Korean J Physiol Pharmacol
Vol 2: 461–469, August, 1998

Pharmacological Characterization of Synthetic Tetrahydroisoquinoline Alkaloids, YS 51 and YS 55, on the Cardiovascular System

Ki-Churl Chang, Young-Jin Kang, Young-Soo Lee, Won-Seog Chong, Hye-Sook Yun-Choi[1], Duck-Hyong Lee[2], and Jae-Chun Ryu[3]

Department of Pharmacology, and Cardiovascular Research Institute, College of Medicine, Gyeongsang National University, Chinju 660–280, Korea; [1]Natural Product Research Institute, Seoul National University, Seoul, Korea; [2]Department of Chemistry, Sogang University, Seoul, Korea; and [3]Korea Institute of Science and Technology, Seoul, Korea

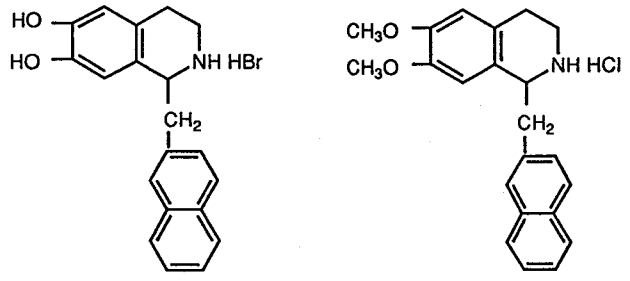
Tetrahydroisoquinoline (THI) alkaloids can be considered as cyclized derivatives of simple phenylethylamines, and many of them, especially with 6,7-disubstitution, demonstrate relatively high affinity for catecholamines. Two -OH groups at 6 and 7 positions are supposed to be essential to exert β-receptor activities. However, it is not clear whether -OH at 6,7 substitution of THIs also shows α-adrenoceptor activities. In the present study, we investigated whether -OH or $-OCH_3$ substitutions of 6,7 position of THIs differently affect the α_1-adrenoceptor affinity. We synthesized two 1-naphthylmethyl THI alkaloids, 1-β-naphthylmethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline HBr (YS 51) and 1-β-naphthylmethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline HCl (YS 55), and their pharmacological actions on α_1-adrenoceptor were compared. YS 51 and YS 55, concentration-dependently relaxed endothelium-denuded rat thoracic aorta precontracted with phenylephrine (PE, 0.1 μM) in which pEC_{50} were 5.89+0.21 and 5.93+0.19, respectively. Propranolol (30 nM) did not affect the relaxation-response curves to YS 51 and YS 55. Concentration-response curves to PE were shifted to right by the pretreatment with YS 51 or YS 55. The pA_2 values of YS 51 and YS 55 showed 6.05+0.24 and 5.88+0.16, respectively. Both probes relaxed KCl (65.4 mM)-contracted aorta and inhibited $CaCl_2$-induced contraction of PE-stimulated endothelium-denuded rat thoracic aorta in Ca^{2+}-free solutions. In isolated guinea pig papillary muscle, 1 and 10 μM YS 51 increased contractile force about 4- and 8- fold over the control, respectively, along with the concentration-dependent increment of cytosolic Ca^{2+} ions. While, 10 μM YS 55 reduced the contractile force about 50 % over the control and lowered the cytosolic Ca^{2+} level, in rat brain homogenates, YS 51 and YS 55 displaced [^{3}H]prazosin binding competitively with *Ki* 0.15 and 0.12 μM, respectively. However, both probes were ineffective on [^{3}H]nitrendipine binding. Therefore, it is concluded that two synthetic naphthylmethyl-THI alkaloids have considerable affinity to α_1-adrenenoceptors in rat aorta and brain.

Key Words: Tetrahydroisoquinoline; α_1-adrenoceptor, Guinea pig papillary muscle; Rat aorta, YS 51, YS 55, Cytosolic Ca^{2+}

INTRODUCTION

Alpha (α)-adrenoceptors mediate many important physiological functions, and the development of α-adrenoceptor antagonists is important in clinical medicine, particularly for the treatment of cardiovascular disorders. Tetrahydroisoquinoline (THI) alkaloids belong to a group of naturally occurring pharmacologically active compounds, because THIs can also be considered as cyclized derivatives of simple phenylethylamines. Since some of the THIs demonstrate

Corresponding to: Ki Churl Chang, Department of Pharmacology, College of Medicine, Gyeongsang National University, 92 Chilam-dong, Chinju 660-280, Korea. (Tel) +82-591-50-8741 (Fax) +82-591-759-0609, e-mail: kcchang@nongae.gsnu.ac.kr



YS 51 YS 55

Fig. 1. Chemical structure of YS 51 and YS 55.

relatively high affinity for both α-adrenoceptor (Osswald et al, 1975; Glusa et al, 1990; Ivorra et al, 1992; Chulia et al, 1994) and β-adrenoceptor (Iwasawa & Kiyomoto, 1967; Asoke et al, 1985; Chang et al, 1994a; Lee at al, 1994; Lin et al, 1996), THIs can be regarded as potential sources to develop new pharmacological agents acting on α and β-adrenoceptors. So far, it has been reported that -OH or -OCH_3 groups at 6,7 positions of THIs are highly active in the cardiovascular system (Iwasawa & Kiyomoto, 1967; Asoke et al, 1985; King et al, 1988; Chang et al, 1992; Shin et al, 1993, Chang et al, 1994a, 1994b; Chang, 1995; Lin et al, 1996). It has been suggested that compounds having two -OH groups at 6 and 7 positions of THI are essential to exert β-receptor activities (Iwasawa & Kiyomoto, 1967; Chang et al, 1994a; Lee at al, 1994; Lin et al, 1996). However, it is not clear whether -OH at 6,7 substitution of THIs also shows α-adrenoceptor activities. In relation to this question, we synthesized two THI derivatives having either -OH or -OCH_3 at 6 and 7 positions, i.e., 1-β-naphthylmethyl-6,7-dihydroxy-1,2,3,4-tetrahydroi soquinoline HBr (YS 51) and 1-β-naphthylmethyl-6, 7-dimethoxy-1,2,3,4-tetrahydroisoquinoline HCl (YS 55) (Fig.1). Then, we compared the pharmacological properties of these compounds on α_1 -adrenoceptors. The following studies were conducted: functional studies of isometric tension measurements using isolated rat aorta; measurements of cytosolic Ca^{2+} concentration and contractile force using guinea pig papillary muscle; and receptor binding studies with [^{3}H] prazosin and [^{3}H] nitrendipine using rat brain homogenates, since some of THIs have strong affinity for dihydropyridine binding sites (Dong et al, 1992).

METHODS

Synthesis of YS 51 and YS 55

N-(3',4'-Dimethoxyphenylethyl)-β-naphthylacetamide was prepared by condensating 3,4-dimethoxyphenylethylamine with 2-naphthyl acetic acid. Cyclodehydration of the above amide (3.5 g) was processed with phosphorus pentachloride (2.7 g) in $CHCl_3$ yielding 1-β-naphthyl-6,7-dimethoxy-3,4-dihydroisoquinoline HCl (3.3 g). Reduction of this dihydroisoquinoline (3 g) with $NaBH_4$ in ethanol provided 2.6 g of 1-β-naphthyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquin oline HCl. (YS 55) (Yun-Choi et al, 1986). Demethylation from YS 55 (2.5 g) was achieved by refluxing YS 55 with the mixture of acetic acid (4 ml) and 47% HBr (3 ml) yielding 1.9 g of 1-β-naphthylmethyl-6, 7-dihydroxy-1,2,3,4-tetrahydroisoquinoline HBr (YS 51).

Effects of YS 51 and YS 55 on vascular smooth muscle

Male Sprague Dawley rats (250~300 g) were anesthetized with pentobarbital sodium (35 mg/kg, i.p). Thoracic aorta was rapidly removed from rats. Transverse endothelium-denuded rings (2.5 mm wide) were prepared, and isometric contraction was measured as previously described (Chang et al, 1993). Each ring was briefly mounted vertically at 1 g resting tension in a 10 ml organ bath, containing Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $NaHCO_3$, 24.9; glucose, 10; EDTA, 0.03. The bath solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Relaxation to YS 51 and YS 55 was assessed in vessels contracted with sub-maximal concentration of phenylephrine (PE, 0.1 μM) or KCl (65.4 mM). Experiments were performed in the presence of indomethacin (10 μM) to prevent the potential effects of prostanoids. Some experiments were performed separately in the presence of propranolol (30 nM) to examine the vasodilatory mechanism of the probes. Isometric tension was measured through force displacement transducer (FT-03) and recorded on a Grass physiograph. Cumulative concentration-response curves to YS 51 and YS 55 were formed and the half-maximally effective concentration was determined and expressed in pEC_{50} (negative logarithm of EC_{50}) value. For contraction study, concentration-response data were obtained by cumulative

addition of PE to the tissue bath. Test drugs were added to the tissue bath 20 min before the response to PE was tested. The degree of relaxation was expressed by calculating the level of contraction due to PE (0.1 μM) in percentage terms. When testing constrictor shows responses, contractile responses were expressed in percentage of the contractile response to KCl (65.4 mM).

pA_2 analysis

pA_2 analysis was performed by using a computer program Schild plot (Arunlaksa & Schild, 1959). The concentration of agonist necessary to give a half-maxima; response in the presence of each concentration of antagonist was divided by the concentration giving a half-maximal response in the absence of antagonist in order to determine the dose ratio (DR). Data were plotted as the -log (antagonist concentration) (M) vs. the log (DR-1). When DR was 2, the -log (antagonist concentration) was taken as the pA_2 value from the Schild plot.

Membrane preparation for receptor binding study

Cortical tissues were homogenized in 20 volumes of assay buffer (50 mM Tris, 5 mM $MgSO_4$, 1 mM EDTA, 1 mM ascorbic acid, pH 7.7, 25°C), using an Ultraturrax homogenizer (13,500 rpm, 15s×2). The homogenate was centrifuged 3 times for 15 min at 35,000 x g, and the pellet was resuspended each time in 20 volumes of assay buffer. After the final centrifugation, the supernatant was aspirated, and pellet was stored at −70°C. The frozen pellet was resuspended in assay buffer just before the binding assay was performed. The final tissue concentration in the binding assay was 5 mg/ml.

Receptor binding study

Binding experiments were performed as previously described (Chang & Hahn 1995). In equilibrium binding studies, different concentrations of [^{3}H] prazosin (50 pM to 2 nM) or [^{3}H]nitrendipine (100 pM-8 nM) were incubated with membrane in a shaking water bath at 25°C for 30 min. The reaction was stopped with 10 ml ice-cold washing buffer (50 mM Tris, pH 7.7, 25°C), and the incubation mixture was immediately filtered through Whatman GF/C glass microfiber filters in vacuum. Wet filters were placed in mini-vials, to which scintillation cocktail was added, and the vials were shaken for two hours and then counted. Competition binding assays were performed to determine drug affinity at corresponding receptors. The appropriate radioligand (200 pM [^{3}H] prazosin or 500 pM [^{3}H]nitrendipine) and a wide range of concentration of YS 51 or YS 55 (in triplicate) were incubated with the receptor preparation (brain homogenates) for 30 min at 25°C. Nonspecific binding was defined as binding in the presence of 1 μM phentolamine and 1 μM of nifedipine, respectively.

Intracellular Ca^{2+} change and contraction in guinea pig papillary muscle

Intracellular Ca^{2+} levels were measured simultaneously and the degree of muscle contraction as described by Sato et al (1988), using a fluorescent Ca^{2+} indicator, fura-PE3. The guinea pig papillary muscle was loaded with 5 mM acetoxymethyl ester of fura-PE 3 for 2 hours in the presence of 0.02% cremophore EL by using the Langendorff method at 30°C and then placed in a tissue at 35°C. The muscle was mounted to measure the isometric tension and illuminated alternatively (48 Hz) with 340 nm and 380 nm light. 500 nm emission was detected with a fluorimeter (CAM-230, JASCO, Tokyo, Japan). The amount of 500 nm fluorescence induced by the 340 nm excitation (F380) was measured, and the ratio of fluorescence of R340/380 was calculated. In some experiments, fluorescence in muscle without fura-PE 3 loading was also examined to monitor the effects of test compounds on endogenous fluorescence.

Ca^{2+}-induced contraction

In Ca^{2+}-induced contraction experiments, the bathing fluid was replaced with a Ca^{2+}-free salt solution for 20 min. After that, PE (3 μM) was added. 15 min later, Ca^{2+} was cumulatively added to the bath in concentrations from 0.1 to 5 μM. To investigate the effects of YS 51 and YS 55 on Ca^{2+}-induced contraction, different concentrations (1, 3, 10 μM) of each compound were added to the bath 15 min prior to PE in the Ca^{2+}-free solution. The contractile responses were expressed percentage by calculating PE (3 μM)-induced contractions obtained in normal Krebs-Ringer Bicarbonate solutions in percentage terms.

Drugs

Phenylephrine (PE) hydrochloride, propranolol hydrochloride, and indomethacin were purchased from Sigma Co. Ltd. (USA). Pentobarbital sodium was obtained from Abbott laboratories (USA). 3,4-Dimethoxyphenylethylamine and 2-naphthyl acetic acid were from Aldrich Chem. Co. (USA). [^{3}H] prazosin and [^{3}H] nitrendipine were purchased from Amersham (U.K.).

Data analysis

Results are expressed in mean±S.E.M. of n experiments. The statistical evaluation was made using student t-test for unpaired samples where $P<0.05$ was considered significant. In binding studies, competition displacement curves were made using the iterative nonlinear least-square curve fitting program in each experiment with triplicates at each ligand concentration (Lundon Software Inc., Chagrin Falls, Ohio, USA).

RESULTS

Analytical data of YS 51 and YS 55

YS 51: m.p. 224~227°C (EtOH, iPrOH); ^{1}H-NMR (Varian FT 80A) (DMSO-d_6) δ 2.84~3.62 (6H, m), 4.73 (1H,t, J=6.9 Hz), 6.58 (2H,s), 7.45~7.98 (7H, m), 8.77 (1H,s), 9.03 (1H,s).

YS 55: m.p. 188~190°C (EtOH); ^{1}H-NMR (Varian FT 80A)(DMSO-δ_6) 2.85~3.63 (6H, m), 3.42 (3H, s OCH_3), 3.73 (3H, s OCH_3), 4.76 (1H,t, J=7.0 Hz), 6.55 (1H,s), 6.79 (1H,s), 7.44~8.29 (7H, m), 9.26 (1H,b).

Relaxation responses of YS 51 and YS 55 on PE and KCl-induced contraction

Relaxation responses of YS 51 and YS 55 were evaluated against PE-induced contraction in endothelium-denuded rat thoracic aorta. YS 51 and YS 55 relaxed PE-contracted rat aorta concentration-dependently (Fig. 2A). Both compounds relaxed the aorta at concentration ranges from 0.1 μM to 10 μM. These compounds also relaxed KCl-contracted aorta concentration-dependently (Fig. 2B). When compared with the relative potency in terms of pEC_{50} on PE-induced

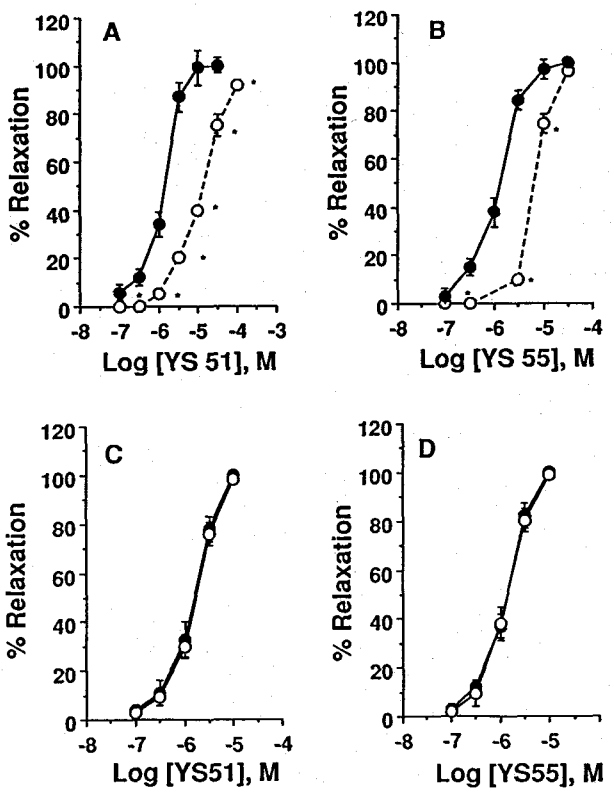


Fig. 2. Concentration-response curves to YS 51 (A) and YS 55 (B) in rat endothelium-denuded thoracic aorta precontracted with PE (●) or KCl (○). Effects of YS 51 (C) and YS 55 (D) on PE-contracted rat endothelium denuded aorta in the absence (●) or presence of 30 nM propranolol (○) for 20 min. All experiments were done in the presence of indomethacin (10 μM). Data are expressed as mean±s.e.m. of at least 3 experiments. *indicates significantly different ($P<0.05$) (PE vs. KCl).

contractions YS 51 and YS 55 showed 5.89±0.21 and 5.93±0.19, respectively. On the other hand, in KCl-induced contractions the potency YS 51 and YS 55 measured 5.16±1.1 and 5.37±0.8, respectively. Even though these drugs relaxed the contractile responses to the PE and KCl, analysis of EC_{50} (PE)/ EC_{50} (KCl) ratio indicated that these drugs exhibited greater inhibition of the contractile response induced by PE a by KCl. Propranolol (30 nM) did not affect the concentration-response curves of both YS 51- and YS 55-induced relaxation responses to PE-contracted aortas (Fig. 2C).

Effects of pretreatment of YS 51 and YS 55 on PE-induced contraction

Alpha$_1$-adrenoceptor antagonistic properties of YS

51 and YS 55 were evaluated against PE-induced contraction in endothelium-denuded rat thoracic aorta. Pretreatment with YS 51 or YS 55 shifted the PE-induced concentration-response curves to the right (Fig. 3). Schild analysis indicated that YS 51 and YS 55 had a considerable affinity for the α_1-adrenoceptor, in which pA_2 values for YS 51 and YS 55 were 6.05 and 5.88, respectively, and the slope was 1.23 and 1.18 for YS 51 and YS 55, respectively.

Effects on PE-induced contraction in Ca^{2+}-free solution

To study the action of YS 51 and YS 55 on Ca^{2+} influx, their effects on contractions evoked by Ca^{2+} at different concentration levels were observed in muscles stimulated with 3 μM PE. The magnitude of tension development corresponded with the increase of Ca^{2+} in the medium. PE-induced phasic contraction in Ca^{2+}-free media was inhibited to different

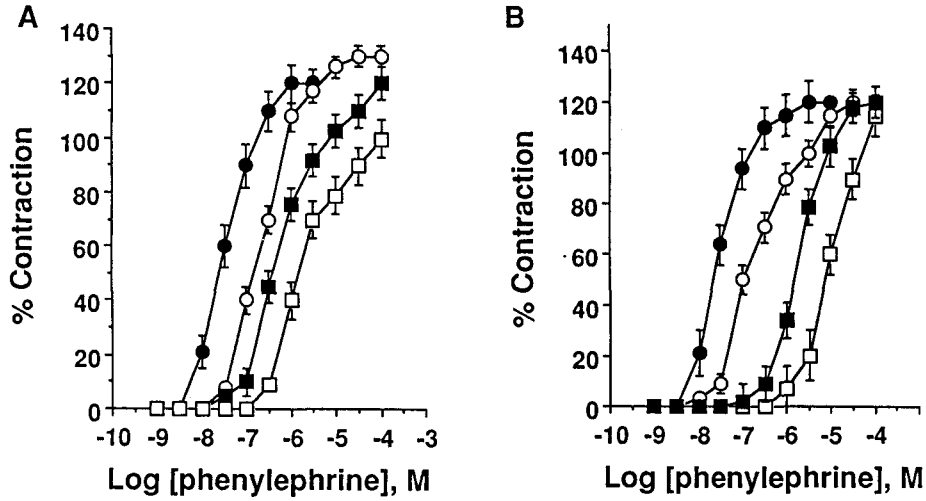


Fig. 3. Concentration-response curves to PE in rat aortic rings incubated in the absence (●) or presence of 1 μM (○), 3 μM(■) and 10 μM (□) of YS 51 (A) and YS 55 (B) for 20 min. Experiments were done in the presence of indomethacin (10 μM). Data are expressed as mean±s.e.m. of at least 3 experiments.

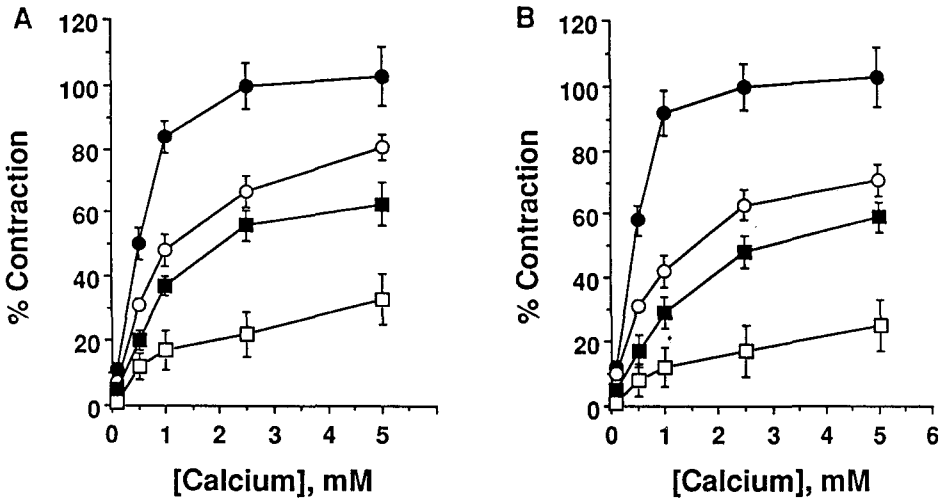


Fig. 4. Ca^{2+}-induced contraction of PE-stimulated rat endothleium-denuded rat aorta, which was incubated in the absence (●) or presence of 1 μM (○), 3 μM (■) and 10 μM (□) of YS 51 (A) and YS 55 (B) for 15 min in Ca^{2+}-free solution before addtion of Ca^{2+} cumulatively.

degrees depending on the pretreatment of YS 51 and YS 55. For example, both 1μM YS 51 and YS 55 inhibited the contration about 40 % and at higher concentrations (100μM), contraction was inhibited completely. Both YS 51 and YS 55, concentration-dependently, inhibited Ca^{2+}-induced contraction in Ca^{2+} -free media (Fig. 4).

Binding studies

To test whether the inhibitive effects of YS 51 and YS 55 on α_1 -adrenoceptor-mediated contraction were due to competition at this receptor, we examined the abilities of these compounds to displace [^{3}H] prazosin binding in the rat cerebral cortical membranes, where α-adrenoceptor is abundant (Glossmann & Hornun, 1980). Also examined was whether these probes have affinity for [^{3}H]nitrendipine binding sites, since some THIs were found to inhibit [^{3}H] nitrendipine binding to rat cerebral cortical membrane (Dong et al, 1992). Prazosin bounds specifically to the α_1-adrenoceptor with K_d 133.5$\pm$8.91 pM and Bmax 15.15$\pm$0.64 fmol/mg tissue (data not shown). After confirming that brain tissue is suitable for the binding assay, test drugs were evaluated whether they have α_1-adrenoceptor affinity. As shown in Fig. 5A, YS 51 and YS 55 showed almost the same degree of affinity for the α_1-adrenoceptor of rat brain with K*i* values measuring 0.15 and 0.12 μM, respectively. However, they had no appreciable affinity for the nitrendipine binding sites (Fig. 5B).

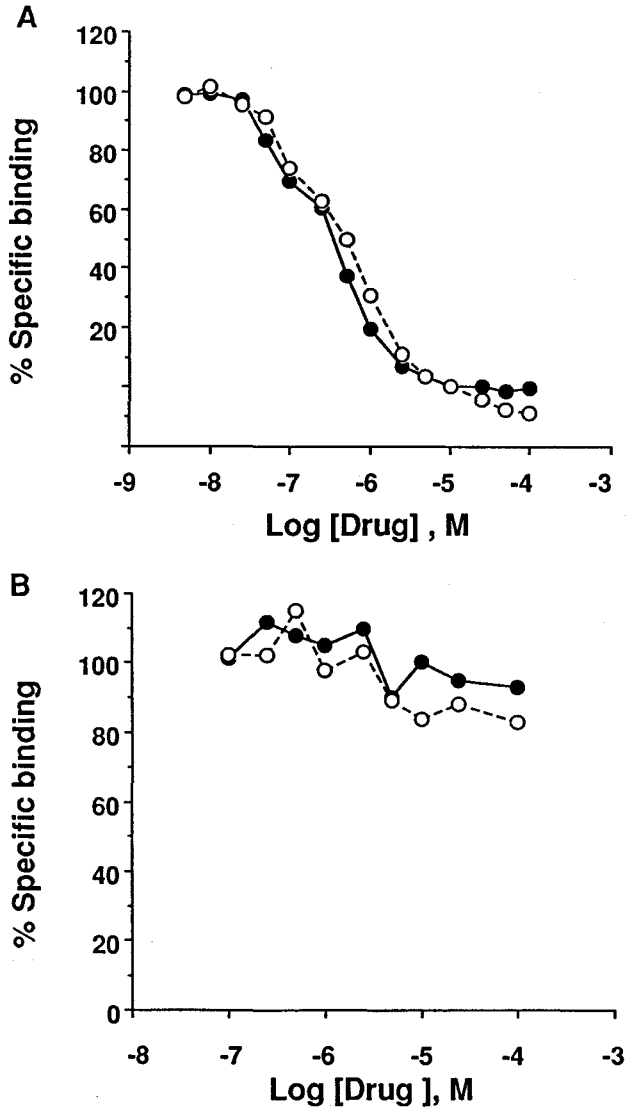


Fig. 5. Competition curves of YS 51 (●) and YS 55 (○) for [^{3}H]-prazosin (A) and [^{3}H]nitrendipine (B) binding to rat cortical membranes. The results indicated are means of 3 experiments performed in triplicate and are expressed as percentages of specific binding.

Intracellular Ca^{2+} and tension in guinea pig papillary muscle

As shown in Fig. 6, in guinea pig papillary muscle, YS 51 increased cardiac inotropic force and cytosolic free Ca^{2+} in a concentration-dependent manner. That is, 1 μM and 10 μM YS 51 caused 400% and 800% increase in contractile force, which also increased the intracellular free Ca^{2+} to 122% and 133%, respectively, while 10 μM YS 55 decreased the cardiac contractile force over 50% of control and simultaneously lowered approximately 40% of the cytosolic Ca^{2+} (Fig. 6).

DISCUSSION

The present study clearly demonstrates that both YS 51 and YS 55 have a considerable affinity for α_1-adrenoceptor and the results of functional studies in rat aorta and receptor binding study in rat brain. Since, none of these compounds showed vascular smooth muscle contraction, these chemicals are likely to act as an α_1-antagonist. In fact, these compounds inhibited contraction induced by α_1-adrenoceptor agonist, PE, in which the inhibitory fashion was competitive. The pA_2 values for YS 51 and YS 55 were 6.05 and 5.88, respectively, and the slopes of YS 51 and YS 55 were 1.23 and 1.18. respectrely, which are

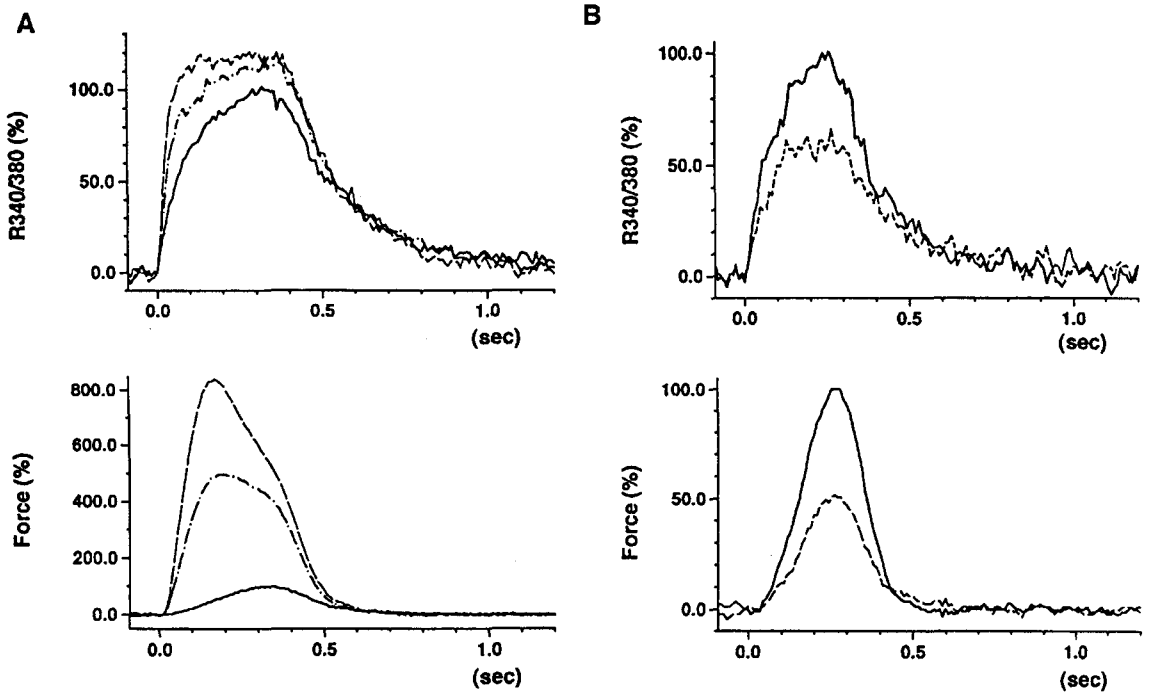


Fig. 6. Typical tracing showing changes in cytosolic Ca^{2+} and contratile force in the absence (—) and presence of 1 μM (- · -) and 10 μM (— —) YS 51 (A) or 10 μM (— —) YS 55 (B) in isolated guinea pig papillary muscle. Changes in cytosolic Ca^{2+} and contraction were measured simultanelusly in the tissues loaded with a fluorescent Ca^{2+} indicator, fura-PE3.

not different from unity. However, it can be speculated that β-adrenoceptor activation may be responsible for vascular relaxation, because it has been reported that two -OH at 6,7- position of THIs are essential to show β-adrenoceptor activation, and that YS 49, steric isomer of YS 51, showed β-adrenoceptor activation in rabbit and rat (Lee et al, 1994). When the relaxation response of YS 51 and YS 55 in the presence of propranolol was tested, propranolol did not affect the concentration-response curve to these drugs. Therefore, it is certain that vasodilatory actions of YS 51 and YS 55 are not, mediated by β-adrenoceptor in the vascular smooth muscle. Thus, it is speculated that the mechanism of the relaxation of YS 51 and YS 55 is possibly resulted from α_1-adrenoceptor blockade. Further supporting evidences for these probes having α_1-antagonistic properties came from the receptor binding experiments. These probes displaced the [^{3}H] prazosin binding competitively with K*i* values at 0.15 and 0.12 μM for YS 51 and YS 55, respectively. Previously, we reported that substitution of -OCH_3 groups for 6,7-OH groups of higenamine, GS 389, resulted in total loss of β-adrenergic activity and showed α-blocking activity in rat thoracic aorta (Chang et al, 1992). In rabbit aorta, another 6,7-dimethoxy-THI showed α-adrenolytic activities (Glusa et al, 1990), suggesting the importance of -OCH_3 at 6,7-position of THIs in exerting a-antagonistic action. As shown in the present study, -OH groups at 6,7 positions of THI showed almost the same affinity as that of -OCH_3 for the binding to α-adrenoceptors. On the other hand, some α-blockers reported to possess Ca^{2+}-channel blocking activity (Atlas & Adler, 1981; Chulia et al, 1994). Many THI compounds are reported to possess Ca^{2+} channel blocking actions (King et al, 1988; Triggle et al, 1989; Lacroix et al, 1991; Dong et al, 1992; Chang et al, 1994b), and some of them have selective affinity for dihydropyridine Ca^{2+} binding sites (Dong et al, 1992). So, we studied whether these probes have Ca^{2+}-antagonistic action or have affinity to diphydropyridine binding sites. The present data indicated that YS 51 and YS 55 possess Ca^{2+}-antagonistic action but have no affinity for dihydropyridine binding site. Therefore, at least these compounds are not involved in dihydropyridine-sensitive Ca^{2+} channel. In this regard, these compounds behave like (±) laudanosine, benzyl-THI, which showed an α_1-selective adreno-

ceptor blocking action along with nitrendipine-insensitive Ca^{2+} channel blocking action (Chulia et al, 1994). Resently, it has become clear that at least three subtypes of the α_1-adrenoceptors exist, which are now designated as α_{1A}- (formerly α_{1C}), α_{1B}-, and α_{1D} (formerly α_1a/d) (Hieble et al, 1995; Michel et al, 1995). The α_{1A}-adrenoceptor subtype requires the influx of Ca^{2+} through dihydropyridine-sensitive channels to cause smooth muscle contraction, but the α_{1B}-adrenoceptor subtype stimulates the formation of inositol phosphate to cause contraction that is independent of extracellular Ca^{2+} influx through dihydropyridine-sensitive channels (Han et al, 1987). Thus, further investigations should be carried out to answer what subtytype of α_1-adrenoceptor is involved in the action of these probes. Of particular interest, as expected, was the observation that YS 51 showed strong positive inotropic activities while YS 55 was devoid of positive inotropic action. When measured cytosolic Ca^{2+} in isolated guinea pig papillary muscles was measured with these probes, YS 51 increased, cytosolic Ca^{2+}, while YS 55 decreased it. Recently, we reported that GS 386, another 6,7-dimethoxy-THI compound, decreased Ca^{2+} currents in isolated rabbit cardiac myocyte when measured by a whole cell patch-clamp technique (Chang et al, 1994), which correlates that dimethoxyl substitutions at 6,7 carbons of THIs results in lower cytosolic Ca^{2+} in cardiac cells. It is well known that stimulation of adenylyl cyclase activity by β-adrenoceptor agonists (Kameyama et al, 1985; Fischmeister & Hartzell 1986) or by forskolin (Kameyama et al, 1986; Hartzell & Fischmeister 1987) leads to the stimulation of inward Ca^{2+} current in heart preparations. Thus, the action of YS 51, like its isomer YS 49, may involve the increase of cyclic AMP within the cardiac cells due to β-adrenoceptor activation, which requires further studies. Since the vasodilating action of YS 51 was not involved in β-adrenoceptor activation in vascular smooth muscle (β_2), it is yet to be determined whether YS 51 possess β_1-selective agonistic effects along with α-blocking activities. In summary, this study provides an evidence that the two synthetic THI compounds, YS 51 and YS 55, have a similar degree of affinity for [^{3}H]-prazosin binding sites, and this may account for α_1-adrenoceptor antagonist and calcium antagonist properties (through dihydropyridine-insensitive calcium channel) in rat aorta and brain.

ACKNOWLEDGMENTS

The authors would like to express sincere gratitude to Prof. Karaki, H, Tokyo University, for measuring the intracellular Ca^{2+} and tension. This work was supported by a grant from KOSEF through the Korea-Japan joint research program (1994) and the Ministry of Health and Welfare (1997).

REFERENCES

Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. *Br J Pharmacol* 14: 48−58, 1959

Asoke M, Stephen SN, Hebatalla MA, Sayed AA, Jane C, Douglas JS, Duana DM, Dennis RF. Effects of trimetoquinol analogs for antagonism of endogenous/thromboxane A_2-mediated responses in human platelet and rat aorta. *J Pharmacol Exp Ther* 232: 1−9, 1985

Atlas D, Adler M. α-adrenergic antagonists as possible calcium channel inhibitors. *Proc Natl Acad Sci USA* 78: 1237−1241, 1981

Chang KC, Hahn KH. Is α-adrenoceptor blockade responsible for atropine flush? *Eur J Pharmacol* 284: 331−33, 1995

Chang KC. Further investigation of the mechanism of action of GS 389: A thromboxane A_2 antagonistic action in platelet and vascular smooth muscle. *Br J Pharmacol* 114: 272P, 1995

Chang KC, Chong WS, Lee IJ. Different pharmacological characteristics of structurally similar benzylisoquinoline analogs, papaverine, higenamine, and GS 389, on isolated rat aorta and heart. *Can J Physiol Pharmacol* 72: 327−334, 1994a

Chang KC, Park CO, Hong SG. Effects of GS 386, a novel dihydroisoquinoline compound, on rabbit atrial myocytes and rat aorta. *Drug Dev Res* 33: 454−459, 1994b

Chang KC, Park CW, Lim JK. Pharmacological evaluation of GS 389, a novel tetrahydroisoquinoline analog, related to higenamine on vascular smooth muscle. *Life Sci* 51: 67−74, 1992

Chang KC, Chung SY, Chong WS, Suh JS, Kim SH, Noh HK, Seong BW, Ko HJ, Chun KW. Possible superoxide radical-induced alteratio of vascular reactivity in aortas from streptozotocin-treated rats. *J Pharmacol Exp Ther* 266: 992−1000, 1993

Chulia S, Ivoraa MD, lugnier C, Vila E, Noguera MA, D'ocon P. Mechanism of the cardiovascular activity of laudanosine: comparison with papaverine and other benzylisoquinolines. *Br J Pharmacol* 113: 1377−1385, 1994

Chulia S. The effect of s-(+)-boldine on the alpha$_1$-adre-

noceptor of the guinea pig aorta. *Br J Pharmacol* 119: 1305−1312, 1996

Dong H, Lee CM, Huang WL, Peng SX. Cardiovascular effects of substitued tetrahydroisoquinolines in rats. *Br J Pharmacol* 107: 262−2681, 1992

Fischmeister R, Hartzell HC. Mechanism of action of acetylcholine on calcium current in single cells from frog ventricle. *J Physiol (London)* 376: 183−202, 1986

Glossmann H, Hornun R. α-receptor in rat brain: sodium changes the affinity of agonists for prazosin sites. *Eur J Pharmacol* 61: 407−408, 1980

Glusa E, Grunner H, Hagen A, Lohmann D, Foken H. Synthesis and alpha-adrenolytic activities of 2-(1,4-benzodioxan-2-yl-methyl)-6,7-dimethoxy-1,2,3,4-tetrah ydroisoquinoline derivatives. *Pharmzie* 45: 408−410, 1990

Han C, Abel PW, Minneman KP. Alpha$_1$-adrenoceptor subtypes linked to different mecahnism for increasing intracelluar Ca^{2+} in smooth muscle. *Nature* 329: 333−335, 1987

Hartzell HC, Fischmeister R. Effects of forskolin and acetylcholine on calcium current in sing heart cells. *Nature (London)* 323: 273−275, 1987

Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz R J, Minneman KP, Ruffolo RRJ. International union of pharmacology. X. Recommendation for nomenclature of α_1 adrenoceptors: Concensus update. *Pharmacol Rev* 47: 267−270, 1995

Ivorra MD, Lugnier C, Schott C, Catret M, Nogera A, Anselmi E, D'ocon P. Multiple actions of glaucine on cyclic nucleotide phosphodiesterases, α_1-adrenoceptor and benzothiazepine binding site at the calcium channel. *Br J Pharmacol* 106: 387−394, 1992

Iwasawa Y, Kiyomoto A. Studies on tetrahydroisoquinoline (THI) 1. Bronchodilator activity and structure-activity relationship. *Jpn J Pharmacol* 17: 143−152, 1967

Kameyama M, Hescheler J, Hofmann F, Trautwein W. Modulation of Ca^{++} current during the phosphorylation cycle in the guinea pig heart. *Pfluegers Arch* 407: 123−128, 1986

Kameyama M, Hofmann F, Trautwein W. On the mechanism of β-adrenergic regulation of the Ca^{++} channel in the guinea-pig heart. *Pfluegers Arch* 405: 285−293, 1985

King VF, Garcia ML, Himmel D, Reuben JP, Lam YT, Pan J, Han G, Kaczorowski GL. Interqaction of tetrandrine with slowly inactivating calcium channels. *J Biol Chem* 263: 2238−2244, 1988

Lacroix P, Linee P, Forest MC. Diproteverine (BRL 40015): a new type of calcium antagonist with potential antianginal properties. *Eur J Pharmacol* 192: 317−327, 1991

Lee YS, Kim CH, Yun-Choi HS, Chang KC. Cardiovascular effect of a naphthylmethyl substituted tetrahydroisoquinoline, YS 49, in rat and rabbit. *Life Sci* 55: PL 415−420, 1994

Lin CH, Yang CM, Chen CM, Ko FN, Teng CM. Pharmacological characteristics of BDTI, a new isoquinoline-derived beta$_2$-adrenoceptor agonist, in canine trachea and rat heart. *Pharmacology* 53: 19−27, 1996

Michel MC, Kenny BA, Schwinn DA.Classification of α_1-adrenoceptor subtypes. *Naunyn-Schmiedeberg Arch Pharmacol* 352: 1−10, 1995

Osswald W, Polonia J, Polonia MA. Preparation and pharmacological activity of the condensation product of adrenaline with adetaldehyde. *Naunyn-Schmiedeberg Arch Pharmacol* 289: 275−290, 1975

Sato K, Ozaki H, Karaki H. Changes in cytosolic calcium level in vascular smooth muscle strip measured simultaneouslywith contraction using fluorescent calcium indicator fura 2. *J Pharmacol Exp Ther* 246: 294−300, 1988

Shin YM, Romstedt KJ, Miller DD, Feller DR. Interactions of noprostanoid trimetoquinol analogs with thromboxane A_2/prostaglandin H_2 receptors in human platelets, rat vascular endothelial cells and rat vascular smooth muscle. *J Pharmacol Exp Ther* 267: 1017−1023, 1993

Triggle DJ, Langs DA, Janis RA. Ca-channels ligands: structure-function reletionships of the 1,4-dihydropyridines. *Med Res Rev* 9: 123−180, 1993

Yun-Choi HS, Paik SW, Lee KR. Platelet anti-aggregating activities of higenamine analogs. *Yakhak Hoeji* 30: 245−252, 1986