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Comparison of Inodilator Effect of Higenamine, YS49, YS51, Tetrahydroisoquinoline Analogs, and Dobutamine in the Rat

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Tetrahydroisoquinoline (THI) alkaloids can be considered as cyclized derivatives of simple phenylethylamines. Many of them, especially with 6,7-disubstitution, demonstrate a relatively high affinity for catecholamines. Present study examines the pharmacological action of limited series of THI, using rats' isolated atria and aorta. In addition, a [^{3}H] prazosin displacement binding study with THI compounds was performed, using rat brain homogenates to investigate whether these probes have α-adrenoceptor affinity. We also compared the vascular relaxation potency of these probes with dobutamine. YS 49, YS 51, higenamine and dobutamine, concentration-dependently, relaxed endothelium-denuded rat thoracic aorta precontracted with phenylephrine (PE, 0.1 μM) in which pEC_{50} were 5.56±0.32 and 5.55±0.21, 5.99±1.16 and 5.57±0.34, respectively. These probes except higenamine also relaxed KCl (65.4 mM)-contracted aorta. In isolated rat atria, all THIs and dobutamine increased heart rate and contractile force. In the presence of propranolol, the concentration response curves of YS 49 and YS 51 shifted to the right and resulted in pA_2 values of 8.07±0.84 and 7.93±0.11, respectively. The slope of each compound was not deviated from unity, indicating that these chemicals are highly competitive at the cardiac β-adrenoceptors. YS 49, YS 51, and higenamine showed α-adrenoceptor affinity in rat brain, in which the dissociation constant (K_i) was 2.75, 2.81, and 1.02 μM, respectively. It is concluded, therefore, that THI alkaloids have weak affinity to α_1-adrenoceptors in rat aorta and brain, respectively, while these probes show relatively high affinity for cardiac β-adrenoceptors. Thus, these chemicals may be useful in the treatment of congestive heart failure.

Key Words: Tetrahydroisoquinoline, Inotropic action, Vasodilatation, Rat, Adrenoceptor

INTRODUCTION

In recent years, considerable advances have been made in the treatment of cardiac failure and in the understanding of the mechanisms underlying the syndrome. Although vasodilator therapy was for a long time thought likely to be of benefit in the treatment of heart failure, the modern era of treatment really began with the trials that demonstrated improved mortality with sustained therapy (Cohn et al, 1986; Consensus Trial Study Group, 1987), even when used in addition to conventional therapy (Consensus Trial Study Group, 1987; The SOLVD Investigators, 1991). Digitalis glycosides have been the principal agents used to treat congestive heart failure for more than a century. However, their narrow margin of safety and potential arrythmogenicity have led to increased efforts to develop safer therapeutic agents. Sympathomimetic agents such as dopamine and dobutamine have been introduced in the treatment of congestive

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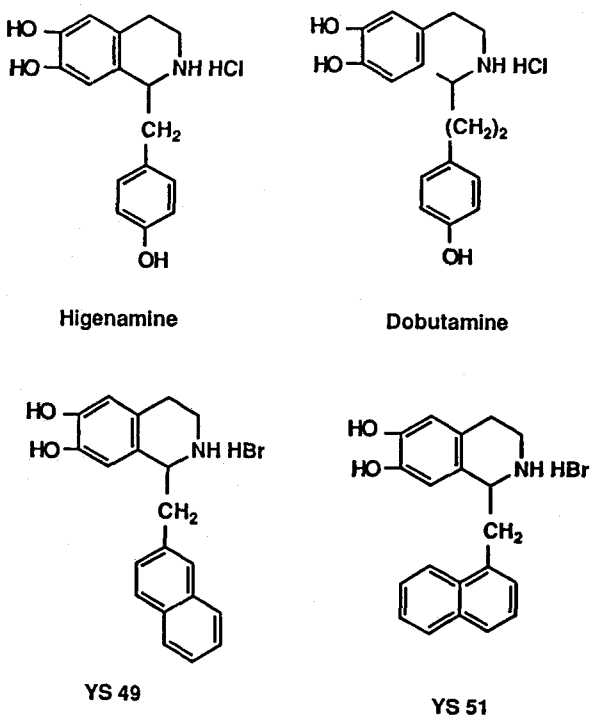

Fig. 1. Chemical structures of tetrahydroisoquinoline analogs and dobutamine.

heart failure, but their use is limited by their oral ineffectiveness and by tachyphylaxis of the β-adrenergic receptor. The newer approaches to the treatment of heart failure take greater account of the neurohormonal and peripheral adaptive changes, which may be regarded as the consequences of the ventricular dysfunction and associated hemodynamic impairment (Francis, 1990). Thus, the development of non -catecholamine and non-glycoside cardiotonic agents may be desirable. Tetrahydroisoquinoline (THI) compounds are possible candidates for non-glycoside and non-catecholamine type of inodilators. (Iwasawa & Kiyomoto, 1967; Asoke et al, 1985; Chang & Chong, 1991; Lacroix et al, 1991; Dong et al, 1992). Chang et al (1986) have synthesized higenamine, an active ingredient of Aconite tuber, of which cardiotonic action mechanism was due to the stimulation of cardiac β-receptor (Park et al, 1984). GS 389, higenamine derivative, has been reported to inhibit the contractile responses of rat aorta to α_1 -adrenoceptor stimulation (Chang et al, 1992). Furthermore, certain THI compounds (Fig. 1) have a great potential to develop as a new type of inodilator (Chang et al, 1992; Lee et al, 1994). YS 49, another THI analog, may represent a new class of drugs with a potential application in congestive heart failure (Lee et al, 1994). In this context, the present study investigated the cardiovascular activity of a limited series of newly synthesized THIs such as YS 49, YS 51 and higenamine, using isolated rat atria and aorta. Moreover, some of their phamacological actions were compared with those of dobutamine, a drug currently in use for the heart failure.

METHODS

Materials

Acetylcholine, indomethacin, phenylephrine, phentolamine, propranolol and prazosin were purchased from Sigma Chemical Company (St. Louis, MO). [^{3}H]prazosin (specific activity 78 Ci/ mmol) was obtained from New England Nuclear (U.S.A.). Lilly Pharmaceutical Co. (U.S.A.) kindly donated (±) Dobutamine. All the other chemicals and reagents used were of the highest quality available.

General

Experiments were carried out on thoracic aortas and hearts from Sprague-Dawley rats of either sex weighing 250~350 g. Rats were anesthesized with ketamine (15 mg/kg) and xylazine (75 mg/kg) by injecting intramuscular route (IM). Thoracic aortas and hearts were removed and placed in physiological salt solution. Rat aortas were dissected according to the method described by Chang et al (1992). Experiments were done in the presence of indomethacin (10 μM) to prevent prostanoids action from vessels. Preliminary study indicated that indomethacin did not affect vasodilatory action of test compounds (data not shown). The endothelium was deliberately removed by inserting wooden stick into the intimal surface. Successful removal of the endothelium was confirmed by the inability of tissues to relax in response to acetylcholine in rat aorta. Rat heart preparations were made according to the method described by Park et al (1984). The tissues were set up at 37°C in a 10 ml volume muscle chamber, in which 95% O_2-5% CO_2 was supplied. Normal Krebs-Ringer bicarbonate solution had 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; and EDTA, 0.03. Isometric tensions were recorded on Grass physiograph (model 7E) via force transducer

FT-03. The initial tension was adjusted to 1 g with equilibration for more than 90 min and washed at every 20 min intervals. Cumulative concentration response curves, with 0.5 log unit intervals in concentration, were utilized to quantitate the sensitivity of tissue to drugs.

Measurement of vasorelaxation and vasocontraction

For the measuring of vasorelaxation, contractions were obtained by adding phenylephrine (PE) in Krebs-Ringer bicarbonate solutions or by changing the bath fluid with 65.4 mM potassium (K^+), which was made by substituting equimolar concentration of sodium from the Krebs-Ringer bicarbonate solutions. After reaching the plateau of contraction, test substances were cumulatively added.

Assay for [^{3}H]prazosin competition binding

Binding experiments were performed as described previously (Chang & Hahn, 1995). In equilibrium binding studies, different concentrations (50 pM to 2 nM) of [^{3}H]prazosin 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 immediately filtered through Whatman GF/C glass microfiber filters under 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 experiments were performed as above, but 200 pM [^{3}H]prazosin and various concentrations of probes were used. Using the iterative nonlinear least-square curve fitting program, data of saturation and competition experiments were analyzed (Lundon Software Inc., Chagrin Falls, Ohio, USA).

Measurement of inotropic and chronotropic action

For the testing of inotropic action, field stimuli were applied in left atria through two parallel platinum electrode which were placed along both sides of the whole length of the preparation. Square wave pulses of 1 m sec duration with threshold intensity sufficient to elicit contractions were generated through Narco SI-10 stimulator. Heart rates were measured from the spontaneously beating right atria.

pA_2 analysis

The pA_2 analysis was performed by computer program according to Schild plot (Arunlakshana & Schild, 1959).

Statistical analysis

Results are expressed in mean$\pm$S.E. for n separate experiments. The statistical evaluation was made using one-way analysis of variance. P values smaller than 0.05 were considered significant. The concentration of agents which produced 50% of the maximal relaxation (IC_{50}) was estimated from the log concentration effective curves in each tissue.

RESULTS

Effects on phenylephrine and high K^+-induced contraction

In order to examine the relaxation effects of THI analogs and dobutamine, endothelium-denuded tissues were contracted with 0.1 μM PE. All compounds including dobutamine, relaxed rat aorta, concentration-dependently. The relaxation pattern was almost the same with respect to onset of action as well as duration of action in PE-induced contraction (data not shown). THIs-induced vasodilatation to high K^+ was different from that to PE. High K^+-induced contraction was the most resistant to higenamine. Concentration-response curves of these drugs were presented in Fig. 2. Their relative potencies were also compared in Table 1.

Effects on heart preparations

All compounds, dose-dependently, increased isometric tension. The concentration range for inotropic action was 0.1 μM to 10 mM (Fig. 3a). It should be noted that higenamine and dobutamine are easily washed out, but YS 49 and YS 51 are not washed out easily. This may reflect the lipophilicity of the drug. For the chronotropic action, all probes also showed positive chronotropic action. The order of potency for positive inotropic effect (PIE) was dobutamine > higenamine > YS 49 > YS 51 (Fig. 3a), while for the positive chronotropic effect (PCE) was higenamine > YS 49 > YS 51 > dobutamine (Fig. 3b).

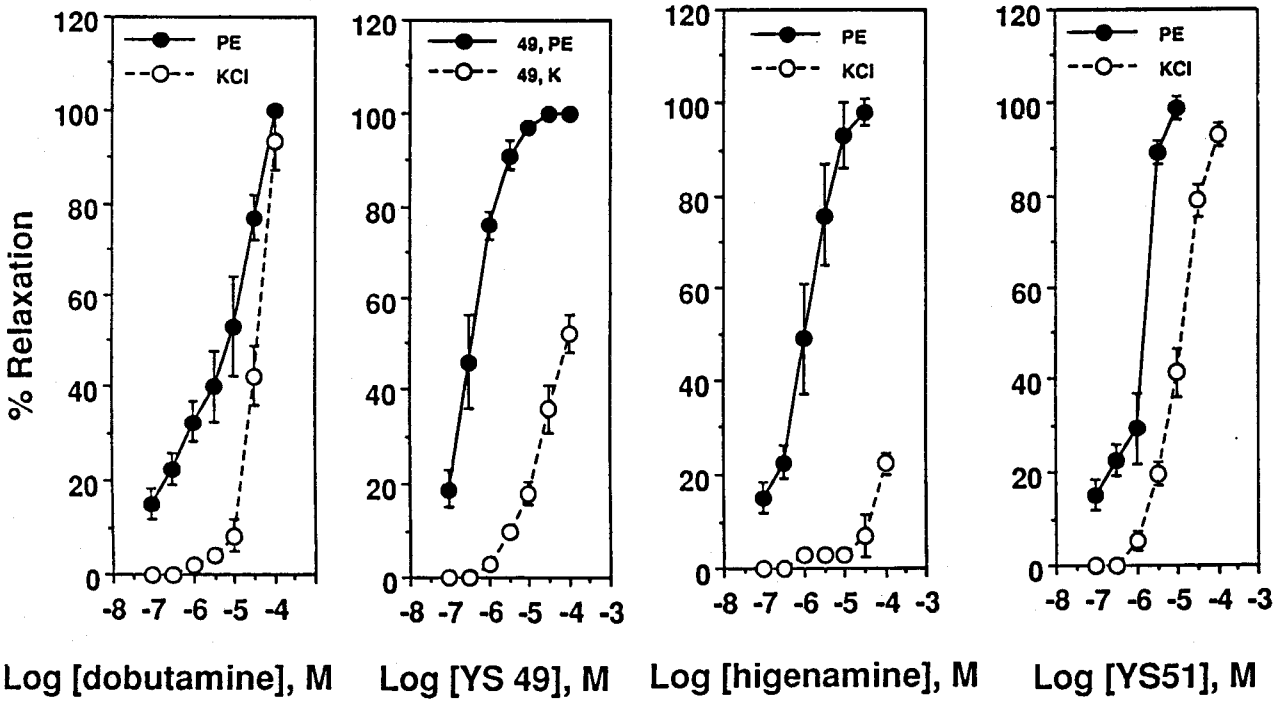

Fig. 2. Cumulative concentration response curves of YS 49, YS 51, higenamine and dobutamine, on PE-induced contraction in endothelium-denuded rat thoracic aorta. Results are expressed as percentages of relaxation to maximum contraction of 0.1 μM PE and each point represents the mean values±SE of 3 experiments.

Table 1. The comparison of relaxation effects of some tetrahydroisoquinoline analogs to the different agonist induced contraction in rat aorta

Compounds	pIC_{50} [a] + S.E	
	K^+-Contraction	PE-Contraction
YS 49	4.08±0.11[b] (9)[c]	5.56±0.32 (12)
YS 51	4.91±1.22 (8)	5.55±0.21 (12)
Higenamine	2.75< ** (8)	5.99±1.16 (8)
Dobutamine	4.62±0.49 (8)	5.57±0.34 (12)

a: - log IC_{50} (M), b: mean±S.E, c: number of experiments. **P<0.05 compared with PE

pA_2 analysis for inotropic action

To understand the mechanism of action of the positive inotropic action, three different concentrations of propranolol were used. Fig. 4a shows the concentration-response curves of the positive inotropic action of YS 49 in the presence or absence of different concentrations of propranolol. In Fig. 4b, the effects of YS 51 on the positive inotropic action was also depicted. As shown in these graphs, the concentration-response curves of YS 49 and YS 51 were shifted parallel to the right, from which dose ratio can be calulated, and pA_2 analysis were done using computer program. Higenamine experiments revealed that the concentration-response curves also shifted to the right (data not shwon). The result indicated that pA_2 value of higenamine was 8.24±0.22; YS49 was 8.07 ±0.84; and YS 51 was 7.93±0.11. The slope of each compound was not deviated from 1, indicating that these drugs are highly competitive at the cardiac β-receptors (Table 2).

Effects on [^{3}H]prazosin binding

It is indicated that THI probes may have affinity to α-adrenoceptor. To test this, [^{3}H] prazosin binding experiment was performed in rat cerebral cortex, where α-adrenoceptor is abundant (Glossmann & Hornun, 1980; Greenberg et al, 1976). Prazosin bound specifi-

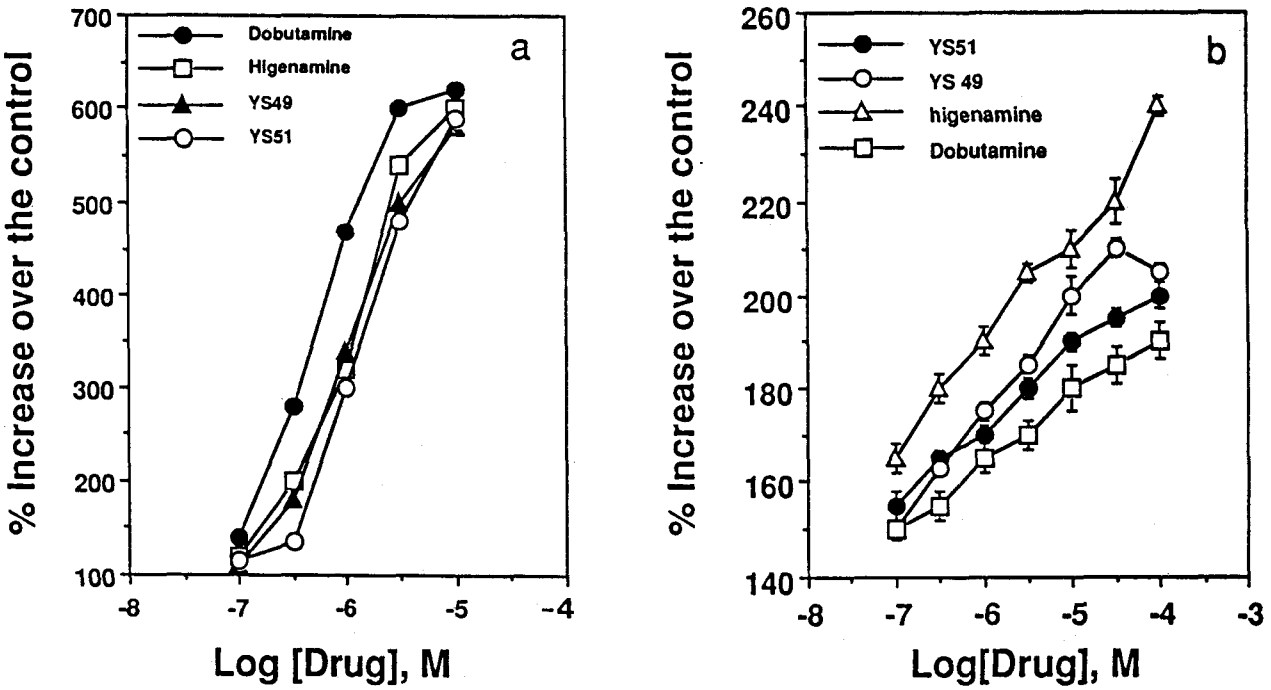

Fig. 3. Comparison of inotropic action(a) and chronotropic action (b) of YS 49, YS 51, higenamine and dobutamine in isolated rat atria. Results are expressed as percent increment over the control, which was regarded as 100%. Each point represents mean±SE of at least 3 experiments.

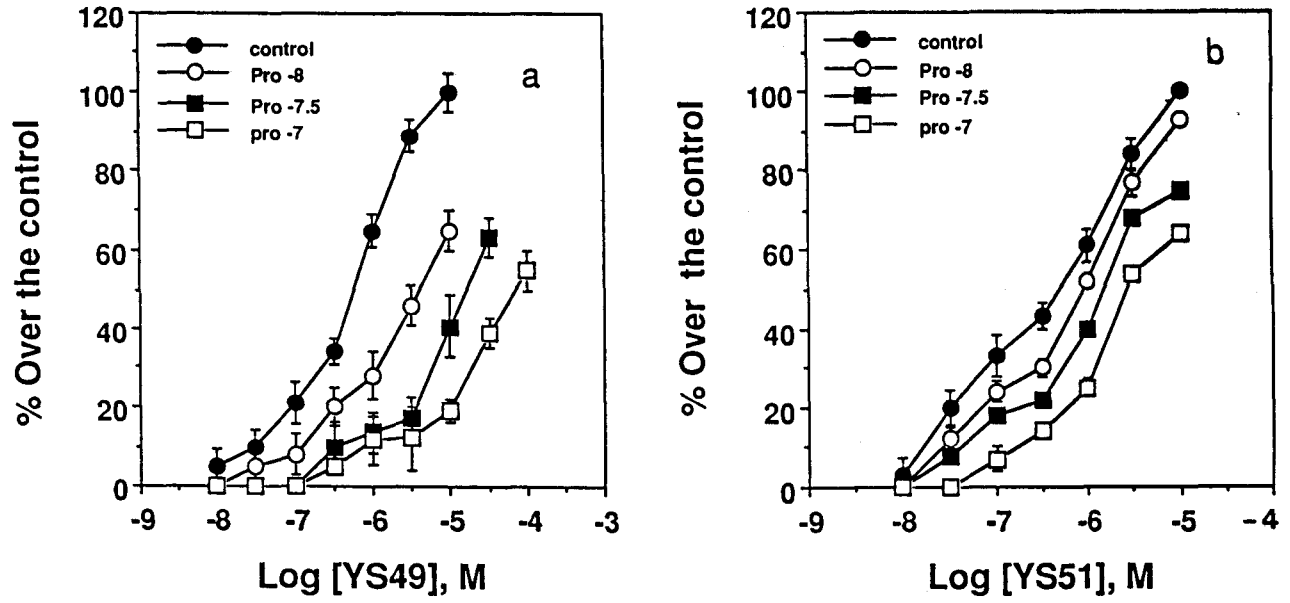

Fig. 4. The concentration-response curves of YS 49 (a, left) and YS 51 (b, right) in the presence of three different concentrations of propranolol in isolated rat left atria. Each point represents mean±SE of 3 different experiments.

cally to the α-adrenoceptor with K_d 133.5±8.91 pM and B_{max} 15.15±0.64 fmol/mg tissue. After confirming that brain tissue is suitable for the binding assy, test drugs were evaluated whether they have α-adrenoceptor affinity. As shown in Fig. 5, all probes had considerable affinity for the brain α-adrenoceptors. The dissociation constants (K_i) of higenamine, YS49, and YS 51 were 1.26, 0.27 and 0.15 μM, respectively.

Table 2. Effects of higenamine, YS 49 and YS 51 on pA_2 against propranolol in isolated rat left atria

Compound	pA_2	slope
higenamine	8.24±0.22	1.27
YS 49	8.07±0.84	1.08
YS 51	7.93±0.11	1.64

Table 3. Comparison of dissociation constant (K_i) in rat brain and IC_{50} in rat aorta for the α-receptor

Compound	K_i (M)	IC_{50} (M)
higenamine	1.26×10^{-6}	1.02×10^{-6}
YS 49	0.27×10^{-6}	2.75×10^{-6}
YS 51	0.15×10^{-6}	2.81×10^{-6}

The K_i value and IC_{50} against α-receptor obtained from the rat brain and aorta were compared (Table 3).

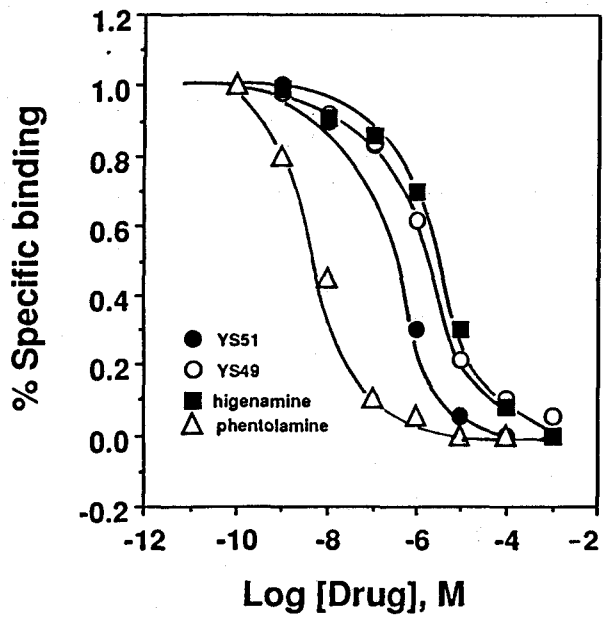

Fig. 5. Displacement of [^{3}H] prazosin binding by YS 49, YS 51 and higenamine in rat cerebral cortex.

DISCUSSION

In the present study, comparison has been made for the cardiac inotropic and chronotropic action and vasodilating action with different THIs and dobutamine, which are quite similar in their structure, using isolated rat heart and aorta. The present results demonstrated that YS 49, YS 51, higenamine, and dobutamine, dose dependently, relaxed PE-induced contraction. When compared the potency among THIs on PE- and high K^+-induced contraction in rat aorta, they were equipotent to dobutamine in terms of IC_{50} values. To PE-induced contraction, higenamine was more potent than dobutamine, while to high K^+-induced contraction, dobutamine was more potent than higenamine. Because PE produces contraction utilizing both Ca^{2+} from intracellular stores and extracellular site (Itoh et al, 1987), while K^+ induces contraction using only extracellular one (van Breemen 1969; van Breemen & McNaughton, 1970), the potency difference in each situation can be understood. That is, the different mechanisms in the handling of Ca^{2+} by the two agonists may be responsible for the different potency within the tested drugs. Even though their inhibitory potencies are different, they all suppressed high K^+-induced contraction. These findings suggest they all have Ca^{2+} antagonistic effect and/or decreasing Ca^{2+} sensitivity. This effect may be a secondary effect resulted from the increment of cyclic nucleotides. It is well known that cAMP can relax the vascular smooth muscle and stimulate the cardiac muscle (Ahluwalia & Rhoads, 1982). Higenamine increases cAMP through β-agonistic action in heart (Chang et al, 1986, Park et al, 1984) and in tracheal smooth muscle (Yoon et al, 1986). Phosphodiesterase inhibitors can induce muscle relaxation by decreasing Ca^{2+} influx (Huddart & Saad, 1980) through voltage sensitive (Brading et al, 1983; Fujioka, 1984) or receptor operated channels, or by inhibiting the release of intracellularly stored Ca^{2+} (Fujioka, 1984). YS 49, YS 51, higenamine, and dobutamine exerted positive inotropic and chronotropic action. The mechanism of these actions was partly responsible for the β-adrenoceptor activation of cardiac muscle, since effects of YS 49 were inhibited by the presence of propranolol (Lee et al, 1995). Higenamine also possesses β-adrenoceptor stimulating activity in rabbit heart (Park et al, 1984). Dobutamine is used for cardiac failure patients because of its positive inotropic action due to cardiac β-adrenoceptor activation (Ruffolo & Morgan, 1984). The positive inotropic action of YS 51 was also inhibited by the presence of propranolol. So it is reasonable to speculate that cardiac stimulating ef-

fects of YS 51 can be attributable, in part, to β -adrenoceptor activation. However, in vascular relaxation study and receptor binding study, YS 51 revealed to possess an α-adrenoceptor antagonistic property. Other compounds also have considerable affinity to brain and vascular smooth muscle α -adrenoceptors (Table 3). It is believed that Ca^{2+} can be differently regulated by the presence of receptors for the given tissues on which drug acts. So, it can be speculated that THIs, such as YS-series and higenamine, have affinity not only for β_1-adrenoceptors in the heart but also for α-adrenoceptor in the vascular smooth muscle. Chang et al (1994) suggested that cAMP may not be responsible for the different action of Ca^{2+} handling between heart and vascular smooth muscle in the case of higenamine. Other factor (s) appear to be involved in the different action of cardiac muscle reactivity. There is a possibility that higenamine has α-receptor blocking action, which shares with calcium antagonistic action (Chang et al, 1994). The report from Atlas and Adler (1981) further supports this idea because they suggested that α-adrenergic antagonists were possible calcium channel inhibitors in brain cells. In cardiac muscle and arterial smooth muscle no correlation was found between the papaverine-induced increase of intracellular cAMP and the relaxation (Reinhardt et al, 1977; Fujioka, 1984). In vascular smooth muscle, regardless of the mechanism, it appears to involve both cAMP and cGMP. Cyclic AMP and cGMP may contribute to vascular smooth muscle relaxation by similar mechanisms, involving phosphorylation of calcium-binding proteins. Evidence exists that vasodilators, which elevate cGMP levels, also phosphorylate common proteins in vascular smooth muscle (Rapoport et al, 1983) and that these effects are sometimes similar to those observed with vasodilators, which elevate cAMP levels (Rapoport et al, 1982). Indeed, YS-series and higenamine showed affinity for α-adrenoceptor in rat cerebral cortex. Dobutamine is well recognized to possess both α-and β-adrenoceptor affinity (Ruffolo & Morgan, 1984). All THIs had positive inotropic and chronotropic actions but relaxed vascular smooth muscle. All had an affinity for the α-adrenoceptor, which may be responsible for vasodilatation; however, this may not be enough to explain the cardiac action. So, cardiac muscle and vascular smooth muscle may be regulated differently in the handling of calcium ion by these compounds depending upon the receptors of the tissues on which drugs act. These drugs may be beneficial for the congestive heart failure in reducing afterload and incresing cardiac inotropic action.

In summary, cardiovascular activities of some THIs and dobutamine have been investigated using isolated rat aorta, and atria in the present study. All THIs tested relaxed PE-contracted rat aorta and the relative potency of these probes were almost the same as that of dobutamine. Higenamine, YS 49, and YS 51 showed α-adrenoceptor affinity in rat aorta and brain in which their pIC_{50} for aorta was compatible to those of K_i of brain. The positive inotropic effect of THIs are attributable to cardiac β-adrenoceptor activation. This conclusion was based on the results of propranolol study in vitro and invivo. It is concluded that these substances may have a beneficial action on the congestive heart failure by reducing afterload to the heart.

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REFERENCES

Ahluwalia GS, Rhoads AR. Selective inhibition of cyclic AMP and cyclic GMP phosphodiesterases of cardiac nuclear fraction. *Biochem Pharmacol* 31: 665 −669, 1982

Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. *Br J Pharmacol* 14: 48−52. 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 A2-mediated responses in human platelet and rat aorta. *J Pharmacol Exp Ther* 232: 1−9, 1985

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

Brading AF, Burdyga TV, Scrippnyuk ZD. The effects of papaverine on the electrical and mechanical activity of the guinea pig ureter. *J Physiol* 334: 79−89, 1983

Chang KC, Chong WS. GS389: A possible cGMP-specific phosphodiesterase inhibitor with Ca++ antagonistic property. In International congress of new drug development. Edited by WS Woo Han Rim Won Press, Seoul, p187, 1991

Chang KC, Hahn KH. Is a-adrenoceptor blockade

responsible for atropine flush? *Eur J Pharmacol* 284: 331−334, 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, 1994

Chang KC, Lim JK, Park CW. Synthesis of higenamine and its cardiovascular effects in rabbit: Evidence for b-adrenoceptor agonist. *Kor J Pharmacol* 22: 96−104, 1986

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

Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, Dunkman S, Jacobs W, Francis GS, Flohr KH, Goldman S, Cobb FR, Shah PM, Saunders R, Fletcher RD, Loeb HS, Hughes VC, Baker B. Effects of vasodilator therapy on mortality in chronic congestive cardiac failure. Results of a Veterans Administratiooon Co-operative study. *New Engl J Med* 314: 1547−1552, 1986

Consensus Trial Study Group. Effects of enalapril on mortality in severe congestive cardiac failure. Results of the North Scandinavian Enalapril Survival Study (CONSENSUS). *New Engl J Med* 316: 1429−1435, 1987

Dong H, Lee CM, Huang WL, Peng SX. Cardiovascular effects of substitued tetrahydroisoquinolines in rats. *Br J Pharmacol* 107: 262−268, 1992

Francis GS. Neuroendocrine activity in congestive heart failure. *Am J Cardiol* 66: 33D−39D, 1990

Fujioka M. Lack of causal relationship between the vasodilatator effect of papaverine and cyclic AMP production in the dog basilar artery. *Br J Pharmacol* 83: 16−18, 1984

Glossman H, Hornun R. a-Receptor in rat brain: sodium changes the affinity of agonists for prazosin sites. *Eur J Pharmacol* 61: 407−412, 1980

Greenberg DA, U Prichard DC, Snyder SH. Alpha- adrenergic receptor binding in mammalian brain: differential labelling of agonist and antagonist states. *Life Sci* 192: 69−73, 1976

Huddart H, Saad KHM. Papaverine-induced inhibition of electrical and mechanical activity and calcium movements of rat ileal smooth muscle. *J Exp Biol* 86: 99−114, 1980

Itoh T, Satoh S, Ishimatsu T, Fujiwara T, Kanmura Y. Mechanism of flunarizine induced vasodilation in the rabbit mesenteric artery. *Cir Res* 61: 446−454, 1987

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

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

Park CW, Chang KC, Lim JK. Effects of higenamine on isolated heart adrenoceptor of rabbit. *Arch Int Pharmacodyn* 167: 279−288, 1984

Rapoport RM, Drazinin MB, Murad F. Sodium nitroprusside-induced protein phosphorylation in intact rat aorta is mimicked by 8-bromo cyclic GMP. *Proc Natl Acad Sci USA* 79: 6470−6474, 1982

Rapoport RM. Drazinin MB, Murad F. Endothelium -dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* 306: 174−176, 1983

Reinhardt D, Roggenbach W, Brodde OE, Schfmann HJ. Influence of papaverine and isoprenaline on contractility and cyclic AMP level of left guinea pig atria at different rates of beat. *Naunyn-Schmiedeberg's Arch Pharmacol* 299: 9−15, 1977

Ruffolo RRJr, Morgan EL. Interaction of the novel inotropic agent, ASL-7022, with alpha and beta adrenoceptors in the cardiovascular system of the pithed rat: Comparison with dobutamine ande dopamine. *J Pharmacol Exp Ther* 229: 364−371, 1984

SOLVD Investigators. Effects of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *New Engl J Med* 327: 685−691, 1991

van Breemen C. Blockade of membrane calcium fluxes by lanthanum in relation to vascular smooth muscle contractility. *Arch Int Physiol Biochem* 77: 710−716, 1969

van Breemen C, McNaughton E. The separation of cell membrane calcium transport from extracellular Ca2+ exchange in vascular smooth muscle. *Biochem Biophys Res Commun* 39: 567−574, 1970

Yoon HI. Studies on the bronchodilator effect of higenamine in isolated guinea-pig tracheal smooth muscle. Ph.D. Dissertation, Seoul National University, 1986