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ASSESSMENT OF THE SELECTIVITY OF OPC-2009, A NEW β_2-ADRENOCEPTOR STIMULANT, BY THE USE OF THE BLOOD-PERFUSED TRACHEA *in situ* AND OF THE ISOLATED BLOOD-PERFUSED PAPILLARY MUSCLE OF THE DOG

N. HIMORI & N. TAIRA

Department of Pharmacology, Tohoku University School of Medicine, Sendai 980, Japan

1 The potency and selectivity of 5-(1-hydroxy-2-isopropylamino)butyl-8-hydroxy carbostyril hydrochloride hemihydrate (OPC-2009), a new β_2-adrenoceptor stimulant, was compared with those of isoprenaline, trimetoquinol and salbutamol by the use of blood-perfused tracheal preparations *in situ* and of blood-perfused papillary muscle preparations of the dog. All drugs were injected intra-arterially.

2 All the four drugs decreased tracheal intraluminal pressure (tracheal relaxation) and increased tracheal blood flow in a dose-dependent manner. The four drugs produced a dose-dependent increase in developed tension of papillary muscles. In both preparations the duration of action of isoprenaline and salbutamol was short, whereas that of OPC-2009 and trimetoquinol was long. These effects were antagonized by propranolol.

3 Dose-response curves to the four drugs for tracheal relaxation were almost parallel. OPC-2009 was 2.4 times more potent, and trimetoquinol and salbutamol were 2.2 and 6.2 times less potent than isoprenaline in causing tracheal relaxation.

4 Dose-response curves to the four drugs for tracheal vasodilatation were also parallel. OPC-2009, trimetoquinol and salbutamol were 3.9, 6.7 and 23 times less potent than isoprenaline.

5 Slopes of the dose-response curves to the four drugs for increased developed tension were not parallel; that of OPC-2009 was the least steep, whereas that of isoprenaline was the steepest. Trimetoquinol, salbutamol and OPC-2009 were about 18, 570 and 2400 times less potent than isoprenaline.

6 Selectivity calculated from relative potencies indicate that OPC-2009 was about 6000 times, salbutamol about 92 times and trimetoquinol about 8.2 times more selective than isoprenaline for tracheal smooth muscle as compared to ventricular muscle.

7 The high potency and selectivity of OPC-2009 for tracheal smooth muscle and its long duration of action suggest its potential usefulness for treatment of bronchial asthma.

8 The present results are also compatible with the concept that β_1-adrenoceptors in cardiac muscle and β_2-adrenoceptors in tracheal and vascular smooth muscle can be distinguished. Furthermore, the results revealed that the β-adrenoceptors mediating tracheal relaxation and vasodilatation may also be different.

Introduction

A β-adrenoceptor stimulant, isoprenaline is one of the most popular bronchodilators but has certain disadvantages. They consist firstly of a rather short duration of action owing to tissue uptake (Gryglewski & Vane, 1970), rapid biotransformation to inactive metabolites (Ross, 1963; Conway, Minatoya, Lands & Shekosky, 1968), or both (Morgan, Sandler, Davies, Conolly, Paterson & Dollery, 1969), and secondly of marked side effects due to its action on cardiovascular β-adrenoceptors. This has stimulated search for bronchodilators that are more selective for bronchial than for cardiovascular β-adrenoceptors and longer lasting in their action. Trimetoquinol (Iwasawa & Kiyomoto, 1967; Sato, Yamaguchi & Kiyomoto, 1967), soterenol (Dungan, Cho, Gomoll, Aviado & Lish, 1968), salbutamol (Hartley, Jack, Lunts &

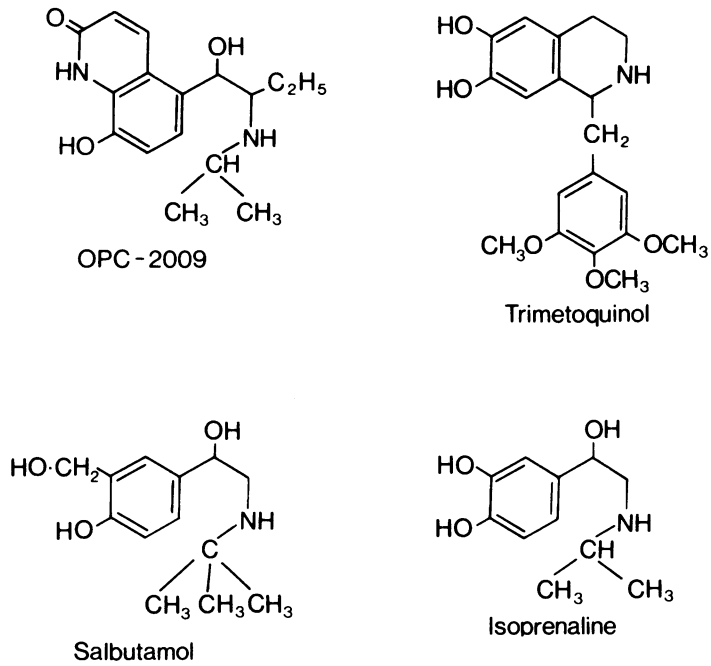

Figure 1 Structures of 5-(1-hydroxy-2-isopropylamino)butyl-8-hydroxy carbostyril (OPC-2009), trimetoquinol, salbutamol and isoprenaline.

Ritchie, 1968; Brittain, Farmer, Jack, Martin & Simpson, 1968; Cullum, Farmer, Jack & Levy, 1969), rimiterol (Bowman & Rodger, 1972), Th1165a (Giles, Williams & Finkel, 1973) and carbutenol (Wardell, Colella, Shetzline & Fowler, 1974) have been developed as such drugs. Lands, Arnold, McAuliff, Luduena & Brown (1967a) and Lands, Luduena & Buzzo (1967b) have proposed that β-adrenoceptors may be pharmacologically differentiated into β_1-adrenoceptors in cardiac muscle and β_2-adrenoceptors in bronchial and vascular smooth muscle. Thus, the broncho-dilators listed above can be classified as selective β_2-adrenoceptor stimulants. Recently, Yoshizaki, Tanimura, Tamada, Yabuuchi & Nakagawa (1976) synthesized a powerful bronchodilator having a β-adrenoceptor stimulant action, 5-(1-hydroxy-2-isopropylamino)butyl-8-hydroxy carbostyril hydrochloride hemihydrate, OPC-2009 (Figure 1). OPC-2009 appears highly selective for β_2-adrenoceptors so far as its selectivity for β_2- v β_1-adrenoceptors is assessed in guinea-pig isolated tracheae and atria (Yoshizaka *et al.*, 1976). In general, however, the selectivities of β_2-adrenoceptor stimulants for β_2- v β_1-adrenoceptors depend upon animal species used for assessment and upon experimental procedures (Brittain, Jack & Ritchie, 1970).

In view of these, we attempted the present experiments to assess the potency and selectivity of OPC-2009 for β_2- v β_1-adrenoceptors in dog tracheal and vascular smooth muscle and ventricular muscle. For this purpose, we used the blood-perfused dog trachea *in situ* (Himori & Taira, 1975; 1976) which allows the injection of drugs selectively to the tracheal vascular bed and the simultaneous observation of responses of vascular and tracheal smooth muscle. We also used the blood-perfused papillary muscle preparation of the dog (Endoh & Hashimoto, 1970). Isoprenaline was used as a reference β-adrenoceptor stimulant in all experiments, and trimetoquinol and salbutamol were included for comparison.

Methods

Blood-perfused tracheal preparations in situ

Fourteen mongrel dogs of either sex, weighing 12–16.5 kg were anaesthetized with pentobarbitone sodium initially at a dose of 30 mg/kg, intravenously and during the experiment, anaesthesia was maintained by continuous infusion of the same anaesthetic at a rate of 5 mg kg^{-1} h^{-1}. The upper cervical region was incised in the midline, and the left and right superior thyroid arteries were dissected out. Muscular, pharyngeal and cricothyroid branches which derive from the superior thyroid arteries but do

not supply the trachea were all ligated. After the animal had been given heparin sodium at a dose of 500 units/kg, intravenously, two arms of a Y-shape cannula were inserted into the left and right superior thyroid arteries. Arterial blood led from the right femoral artery was directed to the Y-shape cannula by the use of a peristaltic pump (Harvard Apparatus, Model 1215). Constant pressure perfusion was accomplished by shunting a fraction of the blood to the femoral vein through a Starling pneumatic resistance set in parallel with the perfusion circuit. The perfusion pressure was monitored at a side arm of the perfusion circuit and the systemic blood pressure was measured at the femoral artery by an individual pressure transducer (Nihon Kohden, MPU-0.5). The perfusion pressure was adjusted initially to give a value approximating the mean systemic blood pressure and kept constant throughout the experiment. The blood flow through the perfused arteries was measured with an electromagnetic flow meter (Nihon Kohden, MF-46-3) set just proximal to the Y-shape cannula. During the experiment heparin sodium (100 units/kg) was given at hourly intervals.

Responses of tracheal smooth muscle were measured as changes in the intraluminal pressure of a water-filled cuff (5 cm in length) of a tracheal tube introduced into the trachea via the mouth. The water-filled cuff was located from 3 to 8 cm caudal to the larynx and connected to a pressure transducer (Nihon Kohden, LPU-0.1) through polyethylene tubing. The resting intraluminal pressure was adjusted to 25–35 cmH_2O. Details of the preparation have been given by Himori & Taira (1976).

Blood-perfused papillary muscle preparation

Nine preparations of the anterior papillary muscle of the right ventricle of the heart were obtained from mongrel dogs of either sex, weighing 9.5–14 kilograms. The preparations were placed in a water jacket warmed at 38–39°C and perfused through the cannulated anterior septal artery at a constant pressure of about 100 mmHg with blood from the right carotid artery of a donor dog. Constant pressure perfusion was achieved by the use of a peristaltic pump (Harvard Apparatus, Model 1215) and a Starling pneumatic resistance set in parallel with the preparation. The venous blood from the preparation and blood passing through the pneumatic resistance was collected by a funnel and returned to the donor dog through the right external jugular vein. The papillary muscle preparations were driven with square-wave pulses of about twice the threshold voltage (0.6–1.4 V) and 5 ms duration at a rate of 2 Hz delivered by an electronic stimulator (Nihon Kohden, MSE 3) via bipolar silver electrodes sutured near the base of the papillary muscle. Isometric contractions of the papillary muscle loaded with a weight of about 1.5 g, were picked up with a force-displacement transducer (Grass, FTO3B) and recorded on a recticorder (San-ei Instrument, Rectiholiz 8S). The blood flow through the anterior septal artery was monitored with an electromagnetic flow meter (Nihon Kohden, MF-46-3).

Donor dogs were anaesthetized initially with pentobarbitone sodium, 30 mg/kg, intravenously and with supplemental doses of about 4.5 mg kg^{-1} h^{-1}, intravenously. Heparin sodium, 500 units/kg, intravenously was given initially and 100 units/kg, intravenously was added at hourly intervals. Details of this preparation have been given by Endoh & Hashimoto (1970).

Drugs used in this study were (−)-isoprenaline hydrochloride (Nikken Kagaku), (±)-OPC-2009 (Otsuka), (±)-propranolol hydrochloride (ICI), (±)-salbutamol sulphate (Leiras) and (±)-trimetoquinol hydrochloride (Tanabe). All these drugs were dissolved in 0.9% w/v NaCl solution (saline) and diluted to desired concentrations with saline. Drug solutions in a volume of 30 µl (in 4 s) were injected by the use of microsyringes into rubber tubing just proximal to the cannulated arteries. Doses of drugs used refer to their bases. Two to four kinds of drugs were administered to each preparation in which the order of drug administration was randomized.

Values in the text are arithmetic means ± s.e. (unless otherwise stated). Differences in mean ED values between agonists were analysed by Student's t test and taken to be significant when $P < 0.05$. Parallelism of dose-response curves was analysed by the use of analysis of covariance techniques described by Snedecor & Cochran (1967). The dose-response curves were treated as linear regressions and analysed for similarities in slope. The criterion for significance was $P < 0.05$.

Results

Blood-perfused tracheal preparations in situ

The average blood flow through the tracheal vascular bed of the 14 dogs was 9.2 ± 0.7 ml/min at the average perfusion pressure of 135 ± 4.7 (s.d.) mmHg. The average resting intraluminal pressure of the trachea was 29 ± 2.1 cmH_2O ($n = 14$).

Single intra-arterial injections of isoprenaline (3 ng–0.3 µg) produced a decrease in intraluminal pressure of the trachea, viz., tracheal relaxation and an increase in blood flow through the tracheal vascular bed, that is, vasodilatation in a dose-dependent manner. Figure 2 (top panel) shows one such experiment. Both tracheal and vascular responses to isoprenaline were reproducible and the preparations were sensitive enough to respond to intra-arterial doses of isoprenaline as small as 1–3 ng. Single intra-arterial injections of OPC-2009 (3 ng–0.1 µg), trimetoquinol (0.01–0.3 µg) and

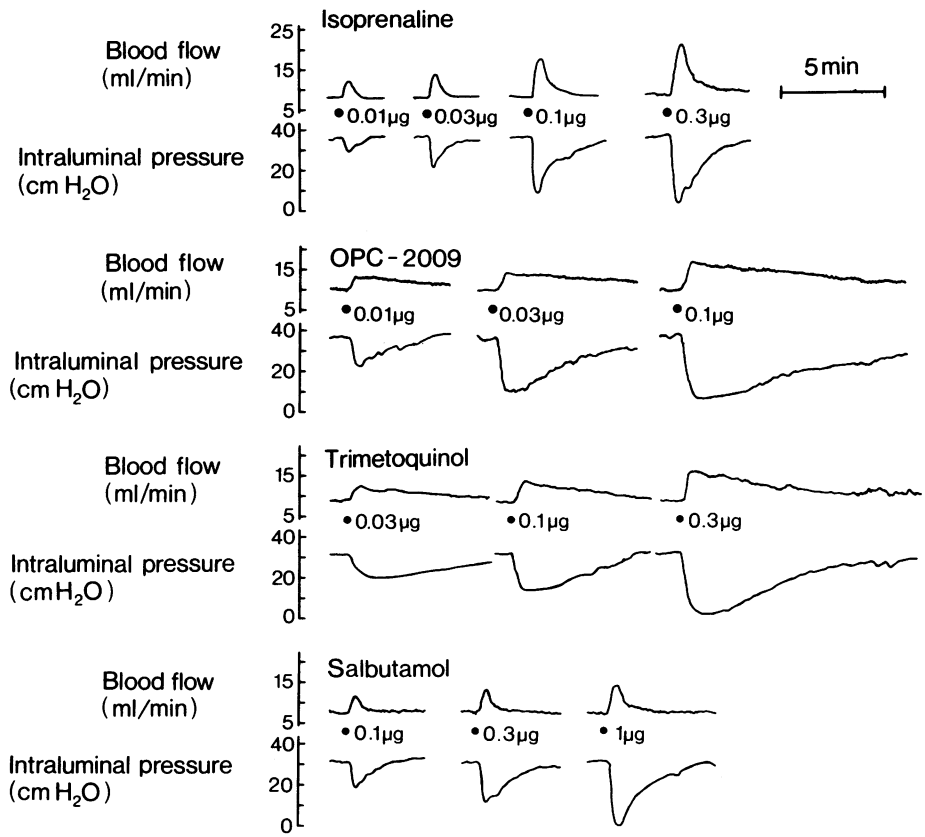

Figure 2 Responses of the tracheal vascular bed (blood flow) and musculature (intraluminal pressure) to intra-arterial isoprenaline, OPC-2009, trimetoquinol and salbutamol.

salbutamol (0.03–1 μg) also produced tracheal relaxation and vasodilatation in a dose-dependent manner (Figure 2). Dose-response curves to four compounds for tracheal relaxation and vasodilatation are shown in Figure 3. The dose-response curves to OPC-2009, trimetoquinol and salbutamol for tracheal relaxation were almost parallel to that of isoprenaline; slopes of these curves were not significantly different (Table 1). The same was true for the dose-response curves for tracheal vasodilatation. When compared in doses reducing the intraluminal pressure by 15 cmH_2O, ($ED_{15\,cmH_2O}$), OPC-2009 was 2·4 times more potent, and trimetoquinol and salbutamol were 2.2 and 6.2 times less potent than isoprenaline in producing tracheal relaxation (Table 2). When compared in doses increasing blood flow by 5 ml/min ($ED_{5\,ml/min}$), OPC-2009, trimetoquinol and salbutamol were 3.9, 6.7 and 23 times less potent than isoprenaline in causing tracheal vasodilatation (Table 2). As can be clearly seen in Figure 2, both tracheal relaxant and vasodilator effects of intra-arterial isoprenaline and salbutamol were brief, whereas those of intra-arterial OPC-2009 and trimetoquinol lasted longer. The tracheal relaxant and vasodilator effects of the four drugs were all antagonized by propranolol (60 μg, i.a.).

Blood-perfused papillary muscle preparations

The basal developed tension of the nine papillary muscles was 5.1 ± 0.3 g and the basal blood flow through the anterior septal artery was 6.2 ± 0.6 ml/minute.

Single intra-arterial injections of isoprenaline (300 pg–0.01 μg), trimetoquinol (3 ng–0.3 μg), salbutamol (0.3–10 μg) and OPC-2009 (0.1–10 μg) all produced a dose-dependent increase in developed tension, i.e., a positive inotropic effect. Typical experiments are shown in Figure 4 and dose-response curves for peak increase in developed tension are shown in Figure 5. As can be seen in Table 1 and

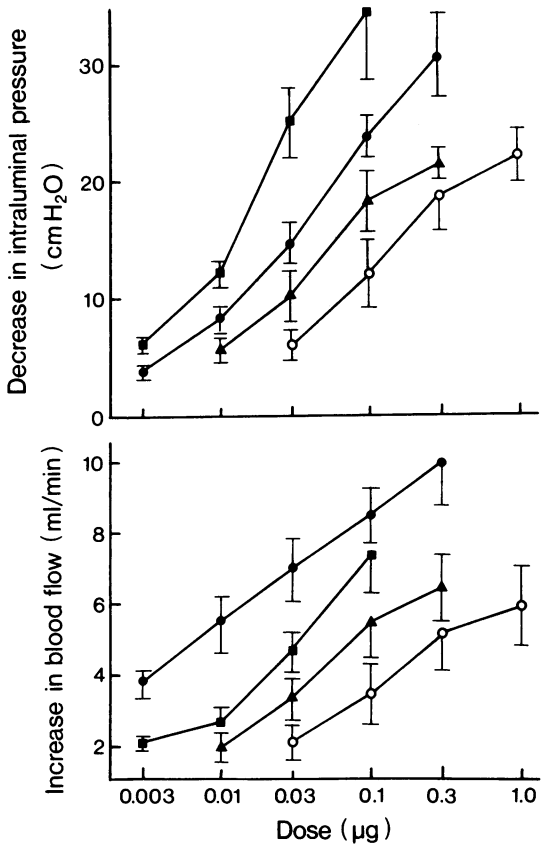

Figure 3 Dose-response curves for decrease in intraluminal pressure of and increase in blood flow through the trachea to intra-arterial isoprenaline (●), OPC-2009 (■), trimetoquinol (▲) and salbutamol (○). Each point represents the mean of 7–13 observations on 14 animals. Vertical bars show s.e. mean.

Figure 5 the slope of the dose-response curves for OPC-2009 was less steep than those for isoprenaline, trimetoquinol and salbutamol. Even with increasing doses of OPC-2009 above 10 μg, the effect of OPC-2009 did not appear to attain that of 0.01 μg of isoprenaline. When compared in doses increasing the developed tension by 2g (ED_{2g}, about 40% of the basal developed tension), trimetoquinol, salbutamol and OPC-2009 were approx. 18, 570 and 2400 times less potent than isoprenaline in increasing force of contraction of ventricular muscle (Table 2). Like the effects on the blood-perfused tracheal preparation, the positive inotropic effects of isoprenaline and salbutamol had a briefer duration of action than those of trimetoquinol and OPC-2009. The positive inotropic effects of the four drugs were antagonized by propranolol (3 μg, i.a.).

Table 1 Slopes of the dose-response curves shown in Figures 3 and 5

Tracheal smooth muscle

	Slope
Isoprenaline (Iso)	15.5
OPC-2009 (OPC)	20.3
Trimetoquinol (Tri)	11.5
Salbutamol (Sal)	11.3
OPC/Iso	$F_{1,55}=1.87$
Tri/Iso	$F_{1,56}=1.51$
Sal/Iso	$F_{1,56}=1.34$

Tracheal vasculature

	Slope
Iso	2.7
OPC	3.9
Tri	3.2
Sal	2.6
OPC/Iso	$F_{1,53}=0.26$
Tri/Iso	$F_{1,61}=0.11$
Sal/Iso	$F_{1,61}=0.01$

Papillary muscle

	Slope
Iso	3.9
Tri	2.4
Sal	3.2
OPC	2.4
OPC/Iso	not calculated because non-parallelism is so evident
Sal/iso	$F_{1,48}=0.59$
Tri/Iso	$F_{1,44}=4.95$

Selectivity for β-adrenoceptors of tracheal smooth muscle v *those of cardiac muscle*

As a measure of selectivity of the four drugs for β-adrenoceptors of tracheal smooth muscle *v* those of cardiac muscle, a ratio of ED_{2g} (the dose increasing the developed tension of papillary muscle by 2g) to $ED_{15\ cmH_2O}$ (the dose reducing the intraluminal pressure of the trachea by 15 cmH_2O) is calculated for each drug, being expressed as a value relative to that of isoprenaline which was taken as 1. The larger the value, the more selective is the action on β-adrenoceptors of tracheal smooth muscle. As shown in Table 2, the values were in descending order: OPC-2009 ≫ salbutamol > trimetoquinol > isoprenaline, being approximately 6000, 92, 8.2 and 1.

Discussion

In the present experiments tracheal relaxation and vasodilatation, and an increase in developed tension of papillary muscles in response to OPC-2009 were all antagonized by suitable doses of propranolol as were those to isoprenaline, salbutamol and trimetoquinol. Thus. OPC-2009 is a β-adrenoceptor stimulant and

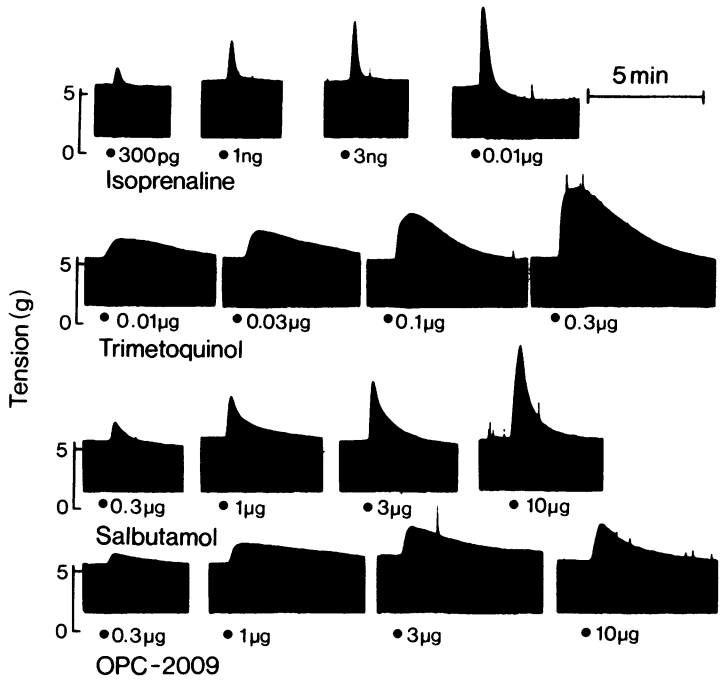

Figure 4 Effects of isoprenaline, trimetoquinol, salbutamol and OPC-2009 injected into the anterior septal artery on the developed tension of blood-perfused dog papillary muscles.

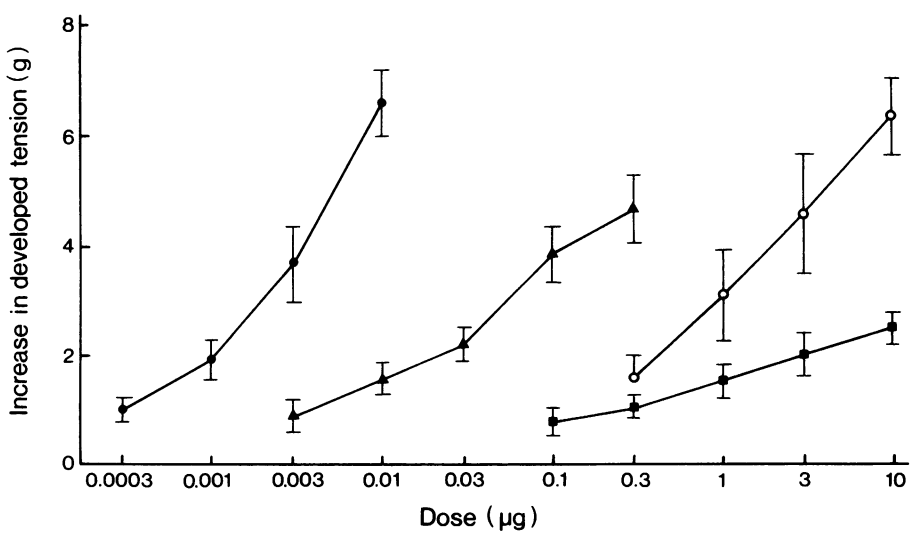

Figure 5 Dose-response curves for increase in developed tension of dog papillary muscles to intra-arterial isoprenaline (●), trimetoquinol (▲), salbutamol (○) and OPC-2009 (■). Each point represents the mean of 6–8 observations on 9 animals. Vertical bars show s.e. mean.

Table 2 Potencies and selectivity of isoprenaline, OPC-2009, trimetoquinol and salbutamol for β-adrenoceptors as determined in blood-perfused dog tracheal and papillary muscle preparations

β-Adrenoceptor stimulants	Tracheal				Papillary muscle		
	Musculature $ED_{15\,cmH_2O}$ (μg)	Relative potency	Vasculature $ED_{5\,ml/min}$ (μg)	Relative potency	ED_{2g} (μg)	Relative potency	Selectivity for tracheal musculature
Isoprenaline	0.037 ± 0.006 (13)	1	0.018 ± 0.006 (13)	1	0.0014 ± 0.0003 (8)	1	1
OPC-2009	0.015 ± 0.002* (8)	0.4	0.07 ± 0.03* (8)	3.9	3.4 ± 1.0* (7)	2400	6000
Trimetoquinol	0.08 ± 0.02* (8)	2.2	0.12 ± 0.03* (8)	6.7	0.025 ± 0.005* (6)	18	8.2
Salbutamol	0.23 ± 0.08* (7)	6.2	0.41 ± 0.20* (7)	23	0.8 ± 0.2* (7)	570	92

Doses are means ± s.e. mean. Number of preparations is shown in parentheses.
$ED_{15\,cmH_2O}$: Dose producing a decrease in intraluminal pressure of 15 cmH_2O. $ED_{5\,ml/min}$: Dose producing an increase in blood flow of 5 ml/minute. ED_{2g}: Dose producing an increase in developed tension of 2 grams. Selectivity for tracheal musculature is calculated by the formula, $ED_{2g}/ED_{15\,cmH_2O}$ and expressed as a value relative to that of isoprenaline which is taken as 1.
* Significantly different from corresponding ED values of isoprenaline.

this is consistent with the conclusion drawn by Yoshizaki *et al.* (1976) for its action in guinea-pig tracheae and atria. In blood-perfused tracheal preparations the dose-response curves to OPC-2009, trimetoquinol and salbutamol for tracheal relaxation were almost parallel to that for isoprenaline. When the potencies of OPC-2009, trimetoquinol and salbutamol relative to that of isoprenaline were compared on the basis of doses decreasing the tracheal intraluminal pressure by 15 cmH_2O ($ED_{15\,cmH_2O}$), OPC-2009 was 2.4 times more potent and trimetoquinol and salbutamol were 2.2 and 6.2 times less potent than isoprenaline in causing tracheal relaxation (Table 2). The values for salbutamol and for trimetoquinol in the present experiments are in good agreement with those obtained previously in the ginea-pig isolated trachea (Brittain *et al.*, 1968; Cullum *et al.*, 1969; Farmer, Kennedy, Levy & Marshall, 1970). Thus the use of the present preparations for assessment of potencies of β-adrenoceptor stimulant bronchodilators is valid. It is worth noting that in the present preparations OPC-2009 was about 2.4 times as potent as isoprenaline in causing tracheal relaxation (Table 2).

In blood-perfused papillary muscle preparations the dose-response curves to the four drugs for increase in developed tension were not parallel. The slopes of the curves for salbutamol, trimetoquinol and OPC-2009 were less steep than that for isoprenaline in that order. Thus, it is not easy to determine their positive inotropic potencies relative to that of isoprenaline. Based on doses producing an increase in developed tension by 2 g (ED_{2g}) trimetoquinol, salbutamol and OPC-2009 were approx. 18, 570 and 2400 times less potent than isoprenaline in producing the positive inotropic effect on ventricular muscle of the dog heart. The value obtained for trimetoquinol is roughly equal to that determined on force of contraction of the heart *in situ* of open-chest dogs (Sato *et al.*, 1967). However, the value obtained for salbutamol does not agree with those reported in guinea-pig isolated left atria (Cullum *et al.*, 1969; Farmer *et al.*, 1970; Brittain *et al.*, 1970). The discrepancy between the values for salbutamol may be due to differences in animal species used, heart muscle (atrial or ventricular), experimental conditions (*in vitro* and *in vivo*), and the way in which dose-response curves were constructed. Indeed, in the present experiments no attempt has been made to obtain the maximum contractile response, because large doses tended to produce arrhythmias and a decrease in developed tension after an increase which resulted in deterioration of the preparations. It is noteworthy that OPC-2009 was about 2400 times less potent than isoprenaline in producing the positive inotropic effect on ventricular muscle of the dog heart.

Selectivity for β-adrenoceptors in tracheal smooth muscle as compared with those in ventricular muscle calculated from $ED_{15\,cmH_2O}$ and ED_{2g} was 6000 for OPC-2009, 92 for salbutamol and 8.2 for trimeto-

quinol. The high selectivity of OPC-2009 for β-adrenoceptors in tracheal smooth muscle taken together with its high potency in this preparation and its long duration of action suggests its potential usefulness for treatment of bronchial asthma. The present results showing a difference in selectivity for β-adrenoceptors in tracheal smooth muscle and ventricular muscle are also consistent with the proposal put forward by Lands *et al.* (1967a, b) that at least two types of β-adrenoceptors can be distinguished; β_1-type in cardiac muscle and β_2-type in bronchial and vascular smooth muscle.

In the tracheal vascular bed the dose-response curves to the four drugs for vasodilatation were almost parallel, and OPC-2009, trimetoquinol and salbutamol were 3.9, 6.7 and 23 times less potent than isoprenaline in causing tracheal vasodilatation. Thus, the relative potencies of the four drugs in producing tracheal vasodilatation are different from those in producing tracheal relaxation in which OPC-2009 was 2.4 times more potent and trimetoquinol and salbutamol were 2.2 and 6.2 times less potent than isoprenaline. OPC-2009 was 9.4 times, salbutamol 3.7 times and trimetoquinol 3.1 times more selective for tracheal smooth muscle than isoprenaline as compared to vascular smooth muscle. This indicates that OPC-2009 would have less hypotensive side effects than the other drugs. However, it would still be premature to draw such a conclusion since at present no information is available as to whether β-adrenoceptors in the whole vascular bed are homogeneous or not. The present results indicate that β-adrenoceptors mediating tracheal relaxation are different from those mediating vasodilatation in the dog trachea, as already suggested by previous workers (Bristow, Sherrod & Green, 1970; Wasserman & Levy, 1974, Wardell *et al.*, 1974) in various mammalian species.

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