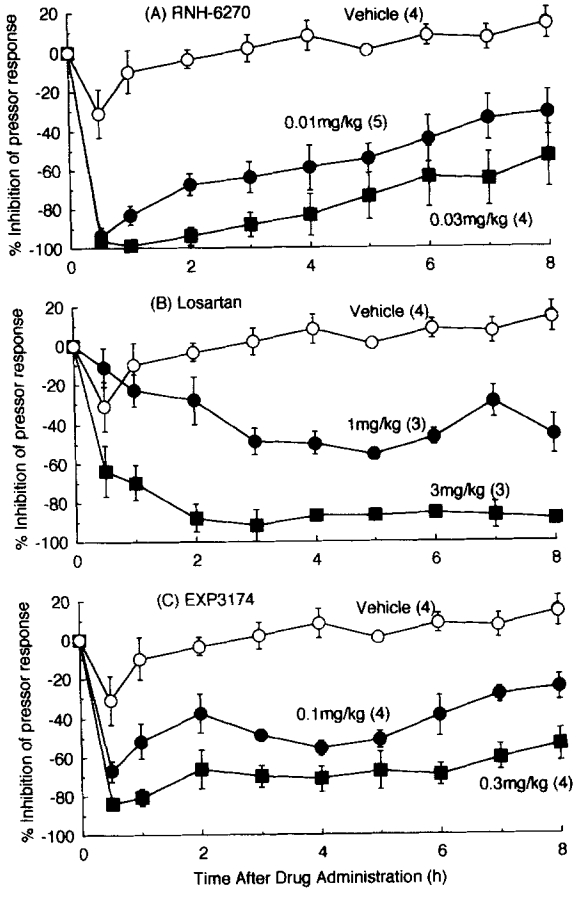

Fig. 7. Time course for inhibition of angiotensin II (50 ng/kg) pressor response after intravenous administration of RNH-6270 (A), losartan (B) or EXP3174 (C) in conscious rats. Values are means ± S.E.M. The number of experiments is shown in parentheses.

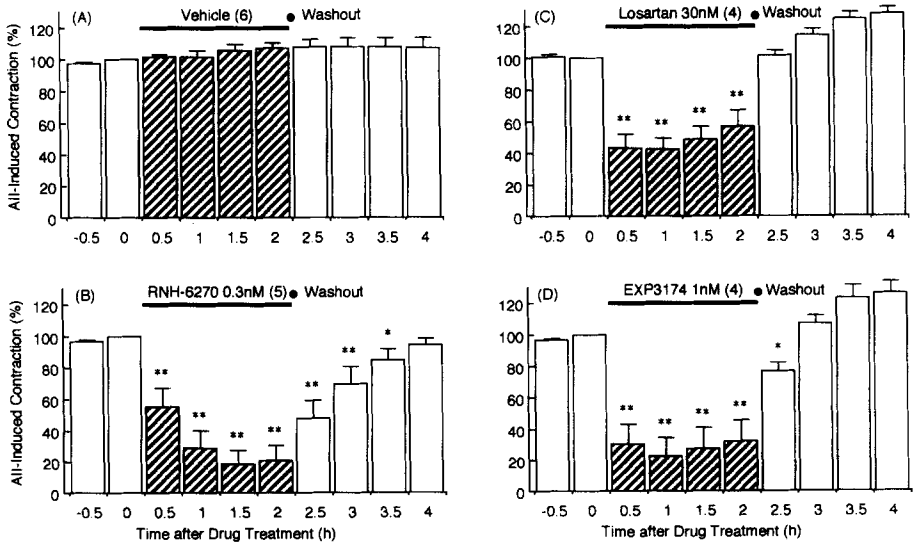

Fig. 6. Time course of angiotensin II-induced contraction during drug exposure and after removal of drugs. Aortas were exposed to vehicle (A), RNH-6270 (B), losartan (C) or EXP3174 (D). Values are means ± S.E.M. The number of experiments is shown in parentheses. Asterisks indicate a statistically significant difference from the corresponding values of the vehicle-treated group ($^{*}P < 0.05$, $^{**}P < 0.01$).

RNH-6270 lacks intrinsic agonistic activity. Fig. 7 shows the time course for inhibition of the angiotensin II pressor response after intravenous administration of RNH-6270, losartan or EXP3174. A maximal inhibition was achieved within 1 h after administration of RNH-6270 at doses of 0.01 and 0.03 mg/kg, i.v. The inhibition gradually decreased thereafter, but the angiotensin II pressor response did not return to pre-administration levels in 8 h. Losartan, at doses of 1 and 3 mg/kg, i.v., caused a maximal inhibition 3 h after administration, and the inhibition was still observed 8 h after dosing.

CS-866 or losartan was orally administered in conscious normotensive rats (Fig. 8). CS-866 at 0.1 mg/kg caused a maximal inhibition of the angiotensin II pressor response 2 h after administration, whereas losartan at 10 mg/kg produced a gradual inhibition with a maximal inhibition 5 h after administration. These two drugs inhibited the angiotensin II pressor response for 8 h.

3.4. Angiotensin II-induced pressor response in anesthetized rats

In normotensive anesthetized rats, CS-866 (0.01 mg/kg) or losartan (1 mg/kg) was intravenously administered with or without SK&F-525A, a P-450 inhibitor (Fig. 9). CS-866 at 0.01 mg/kg and losartan at 1 mg/kg inhibited the angiotensin II pressor response to the same degree (70–80%). The maximal inhibition was achieved within 1 h after CS-866 administration,

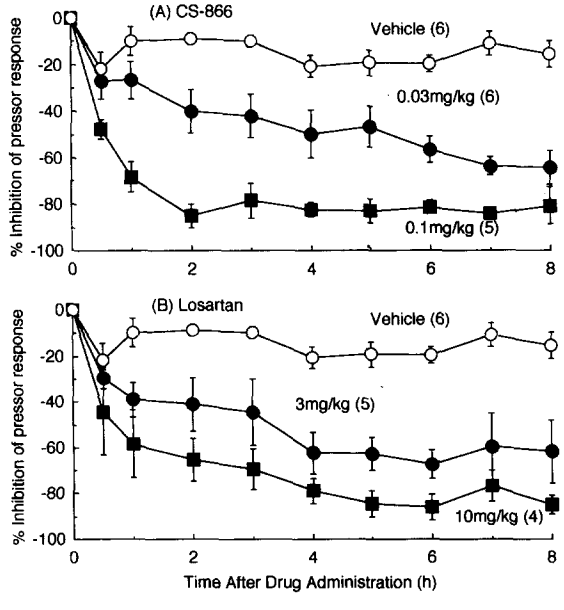

Fig. 8. Time course for inhibition of the angiotensin II (50 ng/kg) pressor response after oral administration of CS-866 (A) or losartan (B) in conscious rats. Values are means ± S.E.M. The number of experiments is shown in parentheses.

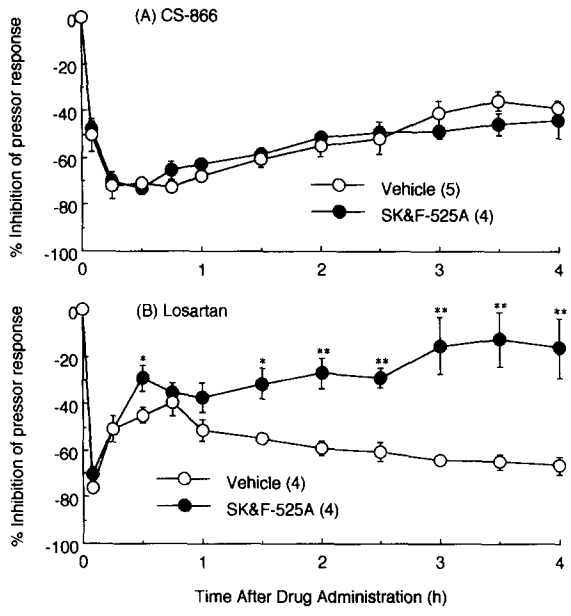

Fig. 9. Inhibitory effect of CS-866 (A) or losartan (B) on the angiotensin II pressor response with or without SK&F-525A in anesthetized rats. Values are means ± S.E.M. The number of experiments is shown in parentheses. Asterisks indicate a statistically significant difference from the corresponding values of the vehicle-treated group ($^{*}P < 0.05$, $^{**}P < 0.01$).

and the inhibition gradually decreased thereafter. Losartan exhibited a biphasic inhibitory effect. The maximal inhibition was achieved immediately after losartan administration, and the inhibition gradually decreased. The inhibitory effect of losartan became greater again 1 h after losartan administration. SK&F-525A did not alter the effect of CS-866 (Fig. 9A), but suppressed the late phase of the angiotensin II inhibitory effect of losartan (Fig. 9B).

4. Discussion

The results of recent binding studies with specific angiotensin receptor antagonists indicated the existence of two angiotensin receptor subtypes (AT_1 and AT_2) in a variety of tissues (Bumpus et al., 1991). Losartan is a selective antagonist for the angiotensin AT_1 receptor subtype, and PD-123,177 is selective for the angiotensin AT_2 receptor subtype (Chiu et al., 1989). In this study, RNH-6270, like losartan, inhibited [^{125}I]angiotensin II binding in bovine adrenal cortical membranes, but did not inhibit the binding in bovine cerebellar membranes. These results suggest that RNH-6270 is selective for angiotensin AT_1-type receptors. The major pharmacological actions of angiotensin II, such as contraction of vascular smooth muscle cell, aldosterone release from the adrenal gland, cell proliferation and hypertrophy of cardiovascular tissues, etc.,

are mediated by angiotensin AT_1 receptors (Wong et al., 1990; Bunkenburg et al., 1992). Therefore, RNH-6270 may be useful not only for hypertension, but also for proliferative disorders, such as cardiac hypertrophy, vascular intimal thickening and a certain type of nephritis.

In isolated guinea pig aorta, losartan caused a parallel rightward shift of the dose-response curve for angiotensin II. In contrast, RNH-6270 and EXP3174 reduced the maximal response of the dose-response curve, but did not cause a parallel shift to the right, suggesting noncompetitive antagonism. RNH-6270, however, inhibited the binding of angiotensin II in a competitive manner in receptor binding studies. Furthermore, RNH-6270 did not alter the contractile responses to phenylephrine and KCl (Fig. 5). These data indicate that RNH-6270 is a selective antagonist for angiotensin receptors and that the reduction of maximum contraction does not result from the nonspecific vasodilator action. It is well known that some receptor antagonists which bind covalently to receptors or slowly dissociate from receptors are able to reduce the maximal response of dose-response curves. Our kinetics study revealed that RNH-6270 showed a slow onset and slow disappearance of its angiotensin II inhibitory effect, whereas losartan showed a fast effect. These data suggest that RNH-6270 has slow kinetics at angiotensin receptors and that this feature of RNH-6270 is related to the reduction of the maximum response to angiotensin II. Further investigations are needed to elucidate the mechanism for the reduction of the maximum response induced by RNH-6270.

RNH-6270 exhibited potent inhibitory effects in the contraction study ($pD'_2 = 9.9$) compared to those in the binding study ($IC_{50} = 7.7$ nM). In the receptor binding study, [^{125}I]angiotensin II and RNH-6270 were mixed with membranes at the same time. In the contraction study, however, vascular tissues were pre-treated with RNH-6270 before angiotensin II was added. The slow kinetics of RNH-6270 at angiotensin receptors may explain the difference of the potency between different experimental conditions. Many drugs bind to serum proteins, mostly to serum albumin. The activities of some angiotensin receptor antagonists, such as EXP3174, are reported to be reduced by bovine serum albumin (Chiu et al., 1991). RNH-6270 was about 6 times less potent in inhibiting the [^{125}I]angiotensin II binding in an assay buffer containing 0.5% bovine serum albumin than in one containing 0.05% bovine serum albumin (data not shown). Therefore, binding to bovine serum albumin may also account for the inconsistency between the results of the contraction study (bovine serum albumin-free) and the binding study (0.05% bovine serum albumin).

The active forms, RNH-6270 or EXP3174, caused a maximal angiotensin II inhibition immediately after intravenous administration, whereas intravenously administered losartan gradually produced the angiotensin II inhibitory effects, and the inhibition reached its maximum 3 h after administration, indicating the slower onset of the effect of losartan (Fig. 7B). It is known that losartan is oxidized and converted gradually to the active metabolite, EXP3174 (Stearns et al., 1991). Indeed, a P-450 inhibitor, SK&F-525A, suppressed the angiotensin II inhibitory effect of losartan, but not that of CS-866 (Fig. 9). This suggests that when orally administered in clinical settings, the angiotensin II antagonistic action of losartan, unlike that of CS-866, may vary from one patient to another due to individual variations in oxidase activity.

In summary, we have demonstrated that RNH-6270 is a potent and AT_1-selective angiotensin receptor antagonist and that the ester form, CS-866, has a long-lasting angiotensin II inhibitory action after oral administration. These drugs, therefore, are potential candidates as antihypertensive agents which are effective on a once-daily dose regimen.

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