Isoeugenolol: A Selective β_1 -Adrenergic Antagonist With Tracheal and Vascular Smooth Muscle Relaxant Properties[†]

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ABSTRACT—Isoeugenolol (1.0, 3.0, 5.0 mg/kg, i.v.) produced a dose-dependent bradycardia and a decrease in blood pressure in anesthetized Wistar rats. Isoeugenolol inhibited the tachycardia effects induced by (–)isoproterenol, but had no blocking effect on the arterial pressor responses induced by (–)phenylephrine. In isolated guinea pig tissues, isoeugenolol antagonized (–)isoproterenol-induced positive inotropic and chronotropic effects on the atria and tracheal relaxations in a concentration-dependent manner. The apparent pA_2 values for isoeugenolol on right atria, left atria and trachea were 7.63 ± 0.03 , 7.89 ± 0.12 and 6.12 ± 0.05 , respectively, indicating that isoeugenolol was a highly selective β_1 -adrenoceptor blocker. On the other hand, isoeugenolol produced a mild direct cardiac depression at high concentration and was without intrinsic sympathomimetic activity (ISA). In isolated rat thoracic aorta, isoeugenolol relaxed more potently the contractions induced by (–)phenylephrine (10 μ M) and 5-HT (10 μ M) than those by high K^+ (75 mM). In isolated guinea pig trachea, isoeugenolol attenuated the carbachol (1 μ M)-contracted trachea more significantly than those contracted with high K^+ . Furthermore, the binding characteristics of isoeugenolol and various β -adrenoceptor antagonists were evaluated in [³H]CGP-12177 binding to rat ventricle, lung and interscapular brown adipose tissue (IBAT) membranes. The $-\log IC_{50}$ values of isoeugenolol for predominate β_1 -, β_2 - and β_3 -adrenergic receptor sites were 5.82 ± 0.09 , 4.74 ± 0.05 and 4.73 ± 0.12 , respectively. In conclusion, isoeugenolol was found to be a highly selective β_1 -adrenoceptor antagonist with tracheal and vascular smooth muscle relaxant activities, but was devoid of α -adrenoceptor-blocking action.

Keywords: Isoeugenol, β_1 -Adrenoceptor antagonist, Smooth muscle relaxation, Interscapular brown adipose tissue, Radioligand binding study

β -Adrenoceptor blockers are an important class of drugs in the management of patients with cardiovascular diseases. These drugs have been shown to reduce mortality in hypertension (1) and prolong survival in patients with ischemic heart diseases (2). β -Blockers retain their position among basic therapies for numerous other cardiovascular and non-cardiovascular conditions, including arrhythmias, hypertrophic cardiomyopathy, migraine, glaucoma and thyrotoxicosis. Such β -blockers share the common feature of being competitive antagonists on the β -adrenoceptors. However, they differ in

many aspects of pharmacological properties such as potency, selectivity for β_1 - or β_2 -receptors, intrinsic sympathomimetic activity (ISA), membrane stabilizing activity (MSA), and α -blocking characteristics (3). In this study, we used two kinds of experimental animals. Isolated guinea pig atria and trachea were used to estimate the selectivity of the test agents between β_1 - and β_2 -receptors, in a rational animal model as described by Harms (4). Additionally, rat brown adipose tissues were used to evaluate the binding affinity of the test agents for predominant β_3 -adrenergic receptor (AR) sites. Previous reports have demonstrated that the β_3 -AR is abundantly expressed in rat brown and white adipose tissues (5, 6). Ventricle and lung, as well as brown adipose tissues, were dissected from the same rat and were also used for the binding experiments.

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Fig. 1. Chemical structure of isoeugenolol.

β -Adrenergic antagonists and particularly those without cardiac selectivity carry considerable risk in patients with bronchospastic disease because they may block β_2 -receptors in therapeutic doses. In the airway system, application of a β -AR blocker with tracheal relaxant activity may reduce the risk of asthmatic attack in patients with bronchospastic disease. However, pulmonary effects such as dyspnea, airway obstruction, and pulmonary failure have also occurred in the administration of β -AR blockers (7, 8). These facts have led many investigators to search for newer β -adrenergic blocking agents that would be able to lessen adverse effects. Therefore, a new generation of β -blockers with a variety of additional characteristics are now emerging; the most interesting of these are β -blockers with vasodilating activity, achieved by a variety of mechanisms including α -adrenoceptor blockade, β -adrenoceptor agonism, and a dilator action independent of the α - and β -receptors (9–11).

Recently, various vanilloid type β -AR blockers derived from 3-methoxy-4-hydroxy benzyl compounds such as vanillin, zingerone, capsaicin, eugenol, etc. have been investigated in our laboratory (12–18). Isoeugenolol (1-(isopropylamino)-3-[(4-propenyl-2-methoxy)propoxy]-2-propanol) (Fig. 1) was synthesized from isoeugenol designed to display its tracheal relaxant β -AR antagonistic activity. Isoeugenol and its structural isomer eugenol, the major constituents of clove or nutmeg oil, have been shown to inhibit lipid peroxidation (19) and platelet aggregation induced by arachidonic acid (20). In our previous study, we found that eugenolol is a eugenol-derived β -AR blocker with partial β_2 agonist and calcium mobilization inhibition associated vasorelaxant activities (13). Developing a β -blocker such as eugenolol with partial β_2 agonist and vasorelaxant activities still has a striking clinical advantage. For this reason, we designed a novel vanilloid type β -blocker, isoeugenolol. Although isoeugenolol was without partial β_2 agonist activity, it still possessed vasorelaxant activity, and it particularly had tracheal relaxant activity. The aim of this study was to investigate the various pharmacological characteristics of isoeugenolol such as its binding affinity on the β -recep-

tors, its β -AR blocking action, its ISA, and its tracheal and vascular smooth muscle (VSM) relaxant activities.

MATERIALS AND METHODS

Animals

Wistar rats and guinea pigs (Hartley), provided from Experimental Animal Center at Cheng-Kung University Medical College, were housed under constant temperature and controlled illumination (light on between 7:30 and 19:30). Food and water were available ad libitum. The study was approved by the Animal Care Committee of the Kaohsiung Medical College.

Drugs and chemicals

(-)[3 H]CGP-12177 (44.5 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA, USA). (-)Isoproterenol bitartrate, (-)propranolol HCl, (-)phenylephrine HCl, ($\pm$)atenolol, mecamlamine HCl, phenoxybenzamine HCl and reserpine were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). ICI 118,551 was obtained from Research Biochemical International (Natick, MA, USA). All other reagents used were from E. Merck (Darmstadt, Germany). ($\pm$)Isoeugenolol (synthesized in this laboratory) was solubilized in 10% absolute alcohol plus 10% Tween 80 and then diluted with distilled water.

Measurement of blood pressure and heart rate

The experiments were accomplished as previously described (16). In brief, male Wistar rats weighing 250–300 g were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Following tracheal cannulation, systemic arterial blood pressure and heart rates were recorded from the femoral artery with a pressure transducer (Model P10EZ; Spectramed, Oxnard, CA, USA). The body temperature was maintained at 37°C by an electric heating pad. A femoral vein was cannulated for intravenous administration of drugs.

β - and α -adrenergic responses

Rats were pretreated with mecamlamine (5 mg/kg, i.v.), a ganglion blocking agent, to ensure a uniform initial heart rate. Isoproterenol (0.5 mg/kg) was administered via a femoral vein and the resultant tachycardia recorded as the control. A single dose of isoeugenolol was then administered intravenously. After 10 min, a further injection of isoproterenol was given. Additionally, rats were pretreated with reserpine (5 mg/kg, i.p.) 24 hr prior to the injection of (-)phenylephrine (10 μ g/kg, i.v.), followed 15 min later by the intravenous injection of a single dose of isoeugenolol (5 mg/kg) or propranolol (1 mg/kg). After 10 min, a further

injection of (–)phenylephrine was given.

Isolated right and left atria

The atrial strips were prepared as previously described (16). Guinea pigs (350–500 g) were sacrificed after mild anesthesia with ether and their hearts were quickly excised. Right and left atria were dissected and mounted in a 10-ml organ bath. The frequency of the right atrium was measured by a tachometer (Model S77-26; Coulbourn, Allentown, PA, USA) connected to a high-speed videograph (AT L19-69, Coulbourn). Left atria were driven at 2-sec intervals via two platinum electrodes placed at either side of the atrium. The experiments were carried out at 37°C in a Krebs solution of the following composition: 113 mM NaCl, 4.8 mM KCl, 2.2 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.2 mM MgCl_2 , 25 mM NaHCO_3 , 11.0 mM dextrose, bubbled with a 95% O_2 + 5% CO_2 mixture. Right and left atria were prestretched to a baseline tension of 0.2 and 0.5 g, respectively, and equilibrated for 90 min. Cumulative concentration-response curves to the positive inotropic and chronotropic effects of isoproterenol were obtained in the absence and presence of various concentrations of a test agent. The test agent was incubated with the atrium 30 min before isoproterenol was added.

Isolated tracheal strips

Guinea pig tracheal strips were suspended in organ baths filled with 20 ml of Krebs solution (37°C) and aerated with 95% O_2 + 5% CO_2 as previously described (16). To achieve a steady spontaneous tone level, an initial tension of 2 g was applied for 1 hr. Cumulative concentration-response curves to the relaxant effects of isoproterenol were obtained in the absence and presence of a test agent (1 hr incubation time). Additionally, we observed the effects of isoeugenolol on the amplitude of contractions evoked by carbachol (1 μM) or 75 mM K^+ .

Intrinsic sympathomimetic activity

Guinea pigs were reserpinized as previously described (16). Isolated right and left atria, and tracheal strips were studied. The concentration-response curve was obtained by cumulative addition of (–)isoproterenol, propranolol or isoeugenolol. To assess the ISA of isoeugenolol on tracheal strips, the method described by Tesfamariam and Allen (21) was employed. Cumulative concentration-response curves to the relaxant effects of isoeugenolol were determined in the absence or presence of ICI 118,551 (0.1–10 nM) for 1 hr.

Isolated thoracic aorta

Rats thoracic aorta were cut into 3–4 mm wide transverse rings, which were then mounted at 1-g resting ten-

sion in a 10 ml organ bath and bathed in an aerated physiological solution (118 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 24 mM NaHCO_3 , 11 mM glucose) at 37°C. Preparations were allowed to equilibrate for 1 hr. Phenylephrine (10 μM), 5-HT (10 μM) or 75 mM K^+ was then added to the bath to induce contractions. When the response achieved maximal tension, isoeugenolol was added to the bath to produce relaxations. The protocol described by Chen et al. (13) was used to study the effects of isoeugenolol on phenylephrine-sensitive intracellular Ca^{2+} release and influx. Isoeugenolol was incubated in EGTA-physiological solution for 10 min before the addition of phenylephrine.

Receptor binding experiments

Wistar rats (400–600 g) were sacrificed by decapitation. Ventricle, lung and interscapular brown adipose tissue (IBAT) were removed and prepared as previously described (16). Protein content was determined by Bradford's method (22). The binding assay of [^3H]CGP-12177 was carried out as described by Muzzin et al. (23). For saturation studies, [^3H]CGP-12177 (0.1–100 nM) and prepared membranes (100–200 μg) were incubated for 60 min at 25°C with or without the addition of 10 (ventricle and lung) or 100 μM (IBAT) propranolol, in 75 mM Tris HCl buffer containing 25 mM MgCl_2 , to obtain a final volume of 250 μl . In competitive binding experiments, the competing agent was added directly to the incubation mixture. The incubation mixture was rapidly filtered by vacuum through Whatman GF/C glass fiber filters. The filters were washed three times with 5 ml of ice-cold assay buffer and dried prior to adding 5 ml of Triton-toluene-based scintillation fluid. Membrane-bound [^3H]CGP-12177 trapped in the filters was counted in a Beckman LS 6500 scintillation system. In each experiment, non-specifically bound [^3H]CGP-12177 was determined by incubating membrane protein and [^3H]CGP-12177 with 10 or 100 μM propranolol. Specific binding was thus obtained by deducting this value from the total binding of [^3H]CGP-12177 for each sample.

Analysis and statistical evaluation of data

The potency of β -adrenergic antagonists was estimated according to Arunlakshana and Schild (24). Concentration ratios (CR), EC_{50} in the presence of antagonist/ EC_{50} in the absence of antagonist, were corrected for any change in sensitivity to the agonist that was not due to the presence of the antagonist. The correction factor was determined as described by O'Donnell and Wanstall (25). The pA_2 values were calculated from the equation $\text{pA}_2 = \log (\text{CR} - 1) - \log [\text{B}]$. The K_d , B_{max} and K_i values were determined by Scatchard analysis using the non-linear

curve-fitting LIGAND program (26).

Statistical results are expressed as the mean $\pm$ S.E.M. Significant differences were determined by the independent and paired Student's *t*-test in unpaired and paired samples, respectively. Whenever a control group was compared with more than one treated group, one-way ANOVA or two-way repeated measures ANOVA was used. When the ANOVA manifested a statistical difference, the Dunnett's or Student-Newman-Keuls test was applied. Analysis of the data and plotting of the figures were done with the aid of software (SigmaStat and SigmaPlot, Version 5.0 (Jandel, San Rafael, CA, USA) and GraphPad PRISM™, Version 2.0 (GraphPad Software, San Diego, CA, USA)) run on an IBM compatible computer and a Power Macintosh.

RESULTS

Effects of isoeugenolol on blood pressure and heart rate

Acute intravenous injection of isoeugenolol (1.0, 3.0, 5.0 mg/kg) produced an initial transient fall that was followed by a sustained fall in blood pressure and a dose-dependent decrease in heart rate in pentobarbital-anesthetized Wistar rats (Fig. 2). However, propranolol produced a temporary rise in blood pressure and a decrease in heart rate (data not shown). In ganglion-blocked anesthetized rats, i.v. injection of isoeugenolol (1.0, 3.0, 5.0 mg/kg) clearly showed a significant decrease in isoproterenol-induced cardioaccelerator responses (Fig. 3). In reserpinized rats, phenylephrine (10 μ g/kg) was injected intravenously before and after i.v. injection of isoeugenolol (5.0 mg/kg) or propranolol (1.0 mg/kg); the pressor response to phenylephrine (57.0 ± 4.5 mmHg) was not inhibited by isoeugenolol (55.6 ± 4.8 mmHg) and propranolol (54.3 ± 5.1 mmHg).

Effects of isoeugenolol on β_1 -adrenoceptor activity

Isoeugenolol antagonized the isoproterenol-induced positive chronotropic activity on the isolated guinea pig right atrial strips. In addition, isoeugenolol (0.1–10 μ M) caused a dose-dependent parallel shift to the right of the isoproterenol concentration-response curves (Fig. 4A). In electrically driven guinea pig left atrial strips, isoeugenolol also antagonized isoproterenol-induced positive inotropic actions and produced dose-dependent rightward shifts of the cumulative concentration-response curves to isoproterenol (Fig. 4B). The apparent pA_2 values and slopes of regression lines for isoeugenolol, atenolol, metoprolol and propranolol are indicated in Table 1.

Effects of isoeugenolol on β_2 -adrenoceptor activity

Isoeugenolol (0.1–10 μ M) competitively antagonized

the isoproterenol-induced relaxation of the tracheal strips with spontaneous tone. Furthermore, isoeugenolol produced parallel shifts to the right of the agonist concentration-response curves (Fig. 4C). The apparent pA_2 values and slopes of regression lines for isoeugenolol,

Fig. 2. Effects of isoeugenolol (1.0 mg/kg, ●; 3.0 mg/kg, ▽; 5.0 mg/kg, ▼) on mean arterial blood pressure (MBP) and heart rate (HR) in Wistar rats, anesthetized with pentobarbital. Saline was used as the control (○). Vertical bars represent the change from the baseline value, which was 115 ± 10 mmHg and 375 ± 21 bpm for MBP and HR, respectively. Each point represents the mean $\pm$ S.E.M. of eight rats. *Significantly different from the control, $P < 0.05$ (two way repeated measures ANOVA followed by Student-Newman-Keuls test).

atenolol, metoprolol and propranolol are indicated in Table 1.

β_1/β_2 selectivity of isoeugenolol

The β_1/β_2 -selectivity ratio was estimated from the anti-logarithm of the difference between the mean pA_2 values obtained from the right atria and trachea. Isoeugenolol was 32.4 times more potent on right atria than on trachea; i.e., was highly selective for β_1 -adrenoceptors. Likewise, atenolol and metoprolol exhibited high selectivity for β_1 -adrenoceptors. However, propranolol was only 1.9 times more potent on right atria than on trachea and was, therefore, considered to be non-selective (Table 1).

Lack of ISA of isoeugenolol

In the reserpinized guinea pigs experiments, various effects of isoeugenolol, propranolol and isoproterenol on the frequency of right atria and tension developed by left atria were observed and shown in Fig. 5. Isoproterenol produced concentration-dependent increases in heart rate and contractility with a maximum increase at $1 \mu\text{M}$. Isoeugenolol and propranolol did not increase heart rate or contractility, but produced negative inotropic and chronotropic effects at $>1 \mu\text{M}$. These depressant effects of propranolol usually increased steeply with concentration, leading to arrest or inexcitability of the atrial tissues at concentrations above $100 \mu\text{M}$ (Fig. 5: A and B). On the other hand, isoeugenolol had nearly no intrinsic relaxant effects on the tracheal strips; however, it did simply relax the tracheal tissues at $\geq 100 \mu\text{M}$. At such concentrations,

Fig. 3. Effects of intravenous injection of isoproterenol ($0.5 \mu\text{g/kg}$) in causing a tachycardia before ($\square$) and after ($\blacksquare$) i.v. injection of isoeugenolol (A, 1.0 mg/kg ; B, 3.0 mg/kg ; C, 5.0 mg/kg) in the ganglion-blocked anesthetized rats. Vertical bars represent the change from the baseline value, which was $278 \pm 15 \text{ bpm}$ for HR. Each value represents the mean $\pm$ S.E.M. of six rats. * $P < 0.05$ (paired Student's t -test).

the relaxant effects of isoeugenolol were not affected by pretreatment with the β_2 -adrenergic antagonist ICI 118,551 (0.1 – 10 nM) (data not shown) and were thus in contrast to the previous results with eugenolol (13).

Fig. 4. Effects of isoeugenolol on responses in guinea pig atria and trachea. Mean cumulative concentration-response curves are shown for the positive chronotropic responses to ($-$)isoproterenol in spontaneously-beating guinea pig right atria (A), positive inotropic responses to ($-$)isoproterenol in electrically driven guinea pig left atria (B) and relaxant effects to ($-$)isoproterenol in guinea pig spontaneous tone tracheal strips (C) in the absence ($\circ$) or presence ($0.1 \mu\text{M}$, $\bullet$; $1 \mu\text{M}$, ∇ ; $10 \mu\text{M}$, $\blacktriangledown$) of isoeugenolol. Each point represents the mean $\pm$ S.E.M. of eight individual experiments.

Table 1. β -Adrenoceptor blocking potency and β_1/β_2 -selectivity of isoeugenolol and other β blockers on guinea pig in vitro preparations

β Blocker	β_1		β_2	β_1/β_2 -Selectivity ratio
	pA ₂ value* Right atrium (slope)	pA ₂ value* Left atrium† (slope)	pA ₂ value* Trachea† (slope)	
Isoeugenolol	7.63±0.08 (0.92±0.05)	7.89±0.12 (0.82±0.02)	6.12±0.05 (0.86±0.06)	32.4
Atenolol	7.50±0.03 (0.97±0.10)	7.31±0.05 (0.85±0.03)	5.95±0.06 (0.90±0.07)	35.5
Metoprolol	7.85±0.09 (0.95±0.07)	7.76±0.04 (0.87±0.09)	6.50±0.07 (0.85±0.04)	22.4
Propranolol	8.46±0.06 (0.95±0.04)	8.39±0.09 (0.81±0.05)	8.19±0.12 (0.92±0.08)	1.9

The pA₂ values and slope values were calculated from individual Schild plots by regression analysis. The β_1/β_2 -selectivity ratio was obtained from the antilogarithm of the difference between the mean pA₂ values obtained from right atrium and trachea.

*Each pA₂ value was the mean±S.E.M. of six to eight experimental results. †pA₂ values were obtained from the formula $pA_2 = [\log (CR - 1) - \log \text{molar concentration antagonist}]$ using CR values that were adjusted for changes in tissue sensitivity.

Vasorelaxant effects of isoeugenolol

In isolated rat thoracic aorta, isoeugenolol (1, 10, 100 μ M) diminished the VSM tonic contractions induced by (–)phenylephrine (10 μ M), 5-HT (10 μ M) and high K⁺ (75 mM) in a concentration-dependent manner. The vasorelaxant response of isoeugenolol was more potent in (–)phenylephrine- and 5-HT-contracted than in high K⁺-contracted aorta preparations, whereas its VSM relaxant effect produced no significant differences in (–)phenylephrine- and 5-HT-induced contractions (Fig. 6). Furthermore, isoeugenolol (1, 10, 100 μ M) pretreatment was shown to attenuate both phasic and tonic contractions produced by (–)phenylephrine. However, equimolar concentrations of isoeugenolol lowered the phasic contraction more potently than the tonic one (Fig. 7).

Effects of isoeugenolol on carbachol or KCl-contracted tracheal smooth muscle

Guinea pig tracheal strips pre-contracted by carbachol (1 μ M) were found to relax in a dose-dependent manner upon isoeugenolol (50, 100, 500 μ M) application. In addition, isoeugenolol also produced relaxation of tracheal strips pre-contracted by high K⁺. The relaxing action of isoeugenolol on the trachea was more potent in carbachol-induced than excess [K⁺]_o-induced contractions at concentrations between 50 and 100 μ M. However, at concentrations up to 500 μ M, isoeugenolol caused tracheal relaxation below the basal tension and achieved 140% relaxation both in carbachol- and high K⁺-contracted preparations (Fig. 8).

Effects of isoeugenolol on radioligand binding studies

[³H]CGP-12177 did bind predominantly to β_1 -, β_2 - and β_3 -AR sites in rat ventricle, lung and IBAT membranes in a saturable manner, respectively. Scatchard analysis was used to determine the affinity and number of binding sites. The K_d for [³H]CGP-12177 binding to the rat β_1 - and β_2 -ARs was 0.16±0.03 and 1.21±0.15 nM, respectively, and the maximum binding capacity (B_{max}) was 43.1±2.6 and 294.5±20.4 fmol/mg protein at 25°C, respectively. In addition, Scatchard analysis of the rat IBAT membrane preparations indicated the existence of two populations of [³H]CGP-12177 binding sites. Their K_d and B_{max} values were determined by non-linear regression analysis using the LIGAND program. Estimated K_d and B_{max} values of the high affinity [³H]CGP-12177 binding sites were 0.83±0.19 nM and 137.5±14.6 fmol/mg protein, respectively, whereas the low affinity [³H]CGP-12177 binding sites had values of 40.21±8.30 nM and 607.1±70.6 fmol/mg protein, respectively. The binding affinity of isoeugenolol and various β -adrenergic blockers for predominant β_1 -, β_2 and β_3 -AR sites expressed as –log IC₅₀ values are indicated in Table 2. Isoeugenolol, atenolol and metoprolol were shown to have a high affinity for β_1 -AR sites, but propranolol was shown to lack specific affinities for β_1 - or β_2 -ARs. Moreover, propranolol bound to rat IBAT β_3 -AR more potently than isoeugenolol and metoprolol. However, atenolol did not bind significantly to rat IBAT β_3 -AR.

Fig. 5. Stimulant and depressant effects of (–)isoproterenol (○), propranolol (●) and isoeugenol (▽) on frequency of spontaneously-beating right atria, tension developed by left atrial strips driven at 2-sec intervals and spontaneous tone of tracheal strips from reserpinized guinea pigs. Cumulative concentration-response curves were determined for the three agents identified in panels A and B. All measurements are expressed as mean percentages of the control frequency or force in the same preparation. Each point shows the mean $\pm$ S.E.M. of six individual experiments.

DISCUSSION

Isoeugenol differs from classical β -blockers such as propranolol because of its trachea and VSM relaxant activities. In the *in vivo* study, the biphasic depressor activity of isoeugenol, unrelated to α -adrenoceptor blocking, was in great contrast to the pressor response obtained in propranolol shown by Dasgupta (27) and our previous reports (12–18). Furthermore, vascular β_2 -adrenoceptors-associated ISA was also not found in the hypotensive activity of isoeugenol. The precise mechanism for this paradoxically hypotensive action remains to be elucidated.

Harms (4) demonstrated that the guinea pig atrium and trachea seem to be fairly good models for the study of

Fig. 6. The vasorelaxant effects of isoeugenol (A, 1 μ M; B, 10 μ M; C, 100 μ M) on (–)phenylephrine (PE, □), high K^+ (KCl, ■) and 5-HT (▨)-induced contractile responses in rat thoracic aorta. Each value represents the mean $\pm$ S.E.M. of six separate preparations. *Significantly different from the control, $P < 0.05$ (ANOVA followed by Dunnett's test). †Significant difference between responses of PE- and KCl-induced contractions ($P < 0.05$ by unpaired *t*-test). ‡Significant difference between responses of 5-HT- and KCl-induced contractions ($P < 0.05$ by unpaired *t*-test). NS: Non-significant difference between responses of PE- and 5-HT-induced contractions.

Fig. 7. Effects of isoeugenol (A, control; B, 1 μ M; C, 10 μ M; D, 100 μ M) on phenylephrine (PE)-induced phasic contractions in EGTA-physiological solution (□) and on tonic contractions induced by the reintroduction of $CaCl_2$ (■). Contractile forces are expressed as percentages of PE-induced maximum responses of the control group. Each value represents the mean $\pm$ S.E.M. of six separate preparations. *Significantly different from the control, $P < 0.05$ (ANOVA followed by Dunnett's test). †Significant difference between responses of PE-induced phasic and tonic contractions ($P < 0.05$ by paired *t*-test).

Fig. 8. The tracheal relaxant effects of isoeugenol (A, 50 μ M; B, 100 μ M; C, 500 μ M) on carbachol ($\square$)- and high K^+ (KCl, $\blacksquare$)-induced contractile responses in guinea pig trachea. Each value represents the mean $\pm$ S.E.M. of six separate preparations. *Significantly different from the control, $P < 0.05$ (ANOVA followed by Dunnett's test). †Significant difference between responses of carbachol- and KCl-induced contractions ($P < 0.05$ by unpaired t -test).

β_1 - or β_2 -adrenoceptor selectivity. Isoeugenol was found to antagonize competitively any increases in beating rate and force of contraction brought about by isoproterenol, suggesting that isoeugenol possessed β_1 -adrenoceptor antagonist activity. Similarly, isoeugenol's competitive antagonism of any relaxation of the trachea preparation induced by isoproterenol implied that isoeugenol possessed β_2 -adrenoceptor antagonist action. The ratio of β_1 vs β_2 selectivity for isoeugenol, atenolol, metoprolol and propranolol were 32.4, 35.5, 22.4 and 1.9, respectively. This result suggested that isoeugenol, atenolol and metoprolol were highly selective β_1 -blockers. However, propranolol was a non-selective β -adrenoceptor blocker.

We have demonstrated that isoeugenol and propranolol seemed to be devoid of direct ISA on β_1 -adrenocep-

tors. Furthermore, we found that isoeugenol produced the relaxation of tracheal strips only at high concentrations; this effect was not affected by pretreatment with ICI 118,551, a highly selective β_2 -adrenergic antagonist. So, we suggest that isoeugenol's tracheal relaxant effect was not related to direct stimulation of β_2 -receptors and thus confirm its lack of direct ISA on β_2 -adrenoceptors.

Isoeugenol diminished the (–)phenylephrine- and 5-HT-contracted aorta more significantly than those contracted with high K^+ . The phenylephrine-induced contractile response is biphasic, consisting of a fast and a slow component (28). The fast component has been confirmed to be due to the mobilization of intracellular calcium (29), whereas the slow component was directly dependent on the influx of calcium through receptor-operated Ca^{2+} channels (30); however, in the rat aorta, a contribution of voltage-dependent Ca^{2+} channels (VDC) cannot be excluded (31). Isoeugenol was shown to inhibit the fast phase more significantly than the slow phase, indicating that isoeugenol had stronger actions on phenylephrine-sensitive intracellular Ca^{2+} release than on phenylephrine-induced Ca^{2+} influx. In rat aorta, the activation of the 5-HT₂ receptor by addition of 5-HT induces a biphasic contraction (32). An initial transient contraction produced by IP₃-mediated release of Ca^{2+} from intracellular stores was followed by a slow sustained contraction primarily caused by Ca^{2+} influx through VDC (33, 34). In addition, Marriott (35) has pointed out that high K^+ -induced rat aorta contractions are mainly related to activation of VDC. ISA of β -adrenergic antagonist was suggested as an alternative vasodilating mechanism. Isoeugenol was shown to not have any ISA. Hence, the possibility of direct stimulation of vasodilatory β_2 -adrenoceptors leading to the VSM relaxant effect of isoeugenol can be excluded. From the above results, we suggest that the vasorelaxant effects of isoeugenol might mainly contribute to the change of Ca^{2+} sensitivity of the contractile apparatus.

Isoeugenol attenuated the carbachol-contracted

Table 2. Binding affinity of various β -adrenergic antagonists for predominant β_1 -, β_2 - and β_3 -adrenergic receptor sites in rat ventricle, lung and interscapular brown adipose tissue (IBAT) membranes, respectively, measured with [³H]CGP-12177

β Blockers	Ventricle (mainly β_1)		Lung (mainly β_2)		IBAT (mainly β_3) ^b	
	–log IC ₅₀ ^a	K _i (nM)	–log IC ₅₀	K _i (nM)	–log IC ₅₀	K _i (nM)
Isoeugenol	5.82 $\pm$ 0.09 (1.51)	209	4.74 $\pm$ 0.05 (18.20)	6859	4.73 $\pm$ 0.12 (18.62)	13542
Atenolol	5.75 $\pm$ 0.08 (1.78)	245	4.69 $\pm$ 0.07 (20.42)	7696	NC	—
Metoprolol	6.10 $\pm$ 0.12 (0.79)	110	5.25 $\pm$ 0.10 (5.62)	2120	4.80 $\pm$ 0.15 (15.85)	11526
Propranolol	8.79 $\pm$ 0.17 (0.0016)	0.22	8.75 $\pm$ 0.21 (0.0018)	0.67	5.65 $\pm$ 0.18 (2.24)	1628

^aNegative logarithm of molar concentration required to displace half of the radiolabeled ligand from specific sites. The values are the mean $\pm$ S.E.M. of the three separate experiments performed in triplicate. The IC₅₀ values are expressed in parentheses in μ M. ^bThe results indicate the low affinity binding sites. K_i: inhibition constant. NC: not calculated, because the 50% displacement of [³H]CGP-12177 was not achieved at concentrations up to 10^{–4} M.

trachea more significantly than those contracted with high K^+ . In airway smooth muscle, sources for increased $[Ca^{2+}]_i$ during contraction include release of Ca^{2+} from intracellular stores and influx of Ca^{2+} via VDC. The relative contribution of each of these sources of Ca^{2+} depends on the stimulus used to produce the contraction and differs for the initiation and maintenance phases of contraction (36). KCl produces contraction by depolarizing the muscle membrane, thereby activating Ca^{2+} entry through VDC for both initiation and maintenance of contraction (37). For muscarinic agonist-induced contractions, initiation is associated with release of Ca^{2+} from intracellular stores, whereas the maintenance phase is also thought to require sustained entry of Ca^{2+} through VDC (38), although we did not measure the effects of isoeugenolol on $[Ca^{2+}]_i$ at all. However, it seems possible that isoeugenolol may inhibit tracheal smooth muscle contraction through suppressing the Ca^{2+} sensitivity of the contractile machinery. Further study on this Ca^{2+} sensitivity of the contractile machinery is still necessary.

Isoeugenolol was found to inhibit $[^3H]CGP-12177$ binding to rat ventricle, lung and IBAT membrane preparations. Scatchard analysis of saturation curves of ventricle and lung membrane preparations were linear, suggesting a single population of binding sites in the tissues. In this report, the K_d value obtained in the rat ventricle membrane is in a good agreement with the K_d reported by Porzig et al. (39). Nevertheless, the K_d value obtained in the rat lung membrane is approximately fivefold higher than that reported by Pauwels et al. (40). The discrepancy between the two K_d values can not be readily explained. Likewise, Scatchard analysis of the IBAT saturation curve using the LIGAND program indicated the presence of two populations of binding sites. These estimated K_d values are in close agreement with those reported by Muzzin et al. (23). Muzzin et al. (23) suggested that the low affinity binding sites in IBAT represents the β_3 -AR, whereas the high affinity binding sites represents the β_1/β_2 -AR subtypes. From the binding results, isoeugenolol, atenolol and metoprolol were clearly demonstrated to possess high affinity for β_1 -AR sites. However, no difference was found in the affinities of propranolol for β_1 - and β_2 -ARs. This result was consistent with the β_1/β_2 -selectivity ratio of those β -blockers obtained from the in vitro functional study. In addition, the order of inhibition for $[^3H]CGP-12177$ binding to the rat IBAT β_3 -AR by such β -blockers was propranolol > metoprolol $\approx$ isoeugenolol, but atenolol was shown to bind very little to IBAT β_3 -AR.

In vivo and in vitro pharmacological investigations have shown that isoeugenolol is a potent and highly selective β_1 -adrenergic antagonist with tracheal and VSM relaxant activities, but is devoid of ISA and α -adreno-

ceptor blocking action. Therefore, we suggest that isoeugenolol with tracheal smooth muscle relaxation may be clinically valuable to diminish unwanted pulmonary adverse effects and asthmatic attacks in patients with bronchospastic diseases that occur in the administration of non-selective β -blockers.

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