J. Pharm. Pharmac., 1978, 30, 273–279 Received December 5, 1977

Actions of procaterol (OPC-2009), a new β_2-adrenoceptor stimulant, on pulmonary resistance, contractions of the soleus muscle, and cardiovascular system of the anaesthetized cat

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The β-adrenoceptor stimulant actions of procaterol hydrochloride [5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate] were compared with those of isoprenaline, orciprenaline and salbutamol on pulmonary resistance, contractions of the soleus muscle, heart rate and diastolic blood pressure in the anaesthetized cat. All four drugs reduced the 5-HT-induced increase in pulmonary resistance, decreased the tension of incomplete tetanic contractions of the soleus muscle and the diastolic blood pressure, and increased the heart rate in a dose-related manner. The duration of the bronchodilator action of procaterol was far longer than that of orciprenaline or salbutamol while isoprenaline had the shortest duration. Procaterol was about 1·5 times more potent, and orciprenaline and salbutamol about 80 and 10 times less potent than isoprenaline in reducing the 5-HT-induced increase in pulmonary resistance and in decreasing the tension of incomplete tetanic contractions of the soleus muscle. Procaterol, orciprenaline and salbutamol were 3·5, 91·9 and 43·9 times less potent than isoprenaline in increasing the heart rate. Procaterol, orciprenaline and salbutamol were 3·4, 130·2 and 12·9 times less potent than isoprenaline in decreasing the diastolic blood pressure. Calculation of selectivity for bronchial *vs* cardiac β-adrenoceptors indicates that procaterol and salbutamol have a similar degree of selectivity for β_2-adrenoceptors mediating the bronchodilation, and that orciprenaline was an essentially unselective stimulant. Furthermore, the results support the suggestion that at present it is not possible to separate the bronchodilating and tremor-enhancing properties of β-adrenoceptor stimulants.

Sympathomimetic amines are used for the treatment of reversible air way obstruction. Isoprenaline, one of the popular bronchodilators, has certain drawbacks which consist of marked side effects due to stimulation of β-adrenoceptors in the cardiovascular system, a short duration of action, and a lack of oral activity. Lands, Luduena & Buzzo (1967b) and Lands, Arnold & others (1967a) proposed a division of β-adrenoceptors into a β_1-group in cardiac muscle and a β_2-group in bronchial muscle, skeletal muscle and vascular smooth muscle. The drawbacks of isoprenaline have led to the synthesis of compounds with selectivity for β_2-adrenoceptors. Salbutamol (Brittain, Farmer & others, 1968; Cullum, Farmer & others, 1969), terbutaline (Bergman, Persson & Wetterlin, 1969), and carbuterol (Wardell, Colella & others, 1974) are such drugs. These β_2-adrenoceptor stimulants when given systemically can produce effective bronchodilation in man with relatively little cardiac stimulation (Legge, Gaddie & Palmer, 1971), but they increase skeletal muscle tremor in some subjects (Freedman, 1971; Epstein, Barnard & Zsotér, 1973; Marlin & Turner, 1975; Watson & Richens, 1974). Bowman & Nott (1970) have suggested that cat soleus muscle would provide a useful model for detecting the likelihood of this side effect and that the β-adrenoceptors in soleus muscle can be classified as of the β_2-type. Subsequent studies (Bowman & Rodger, 1972; Rodger, 1973; Apperley, Daly & Levy, 1976) have shown that it may not be possible to differentiate β_2-adrenoceptors in soleus muscle from those in bronchial smooth muscle.

Recently, Yoshizaki, Tanimura & others (1976) synthesized a potent bronchodilator, 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate, procaterol hydrochloride. Procaterol is highly selective for β_2-adrenoceptors as judged from results obtained on the bronchial and cardiovascular systems of dog (Himori & Taira, 1977; Yabuuchi, Yamashita & Tei, 1977) and in guinea-pig isolated trachea and atrium (Takayanagi, Kondo & others, 1977). Furthermore, the bronchodilator effect of procaterol is longer lasting than that of salbutamol in anaesthetized dogs (Yabuuchi & others, 1977) and in blood

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perfused tracheal preparations of dog *in situ* (Himori & Taira, 1977). However, the selectivity of β-adrenoceptor stimulants for β_2- *vs* β_1-adrenoceptors can differ depending upon the animal species and experimental conditions (*in vitro vs in vivo*) used (Bowman & Raper, 1976).

In view of this, the β-adrenoceptor stimulant properties of procaterol on the bronchial, skeletal and cardiovascular systems of cats were investigated in comparison with those of isoprenaline, orciprenaline and salbutamol.

MATERIALS AND METHODS

Thirty cats of either sex, 2·5 to 4 kg, were anaesthetized with a mixture of α-chloralose (80 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}) injected intraperitoneally. The trachea was intubated and the animal was artificially respired with a respirator (Harvard Apparatus, Model 661) at a rate of 27–30 breaths min^{-1} with an air volume of about 13 ml kg^{-1}. Bilateral vagotomy was carried out in all animals to eliminate vagal reflexes. The systemic blood pressure was measured from the right carotid or femoral artery by a pressure transducer (San-ei Instrument, MPU–0.5) and the heart rate by a cardiotachometer (Data Graph, T–149) triggered by the blood pressure pulse. The effects of test drugs on heart rate and blood pressure were not measured in experiments on pulmonary resistance because the intravenous infusion of 5-hydroxytryptamine (5-HT) increased the heart rate and decreased the blood pressure. All the recordings were made on an ink-writing recticoder (San-ei Instrument, Rectihoriz 8S).

Pulmonary resistance. The method used was essentially the same as that described by Diamond (1967) and Rodger (1974). Air flow rate was measured by a pneumotachograph (San-ei Instrument, Type 9104) and tidal volume by integrating electronically this air flow signal with an integrator (Data Graph, Computer unit IG–110). Transpulmonary pressure was measured by a differential pressure transducer (San-ei Instrument, Type DLPU–0.05–3P), one input of which was connected through polyethylene tubing to a 14 gauge needle inserted at the left fifth or sixth intercostal space into the intrapleural space and the other was connected to the side arm of the tracheal cannula. Pulmonary resistance was calculated electronically with an analogue computer (Data Graph) from the signals of air flow rate and transpulmonary pressure at isovolumic points on the tidal volume according to the formula described by Amdur & Mead (1958). Electronically calculated values were confirmed by manual calculation using the flow and pressure values read from the chart. A sustained increase in pulmonary resistance (200–400% of control value) was obtained by infusion of 5-HT (20–40 μg kg^{-1} min^{-1}, i.v.) with an infusion pump (Harvard Apparatus, Model 901). When the effect of 5-HT was stabilized, the test drugs were injected cumulatively as described by Rodger (1974) through a cannula in the femoral vein. Responses to each drug were expressed as a percentage of the maximum response to isoprenaline in each preparation. Dose-response curves to isoprenaline were obtained at 30 min to 1 h intervals until the sensitivity became constant. Two or three dose-response curves were needed to obtain consistent dose-response curves to isoprenaline which had markedly less than a 2-fold difference in sensitivity. 30 min to 1 h later, a dose-response curve to a second drug was determined in each preparation.

Soleus muscle. The left soleus muscle was prepared in essentially the same way as described by Bowman & Nott (1970). The cat was laid prone and the left hind limb was rigidly fixed in a horizontal position by means of two pairs of clamps at the tibia and fibula and the femur. A skin incision was made from the level of the Achilles tendon to the popliteal space and the soleus muscle was dissected free from the neighbouring muscles, e.g. the gastrocnemius and plantaris muscles. Bipolar platinum electrodes were placed on the soleus nerve close to the muscle. By raising up the skin flaps a pool was made and was filled with warm liquid paraffin maintained at about 37° by means of a heating lamp. The soleus nerve was stimulated with rectangular pulses of about twice the voltage required to elicit a maximum twitch (6–10 V) and 100 μs duration by means of an electronic stimulator (MEC, Model ME–6022) and submaximal incomplete tetanic contractions were elicited at a frequency of 8–10 Hz for 1 s every 10 s. The contraction of the soleus muscle was measured isometrically by a force-displacement transducer (San-ei Instrument, Type 45072). Resting tension (30–100 g) was adjusted to give an optimal evoked twitch tension (Bowman & Nott, 1970). The test drugs were injected cumulatively as described by Nott & Raper (1972) through a cannula in the jugular vein. Responses to each drug were expressed as a percentage of the maximum response to isoprenaline in each preparation. Consistent dose-response curves to isoprenaline were obtained

and 30 min to 1 h later dose-response curve to a second drug was determined in each preparation.

Drugs. The drugs used were procaterol hydrochloride [(±)-5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate, OPC-2009] (Otsuka), (−)-isoprenaline hydrochloride (Nikken Kagaku), (±)-salbutamol sulphate (Leiras), (±)-orciprenaline sulphate (Lusochimica), 5-hydroxytryptamine creatinine ulphate (Sigma) and (±)-propranolol hydrochloride (ICI). All drugs were dissolved in 0·9% saline and diluted with 0·9% saline to desired concentrations. Doses of all drugs refer to their salts.

Statistical methods. Values in the text are arithmetic means ± standard errors. The difference between mean values was analysed with Student's *t*-test and taken as significant when P values $< 0{\cdot}05$. Parallelism of dose-response curves was analysed by the use of parallel line assay. The dose-response curves were treated as linear regressions and analysed for similarities in slope. The criteria for significance were P values $< 0{\cdot}05$.

RESULTS

Effects of procaterol, isoprenaline, orciprenaline and salbutamol on pulmonary resistance

The mean resting pulmonary resistance in anaesthetized cats was 17·4 ± 2·0 cm H_2O litre^{-1} s^{-1} (n = 15). The increase in pulmonary resistance induced by 5-HT reached its peak within 15 min of the start of infusion and remained at this level or a level slightly below the initial peak. Procaterol (0·001–1 μg kg^{-1}, i.v.), isoprenaline (0·001–1 μg kg^{-1}, i.v.), orciprenaline (0·1–30 μg kg^{-1}, i.v.) and salbutamol (0·01–10 μg kg^{-1}, i.v.) reduced the 5-HT-induced increase in pulmonary resistance in a dose-related manner. Fig. 1 shows traces from one of experiments obtained with procaterol. The dose-response curves to procaterol, isoprenaline, orciprenaline and salbutamol for reduction of the 5-HT-induced increase in pulmonary resistance were parallel (Fig. 2A). The mean dose of isoprenaline producing 50% of its maximum effect (ED50) was 0·024 ± 0·004 μg kg^{-1} (n = 15). When compared at ED50 values, the activity ratio of procaterol relative to isoprenaline (= 1) was 0·78 ± 0·12. Those of orciprenaline and salbutamol were 80·8 ± 9·82 and 10·2 ± 1·88, respectively. The activity ratios of the four drugs are summarized in Table 1. The peak responses to doses producing about 50% maximum effect were attained within 25–50 s with isoprenaline, orciprenaline or salbutamol, and 60–180 s with procaterol. Times to half recovery from peak effects induced by maximum doses used were about 2·5–4·5 min for isoprenaline, 15–60 min for orciprenaline, 15–30 min for salbutamol and over 60 min for procaterol. Thus, the duration of action of procaterol was longer than that of orciprenaline or salbutamol while isoprenaline had the shortest duration. The sustained effect of procaterol on pulmonary resistance was reversed by propranolol (300 μg kg^{-1}, i.v.) (Fig. 1).

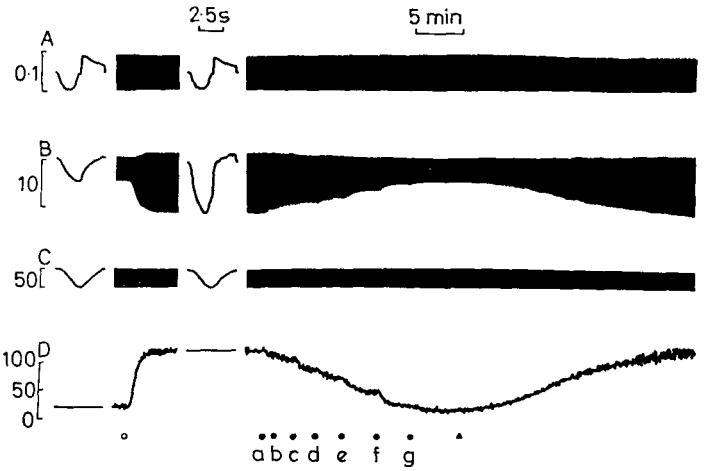

FIG. 1. Effect of procaterol on the 5-HT (30 μg kg^{-1} min^{-1}, ○)-induced increase in pulmonary resistance. Pen recordings were made at two different chart speeds. Doses of procaterol injected intravenously were as follows (μg kg^{-1}); a, 0·001; b, 0·002; c, 0·007; d, 0·02; e, 0·07; f, 0·2; g, 0·7. Propranolol (300 μg kg^{-1}, ▲) was injected during the sustained responses to procaterol. Ordinates: A: Air flow rate (litres s^{-1}); B: Transpulmonary pressure (cm H_2O); C: Tidalvolume (ml); D: Pulmonary resistance (cm H_2O litre^{-1} s^{-1}).

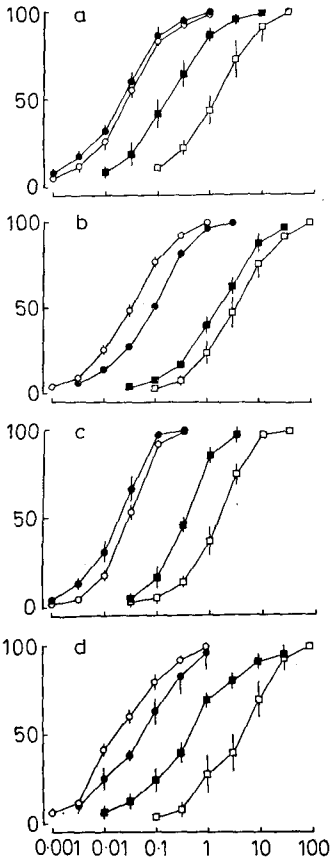

FIG. 2. The cumulative dose-response curves to procaterol (●), isoprenaline (○), orciprenaline (□) and salbutamol (■) for reduction of the 5-HT-induced increase in pulmonary resistance (a), increase in heart rate (b), decrease in tension of incomplete tetanic contractions of the soleus muscle (c) and decrease in diastolic blood pressure (d). Each point represents the mean and vertical bars show s.e. The number of experiments is 15 for isoprenaline and 5 for procaterol, orciprenaline and salbutamol. The responses to each drug were expressed as a percentage of the maximum responses of isoprenaline. Ordinate: % maximum response. Abscissa: Dose of β-stimulants ($\mu g\ kg^{-1}$).

Effects of procaterol, isoprenaline, orciprenaline and salbutamol on soleus muscle, heart rate and diastolic blood pressure

The mean developed tension of incomplete tetanic contractions of the soleus muscle, heart rate and diastolic blood pressure were 846·2 ± 49·8 g (n = 15), 126·8 ± 4·0 beats min^{-1} (n = 15) and 104·9 ± 6·2 mm Hg (n = 15), respectively. Procaterol (0·001–3 $\mu g\ kg^{-1}$, i.v.), isoprenaline (0·001–1 $\mu g\ kg^{-1}$, i.v.), orciprenaline (0·03–100 $\mu g\ kg^{-1}$, i.v.) and salbutamol (0·03–30 $\mu g\ kg^{-1}$, i.v.) decreased the tension and the degree of fusion of incomplete tetanic contractions of the soleus muscle, decreased diastolic blood pressure, and increased the heart rate in a dose-related manner. Fig. 3 shows traces from one of the experiments obtained with procaterol and the data obtained with the four drugs are summarized in Fig. 2b, c and d. The dose-response curves to procaterol, isoprenaline, orciprenaline and salbutamol for decrease in tension of incomplete tetanic contractions of the soleus muscle were parallel and the four drugs caused a similar maximum decrease in tension of incomplete tetanic contractions of the soleus muscle which ranged from 40 to 59% of the original tension. The mean dose of isoprenaline producing 50% maximum decrease (ED50) was 0·030 ± 0·004 $\mu g\ kg^{-1}$. When compared at ED50 values, the activity ratio of procaterol relative to isoprenaline (= 1) was 0·63 ± 0·05. Those of orciprenaline and salbutamol were 76·4 ± 13·60 and 10·5 ± 1·82, respectively. In these experiments, the effect of procaterol was sustained for over 1 h after dosing, although the durations of action of the four drugs were not determined. As shown in Fig. 3, the sustained decrease in the tension and the degree of fusion of incomplete tetanic contractions of the soleus muscle produced by a maximally effective dose (3 $\mu g\ kg^{-1}$) of procaterol was reversed by propranolol (300 $\mu g\ kg^{-1}$, i.v.). Dose-response curves to procaterol for increase in heart rate and decrease in diastolic blood pressure were parallel to those of isoprenaline, orciprenaline and salbutamol, and the four drugs caused a similar maximum increase in heart rate which ranged from 54 to 96 beats min^{-1} and decrease in diastolic blood pressure which ranged from 38 to 74 mm Hg. The mean doses of isoprenaline producing 50% of its maximum effects (ED50) were 0·04 ± 0·007 $\mu g\ kg^{-1}$ for heart rate and 0·03 ± 0·001 $\mu g\ kg^{-1}$ for diastolic blood pressure. When compared at ED50 values, the activity ratios of procaterol, orciprenaline and salbutamol relative to isoprenaline were 3·5 ± 0·22, 91·9 ± 8·92 and 43·9 ± 6·13, respectively, for heart rate and 3·4 ± 0·77, 130·2 ± 25·06 and 12·9 ± 2·85, respectively, for diastolic blood pressure.

Selectivity for β-adrenoceptors mediating the bronchodilation vs *those mediating the increase in heart rate, the decrease in tension of incomplete tetanic contractions of the soleus muscle or the decrease in diastolic blood pressure*

The 'selectivity' of procaterol, orciprenaline and salbutamol for β-adrenoceptors mediating bronchodilation *vs* those mediating the increase in heart rate, the decrease in tension of incomplete

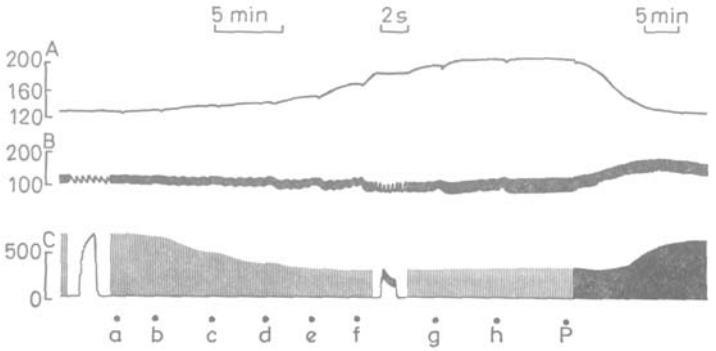

FIG. 3. Effects of procaterol on A: heart rate (beats min^{-1}), B: blood pressure (mm Hg) and C: the tension and degree of fusion of incomplete tetanic contractions of the soleus muscle (g) (9 Hz for 1 s every 10 s). Pen recordings were made at three different chart speeds. Doses of procaterol injected intravenously were as follows ($\mu g\ kg^{-1}$): a, 0·001; b, 0·002; c, 0·007; d, 0·02; e, 0·07; f, 0·2; g, 0·7; h, 2. Propranolol (300 $\mu g\ kg^{-1}$, P) was injected intravenously during the sustained responses to procaterol.

tetanic contractions of the soleus muscle or the decrease in diastolic blood pressure, were calculated from the doses of a drug producing a 50% response of its own maximum (ED50) and then expressed as the ratio of each compound relative to isoprenaline (= 1). These values are shown in Table 1.

The selectivity value of procaterol for β-adrenoceptors mediating the bronchodilation *vs* those mediating the decrease in tension of incomplete tetanic contractions of the soleus muscle was about 1, and was almost equal to those of orciprenaline and salbutamol. On the other hand, the selectivity value of procaterol for β-adrenoceptors mediating the bronchodilation *vs* those mediating the increase in heart rate was 4·5 and was almost equal to that of salbutamol and approximately 4 times larger than that of orciprenaline. The selectivity value of procaterol for β-adrenoceptors mediating the bronchodilation *vs* those mediating the decrease in diastolic blood pressure was 4·4, and was about 3 times larger than those of orciprenaline and salbutamol.

DISCUSSION

In this study, the reduction of the 5-HT-induced increase in pulmonary resistance, the decreases in tension of incomplete tetanic contractions of the soleus muscle and in diastolic blood pressure, and the increase in heart rate in response to procaterol were antagonized by propranolol. Thus, the effects of procaterol on each organ in the cat were attributable to its β-adrenoceptor stimulant action as previously described in dogs (Himori & Taira, 1977; Yabuuchi & others, 1977).

In reducing the 5-HT-induced increase in pulmonary resistance procaterol was 1·5 times more potent than isoprenaline, but orciprenaline and salbutamol were 80 and 10 times less potent than isoprenaline. The relative activities of orciprenaline and salbutamol to isoprenaline determined in this study were almost equal to those obtained by previous investigators (Bowman & Rodger, 1972; Rodger, 1973, 1974; Apperley & others, 1976; Malta & Raper, 1976). Thus, the results indicate that procaterol has the most potent bronchodilator

Table 1. *Activity ratios and selectivity ratios for isoprenaline, orciprenaline, salbutamol and procaterol on pulmonary resistance, soleus muscle, heart rate and diastolic blood pressure in the anaesthetized cat.*

Parameter	Isoprenaline Activity[a] ratio	n[b]	Orciprenaline Activity ratio	n	Selectivity[c] ratio	Salbutamol Activity ratio	n	Selectivity ratio	Procaterol Activity ratio	n	Selectivity ratio
Pulmonary resistance	1	15	80·8 ± 9·82	5	1	10·2 ± 1·88	5	1	0·78 ± 0·12	5	1
Soleus muscle	1	15	76·4 ± 13.60	5	1·0	10·5 ± 1·82	5	1·0	0·63 ± 0·05	5	0·8
Heart rate	1	15	91·9 ± 8·92	5	1·1	43·9 ± 6·13	5	4·3	3·5 ± 0·22	5	4·5
Diastolic blood pressure	1	15	130·2 ± 25·06	5	1·6	12·9 ± 2·85	5	1·3	3·4 ± 0·77	5	4·4

[a] Activity ratio = ED50 of a test drug/ED50 of isoprenaline.
[b] n is the number of experiments.
[c] Selectivity ratio = Activity ratio on soleus muscle, heart rate or diastolic blood pressure/activity ratio on pulmonary resistance.

action in the cat, and are consistent with those obtained previously in dogs (Himori & Taira, 1977; Yabuuchi & others, 1977) and in guinea-pig isolated tracheal muscle (Takayanagi & others, 1977). The duration of bronchodilator action of procaterol was over 60 min, and longer than that of orciprenaline or salbutamol. One of the likely explanations is that procaterol does not include a catechol group and consequently is not a substrate for catechol-*O*-methyltransferase. The low rate of its biological inactivation may also contribute to its long duration of action, although no comparative studies on rates of metabolism and excretion of procaterol, salbutamol and orciprenaline have been done.

In decreasing the tension of incomplete tetanic contractions of the soleus muscle, procaterol was about 1·5 times more potent, and orciprenaline and salbutamol were 75 times and 10 times less potent than isoprenaline. On the other hand, procaterol was about 4 times less potent than isoprenaline in decreasing diastolic blood pressure and in increasing heart rate. Orciprenaline and salbutamol were 130 times and 10 times less potent in decreasing diastolic blood pressure and 90 times and 40 times less potent in increasing heart rate than isoprenaline. The results obtained with isoprenaline, orciprenaline and salbutamol in this study were almost compatible with the results obtained by previous investigators (Bowman & Nott, 1970; Bowman & Rodger, 1972; Rodger, 1973; Apperley & others, 1976; Malta & Raper, 1976).

The above results indicate procaterol to be more active in mediating bronchodilation and decrease in tension of incomplete tetanic contractions of the soleus muscle than in mediating increase in heart rate and decrease in diastolic blood pressure.

The selectivity of procaterol, orciprenaline and salbutamol for β-adrenoceptors mediating bronchodilator action *vs* those mediating decrease in tension of the incomplete tetanic contractions of the soleus muscle was almost 1. The results are consistent with the conclusion of Bowman & Rodger (1972) that β-adrenoceptors mediating decrease in tension of incomplete tetanic contractions of the soleus muscle are closely similar to the β_2-adrenoceptors that mediate bronchodilation (Lands & others, 1967a, b). The present results indicate therefore that procaterol may produce muscle tremor in some patients.

The degrees of selectivity of procaterol and salbutamol for β_2-adrenoceptors mediating bronchodilation *vs* those for β_1-adrenoceptors mediating increase in heart rate were smaller than those obtained in anaesthetized dogs previously (Yabuuchi & others, 1977). These findings are consistent with the view that the selectivity of β_2-adrenoceptor stimulants are smaller in the cat than in other laboratory animals (Bowman & Rodger, 1972; Rodger, 1973; Houston & Rodger, 1974; Davey, Malta & Raper, 1974; Bowman & Raper, 1976; Apperley & others, 1976).

The selectivities for β_2-adrenoceptors mediating bronchodilation *vs* those mediating decrease in diastolic blood pressure were 4·4 for procaterol, 1·6 for orciprenaline and 1·3 for salbutamol. Thus, procaterol was 2·7–4·4 times more selective than isoprenaline, orciprenaline or salbutamol for bronchial smooth muscle compared with vascular smooth muscle. This indicates that procaterol would have less hypotensive side effects than other drugs and that β_2-adrenoceptors mediating bronchodilation are different from those mediating the decrease in diastolic blood pressure in cats, as already suggested by previous investigators (Bristow, Sherrod & Green, 1970; Wasserman & Levy, 1974; Wardell & others, 1974; Himori & Taira, 1977; Yabuuchi & others, 1977) in various mammalian species.

Acknowledgement

We are grateful to Prof. N. Taira, Department of Pharmacology Tohoku University School of Medicine, Sendai, for his advice and suggestion given throughout the experiment and during preparation of the manuscript.

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