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CHARACTERIZATION OF THE RECEPTOR MEDIATING THE ANTI-ANAPHYLACTIC EFFECTS OF β-ADRENOCEPTOR AGONISTS IN HUMAN LUNG TISSUE *in vitro*

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1 The rank order of potency of six β-adrenoceptor agonists as inhibitors of the anaphylactic release of histamine from fragments of passively sensitized human lung *in vitro* was (−)-isoprenaline > (−)-adrenaline > (±)-salbutamol > (−)-noradrenaline > R0363 > H133/22.

2 The β-adrenoceptor antagonists, propranolol, atenolol and H35/25, blocked the response to both (−)-isoprenaline and (±)-salbutamol competively. Each antagonist gave similar pA_2 values with both agonists. pA_2 values were consistenly at the high end of the range expected for interaction at a β_2-adrenoceptor.

3 Practolol did not antagonize isoprenaline in a competitive manner but was a competitive antagonist of salbutamol with a pA_2 at the high end of the range expected for interaction at a β_2-adrenoceptor.

4 Data obtained with agonists are consistent with the receptor being of the β_2-subtype. Data obtained with antagonists indicate a consistently higher affinity for the receptor than observed for the β_2-subtype in other tissues but do not suggest a novel β-adrenoceptor subtype on the mast cell of the human lung.

Introduction

β-Adrenoceptor agonists inhibit the anaphylactic release of histamine and slow reacting substance of anaphylaxis (SRS-A) from human lung tissue (Orange, Kaliner, LaRaia & Austen, 1971; Strandberg, Pegelow, Persson & Sörenby, 1979; Butchers, Fullarton, Skidmore, Thompson, Vardey & Wheeldon, 1979) and from guinea-pig lung (Assem & Schild, 1971; Malta & Raper, 1975; Sörenby, 1975; Forsberg & Sörenby, 1979). The subclassification of β-adrenoceptors into two distinct types termed β_1- and β_2-adrenoceptors (Lands, Arnold, McAuliff, Luduena & Brown 1967) is now generally accepted. However, there is evidence that the β-adrenoceptor present, presumably on the mast cell, which mediates the anti-anaphylactic activity of β-adrenoceptor agonists in guinea-pig lung may differ from both β_1- and β_2-subtypes (Assem & Schild, 1971; Sörenby, 1975; Malta & Raper, 1975). However, no systematic attempt to classify β-adrenoceptors responsible for anti-anaphylactic activity in human lung has been carried out. We have undertaken such a study using a series of β-adrenoceptor agonists and antagonists to determine the subtype involved.

Methods

Anaphylaxis in fragments of human lung

The preparation of sensitized fragments of human lung, challenge of sensitized fragments with antigen and the general technique for determining the response to drugs were carried out by the method of Butchers *et al.* (1979). Histamine was assayed on the guinea-pig isolated ileum by an automated superfusion technique. The Tyrode superfusion solution contained atropine (0.3 μM) propranolol (0.7 μM) and an antagonist of SRS-A, FPL 55712 (Augstein, Farmer, Lee, Sheard & Tattersall, 1973) (0.4 μM). In experiments to determine the relative potencies of β-adrenoceptor agonists the tissue was incubated with the agonist for 10 min before challenge with antigen. In experiments to determine the potency of antagonists, three antagonist concentrations were used, the tissue was incubated with the antagonist for 10 min, the agonist was then added and incubation continued for a further 5 min followed by challenge with the antigen. All incubations were performed in quadruplicate. No uptake blockers or α-adrenoceptor blocking agents were present in the incubations.

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Calculation of results

Dose-response relationships were calculated from the linear portion of the curve by least squares regression analysis. Agonist potencies were expressed as the negative logarithm of the molar concentration of agonist causing 50% inhibition of the release of histamine (pD_2). Antagonist potencies were calculated by the method of Arunlakshana & Schild (1959). Dose-ratios were determined from EC_{50} values obtained by regression analysis.

Drugs

The following agonists were used: (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop); (−)-noradrenaline bitartrate and (−)-adrenaline bitartrate (Sigma); (±)-salbutamol (Allen & Hanburys); (−)-1-(4-hydroxyphenoxy)-3-isopropylamino-2-propanol (H 133/22, a gift from AB Hässle); (±)-1-(3,4-dimethoxyphenethylamino)-3-(3,4-dihydroxyphenoxy)-2-propanol (R0363, Raper, McPherson & Iakovidis, 1978) synthesized by Mr J. Paton and Mr I. Smith, Glaxo Group Research Limited. All agonists were dissolved in 20 μg/ml ascorbic acid and were used immediately.

The following antagonists were used: propranolol (Sigma); practolol and atenolol (ICI Ltd.); H35/25 (±-erythro-2-isopropylamino-1-(4-tolyl)-1-propanol: AB Hässle); FPL 55712 (8-propyl-7-{3-(4-acetyl-2-propyl-3-hydroxyphenoxy)-2-hydroxyproxy}-4-oxo-4H-benzopyran-2-carboxylic acid) synthesized by Dr D. Brickwood, Glaxo Group Research Limited; atropine sulphate (BDH).

Results

(−)-Isoprenaline inhibited the release of histamine maximally at concentrations between 10^{-8} and 10^{-7} M. The maximum degree of inhibition varied from lung sample to lung sample but was always between 70 and 90%. Although in some experiments the other agonists achieved lower maximum inhibition than isoprenaline, none was consistently a partial agonist, indeed statistical analysis of the tendency of noradrenaline to partial agonist activity (Figure 1) showed that this was not significant ($P > 0.05$). The rank order of potency was (−)-isoprenaline > (−)-adrenaline > (±)-salbutamol > (−)-noradrenaline > R0363 > H133/22 (Table 1).

Antagonist potencies (Table 2) were compared by use of both the non-selective agonist, (−)-isoprenaline, and the β_2-selective agonist, (±)-salbutamol. Propranolol was a competitive antagonist of both agonists (mean pA_2 9.33 and 9.01 respectively). The β_2-selective antagonist, H35/25, was also a competitive antagonist of both agonists (mean pA_2 6.97, 7.04) as was the β_1-antagonist, atenolol (mean pA_2 5.97, 6.24). The β_1-selective antagonist, practolol, was a competitive anatagonist of (±)-salbutamol (mean pA_2 5.11) but with (−)-isoprenaline gave an abnormally high pA_2 value (6.71) and a slope for an Arunlakshana & Schild plot consistently less than unity.

Discussion

In this study, isoprenaline inhibited the release of histamine almost completely with a pD_2 (8.78) in general agreement with the results obtained by Assem & Schild (1971) and Orange, *et al.* (1971). It was, however, more than three orders of magnitude less potent than reported by Strandberg *et al.* (1979).

Salbutamol was a full agonist when compared with isoprenaline and both the rank order of potency: isoprenaline > adrenaline > salbutamol > noradrenaline, and the relative potency of these agonists was consistent with an interaction primarily at a β_2-adrenoceptor. Two selective β_1-agonists, R0363 (Raper *et al.*, 1978) and H133/22 (Carlsson, Dahlöf, Hedberg, Persson & Tangstrand, 1977) were respectively more than four and five orders of magnitude less potent than isoprenaline, suggesting that the concentration of β_1-adrenoceptors on the mast cell is negligible. It was initially considered that the tendency of noradrenaline to act as a partial agonist could result from α-agonist activity, which has been shown to potentiate antigen-induced release of histamine. (Kaliner, Orange & Austen, 1972) and weak activity at β_2-adrenoceptors, but experiments showing that phenoxybenzamine, an α-adrenoceptor antagonist, failed to modify inhibition of histamine release by noradrenaline (Butchers & Vardey, unpublished observations) did not support this possibility.

Four β-adrenoceptor antagonists were used in combination with the non-selective agonist, isoprenaline, and the β_2-selective agonist, salbutamol. With the exception of practolol the antagonism was competitive and the antagonists gave similar pA_2 values with both agonists. The pA_2 values for propranolol were at the upper end of the range obtained in a variety of tissues from man and guinea-pig (see Table 3 for references). Atenolol, a β_1-selective antagonist (Barrett, Carter, Fitzgerald, Hull & LeCount, 1973), gave pA_2 values midway between those reported for tissues containing predominantly β_2-adrenoceptors and for tissues containing β_1-adrenoceptors (Table 3). The pA_2 values for H35/25, a β_2-selective antagonist (Levy & Wilkenfeld, 1969) were higher than those found for relaxation and inhibition of antigen-induced release of histamine in guinea-pig lung (Van der Heijden & Zaagsma, 1979).

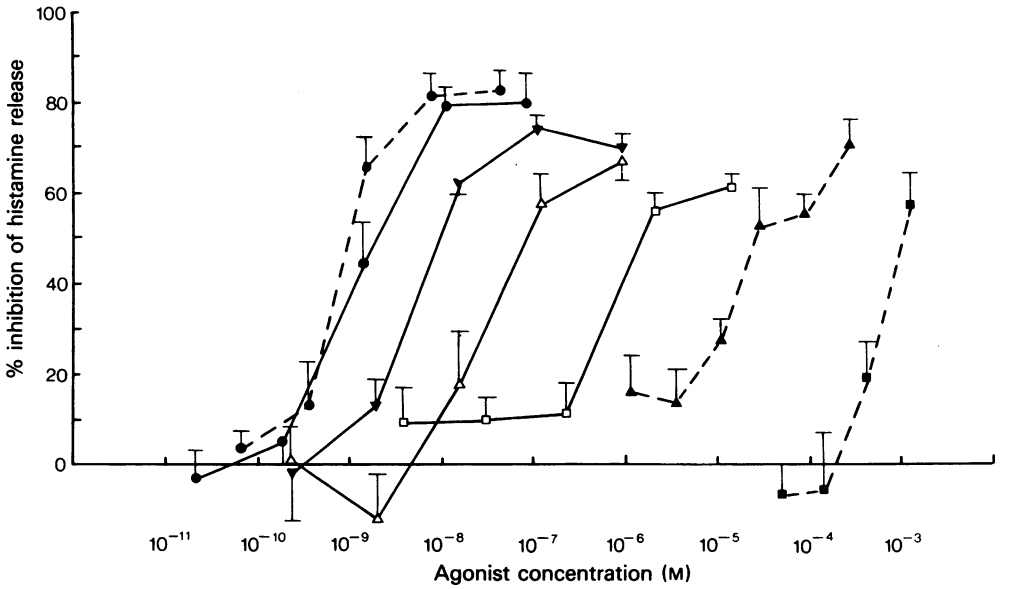

Figure 1 Inhibition of the release of histamine from fragments of passively sensitized human lung by β-adrenoceptor agonists: (●) (−)-isoprenaline; (▼) (−)-adrenaline; (△) (±)-salbutamol; (□) (−)-noradrenaline; (▲) R0363 and (■) H133/22. Points joined by similar (continuous or broken) lines were obtained in the same experiments. Bars indicate s.e. mean.

Practolol, a β_1-selective antagonist (Dunlop & Shanks, 1968), antagonized salbutamol competitively and gave a pA_2 value at the top end of the range for tissues containing predominantly β_2-adrenoceptors (Table 3). It was not a competitive antagonist of isoprenaline and no conclusions can be drawn from the data with this agonist. A similar finding was reported by Coleman & Somerville (1977) for the antagonism of isoprenaline by practolol in membrane preparations from rabbit uterus.

Although the subdivision of β-adrenoceptors into β_1- and β_2-subtypes (Lands, *et al.*, 1967) holds good for most tissues, there are situations where it may be an oversimplication. This has led both to the proposal that several subtypes of β-adrenoceptor exist (Furchgott, 1972) and to the recognition that both β_1- and

Table 1 Inhibition of the release of histamine from fragments of passively sensitized human lung by β-adrenoceptor agonists: comparison with agonist potency in other tissues

	Human lung anaphylaxis				
	$pD_2 \pm$ s.e. mean	(*n*)	EC	EC(β_1-)	EC(β_2-)
(−)-Isoprenaline	8.78 ± 0.08	(11)	1.0	1.0	1.0
(−)-Adrenaline	8.05 ± 0.05	(4)	5.4	10–40	3–15
(±)-Salbutamol	7.22 ± 0.22	(4)	36.3	500 *	10–35
(−)-Noradrenaline	5.87 ± 0.07	(4)	813.3	5–20	60–400
R0363	4.38 ± 0.22	(4)	2.5×10^4	ND	ND
H133/22	2.98 ± 0.08	(4)	6.3×10^5	ND	ND

n = number of separate experiments. ND = not determined. * = partial agonist.
EC = equipotent concentrations, (−)-isoprenaline = 1.0.
EC (β_1-), EC(β_2-) = equipotent concentrations ((−)-isoprenaline = 1.0) in tissues containing predominantly β_1- and β_2- adrenoceptors. Data from Levy & Apperley (1978).

β_2-subtypes can coexist and mediate the same response in a single cell (Levy & Apperley, 1978).

In the present study the pattern of inhibition of histamine release obtained with a range of β-adrenoceptor agonists suggests strongly that the receptor on the mast cell of the human lung is of the β_2-subtype. This is not fully supported by the results obtained with antagonists where there are indications that the receptor may differ in some ways from what is generally accepted as a β_2-adrenoceptor. The high pA_2 values obtained in this study were not found in other human tissues by Harms (1976), nor by Conolly & Greenacre (1977), in human lung parenchyma, and so cannot be a property of human β_2-adrenoceptors in general. The very low potency of the β_1-selective agonists, R0363 and H133/22, suggests the difference is not due to the co-existence of both β_1- and β_2-subtypes on the same cells.

We conclude that the results of this study support the view that the receptor on the mast cell of human lung is a β_2-adrenoceptor in which the affinity for antagonists is increased, presumably by some property inherent in the membrane of this cell rather than a receptor of a novel subtype.

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Table 2 The effect of β-adrenoceptor antagonists on the inhibition of histamine release from passively sensitized fragments of human lung by (−)-isoprenaline and (±)-salbutamol

	Propranolol pA_2 (slope)	*Practolol* pA_2 (slope)	*Atenolol* pA_2 (slope)	*H35/25* pA_2 (slope)
(−)-Isoprenaline	9.35 (1.11)	6.86 (0.52)	5.87 (0.97)	7.10 (1.00)
	9.31 (0.92)	6.83 (0.69)	6.16 (0.97)	6.94 (1.02)
		6.44 (0.73)	5.88 (1.09*)	6.87 (0.91*)
				6.95 (1.06)
(±)-Salbutamol	9.16 (0.95)	4.74 (0.96)	6.16 (0.90)	6.78 (1.18)
	8.86 (0.94)	5.47 (1.00)	6.31 (0.87)	7.43 (0.83)
				6.91 (0.80)

Figures are results from individual experiments performed on different samples of lung. Three concentrations of antagonist were used except (*) where two concentrations only were used.

Table 3 Comparison of mean pA_2 values for β-adrenoceptor antagonists: data for anaphylaxis in human lung compared with data from other human and guinea-pig tissues (isoprenaline as agonist)

System	*Propranolol*	*Practolol*	*Atenolol*	*H35/25*	*Reference*
Human:					
Lung—anaphylaxis	9.33	6.70*	5.97	6.97	This paper
Lung—anaphylaxis (salbutamol as agonist)	9.01	5.11	6.24	7.04	This paper
Atrium—positive inotropy	8.36	6.44	6.95		Harms (1976)
Bronchial muscle—relaxation	8.56	4.65	5.33		Harms (1976)
Guinea-pig:					
Atrium—positive inotropy	8.51	6.50	7.21		Harms (1976)
Atrium—positive chronotropy	8.32	6.49	7.27		Barrett *et al.* (1973)
Tracheal chain—relaxation	8.25	4.87	5.57		Harms (1976)
Lung strip—relaxation		4.91		6.39	Van der Heijden & Zaagsma (1979)
Lung—anaphylaxis		4.70		6.50	
Various tissues with β_1 profile	8.3–8.8	6.5–6.9			Levy & Apperley (1978)
Various tissues with β_2 profile	8.3–9.4	4.6–5.1			

*Slope of Arunlakshana & Schild plot consistently less than unity.

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