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THE β-ADRENOCEPTOR STIMULANT PROPERTIES OF OPC-2009 ON GUINEA-PIG ISOLATED TRACHEAL, RIGHT ATRIAL AND LEFT ATRIAL PREPARATIONS

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1 The β-adrenoceptor stimulant properties of 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate (OPC-2009) were compared with those of isoprenaline and salbutamol on guinea-pig isolated tissues.

2 In producing tracheal relaxation, OPC-2009 was approximately 7 times more potent and salbutamol 5 times less potent than isoprenaline. Both compounds were less potent than isoprenaline in increasing either the rate of beating of isolated right atria or the contractile force of left atria, OPC-2009 being 4 and 127 times and salbutamol being 100 and 700 times less potent on the respective preparations.

3 Selectivity calculated from EC_{50} ratio indicates that OPC-2009 was approximately 26 times and salbutamol approximately 21 times more selective than isoprenaline for tracheal smooth muscle as compared to right atrial muscle, whereas OPC-2009 was approximately 850 times and salbutamol 140 times more selective than isoprenaline for tracheal smooth muscle as compared to left atria.

4 The responses to OPC-2009 on trachea and right atria were not altered by treatment of animals with reserpine 24 h previously. Propranolol was a competitive antagonist of OPC-2009 on these tissues.

5 OPC-2009 at high concentrations competitively antagonized the positive chronotropic and inotropic responses to isoprenaline, indicating that OPC-2009 like salbutamol, may be classified as a partial agonist.

6 The results indicate that the action of OPC-2009 is more selective for tracheal smooth muscle than cardiac muscle and are interpreted in the light of subdivisions of β-adrenoceptors.

Introduction

Sympathomimetic amines are widely used for the treatment of reversible airway obstruction. Isoprenaline has been extensively used since its bronchodilator activity was demonstrated in animals (Konzett, 1940a, b) and in man (Stolzenberger-Seidel, 1940). However, isoprenaline powerfully stimulates β-adrenoceptors in the cardiovascular system, has a short duration of action and is relatively ineffective orally because it is rapidly inactivated by catechol-*O*-methyl transferase (COMT) (Ross, 1963).

Lands and his co-workers (Lands & Brown, 1964; Lands, Arnold, McAuliff, Luduena & Brown, 1967a; Lands, Luduena & Buzzo, 1967b) proposed a division of β-adrenoceptors into a β_1-group in cardiac muscle and a β_2-group in bronchial and vascular smooth muscle. The drawbacks of isoprenaline have led to synthesis of a number of compounds such as salbutamol (Brittain, Farmer, Jack, Martin & Simpson, 1968; Cullum, Farmer, Jack & Levy, 1969) and terbutaline (Bergman, Persson & Wetterlin, 1969) supposed to be more selective for β_2-adrenoceptors in terms of the concept of Lands and his co-workers, or longer-acting than isoprenaline.

Recently, Yoshizaki, Tanimura, Tamada, Yabuuchi & Nakagawa (1976) reported on a novel series of sympathomimetic amines having a carbostyril nucleus. One member of this series, 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate (OPC-2009) (Figure 1) was selected for more detailed pharmacological study, for the preliminary experiments *in vivo* showed it to be a promising compound. The present paper describes the β-adrenoceptor stimulant properties of OPC-2009 in guinea-pig isolated tracheal and atrial preparations in comparison with those of isoprenaline and salbutamol.

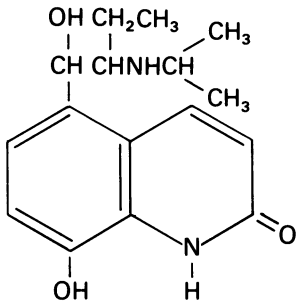

Figure 1 Chemical structure of 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate (OPC-2009).

Methods

Guinea-pig trachea

Male Hartley strain guinea-pigs weighing from 450 to 600 g were used. The trachea was excised and spirally cut preparations (Constantine, 1965) were suspended at a resting tension of 2 g in a 30 ml tissue bath containing Krebs Henseleit solution maintained at 37°C and equilibrated with 95% O_2 and 5% CO_2 for 30 to 60 minutes. Tension changes in the tracheal smooth muscle were recorded isometrically with a force-displacement transducer (San-ei Instrument, Type 45072) on an ink-writing recorder (Nippon Denshi, U-125M).

Phentolamine 10^{-5} M was added to the bathing fluid in order to block α-adrenoceptors. Fifteen minutes later carbamylcholine 10^{-6} M was added to the bathing fluid; this concentration contracted the tissue by 50–70% of its maximum response. Relaxations to the test compounds were obtained in the presence of carbamylcholine which maintained a constant level of tone in the strips for a period of time longer than that required to obtain a concentration-response curve to a test compound (approximately 60 min) which was added cumulatively to the bathing fluid as described by Van Rossum (1963). Responses to each compound were expressed as a percentage of the maximum relaxation in response to isoprenaline in each strip. In each strip isoprenaline concentration-response curves were obtained at about 1 h intervals until the sensitivity became constant. One or 2 h later a concentration-response curve was obtained to a test compound. In a few experiments, the agonistic activities of test compounds were determined in strips obtained from guinea-pigs treated with reserpine (5 mg/kg i.p.) 24 h previously.

In other experiments, consistent concentration-response curves to isoprenaline or OPC-2009 were first established and then the cumulative addition was repeated in the presence of propranolol which had been added 20 min earlier. The procedure was again repeated in the presence of increasing concentrations of propranolol (in the concentration range of 10^{-8} to 10^{-7}M).

The agonistic activity of the test compound was estimated from each concentration-response curve as pD_2 and α (intrinsic activity, maximum response of isoprenaline = 1) values described by Van Rossum (1963), and the antagonistic activity of propranolol was expressed as a pA_2 value (Schild, 1947), calculated according to Arunlakshana & Schild (1959).

Guinea-pig atria

Separate preparations of guinea-pig right and left atria were suspended in 30 ml tissue baths containing Krebs Henseleit solution maintained at 37°C and equilibrated with 95% O_2 and 5% CO_2 for 30 to 60 minutes. The beating rate of the right atria was measured with a cardiotachometer (San-ei Instrument, Type 2130) triggered by contractions which were measured isometrically by a force-displacement transducer (San-ei Instrument, Type 45072).

The left atria were driven with square-wave pulses of about 30% above threshold voltage and 5 ms duration at a rate of 2 Hz, delivered by an electronic stimulator (MEC, Model ME 6022) through bipolar platinum electrodes, essentially the same as those described by Blinks (1966). Contractile force was measured isometrically as with the right atria. The resting tension was 1 g for each preparation. Recordings were made on an ink-writing recticorder (San-ei Instrument, Recti-Horiz 8S).

The test compound was added to the bathing fluid by the cumulative method of drug administration described by Van Rossum (1963). Increases in beating rate were measured as an absolute increase (beats/min) and those of contractile force as an absolute increase in developed tension. In each preparation, isoprenaline concentration-response curves were obtained at intervals of 40 to 60 min until sensitivity became constant, and then a cumulative addition of a test compound was made. In a few experiments, the agonistic activities of test compounds were determined in right atria obtained from guinea-pigs treated with reserpine (5 mg/kg i.p.) 24 h previously.

Further experiments were carried out to evaluate the antagonistic activities of OPC-2009 and salbutamol against the responses to isoprenaline on right or left atrial preparations. In each case reproducible concentration-response curves to isoprenaline were obtained at 40 to 60 min intervals. One of the test compounds was then added to the bathing fluid and after a 30 min contact time a further curve to isoprenaline was established. The procedure was then repeated using a higher concentration of the test compound. Three or four concentration-response

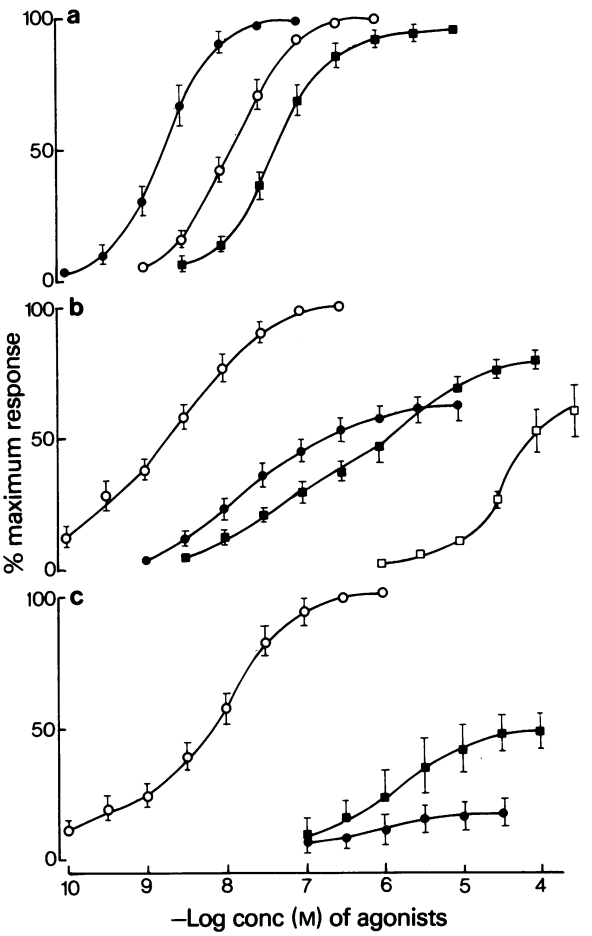

Figure 2 Concentration-response curves for OPC-2009 (●), isoprenaline (○) and salbutamol (■) obtained in (a) tracheal, (b) right atrial and (c) left atrial preparations isolated from guinea-pigs. A curve for tyramine (□) obtained in right atria is also shown. Each point represents the mean of 4–11 preparations. Vertical bars show s.e. mean.

curves to isoprenaline were obtained in the presence of each compound.

As with the tracheal preparations agonistic activities were expressed as pD_2 and α values, and antagonistic activities as pA_2 values.

The drugs used in this study were (±)-5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate (OPC-2009, Otsuka), (±)-isoprenaline hydrochloride (Boehringer Sohn), (±)-salbutamol sulphate (Leiras), tyramine hydrochloride (Wako), carbamylcholine chloride (Sigma), (±)-propranolol hydrochloride (ICI) and reserpine (Daiichi, Apoplon). Isoprenaline and tyramine were dissolved with equimolar ascorbic acid in distilled

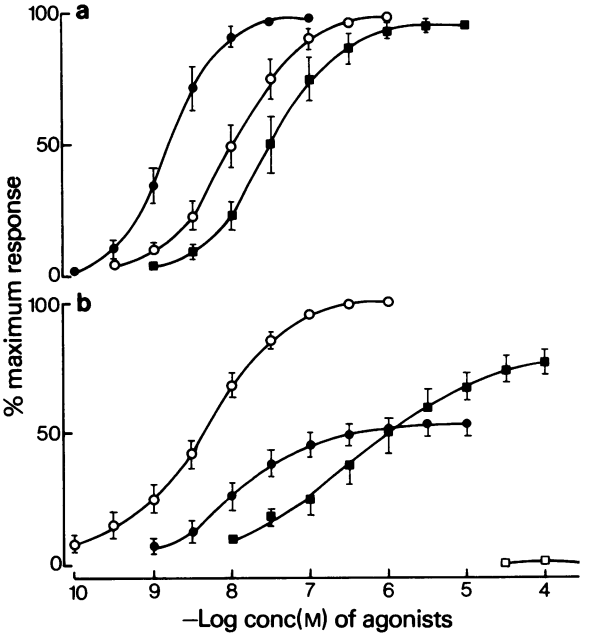

Figure 3 Concentration-response curves for OPC-2009 (●), isoprenaline (○) and salbutamol (■) obtained in (a) tracheal and (b) right atrial preparations isolated from guinea-pigs treated with reserpine (5 mg/kg i.p.) 24 h previously. A curve for tyramine (□) obtained in right atria is also shown. Each point represents the mean of 4–12 preparations. Vertical bars show s.e. mean.

water and other drugs except for reserpine were dissolved in distilled water. Stock solutions were diluted with Krebs Henseleit solution.

Statistical method

Values in the text are geometric means ± s.e. The difference between mean values was analysed by Student's t test and judged to be significant when P values <0.05.

Results

Agonistic activities on isolated trachea

OPC-2009 in concentrations from 10^{-10} to 10^{-7} M, isoprenaline 3×10^{-10} to 10^{-6} M and salbutamol 10^{-9} to 10^{-5} M relaxed tracheal strips contracted by carbamylcholine 10^{-6} M. The concentration-response curve for OPC-2009 was parallel to those for isoprenaline and salbutamol as shown in Figure 2. The pD_2 and α values of these compounds estimated from each concentration-response curve are summarized in

Table 1 pD_2 values, α values, EC_{50} ratios and 'Selectivities' of isoprenaline, OPC-2009 and salbutamol on β-adrenoceptors in isolated tracheal and atrial preparations of guinea-pigs

	Untreated animals			*Reserpine-treated animals*		
	Isoprenaline	*OPC-2009*	*Salbutamol*	*Isoprenaline*	*OPC-2009*	*Salbutamol*
Trachea						
pD_2	7.84 ± 0.08 (11)	8.74 ± 0.10 (5)	7.25 ± 0.10 (6)	7.98 ± 0.16 (10)	8.78 ± 0.10 (5)	7.48 ± 0.15 (5)
α	1	0.99 ± 0.004	0.95 ± 0.03	1	0.99 ± 0.004	0.97 ± 0.02
EC_{50} ratio	–	0.15 ± 0.01	5.15 ± 1.18	–	0.19 ± 0.05	3.6 ± 1.1
Atrial rate						
pD_2	8.45 ± 0.11 (11)	7.86 ± 0.15 (7)	6.49 ± 0.24 (4)	8.45 ± 0.16 (12)	8.07 ± 0.14 (8)	6.45 ± 0.21 (4)
α	1	0.57 ± 0.07	0.73 ± 0.04	1	0.53 ± 0.05	0.76 ± 0.05
EC_{50} ratio	–	3.80 ± 1.13	107 ± 18.6	–	7.05 ± 4.95	155 ± 66.2
Atrial force						
pD_2	8.20 ± 0.14 (8)	6.59 ± 0.27 (4)	5.78 ± 0.26 (4)	Not tested	Not tested	Not tested
α	1	0.14 ± 0.04	0.46 ± 0.07			
EC_{50} ratio	–	127 ± 71.3	707 ± 360			
'Selectivity'						
Trachea/Atrial rate		26	21			
Trachea/Atrial force		847	137			
Atrial rate/Atrial force		33	7			

Values are means ± standard error with number of experiments in parentheses. pD_2 = negative logarithmic molar concentration for 50% maximum response. α = intrinsic activity; maximum response to test compound/maximum response to isoprenaline. EC_{50} ratio = ratio of concentration of test compound to concentration of isoprenaline required to produce 50% of their own maximum response. 'Selectivity' = reciprocal of the ratio of EC_{50} ratios in the respective pairs of tissues.

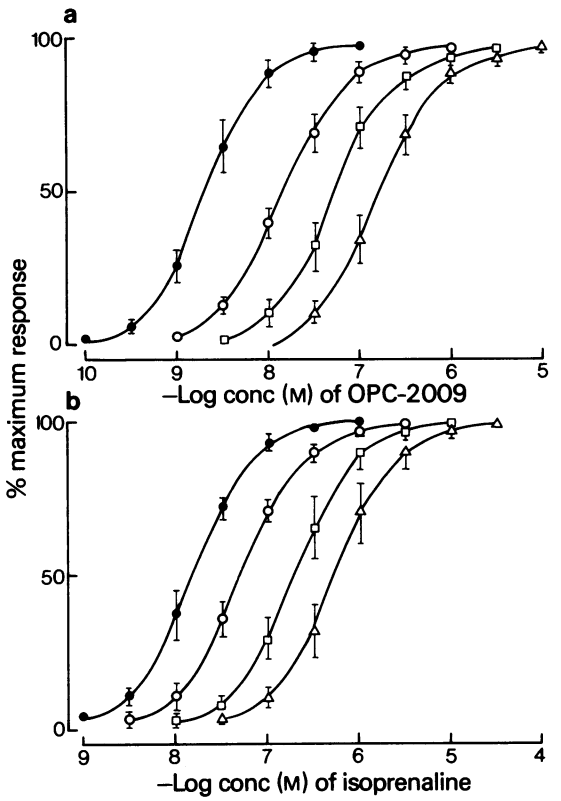

Figure 4 Concentration-response curves for tracheal relaxation to OPC-2009 (a) and isoprenaline (b) in the absence (●) and the presence of propranolol (○; 10^{-8} M; □; 3×10^{-8} M, △; 10^{-7} M) obtained in guinea-pig isolated trachea. Vertical bars show s.e. mean ($n=4$).

Table 1, as are the ratios of the concentrations to produce 50% maximal responses (EC_{50} ratios) which for OPC-2009 and salbutamol relative to isoprenaline (=1) were 0.15 and 5.15 respectively. The responses to OPC-2009, isoprenaline and salbutamol of tracheal strips obtained from guinea-pigs treated with reserpine 24 h previously were not significantly different from those of untreated animals (cf. Figures 2 and 3 and Table 1).

The concentration-response curves for OPC-2009 and isoprenaline on tracheal strips obtained from untreated guinea-pigs were shifted in a parallel fashion to the right in the presence of propranolol in concentrations of 10^{-8} to 10^{-7} M (Figure 4a, b). The pA_2 values of propranolol against OPC-2009 and isoprenaline were 8.67 ± 0.09 ($n=4$, the slope of regression line $= 1.09 \pm 0.08$) and 8.41 ± 0.13 ($n=4$, the slope of regression line $= 1.02 \pm 0.11$), respectively.

Agonistic activities on isolated right atria

OPC-2009, isoprenaline and salbutamol increased the beating rate of right atria obtained from animals either untreated or treated with reserpine (Figures 2 and 3). Tyramine, 10^{-6} to 3×10^{-4} M, increased the rate in atria from untreated animals but not in those from reserpine-treated ones (Figures 2 and 3). Concentration-response curves for these compounds were not parallel; the curves for OPC-2009 and salbutamol were less steep than that for isoprenaline. The maximum response (α value) to OPC-2009 for increase in rate was slightly less than that to salbutamol which was less than that to isoprenaline. The pD_2 values and α values of these compounds estimated from each concentration-response curve are summarized in Table 1. The EC_{50} ratios of OPC-2009 and salbutamol relative to isoprenaline (=1) were 3.8 ± 1.1 and 107 ± 18.6 in right atria from untreated guinea-pigs, and 7.1 ± 5.0 and 155 ± 66 respectively in right atria obtained from animals treated with reserpine. The responses to OPC-2009, 10^{-7} M, were markedly antagonized by propranolol, 10^{-7} M, given 20 min before administration of OPC-2009.

Agonistic activities on isolated left atria

OPC-2009, isoprenaline and salbutamol increased the contractile force of electrically driven left atria of untreated animals (Figure 2). Concentration-response curves for these compounds were not parallel, the curve for OPC-2009 being extremely shallow and that for salbutamol being less steep than that for isoprenaline. The maximum response (α value) to OPC-2009 was very low, being about one-third of that to salbutamol and one-seventh of that to isoprenaline. It was therefore difficult to obtain an accurate pD_2 value for OPC-2009; an approximate estimate is shown in Table 1 for comparison with those for isoprenaline and salbutamol. The EC_{50} ratios of OPC-2009 and salbutamol relative to isoprenaline (=1) were 127 ± 71 and 707 ± 360, respectively. The responses to OPC-2009, 10^{-5} M, were markedly antagonized by propranolol, 10^{-7} M, administered 20 min before exposure to OPC-2009.

Selectivity for β-adrenoceptors of tracheal smooth muscle vs. those of cardiac muscle

Using data from untreated guinea-pigs, the 'selectivity' of OPC-2009 and salbutamol for β-adrenoceptors of tracheal smooth muscle vs. those of right or left atrial muscle were calculated from the concentration of a drug required to produce a response 50% of its own maximum (EC_{50}) and then expressed as the EC_{50} ratio of each compound relative to isoprenaline; these values are shown in Table 1. The selectivity value of OPC-2009 for β-adrenoceptors of tracheal smooth muscle vs. those of right atrial muscle was almost

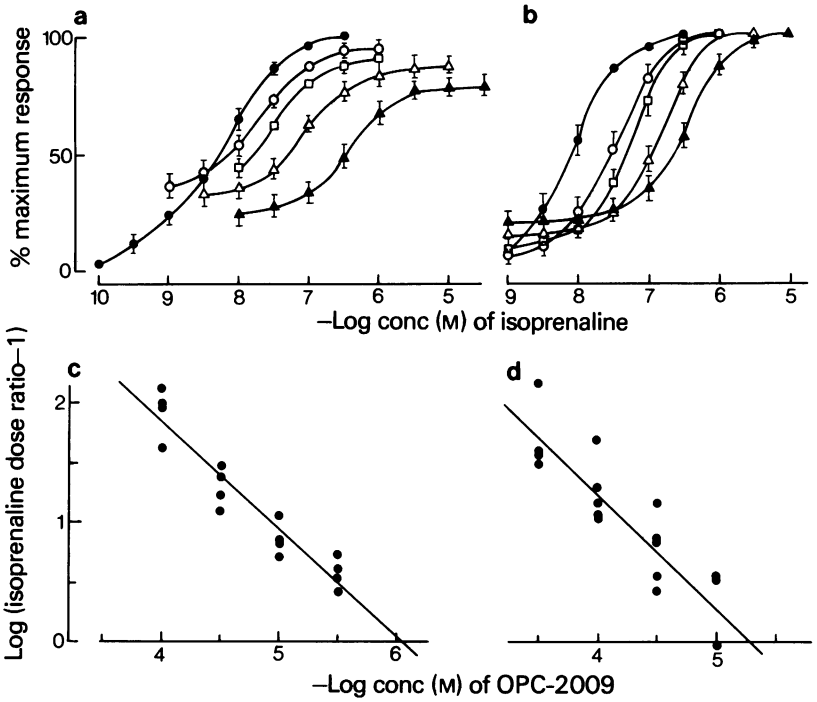

Figure 5 (a) Concentration-response curves to isoprenaline for increase in beating rate of right atria in the absence (●) and the presence of OPC-2009 (○; 3×10^{-6} M, □; 10^{-5} M, △; 3×10^{-5} M, ▲; 10^{-4} M) obtained in right atria. (b) Concentration-response curves to isoprenaline for increase in contractile force of left atria in the absence (●) and the presence of OPC-2009 (○; 10^{-5} M, □; 3×10^{-5} M, △; 10^{-4} M, ▲; 3×10^{-4} M). Relation between the log (isoprenaline dose ratio −1) and the negative logarithm of the molar concentration of OPC-2009 in isolated right atria (c) and left atria (d). Vertical bars show s.e. mean ($n = 3-5$).

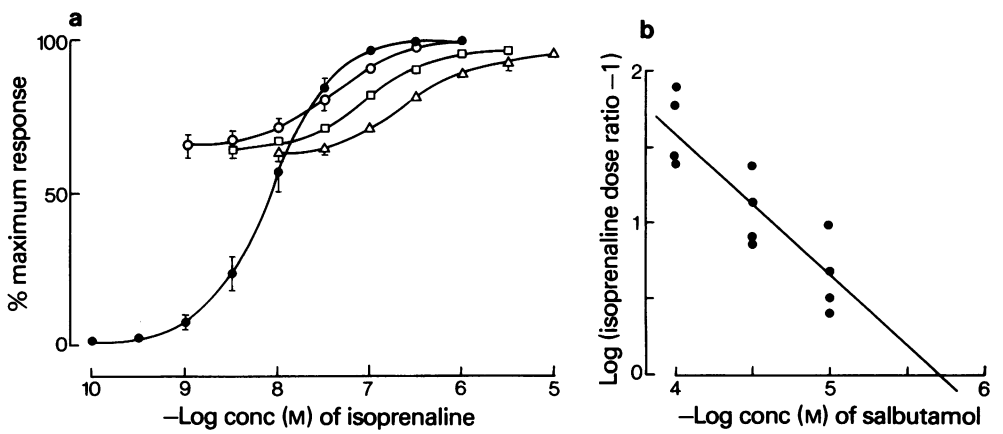

Figure 6 (a) Concentration-response curves to isoprenaline for increase in beating rate of guinea-pig isolated right atria in the absence (●) and the presence of salbutamol (○; 10^{-5} M, □; 3×10^{-5} M, △; 10^{-4} M). (b) Relation between the log (isoprenaline dose ratio −1) and the negative logarithm of the molar concentration of salbutamol obtained from the above data. Vertical bars show s.e. mean ($n = 4$).

equal to that of salbutamol. On the other hand, the selectivity value of OPC-2009 for β-adrenoceptors of tracheal smooth muscle vs. those of left atrial muscle was approximately 6 times larger than that of salbutamol. However, it must be emphasized that this value is markedly influenced by the shallow concentration-response curve to OPC-2009 on left atrial force.

Antagonistic activities in isolated right and left atria

Salbutamol caused an increase in the beating rate of right atria which was maintained for up to 30 minutes. However, OPC-2009 (3×10^{-6} to 10^{-4} M) produced an increase in rate which reached a maximum in 2 to 3 min and gradually declined during the next 30 min to a level 20 to 40% below the peak response. The failure to sustain the peak was more marked with higher concentrations. By contrast, no such attenuation was seen of the increase in contractile force caused by OPC-2009.

Concentration-response curves to isoprenaline for increases in rate of right atria were shifted to the right in the presence of either OPC-2009, 3×10^{-6} to 10^{-4}M, or salbutamol, 10^{-5} to 10^{-4}M (Figure 5a and 6a). The curves to isoprenaline for increase in contractile force of left atria were also shifted to the right in the presence of OPC-2009, 10^{-5} to 3×10^{-4} M (Figure 5b). The relation between the log (isoprenaline dose-ratio − 1) and the negative logarithm of the molar concentration of OPC-2009 or salbutamol is shown in Figures 5c, d and 6b. In these figures, the isoprenaline dose-ratio was estimated from EC_{50} values of isoprenaline in the absence and the presence of OPC-2009 or salbutamol. The pA_2 values of OPC-2009 obtained from experiments on right and left atria were 6.03 ± 0.07 ($n=4$) and 5.28 ± 0.15 ($n=5$), respectively, and the slope of regression line for right and left atria were 0.98 ± 0.03 ($n=4$) and 0.96 ± 0.10 ($n=5$), respectively. The pA_2 value of salbutamol in right atria was 5.71 ± 0.21 ($n=4$) and the slope of the regression line was 0.97 ± 0.14 ($n=4$). These values indicate that OPC-2009 and salbutamol are competitive antagonists of isoprenaline.

Discussion

As clearly demonstrated in this study, OPC-2009 was 4 to 7 times more potent than isoprenaline and approximately 30 times more potent than salbutamol in relaxing tracheal smooth muscle obtained from guinea-pigs, either untreated or treated with reserpine 24 h previously. The concentration-response curve for OPC-2009 was parallel with those for isoprenaline and salbutamol; the curves for OPC-2009 and isoprenaline were shifted in a parallel fashion to the right in the presence of propranolol, and the pA_2 values for propranolol against these two agonists were very similar. Furthermore, the responses to OPC-2009 were not changed by treatment of animals with reserpine. These results indicate that OPC-2009 acts directly on β-adrenoceptors to cause relaxation of tracheal smooth muscle. The relative activity of salbutamol to isoprenaline determined in this study was almost equal to that obtained by previous investigators (Cullum *et al.*, 1969; Farmer, Kennedy, Levy & Marshall, 1970a; Farmer, Levy & Marshall, 1970b).

OPC-2009 increased by similar amounts the beating rate of right atria of guinea-pigs, whether untreated or treated with reserpine, and increased the contractile force of left atria of untreated guinea-pigs (left atria from reserpine-treated guinea-pigs were not tested). However, the maximum response to OPC-2009 was far smaller than that to isoprenaline. Since the positive chronotropic and inotropic responses to OPC-2009 were antagonized by propranolol, 10^{-7} M, and the positive chronotropic responses to OPC-2009 were not changed by treatment of animals with reserpine, they can also be attributed to a direct stimulation of β-adrenoceptors. Other workers have shown that, in isolated atrial preparations of guinea-pigs and rats, the maximum positive inotropic and chronotropic responses to salbutamol are smaller than those to isoprenaline. Salbutamol has, therefore, been classified as a partial agonist (Farmer *et al.*, 1970a; Brittain, 1972; O'Donnell, 1972). In accord with this, salbutamol has been shown to antagonize competitively the positive chronotropic responses to isoprenaline in isolated right atria of guinea-pigs (Raper & Malta, 1973). We have confirmed this finding in the present study. Similarly, it is concluded that OPC-2009 can be classified as a partial agonist because OPC-2009 had small α values and competitively antagonized the positive chronotropic and inotropic responses to isoprenaline in guinea-pig atria. On the other hand, in guinea-pig isolated tracheal preparations, salbutamol and isoprenaline produce almost the same maximum relaxation (Farmer *et al.*, 1970a; O'Donnell, 1972). In this study OPC-2009 produced almost the same maximum tracheal relaxation as did isoprenaline. Therefore, OPC-2009 was much more active on tracheal smooth muscle than on cardiac muscle.

The results can be described in terms of the two kinds of β-adrenoceptors proposed by Lands *et al.* (1967a, b); OPC-2009 is more active on β_2-adrenoceptors in tracheal smooth muscle than on β_1-adrenoceptors in cardiac muscle. Selectivity for β_2-adrenoceptors in tracheal smooth muscle vs. β_1-adrenoceptors mediating an increase in atrial rate was 26 for OPC-2009 and 21 for salbutamol while the figures for bronchial β_2-adrenoceptors vs. β_1-adrenoceptors mediating an increase in atrial contractile force were 847 for OPC-2009 and 137 for salbutamol. Thus, the degree of selectivity of OPC-2009 for β_2-adrenoceptors in tracheal smooth muscle

is greater than that of salbutamol which is the most selective β_2-adrenoceptor stimulant described to date (Cullum *et al.*, 1969; Farmer *et al.*, 1970a, b). Since the selectivity of agonists for β_2-adrenoceptors vs. β_1-adrenoceptors differs according to the animal species and experimental conditions used (Bowman & Raper, 1976), further experiments on other animal species *in vitro* and *in vivo* will be needed to draw a firm conclusion on the selectivity of OPC-2009.

The positive chronotropic effects of OPC-2009 and salbutamol on right atria were greater than the positive inotropic effects on left atria. Selectivity for β-adrenoceptors of right atria vs. those of left atria was 33 for OPC-2009 and 7 for salbutamol. Farmer *et al.* (1970a, b) reported that salbutamol was more effective in increasing the beating rate of right atria than the contractile force of electrically driven left atria as compared to isoprenaline and suggested, therefore, that β-adrenoceptors in the two preparations might differ. The present results with salbutamol confirm those of Farmer *et al.* (1970a, b) and show that the same is true of OPC-2009. Since OPC-2009 and salbutamol can be classified as partial agonist on cardiac muscle, the observed difference in the effectiveness of OPC-2009 and salbutamol on heart rate in comparison with that on contractile force may not necessarily reflect a difference in β-adrenoceptors mediating increase in these two parameters (Furchgott, 1972). However, in this study, the difference in pA_2 values of OPC-2009 between rate and force was 0.75. The value is greater than 0.5 which is required to differentiate receptor types as proposed by Furchgott (1972). These findings may therefore reflect differences in the β-adrenoceptors mediating increase in rate and force.

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