β -ADRENOCEPTORS IN HUMAN LUNG, BRONCHUS AND LYMPHOCYTES

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1 The ability of propranolol, metoprolol, acebutolol, atenolol and practolol to reverse the stimulant effect of 10^{-5} M isoprenaline on lymphocyte cyclic AMP levels was determined. The ratios of doses required to produce 50% inhibition as compared with propranolol were 1:316 for metoprolol 1:1780 for acebutolol and 1:2820 for atenolol. No ratio could be calculated for practolol as it never produced more than 35% inhibition possibly because of its intrinsic sympathomimetic activity. This rank order of antagonist potencies suggest the lymphocyte β -receptor has β_2 selectivity.

2 For peripheral airway smooth muscle isoprenaline, adrenaline and noradrenaline had relative EC_{50} s of 1:25:9290 respectively in the absence of uptake blockade. However, the EC_{50} s changed to 1:15:82 in the presence of neuronal uptake blockade and α -adrenoceptor blockade. These observations are consistent with β_2 selectivity. Salbutamol behaved as a partial agonist on peripheral airway smooth muscle. It was possible to demonstrate competitive antagonism to isoprenaline. Estimates of the dissociation constant of salbutamol were obtained $(6.6 \pm 0.02) \times 10^{-7}$ M, $n=4$, mean $\pm$ s.e. mean).

3 A novel technique for determining the pA_2 is described which is particularly useful on peripheral airway smooth muscle. Values obtained for propranolol and practolol using this technique were:

propranolol pA_2 7.83 ± 0.14 ; slope $= 0.97 \pm 0.05$ ($n=4$),

practolol pA_2 5.62 ± 0.15 ; slope $= 1.17 \pm 0.17$ ($n=4$)

These values support the β_2 selectivity suggested by the agonist studies.

4 The cyclic AMP response of lung parenchyma to β -adrenoceptor agonists and antagonists (in the presence of isoprenaline demonstrated β_2 selectivity. Salbutamol appeared to be a partial agonist when compared with the cyclic AMP response to isoprenaline.

5 The β -adrenoceptors of the lymphocyte, bronchial smooth muscle and lung parenchyma have β_2 selectivity although they differ somewhat quantitatively from each other.

Introduction

Studies of drug action in man or animals may be approached on several different levels, each with its own strengths and weaknesses. However, 'for more refined and exact characterization and differentiation of types of receptors in any one class, experiments with isolated tissue preparations are required' (Furchgott, 1972). In human studies, further constraints arise in relation to the difficulty of obtaining isolated tissues in good condition, particularly if repeated studies within an individual subject or patient are required.

For this reason the use of the peripheral blood

lymphocyte as a readily accessible source of cell surface receptors for hormones and autacoids has attracted increasing attention in recent years (Logsdon, Middleton & Coffey, 1972; Bourne, Melmon & Lichtenstein, 1971; Bourne & Melmon, 1972; Gavin, Mann, Buell & Rath, 1972; Archer, Gordon, Gavin, Lesniak & Roth, 1973).

Before such a model (despite its convenience) can be accepted, it is necessary to establish as extensively as possible the resemblance of the lymphocyte receptors with those of the organ primarily under consideration. Initial studies in this laboratory (Conolly & Greenacre, 1977) have demonstrated the validity of the lymphocyte as a model for studying β_2 -adrenoceptors, the lung being the primary focus of attention.

In this communication we wish to amplify our previous observations on human lung parenchyma, as well as reporting further studies using the

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lymphocyte. In addition, data relating to the pharmacology of central and peripheral human bronchial smooth muscle will be described, including details on a technique for determining pA_2 values for β -adrenoceptor antagonists on this tissue.

Methods

Pharmacological response in lymphocytes and in human lung parenchyma was quantitated by measuring changes in cyclic 3'5' adenosine monophosphate (cyclic AMP) using the methods as previously described (Conolly & Greenacre, 1977).

Human lung tissue was obtained from patients undergoing surgery for bronchial carcinoma. The list of drugs taken within 1 week of operation included:

1. Antibiotics: penicillins, tetracyclines, cotrimoxazole.
2. Sedatives and analgesics: papaveretum, codeine, aspirin nitrazepam.
3. Others: digoxin, diuretics.
4. Preanaesthetic: atropine, hyoscine.
5. Anaesthetic: N_2O , O_2 , halothane.

None of the patients were taking β -adrenoceptor agonists or antagonists, and the patients were free of any historical or spirometric evidence of clinically significant airway obstruction. All tissue (taken from macroscopically normal areas of lung) was obtained within 20 min of resection, was placed in ice cold Krebs solution with the following composition in mM concentration NaCl 121.9, KCl 4.7, KH_2PO_4 1.2, $NaHCO_3$ 22, $CaCl_2$, $MgCl_2$ 1.2, glucose 11.0. The pH was 7.4 when the solution was bubbled with 95% O_2 , 5% CO_2 at 37°C. It was then transferred to our laboratory within 90 min and used immediately. It was dissected free of visible pleura, bronchial and vascular tissue, and chopped into fragments approximately 1 mm³. It was then transferred in aliquots of approximately 200 mg wet weight to tubes containing Hanks balanced salt solution, theophylline (10^{-2} M) and a range of β -adrenoceptor agonists and/or antagonists (see below). They were then incubated for 15 min at 37°C. The reaction was terminated by placing the tubes in boiling water. After cooling, [³H]-cAMP (5000 counts/min) was added for quantitation of recovery. The lung tissue was homogenized and centrifuged and the supernatant taken for cAMP assay. The residual protein was quantitated by the Lowry, Rosebrough, Farr & Randal (1951) method. Where propranolol and practolol were being compared, portions of each specimen of lung tissues were exposed to both drugs. Duplicate samples were used for each dose in every experiment.

Bronchial muscle was prepared at the same time. Distal bronchioles were identified by the passage of a stainless steel probe 1.5 mm diameter, dissected free

of parenchymal tissue and then cut into spiral strips approximately 2 mm in width according to the method of Constantine (1965). When available, strips of bronchial smooth muscle from central airways were also prepared.

It was found empirically that the muscle preparations responded far more consistently if allowed to 'recover' at least overnight at 4°C. Accordingly, they were stored in gassed Krebs solution at 4°C (in a glass stoppered flask) for up to 3 days prior to study. On the day of study they were suspended under an estimated optimal passive tension of 200 mg (Stephens, Meyers & Cherniack, 1968) in a water jacketed organ bath containing constantly oxygenated Krebs solution, and left for 90 min to equilibrate. The strips were attached to the arm of an isotonic optical density wedge strain gauge (Scientific Research Instruments Ltd, Model no. 7043) whose signal was displayed on the chart of a Devices M4 recorder.

Determination of pA_2 values in human bronchial muscle

The usual method of determining the pA_2 value is to perform repeated agonist dose-response curves as successively greater concentrations of antagonist are added (Schild, 1947). It is not easy to follow this protocol with human bronchial muscle, because the time required to generate the necessary family of dose-response curves may exceed the period of reliable viability of the tissue. As an alternative to using several different specimens (with the problems of inter-individual variability) the following technique was adopted.

The bronchi were contracted using 10^{-5} M

Figure 1 Time course of a) contraction (histamine 10^{-5} M, $n=15$) and b) relaxation (isoprenaline 5×10^{-7} M, $n=9$) of human bronchi (mean $\pm$ s.e. mean).

Figure 2 Inhibition of isoprenaline responsiveness in normal human lymphocytes (mean $\pm$ s.e. mean) by propranolol (●), acebutolol (△), practolol (□), metoprolol (■) and atenolol (○).

histamine and a cumulative dose-response was obtained by adding increasing doses of isoprenaline (Figure 7). The strip was then washed and allowed to recover for at least 90 min before the same dose of histamine was reapplied. Relaxation was restored by the addition of a fixed concentration of isoprenaline (10^{-6} M). This relaxation was then reversed incrementally by the cumulative addition of increasing amounts of the β -adrenoceptor antagonist (Figure 7, practolol). By forming the dose ratios of isoprenaline at various isoresponse points along the two curves a Schild plot was constructed (Figure 7b). The pA_2 and slope of the Schild plot were then derived by linear regression.

For this technique to work it is, of course, necessary first to establish that the continuing presence of histamine will, in the absence of other drugs, maintain a constant level of contractility, and that isoprenaline added to a tissue so contracted will

Figure 3 Human bronchial muscle dose-response curves for isoprenaline (●), adrenaline (▲), noradrenaline (□) and noradrenaline with 10^{-5} M imipramine (■) (mean $\pm$ s.e. mean).

Figure 4 a) Isoprenaline and b) adrenaline dose-response curves in the presence (△) and absence (▲) of phenoxybenzamine (10^{-5} M) (mean $\pm$ s.e. mean).

produce a sufficiently sustained relaxation. Data on both these points are shown for 15 separate preparations for histamine and 9 for isoprenaline in Figure 1. To minimize the error in obtaining isoprenaline dose ratios the concentration of isoprenaline, which is subsequently antagonized was chosen to be at the top end of the log linear portion of the initial isoprenaline dose response. This optimized the antagonist's response. Only those strips which gave comparable ($\pm 15\%$) response to 10^{-6} M isoprenaline on the initial and second application could be used in deriving the pA_2 . This effectively excluded two of every three strips used but in doing so, variation between strips' responses and the error associated was minimized.

The results are expressed in terms of the mean and s.e. mean.

Drugs

The final concentrations of drugs used (and their sources) were as follows: theophylline 10^{-2} M (BDH Ltd); ($\pm$) isoprenaline sulphate 10^{-8} to 10^{-4} mol free base l^{-1} (Sigma Ltd); ($\pm$) salbutamol

Figure 5 a) Human peripheral bronchial smooth muscle dose-response curves for isoprenaline (●) and salbutamol (○) in the presence of 10^{-5} M histamine (mean $\pm$ s.e. mean) b) Bronchial smooth muscle dose-response curves for isoprenaline (●) and salbutamol (○) on bronchi with basal tone (mean $\pm$ s.e. mean).

Figure 6 Human bronchial muscle dose-response curves for propranolol (●) and practolol (□) in the presence of 10^{-6} M isoprenaline.

sulphate 10^{-8} to 10^{-4} mol free base l^{-1} (a gift from Allen and Hanbury Ltd); ($\pm$) propranolol hydrochloride 10^{-9} to 10^{-4} mol free base l^{-1} and ($\pm$) practolol 10^{-6} to 10^{-3} mol free base l^{-1} ; ($\pm$) atenolol 10^{-6} to 10^{-3} mol free base l^{-1} (all three were gifts from ICI Ltd); ($\pm$) acebutolol hydrochloride, 10^{-6} to 10^{-2} mol free base l^{-1} (a gift from May and Baker Ltd); ($\pm$) metoprolol tartrate, 10^{-6} to 10^{-2} mol free base l^{-1} (a gift from Geigy Pharmaceuticals Ltd). To ensure solution of practolol and atenolol which were supplied as free base, the drug was dissolved first in 0.5% citric acid. The pH was then adjusted to 6.0 with 0.1N NaOH. Serial dilutions were then made from this stock.

Results

A. Lymphocytes

The ability of four different selective β_1 adrenoceptor antagonists to reverse the stimulant effect of isoprenaline (10^{-5} M) on cyclic AMP levels was compared with propranolol. The results are shown in Figure 2 which represents the mean values obtained from 3–5 separate studies in lymphocytes from four normal subjects. There is a clear separation between the effect of propranolol on the one hand and of metoprolol, acebutolol, atenolol and practolol on the other, the ratios of doses required to produce a 50% inhibition of the action of isoprenaline as compared with propranolol being 1:316 for metoprolol, 1:1779 for acebutolol and 1:2817 for atenolol. No ratio could be calculated for practolol as it never produced more than 35% inhibition.

B. Bronchial smooth muscle

The reversal by isoprenaline of bronchial smooth muscle contraction induced by histamine (10^{-5} M) is shown in Figure 3. The response has the form of a typical sigmoid log dose-response relationship with 50% inhibition (relaxation) occurring at 4.3×10^{-8} M isoprenaline.

Adrenaline was slightly less potent than isoprenaline but more active than noradrenaline in causing relaxation in tissues in which potential uptake mechanisms are not blocked. Adrenaline behaved as a full agonist.

Noradrenaline alone at the highest concentration tested (10^{-3} M) did not cause complete relaxation, although from the shape of the curve it is apparent that its full effect had not been achieved.

The ratio of ED_{50} s for isoprenaline v adrenaline and noradrenaline was 1:25 and 1:9286 respectively.

Figure 7 a) Practolol pA_2 determination on peripheral bronchial smooth muscle. *Inset*: The strip initially contracted with 10^{-5} M histamine (H), then a cumulative isoprenaline (I) dose-response curve is obtained. The strip is washed and left for 90 min to recover and then given histamine 10^{-5} M followed by isoprenaline (10^{-6} M) and increasing concentrations of practolol. Two curves are then constructed; the isoprenaline dose-response in the presence of histamine and the practolol dose-response in the presence of histamine and a fixed dose of isoprenaline. From the iso response points, dose ratios of isoprenaline in the presence and absence of practolol are derived. b) A Schild plot of the data from 7a. The logarithm of the (dose ratio-1) plotted against the negative logarithm of the practolol concentration. Linear regression was used to determine the slope and zero intercept (pA_2) on the abscissa. pA_2 value = 5.8.

Blockade of uptake₁ with imipramine (10^{-5} M) potentiated the effect of noradrenaline so that relative to isoprenaline the ED_{50} ratio became 1:82. The potency of isoprenaline was unaffected by either imipramine or phenoxybenzamine which blocks α -adrenoceptors and both uptake₁ and uptake₂. Adrenaline was potentiated about 2 fold by phenoxybenzamine (Figure 4).

Salbutamol has, in contrast, the appearance of a partial agonist; 50% inhibition did not occur until 1.3×10^{-3} M salbutamol. At the highest concentration tested (10^{-2} M) only 56% relaxation was achieved (Figure 5). The bronchial response to salbutamol was much greater (with respect to the maximal isoprenaline induced response) in strips which had not been contracted with histamine than in those that had been exposed to histamine 10^{-5} M. However, in each case the maximal response to salbutamol was less than the maximal response to isoprenaline. In uncontracted bronchi the response achieved a maximum of 67% of the maximal response to isoprenaline with an EC_{50} of 4.8×10^{-7} M. Furthermore, on the addition of isoprenaline to the salbutamol treated strips, evidence of competitive antagonism of isoprenaline's response by salbutamol was seen. By forming the isoprenaline dose ratios from isoresponse points of the remaining log linear portion of isoprenaline's dose response in the presence of 10^{-4} M salbutamol, estimates of salbutamol's dissociation constant were obtained in

four strips from three lungs. Values ranged from 1.4×10^{-7} to 10^{-6} M with a mean $\pm$ s.e. mean of $(6.6 \pm 0.2) \times 10^{-7}$ M.

The effects of propranolol and practolol on the response to isoprenaline are compared in Figure 6. Although practolol does produce complete antagonism it is much less potent than propranolol, the ratio of the ED_{50} being 1142:1 in favour of propranolol.

An example of the new method for determination of pA_2 is shown in Figure 7, using practolol. In the top left a representative isotonic response to histamine (10^{-5} M) followed by varying increasing concentrations of isoprenaline. After washing and waiting 90 min, histamine followed by isoprenaline (10^{-6} M) was applied and on this the β -adrenoceptor blocker was then added in a cumulative fashion.

The isoprenaline dose-response curve is shown on the left and the practolol dose-response in the presence of 10^{-6} M isoprenaline on the right. Dose ratios obtained by interpolation were used to construct the Schild plot from which pA_2 was derived. Overall values obtained by this method are as follows:

for	propranolol,	$pA_2 = 7.83 \pm 0.14$;
	slope = 0.97 ± 0.05 ($n=4$),	
for	practolol,	$pA_2 = 5.62 \pm 0.15$,
	slope = 1.17 ± 0.17 ($n=4$)	

values quoted being the mean and s.e. mean.

Figure 8 Cyclic AMP response of human lung parenchyma to isoprenaline (●) and salbutamol (○) (mean $\pm$ s.e. mean).

C. Lung parenchyma

The response (increase of cyclic AMP), to isoprenaline and the selective β_2 -adrenoceptor agonist salbutamol is shown in Figure 8, which represents the mean value of four experiments performed in duplicate. It can be seen that isoprenaline causes a five-fold greater increase in cyclic AMP than does salbutamol, and that the isoprenaline peak occurs at a lower concentration (10^{-5} as opposed to 10^{-4} M).

The effect of propranolol and the β_1 selective antagonist practolol on the cyclic AMP response to isoprenaline is shown in Figure 9 which represents the mean values of four experiments. The dose-response curves for the two drugs are parallel, but the concentrations of practolol required to produce the same degree of inhibition are several orders of magnitude greater. The dose ratio of the two drugs at the level of 50% inhibition is 708:1 in favour of propranolol.

Discussion

A. Lymphocytes

The cyclic AMP response observed in the lymphocytes amplifies data reported previously (Conolly & Greenacre, 1977) and is compatible with assignment of its adrenoceptors to the β_2 category. The inability of practolol to achieve more than a minor degree of attenuation of the cyclic AMP response to isoprenaline argues against there being a

dual population of β_1 and β_2 adrenoceptors in the lymphocyte such as has been reported for single tissues elsewhere (Lulich Mitchell & Sparrow, 1976; Carlsson, Åblad, Brondstrom & Carlsson, 1972). The finding that selective β_1 -adrenoceptor antagonists will eventually antagonize the effect of isoprenaline on a β_2 receptor accords with the clinical observations that, especially at higher doses, selectivity is lost, and that this may give rise to unwanted side effects, such as exacerbation of bronchial asthma (Conolly, Kersting & Dollery, 1976). The fact that complete antagonism was not obtained with practolol in the lymphocytes as it was in bronchial smooth muscle and lung parenchyma is possibly because the stimulus in the lymphocytes was 10–15 times higher than that used in the other two tissues. This dose was chosen because of the steepness of the lymphocyte dose-response curve to isoprenaline and the interindividual variability encountered (Conolly & Greenacre, 1977). The flatter configuration of the practolol dose-response curve compared with those of the other selective β_1 -adrenoceptor antagonists may be related to the intrinsic sympathetic activity of practolol.

It is noteworthy that acebutolol which exhibits selectivity in animals but not in man when given intravenously (Briant, Dollery, Fenyvisi & George, 1974) apparently behaves as a selective β_1 -adrenoceptor antagonist in this system.

Salbutamol has previously been reported to act as a partial agonist with respect to cyclic AMP formation in the lymphocyte (Conolly & Greenacre, 1977).

Figure 9 Inhibition of isoprenaline responsiveness in normal lung parenchyma in the presence of propranolol (●) and practolol (□) (mean $\pm$ s.e. mean).

B. *Bronchial smooth muscle*

The relaxant response to isoprenaline has been compared with that of a number of other β -adrenoceptor agonists. Isoprenaline was the most potent agent in the series studied. The ratio of ED_{50} s for isoprenaline v adrenaline was 1 : 17, while for noradrenaline in the absence of any inhibitor of neuronal or non-neuronal uptake it was 1 : 9290. If uptake was blocked with imipramine (10^{-5} M) the effect of noradrenaline was potentiated 113-fold. The effects of isoprenaline were not significantly altered either by imipramine or phenoxybenzamine, though a two-fold potentiation of adrenaline was seen after phenoxybenzamine. For each of these agonists the same degree of relaxation of a histamine induced contraction was ultimately achieved. The order of potency (isoprenaline—adrenaline—noradrenaline) is compatible with classification of the receptor as β_2 (Lands, Arnold, McAuliff, Luduena & Brown, 1967; Furchgott, 1972).

On the other hand, salbutamol behaved as only a partial agonist, producing at most, a 56% inhibition of the contractile response to histamine. These results are in contrast to the data obtained in animals (Cullum, Farmer, Jack & Levy, 1969) in which salbutamol acted as a full agonist, being about 1/10 as active as isoprenaline in causing relaxation. A possible explanation for this discrepancy may lie in the conditions of study used in the two laboratories. If the contractile stimulus applied is not maximal or near maximal, it may be possible for a β -adrenoceptor agonist such as isoprenaline, with a higher degree of efficacy, to produce complete relaxation while occupying only a small fraction of the available receptors. In contrast, an agonist of lesser efficacy may also be able completely to reverse the same contraction, though only after occupying a much larger fraction of the available receptors. Nonetheless, under these conditions it would appear as a full agonist. However, if a more powerful contractile stimulus were to be applied, isoprenaline at a higher concentration could still be capable of producing a complete inhibition by occupying more receptors, whereas for the agent of lower efficacy this would not be possible because more nearly complete receptor occupancy had already been achieved in reversing the initial contraction, and thus the phenomenon of partial agonism would appear (Furchgott, 1972; Buckner & Saini, 1969). This may also explain why in clinical studies not characterized by powerful bronchoconstrictor stimuli, the efficacy of salbutamol as a bronchodilator appears to be so comparable with that of isoprenaline (Warrell, Robertson, Howes, Conolly, Paterson, Berlin & Dillery, 1970).

The values of pA_2 obtained for propranolol and practolol agree fairly closely with results obtained in

other laboratories. The slopes of the Schild plots do not differ significantly ($P > 0.55$ propranolol, $P > 0.4$ practolol) from unity with either drug, which is the expected value for a competitive antagonist under conditions of equilibrium. In view of this, and of the fact that isoprenaline is apparently not affected by any uptake mechanism, this technique would appear to satisfy the requirements for measurement of pA_2 (Furchgott, 1972). In terms of speed and convenience in bronchial muscle, it compares favourably with established methods (Schild, 1947; van Rossum, 1963).

C. *Lung parenchyma*

The results obtained in this tissue are harder to interpret since all of the 20 or more cell types found in the lung (Fishman & Peitra, 1974) are likely to be represented in the tissue fragments. The more than 700-fold greater potency of propranolol as compared with practolol in inhibiting the cyclic AMP response to isoprenaline suggests that β_2 -adrenoceptors predominate. Once again the response to salbutamol is less than that seen following isoprenaline, and the difference is more marked than in the case of the lymphocyte. In view of the data obtained with the β -adrenoceptor blocking drugs which point to at least a predominance of β_2 adrenoceptors, it seems reasonable to regard the poor cyclic AMP response to salbutamol as another example of the phenomenon of partial agonism. At low concentrations salbutamol is close to isoprenaline in inhibiting the release of histamine from sensitized challenged lung, the potency ratio being only 10 : 1 to 100 : 1. However, as the concentration of agonist is increased up to the levels at which (in this experimental design) changes in cyclic AMP become measurable, salbutamol having 'used up' relatively more receptors, produced an inferior response compared with isoprenaline.

The β -adrenoceptor of the human lymphocyte, bronchial smooth muscle and lung parenchyma conform to a pattern of response predicted for a β_2 receptor. These results amplify our previous data and support the use of the lymphocyte as a model for studying β_2 -adrenoceptor function in bronchial asthma. The results obtained with bronchial smooth muscle using imipramine to block neuronal uptake of noradrenaline are of particular interest. They indicate that there is a considerable degree of neuronal uptake of noradrenaline. This finding, which conflicts with the generally held view that terminal bronchioles have little in the way of adrenergic innervation (Richardson & Beland, 1976) will be the subject of a future report.

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