Tracheal Relaxing Effects and β_2 -Selectivity of TA-2005, a Newly Developed Bronchodilating Agent, in Isolated Guinea Pig Tissues

Hideo Kikkawa, Kazuaki Naito and Katsuo Ikezawa

Biological Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-2-50 Kawagishi, Toda, Saitama 335, Japan

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ABSTRACT—Tracheal relaxing effects and β_2 -selectivity of TA-2005 were investigated by functional experiments and radioligand binding assay in guinea pigs in comparison with those of other β -agonists, isoproterenol, procaterol, formoterol and salbutamol. The relaxing activity of TA-2005 on histamine-induced contraction in the isolated trachea was most potent among the five agonists, and it was blocked by a β_2 -selective antagonist (ICI 118,551) but not by a β_1 -selective antagonist (bisoprolol). The potency of the relaxing effect was in the order of TA-2005 ($pD_2 = 9.79$) > formoterol > procaterol > isoproterenol > salbutamol. The positive chronotropic effect of TA-2005 was similar to that of isoproterenol; and it was more potent than those of formoterol, procaterol and salbutamol in the isolated atria. The selectivity for tracheal muscle to atria of these agonists were in the order of procaterol $\geq$ formoterol > TA-2005 > salbutamol $\gg$ isoproterenol. A radioligand binding experiment using guinea pig lung and cardiac ventricle as β_2 - and β_1 -adrenoceptor sources, respectively, has also demonstrated that TA-2005 possesses extremely high affinity ($IC_{50} = 1.04$ nM) and selectivity (38-fold) to β_2 -adrenoceptors. By addition of GTP, the competition curve of [125 I]iodocyanopindolol shifted rightward, indicating the agonist property. These results confirmed that TA-2005 is a highly β_2 -selective agonist that exerts a potent tracheal relaxing effect.

Bronchodilators, especially β_2 -adrenoceptor stimulants have been widely used as agents for the treatment of patients with reversible airway obstruction. Historically, isoproterenol has been the most popular β -adrenoceptor stimulant but it does have some disadvantages such as tachycardia due to its low selectivity towards the airway. In 1967, Lands et al. (1, 2) proposed a classification of β -adrenoceptors into two subtypes, β_1 (mediating the cardiac stimulation) and β_2 (mediating the bronchial and vascular relaxation). Since then, many bronchodilators such as trimetoquinol (3, 4), salbutamol (5), procaterol (6) and formoterol

(7), which are selective for bronchial smooth muscle, have been developed.

Recently, TA-2005 (8-hydroxy-5-[(1R)-1-hydroxy-2-[N-[(1R)-2-(p-methoxy-phenyl)-1-methylethyl]amino]ethyl] carbostyryl hydrochloride, Fig. 1) has been synthesized as a novel bronchodilating agent that possesses β_2 -adrenoceptor stimulating action (H. Kikkawa et al., unpublished data). We investigated the tracheal relaxing effect and β_2 -selectivity of TA-2005 in guinea pigs by pharmacological and radioligand binding studies to characterize its properties; and the effects of TA-2005 were compared with those of other β_2 -adrenoceptor

Fig. 1. The chemical structure of TA-2005.

stimulants, salbutamol, procaterol, formoterol and isoproterenol. As a result, TA-2005 exhibited much more potent tracheal relaxing activity than other tested compounds and high β_2 -selectivity as well.

MATERIALS AND METHODS

Experiments using isolated guinea pig trachea

Male Hartley guinea pigs (250–770 g, Japan SLC) were killed by exsanguination with a blow on the head.

The trachea was dissected and cut into zig-zag strips according to the method of Emerson and Mackay (8). Trachea strips were suspended in 20-ml organ baths filled with Tyrode solution of the following composition: 137.0 mM NaCl, 11.9 mM NaHCO₃, 2.68 mM KCl, 1.89 mM CaCl₂, 1.09 mM MgCl₂, 0.42 mM NaH₂PO₄ and 5.6 mM glucose, which was continuously gassed with 95% O₂–5% CO₂ and maintained at 37°C.

Tension changes of the preparation were recorded isometrically with a strain gauge transducer (UL-10, Shinkoh) on an ink-writing recorder (WI-681G, Nihon Kohden). The preparation was stretched to a resting tension of 1.0 g and was allowed to equilibrate for an hour.

A contraction was induced by the addition of 3×10^{-5} M histamine, which produced a submaximal contraction. Test compounds were added cumulatively in the tonic phase of the contraction. At the end of the experiment, 1×10^{-4} M papaverine was also added to

obtain the maximum relaxation. Agonistic activities of the compounds were estimated by pD₂ (negative log molar concentration that produced 50% relaxation) obtained from each concentration-response curve and by their intrinsic activity (i.a.: ratio of maximum relaxation induced by the compounds to that induced by 1×10^{-4} M papaverine).

In some experiments, tissues were preincubated with β -adrenoceptor antagonists (propranolol, ICI 118,551 (9) or bisoprolol (10)) 30 min before introduction of the compounds.

Experiments using isolated guinea pig right atria

Male Hartley guinea pigs (250–770 g, Japan SLC) were killed by exsanguination with a blow on the head.

Right atrial strips were prepared and suspended in organ baths in the same way as described above except that concentration of glucose in Tyrode solution was 11.1 mM and the temperature was maintained at 32°C. The spontaneous atrial beating rate was measured with a heart rate counter (AT-601G, Nihon Kohden) triggered by atrial contraction, and it was recorded on a Linearcorder (WR3701, Graphtec). Test compounds were also added cumulatively in this experiment. Since the responsibility of the tissue decreased after the treatment with each of the test compounds, the effect of the agonist was normalized by maximum isoproterenol response, which was obtained in the initial isoproterenol run before testing each drug (TA-2005, procaterol, formoterol, salbutamol or isoproterenol). Since several concentration-response curves were biphasic, agonistic activities of the compounds were expressed as pED₂₅ (negative log molar concentration that produced a 25% increase of the maximum response to isoproterenol). Intrinsic activities of compounds were also calculated from the ratio of the maximum response induced by the compounds to that induced by isoproterenol.

In some experiments, tissues were preincubated with a β -adrenoceptor antagonist (ICI 118,551 or bisoprolol) 30 min before introduc-

tion of the compounds.

[¹²⁵I]Iodocyanopindolol binding in guinea pig ventricular and lung membranes

Male Hartley guinea pigs (300–350 g, Japan SLC) were killed by exsanguination with a blow on the head.

The cardiac ventricle and lung were removed and homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5, 25°C) using a Polytron (setting 7–8, 15 sec × 2). The homogenate was centrifuged at $1,500 \times g$ for 10 min, and the supernatant was recentrifuged at $45,000 \times g$ for 20 min. The pellet was homogenized using a Potter type glass Teflon homogenizer with 7–8 passes. The homogenate was centrifuged again at $45,000 \times g$ for 20 min. The final pellet was resuspended in the above buffer and stored in liquid nitrogen until use. The above procedures were conducted at 2–4°C.

The saturation experiments were performed in duplicate by incubating an aliquot of the membrane preparations (ventricle, 50 µg; lung, 5 µg) with various concentrations of [¹²⁵I]iodocyanopindolol (10–200 pM) for 60 min at 25°C (final incubation volume of 300 µl). Incubation was terminated by adding 8 ml of ice-cold 50 mM Tris-HCl buffer. Membrane-bound [¹²⁵I]iodocyanopindolol was separated from free radioligand by filtration using a cell harvester (Brandell, Gaithersburg, MD) over glass fiber filters (Whatman, GF/B). The filters were washed with an additional 8 ml of ice-cold Tris-HCl buffer. The radioactivity of the membrane-bound [¹²⁵I]iodocyanopindolol was determined with a well type gamma counter (ARC-360, Aloka, Japan). Specific binding was defined as the difference between the amount of [¹²⁵I]iodocyanopindolol bound in the absence and presence of 5 µM propranolol.

In the competition experiment, an aliquot of the membrane suspension was incubated with [¹²⁵I]iodocyanopindolol (50–70 pM) and various concentrations of competing drugs. Specific binding was obtained as described above. Specific binding in the ventricular and lung

membranes were about 80 and 90%, respectively, of the total binding, which did not exceed 20% of the total ligand added. In the case of isoproterenol, ascorbic acid (0.1 mM) was included in the incubation mixture to prevent oxidation of the catechol moiety.

In the lung membrane preparation, the experiment was conducted in the presence of an appropriate concentration (10^{-7} M) of the highly β_1 -selective antagonist bisoprolol to suppress β_1 -adrenoceptors which occupy 20% of the total β -adrenoceptors (11, 12). All the experiments were performed both in the presence and absence of 0.2 mM guanosine 5'-triphosphate (GTP). Protein concentration was determined using the Pierce BCA Protein Assay Reagent (Pierce, Rockford, IL).

Materials

[¹²⁵I]Iodocyanopindolol (specific activity, 2,000 Ci/mmol) was purchased from Amersham. TA-2005, procaterol hydrochloride, formoterol fumarate, salbutamol sulphate, and ICI 118,551 hydrochloride were synthesized in the Organic Chemistry Laboratory of Tanabe Seiyaku Co., Ltd. Other drugs used were as follows: (±)-isoproterenol hydrochloride, histamine dihydrochloride (Nacalai Tesque), papaverine hydrochloride (Tokyo Kasei), (±)-propranolol hydrochloride (ICI), guanosine 5'-triphosphate (Merck), and ascorbic acid (Nacalai Tesque). (–)-Bisoprolol fumarate was a gift from E. Merck. All other chemicals used were of reagent grade.

Statistics

Mean pD_2 values are given together with the standard error of the mean (S.E.). pA_2 values were obtained from the Schild equation (13). Selectivities of the agonists between tracheal relaxing and positive chronotropic effects were estimated by the pD_2/pED_{25} ratio in a pharmacological study. Statistical analysis was performed by ANOVA and Dunnett's method, and $P < 0.05$ was considered to be statistically significant.

The equilibrium dissociation constant (K_D) and maximum number of binding sites (B_{max})

for the [125 I]iodocyanopindolol binding to both membrane preparations were determined by Scatchard analysis using a personal computer (Sord M343). IC_{50} s of the radioligand competition curves were calculated by Hill plots. Overall selectivity of the drugs in the binding study for β_2 -adrenoceptor subtypes was assessed by IC_{50} (ventricle)/ IC_{50} (lung) ratio.

RESULTS

Effects on histamine-induced contraction in the isolated guinea pig trachea

TA-2005 concentration-dependently relaxed the histamine-induced contraction of isolated trachea at 3×10^{-10} M or more (Fig. 2). The concentration-response curve for TA-2005 was parallel to those for salbutamol, isoproterenol, procaterol and formoterol (data not shown). The pD_2 value of TA-2005 was 9.79 ± 0.04 (Table 1). Based on the pD_2 value, the relaxing effect of TA-2005 was 309, 275, 14, and 7.2 times more potent than those of salbutamol, isoproterenol, procaterol and formoterol, respectively. The intrinsic activities of all compounds were close to 1.0.

Effects on the isolated guinea pig right atria

Although TA-2005 at 1×10^{-11} M or more increased spontaneous atrial rate in a concentration-dependent manner, the concentration-response curve of TA-2005 was biphasic (Fig. 3). The pED_{25} value of TA-2005 (9.16 ± 0.06) was similar to that of isoproterenol ($pED_{25} = 8.82$) but was 170, 26 and 12 times greater than those of salbutamol, procaterol and formoterol, respectively (Table 1).

The intrinsic activities of TA-2005 and formoterol were close to 0.9, but those of salbutamol and procaterol were smaller than the former (Table 1).

Selectivity for tracheal muscle

Isoproterenol was significantly less potent in the tracheal muscle than in the right atria (selectivity ratio = 0.034). In contrast, the selectivity ratio of TA-2005 was 4.3, indicating that this compound is much more selective to the tracheal muscle than isoproterenol. Procaterol and formoterol showed slightly higher selectivity than TA-2005, while the selectivity ratio of salbutamol was smaller than that of TA-2005.

Fig. 2. TA-2005-induced relaxation in the isolated guinea pig trachea, and effects of (A) ICI 118,551 ($\circ$: 10^{-9} M, $\square$: 10^{-8} M, $\triangle$: 10^{-7} M) or (B) bisoprolol ($\square$: 10^{-8} M, $\triangle$: 10^{-7} M) on it. Points and vertical bars are the mean $\pm$ S.E. ($N = 8$). $\bullet$: control.

Table 1. Comparison of pD₂ values, pED₂₅ values, intrinsic activities (i.a.) and selectivities of β -adrenoceptor agonists in isolated trachea and right atria of guinea pigs

Drugs (N = 8)	Trachea			Right atria			Selectivity pD ₂ /pED ₂₅
	pD ₂ ^{a)} (S.E.)	Potency ratio (Iso = 1)	i.a. ^{b)}	pED ₂₅ ^{c)} (S.E.)	potency ratio (Iso = 1)	i.a. ^{b)}	
TA-2005	9.79 (± 0.04)	275	0.97	9.16 (± 0.06)	2.2	0.89	4.3
Procaterol	8.63 (± 0.06)	19.1	0.97	7.75 (± 0.07)	0.085	0.40	7.6
Formoterol	8.93 (± 0.03)	38.0	0.94	8.09 (± 0.20)	0.19	0.85	6.9
Salbutamol	7.30 (± 0.04)	0.89	0.97	6.93 (± 0.13)	0.013	0.63	2.3
Isoproterenol	7.35 (± 0.05)	1	0.97	8.82 (± 0.03)	1	1.0	0.034

^{a)}Negative log molar concentration that produces a 50% relaxation of isolated trachea. ^{b)}Intrinsic activity.

^{c)}Negative log molar concentration that produces a 25% increase in the atrial rate of the maximum response induced by isoproterenol.

Influence of β -adrenoceptor antagonists on relaxing effect of TA-2005 in the isolated trachea

The cumulative concentration-response curve for the relaxing effect of TA-2005 was shifted to the right by the non-selective β -antagonist propranolol (3.4×10^{-8} M to 3.4×10^{-7} M) (data not shown) and the β_2 -selective antagonist ICI 118,551 (1×10^{-8} M to 1×10^{-7} M) (Fig. 2A). The pA₂ values and the slope factors of the Schild plot for propranolol and ICI 118,551 were 8.35 and 1.11, and 8.73 and 1.15, respectively. On the other hand, the β_1 -selective antagonist bisoprolol (1×10^{-8} M and 1×10^{-7} M) did not affect TA-2005-induced tracheal relaxation (Fig. 2B).

Influence of β -adrenoceptor antagonist on the positive chronotropic effect

Figure 3 shows influences of ICI 118,551 (1×10^{-9} M to 1×10^{-7} M, panel A) and bisoprolol (1×10^{-8} M and 1×10^{-7} M, panel B) on the TA-2005-induced increase in atrial rate. Pretreatment with ICI 118,551 changed the biphasic shape of the concentration-response curve of TA-2005 to a monophasic one by blocking the responses at a lower concentration range (1×10^{-12} M to 1×10^{-8}

M) of TA-2005 (Fig. 3A). On the contrary, bisoprolol did not affect the responses at a lower concentration range of TA-2005, but blocked the responses at a higher concentration range (1×10^{-8} M to 3×10^{-5} M) of TA-2005 (Fig. 3B).

Similar results were obtained with the positive chronotropic effect of salbutamol, but the blocking activities of ICI 118,551 and bisoprolol were more prominent than in the case of TA-2005 (Fig. 4A). In the case of isoproterenol, the cumulative-response curve was shifted to the right by bisoprolol (1×10^{-8} M), but not by ICI 118,551 (10^{-8} M) (Fig. 4B).

Affinity and selectivity for β -adrenoceptor subtypes by radioligand binding

Specific binding of [¹²⁵I]iodocyanopindolol to the guinea pig ventricle was saturable, and Scatchard analysis indicated a single population of binding sites with a K_D of 64.1 ± 4.1 pM (4 experiments) and B_{max} of 20.0 ± 0.3 fmol/mg protein (4 experiments). In the lung membrane, specific binding was also saturable in the presence of 10^{-7} M bisoprolol; K_D and B_{max} were 48.5 ± 2.8 pM (4 experiments) and 399.3 ± 6.9 fmol/mg protein (4 experiments),

respectively. These data were similar to those previously reported (11, 14). The Scatchard plots were linear and the apparent Hill coefficients were unity in both membranes, indicating the absence of cooperative interactions.

Displacement curves for the tested agonists of [125 I]iodocyanopindolol binding to guinea pig lung (in the presence of 10^{-7} M bisoprolol) and the ventricular membranes, which

contain relatively homogeneous populations of β_2 - and β_1 -adrenoceptors, respectively, are depicted in Fig. 5. IC_{50} values and overall β_2 -selectivity ratios in the absence of GTP are shown in Table 2. The potency order of the inhibition of [125 I]iodocyanopindolol binding to the lung membrane was TA-2005 > formoterol > procaterol > isoproterenol > salbutamol. In the ventricular membrane, the

Fig. 3. Positive chronotropic effect of TA-2005 in the isolated guinea pig right atria, and effects of (A) ICI 118,551 ($\circ$: 10^{-9} M, $\square$: 10^{-8} M, $\triangle$: 10^{-7} M) or (B) bisoprolol ($\square$: 10^{-8} M, $\triangle$: 10^{-7} M) on it. Points and vertical bars are the mean $\pm$ S.E. (N = 8). $\bullet$: control.

Fig. 4. Positive chronotropic effect of (A) salbutamol and (B) isoproterenol in the isolated guinea pig right atria, and effects of ICI 118,551 ($\square$: 10^{-8} M) and bisoprolol ($\triangle$: 10^{-8} M) on them. Points and vertical bars are the mean $\pm$ S.E. (N = 8). $\blacktriangle$: control (A), $\blacksquare$: control (B).

Fig. 5. Inhibition of [^{125}I]iodocyanopindolol ($^{125}\text{I}\text{-ICYP}$) binding ($50\text{--}70 \text{ pM}$) to guinea pig ventricular ($\blacksquare$, $\square$) ($\beta_1 \div 100\%$) and the lung ($\bullet$, $\circ$) (in the presence of 10^{-7} M bisoprolol, $\beta_2 \div 100\%$) membrane preparations by β -adrenergic agonists in the presence (closed symbols) and the absence (open symbols) of 0.2 mM GTP. Each point represents the mean of 3–4 experiments.

order of potencies was TA-2005 > isoproterenol > formoterol > procaterol > salbutamol. Selectivity for β_2 -adrenoceptors as judged from the IC_{50} (ventricle)/ IC_{50} (lung) values was in the order of TA-2005 > formoterol > procaterol > salbutamol > isoproterenol. Thus, TA-2005 was most selective for β_2 -adre-

noceptor subtypes among the compounds examined.

Effect of GTP on the β -agonists-induced inhibition of [^{125}I]iodocyanopindolol binding in the lung and the ventricular membranes

Inhibition of [^{125}I]iodocyanopindolol binding

Table 2. Comparison of IC_{50} values and apparent selectivities of β -adrenoceptor agonists determined by binding inhibition studies with [125 I]iodocyanopindolol in the presence and absence of GTP (0.2 mM)

Drugs (No. of exp.)	Guinea pig ventricle (β_1) IC_{50} (nM) ^{b)}		Guinea pig lung ^{a)} (β_2) IC_{50} (nM) ^{b)}		β_2 -Selectivity $IC_{50}H/IC_{50}L$ ^{c)}	
	-GTP	+GTP	-GTP	+GTP	-GTP	+GTP
TA-2005 (3-4)	39.4 (25.2-61.7)	63.3 (39.6-101)	1.04 (0.31-3.55)	2.84 (2.30-3.51)	37.8	22.3
Procaterol (4)	1660 (1370-2020)	1660 (1140-2420)	55.1 (36.7-82.6)	175 (159-193)	30.2	9.5
Formoterol (3-4)	303 (192-477)	459 (287-734)	9.34 (7.56-11.5)	30.8 (24.8-38.2)	32.4	14.9
Salbutamol (3-4)	2620 (1490-4620)	6740 (5240-8670)	422 (339-526)	2400 (1400-4130)	6.2	2.8
Isoproterenol (3-4)	113 (70.9-181)	464 (268-804)	129 (84.3-197)	1030 (707-1500)	0.88	0.45

^{a)}In the presence of 10^{-7} M bisoprolol ($\beta_2 \cong 100\%$). ^{b)}Numbers in parenthesis indicate 95% confidence limits.

^{c)} IC_{50} (ventricle)/ IC_{50} (lung).

to the guinea pig lung and ventricular membranes by β -agonists was studied in the presence of 0.2 mM GTP and was compared with that conducted in the absence of GTP. When 0.2 mM GTP was added to the incubation mixture, as shown in Fig. 5 and Table 2, all radioligand competition curves in the lung membrane (β_2) prominently shifted rightward to lower affinity. IC_{50} values for isoproterenol and salbutamol increased by 700 and 470%, respectively. Those for the other β -agonists increased by 170-230%. Generally, slope factors also increased by the addition of GTP, and those for isoproterenol, salbutamol and procaterol approached to near 1 (data not shown). In the case of TA-2005, however, the slope factor was not affected greatly in the presence of GTP. In the ventricular membrane (β_1), GTP-induced changes in IC_{50} values were less than those in the lung membrane. Though IC_{50} values for isoproterenol and salbutamol increased by 310 and 160%, respectively, those for TA-2005 and formoterol increased by only about 60%.

Figure 6 shows a schematic representation of the effect of GTP on the IC_{50} values in the ventricular and the lung membranes. IC_{50} values for isoproterenol and salbutamol increased in both membrane preparations and

Fig. 6. Schematic representation of the effect of 0.2 mM GTP on IC_{50} values for the inhibition of specific [125 I]iodocyanopindolol binding to the guinea pig ventricular (β_1) and lung (β_2) membranes by β -adrenergic agonists. The horizontal- and vertical-axis represent IC_{50} values for the ventricular and the lung membranes, respectively. Closed and open symbols show affinities of drugs with and without GTP, respectively. Therefore, the length of horizontal and vertical components of the arrows show degrees of increase in IC_{50} values for the ventricular and the lung membranes, respectively. Each point represents the mean of 3-4 experiments.

their arrows were directed to the upper-right. The arrows of TA-2005 and formoterol also were directed the upper-right, while their lengths were shorter than that of the full agonist isoproterenol, indicating their partial agonist property. The arrow of procaterol was directed upward, and its length was shorter than that of isoproterenol.

DISCUSSION

In the isolated trachea, TA-2005 had the most potent relaxing effect among the compounds tested. The effect was 309, 275, 14 and 7.2 times more potent than those of salbutamol, isoproterenol, procaterol and formoterol, respectively. The concentration-response curve for the relaxing effect of TA-2005 was parallel with those of the other reference β -agonists, and it was shifted to the right in the presence of propranolol. The intrinsic activity of TA-2005 in this tissue was close to 1.0, indicating that TA-2005 behaved as a β -adrenoceptor full agonist like isoproterenol.

The curve for TA-2005 was shifted in a parallel fashion to the right by ICI 118,551. The pA_2 value of ICI 118,551 for TA-2005 was 8.73, which is nearly the same as the reported pA_2 values of ICI 118,551 for known β_2 -selective agonists fenoterol (10) and salbutamol (15). The slope factor of the Schild plot for antagonism of ICI 118,551 to the effect of TA-2005 was almost 1.0, indicating competitive antagonism on the same receptor. In contrast, the β_1 -selective antagonist bisoprolol (1×10^{-8} M), at concentrations known to antagonize β_1 -agonistic action selectively (9), did not affect TA-2005-induced tracheal relaxation. These results indicate that the tracheal relaxing effect of TA-2005 is mediated by β_2 -adrenoceptors.

In the isolated right atria, when compared by pED_{25} , the order of the chronotropic effect was TA-2005 $\geq$ isoproterenol $>$ formoterol $>$ procaterol $>$ salbutamol. The maximum positive chronotropic response of TA-2005 was about 80% of that of isoproterenol. The selectivity ratio (tracheal relaxation/chronotropic

action) of TA-2005 was 1.8 and 125 times greater than those of salbutamol and isoproterenol. It was less than those of procaterol and formoterol. Positive chronotropic effects and the selectivity ratio for isoproterenol and salbutamol in this study were practically compatible with those reported previously (6, 16). The maximum positive chronotropic response to TA-2005 was about 80% of that of isoproterenol, and it was larger than those of procaterol and salbutamol. Raper and Malta (17) and Yabuuchi (6) found that salbutamol and procaterol antagonized the positive chronotropic response to isoproterenol competitively in the isolated guinea pig right atria. The antagonistic activity of TA-2005 in the isolated right atria is considered to be smaller than those of salbutamol and procaterol, because of its higher intrinsic activity than these drugs. Mabuterol, which is known as a β_2 -selective agonist (18, 19), has been reported to possess β_1 - and β_2 -adrenoceptor antagonistic activities at higher doses.

We found that the concentration-response curves for TA-2005 in the isolated right atria was biphasic and the chronotropic effect of TA-2005 at the lower concentration range (1×10^{-12} M to 1×10^{-8} M) was blocked by ICI 118,551, but not by bisoprolol. However, the chronotropic effect of TA-2005 at the higher concentration range (1×10^{-8} M to 1×10^{-5} M) was blocked by bisoprolol, but not by ICI 118,551. These results indicate that the chronotropic effect of TA-2005 at the lower concentration range in the guinea pig right atria occurs predominantly through activation of β_2 -adrenoceptors. In contrast, the chronotropic effect of isoproterenol in the same preparation occurs through the activation of β_1 -adrenoceptors only, because the effect of isoproterenol was blocked by bisoprolol, but not by ICI 118,551. These observations were compatible with the data obtained by Johansson and Persson (20).

In order to confirm the airway selective bronchodilating action of TA-2005 demonstrated by functional experiments, we also examined the affinity and selectivity of the com-

pound for β -adrenoceptor subtypes by radioligand binding in comparison with other β -agonists. Though the guinea pig ventricle contains a homogeneous population of β_1 -adrenoceptor, 20% of the β -adrenoceptors are of the β_1 -subtype in lung membranes (11, 12, 21). Thus, we prepared a relatively pure β_2 -adrenoceptor preparation by including highly selective β_1 -antagonist bisoprolol (10^{-7} M) in the lung membrane to suppress β_1 -adrenoceptors. Specific binding in both membrane preparations were saturable, and the Scatchard analysis and Hill plots indicate the validity of our experimental conditions.

TA-2005 exhibited extremely high affinity ($IC_{50} = 1.04$ nM) in the lung membrane. Selectivity of TA-2005 for β_2 -adrenoceptor assessed by the IC_{50} (ventricle)/ IC_{50} (lung) ratio was 38 times, showing the highest selectivity among the β -agonists examined. The selectivity ratio of isoproterenol, a nonselective β -adrenoceptor full agonist, was 0.9. The order of β_2 -selectivity among the agonists did not change in the presence of GTP. Interestingly, the extent of the rightward shift of the inhibition curve of TA-2005 in the lung membrane was much smaller than that of isoproterenol. It seems that TA-2005 has less intrinsic activity than isoproterenol, because the GTP-induced rightward shift has been known to correspond to the intrinsic activities of β -agonists (21–23). The reason for the discrepancy between functional and binding experiments is unknown. The high affinity state of this compound may possess a different efficiency in β -adrenoceptor-adenylate cyclase coupling from that of isoproterenol.

As for the β_2 -selectivity, TA-2005 exhibited relatively higher selectivity in the binding experiment than in the functional experiment. Although the reason for this disagreement is not clear, possibilities are as follows: potent activity of TA-2005 on β_2 -adrenoceptor caused a positive chronotropic effect on the right atria which contains a significant population (23%) of β_2 -adrenoceptors in contrast of the left ventricle (24). Secondly, antagonistic activities of salbutamol (17) and procaterol (6)

in the isolated right atria may have increased the selectivity in functional experiments. In contrast, the antagonistic activity of TA-2005 in the isolated right atria is considered to be smaller than those of salbutamol and procaterol, because TA-2005 has a higher intrinsic activity than these drugs.

In conclusion, our results indicate that TA-2005 is an extremely potent and highly selective β_2 -adrenoceptor agonist, and they suggest that this compound is a potential bronchodilating agent useful for the treatment of patients with reversible airway obstruction.

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REFERENCES

- 1 Lands, A.M., Arnold, A., McAuliff, J.P., Luduena, F.P. and Brown, T.G.: Differentiation of receptor systems activated by sympathomimetic amines. *Nature* **214**, 597–598 (1967)
- 2 Lands, A.M., Luduena, F.P. and Buzzo, H.J.: Differentiation of receptors responsive to isoproterenol. *Life Sci.* **6**, 2241–2249 (1967)
- 3 Iwasawa, Y. and Kiyomoto, A.: Studies on tetrahydroisoquinolines (THI). (I) Bronchodilator activity and structure-activity relationship. *Japan. J. Pharmacol.* **17**, 143–152 (1967)
- 4 Sato, M., Ikezawa, K., Nagao, T. and Kiyomoto, A.: Bronchodilating action of trimetoquinol and its selectivity to bronchial adrenoceptors in anesthetized dog and cat. *Pharmacometrics* **19**, 269–276 (1980)
- 5 Cullum, V.A., Farmer, J.B., Jack, D. and Levy, G.P.: Salbutamol: a new, selective β -adrenoceptive receptor stimulant. *Br. J. Pharmacol.* **35**, 141–151 (1969)
- 6 Yabuuchi, Y.: The β -adrenoceptor stimulant properties of OPC-2009 on guinea-pig isolated tracheal, right atrial and left atrial preparations. *Br. J. Pharmacol.* **61**, 513–521 (1977)
- 7 Ida, H.: Comparison of the action of BD 40A and some other β -adrenoceptor stimulants on the isolated trachea and atria of the guinea pig. *Arzneimittelforschung* **26**, 839–842 (1976)
- 8 Emmerson, J. and Mackay, D.: The zig-zag tracheal strip. *J. Pharm. Pharmacol.* **31**, 798 (1979)

- 9 Brodde, O.-E.: Bisoprolol (EMD 33512), a highly selective β_1 -adrenoceptor antagonist: In vitro and in vivo studies. *J. Cardiovasc. Pharmacol.* **8**, S11, S29–S35 (1986)
- 10 O'Donnell, S.R. and Wanstall, J.C.: Evidence that ICI 118,551 is a potent, highly β_2 -selective adrenoceptor antagonist and can be used to characterize β -adrenoceptor populations in tissues. *Life Sci.* **27**, 671–677 (1980)
- 11 Das, S.K., Sikpi, M.O. and Skolnick, P.: Heterogeneity of β -adrenoreceptors in guinea pig alveolar type II cells. *Biochem. Biophys. Res. Commun.* **142**, 898–903 (1987)
- 12 Engel G., Hoyer, D., Berthold, R. and Wagner, H.: ($\pm$)[125 I]iodocyanopindolol, a new ligand for β -adrenoceptors: identification and quantitation of subclasses of β -adrenoceptors in guinea pig. *Naunyn Schmiedebergs Arch. Pharmacol.* **317**, 277–285 (1981)
- 13 Arunlakshana, O. and Schild, H.O.: Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **14**, 48–58 (1959)
- 14 Aass, H., Hedberg, A., Skomedal, T., Carlsson, E. and Osnes, J.-B.: Characterization of mechanical effects and β -adrenoceptor binding of prenalterol in rat myocardium. *Acta Pharmacol. Toxicol.* **53**, 3–11 (1983)
- 15 Brandt, H.D. and Meyer, C.L.: Apparent affinity values of β -adrenergic blockers in a tissue with a mixture of β -adrenergic receptor subtypes. *Arch. Int. Pharmacodyn.* **289**, 46–59 (1987)
- 16 O'Donnell, S.R.: An examination of some β -adrenoreceptor stimulants for selectivity using the isolated trachea and atria of the guinea pig. *Eur. J. Pharmacol.* **19**, 371–379 (1972)
- 17 Rapcr, C. and Malta, E.: Salbutamol: agonistic and antagonistic activity at β -adrenoceptor sites. *J. Pharm. Pharmacol.* **25**, 661–663 (1973)
- 18 Murai, T., Maejima, T., Sanai, K. and Osada, E.: Pharmacological studies of mabuterol, a new selective β_2 -stimulant I: Bronchodilating effect. *Arzneimittelforschung* **34**, 1633–1640 (1984)
- 19 Akahane, K., Furukawa, Y., Ogiwara, Y., Haniuda, M. and Chiba, S.: β -Adrenoceptor blocking effects of a selective β_2 -agonist, mabuterol, on the isolated, blood-perfused right atrium of the dog. *Br. J. Pharmacol.* **97**, 709–716 (1989)
- 20 Johansson, L.-H. and Persson, H.: β_2 -Adrenoceptors in guinea-pig atria. *J. Pharm. Pharmacol.* **35**, 804–807 (1983)
- 21 Stiles, G.L., Taylor, S. and Lefkowitz, R.J.: β -Adrenergic receptors: Biochemical mechanisms of physiological regulation. *Physiol. Rev.* **64**, 661–743 (1984)
- 22 Kent, R.S., Lean, A.D. and Lefkowitz, R.J.: A quantitative analysis of beta-adrenergic receptor interactions: Resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. *Mol. Pharmacol.* **17**, 14–23 (1979)
- 23 Naito, K., Nagao, T., Ono, Y., Otsuka, M., Harigaya, S. and Nakajima, H.: Effect of GTP on the affinity of denopamine, a new cardiotonic agent, for β -adrenergic receptors of turkey erythrocytes and rat reticulocyte membranes. *Japan. J. Pharmacol.* **39**, 541–549 (1985)
- 24 Hedberg, A., Minneman, K.P. and Molinoff, P.B.: Differential distribution of beta-1 and beta-2 adrenergic receptors in cat and guinea-pig heart. *J. Pharmacol. Exp. Ther.* **212**, 503–508 (1980)