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THE NATURE OF THE β-ADRENOCEPTOR INVOLVED IN THE INHIBITION OF ANTIGEN-INDUCED HISTAMINE RELEASE

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Summary Antigen stimulated release of histamine from chopped sensitized guinea-pig lung is inhibited by the addition of β-adrenoceptor agonists in a manner indicating a β_2 response. Preaddition of β-adrenoceptor antagonists blocks this inhibition in a manner indicating a β_1 response. This apparent dichotomy probably results from a hybrid receptor, though the danger of the use of chemical analogues for classifying receptors is highlighted. Inhibition of histamine release by β-stimulation is shown to be species rather than organ specific.

INTRODUCTION

In 1936 Schild demonstrated that high concentrations of adrenaline inhibited antigen stimulated release of histamine from chopped sensitized guinea-pig lung. (1) Assem and Schild (2) later showed with passively sensitized human lung that inhibition was induced by other catecholamines such as isoprenaline and could be blocked by the β-adrenoceptor antagonist propranolol, indicating that it was a β- rather than an α- adrenergic effect. From the relative potencies of these different stimulants in inhibition of release, they concluded that the response was β_2-like. Sorenby (3) similarly concluded from agonist potencies that the receptor was β_2, other workers have claimed that the receptor is a hybrid (4). The work described below investigates inhibition of the release of histamine from guinea-pig and rat tissues. These experiments attempt to classify the receptors and study any tissue or species specificity.

METHODS

Histamine release from guinea-pig and rat tissues was determined using methods previously described (5)(6). Briefly lungs were removed from the thoracic cavity of guinea-pigs or rats actively sensitized to ovalbumin, chopped and washed free of blood by filtration. Shaved skin was removed

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from the underside of the animal. Gassed Krebs Ringer saline was added and the vial incubated at 37°C for 20 minutes before challenge with antigen. After a further 10 minutes the suspension was filtered and the free histamine determined fluorometrically. Freshly prepared stimulants were added 2 minutes before antigen and blockers 3 minutes before antigen. These times were chosen as giving the maximal effect. All additions were in 30 microlitre volumes of saline.

RESULTS and DISCUSSIONS

Using the above methods we have shown that it was possible to duplicate and extend the experiments of Assem and Schild in guinea-pig lung and show inhibition of release by β-agonists. The relative potencies for these stimulants (compared to isoprenaline) were as follows : isoprenaline (1) ≈ adrenaline (1) > salbutamol (0.1) > noradrenaline (0.01). These ratios are those which might be expected for a β_2 response.

If, however, β-stimulants are used in the presence of β-blockers and the released histamine measured following challenge with antigen it is possible to construct 'Schild plots' and obtain K_b values for the antagonists. Figure 1 shows such a plot for atenolol antagonism of isoprenaline inhibition, the K_b values for heart and uterus are also shown. These values are characteristic of the binding of the blockers to specific β_1- or β_2-receptors and should characterise that receptor. Data summarized in Table I shows results from experiments using isoprenaline and the β_2 selective stimulant, salbutamol, as agonists with β_1 selective blocker, atenolol ('Tenormin'), and β_2 selective blocker, butoxamine, as antagonists. K_b values for these antagonists against isoprenaline in heart and uterus membranes are also shown. It is clear that atenolol has a K_b value which approximates to that in heart membranes rather than the β_2-receptor organ uterus, i.e. there is a β_1 like response. The results with butoxamine are not so clear cut, however unpublished observations with other β_1 and β_2 selective antagonists have given similar results. i.e. an apparent β_1 like response.

When Alderley Park strain rat tissues were used instead of guinea-pig, e.g. skin, lung and isolated mast cells, we were unable to detect any inhibit-

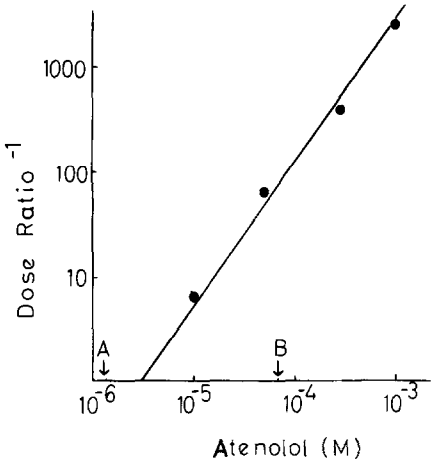

Figure 1 Schild plot for atenolol using isoprenaline as agonist. Inhibition is of antigen induced histamine release from activity sensitized guinea-pig lung. A and B show the K_B values from heart and uterus membranes respectively (10).

Table 1 K_B values for the β-adrenoceptor antagonists, atenolol and butoxamine, completing against β-agonists in the inhibition of antigen stimulated histamine release from guinea-pig lung.

	antagonists $K_B \pm SE$ (M)	
Agonist	Atenolol	Butoxamine
Salbutamol	$3.4 \pm .2 \times 10^{-6}$	$7.3 \pm 2.2 \times 10^{-6}$
Isoprenaline	$3.2 \pm .3 \times 10^{-6}$	$6 \pm 2 \times 10^{-6}$
*Heart Isoprenaline	1.3×10^{-6}	9.2×10^{-6}
*Uterus Isoprenaline	67.9×10^{-6}	2.2×10^{-6}

* Data from Coleman and Somerville (unpublished observations and (10)). Error calculations were made by a least squares analysis - see (10).

ion of histamine release by β-agonists, i.e. adrenaline, isoprenaline or salbutamol, in the concentration range 10^{-9} to 10^{-3}M examples are shown in Table II. These results are in disagreement with those of Nagai (7) who was able to detect 20-50% inhibition of histamine release from sensitized rat lung.

Table II. Inhibition of antigen induced release of histamine from actively sensitized rat and guinea-pig tissue by β-agonists.

Tissue	% Inhibition ± SE	
	Salbutamol (10^{-4}M)	Isoprenaline (10^{-4}M)
Rat skin	0 ± 5	0 ± 5
g.pig skin	40 ± 10	35 ± 9
Rat lung	0 ± 2	0 ± 3
g.pig lung	50 ± 5	45 ± 6

Histamine release from sensitized guinea-pig skin was also shown to be inhibited by β-stimulants in the same way as lung tissue. (See Table II) These results suggest that the inhibition of histamine release from mast cells is species rather than tissue specific and perhaps even strain specific in rats.

The classification with β blockers is at variance with the results obtained with β stimulants. A similar kind of situation has been observed with the β-receptor of fat cells. Lands et al. (8) classified the receptor involved in lipolysis as β_1 on the basis of a study with β-agonists whereas Harms et al. (9) classified it as β_2 using β-antagonists.

We believe that this dichotomy can be resolved by an examination of the structures of the stimulants and blockers used. Isoprenaline, noradrenaline and adrenaline have similar catechol head groups with varying side chains whereas the β blockers, atenolol and butoxamine, have similar side chains but different aromatic head groups.

If the assumption is made that the β receptor has separate binding sites for the non-polar aromatic head groups and the polar side chains, and furthermore that simultaneous binding by an agaonist to both these sites is necessary for activity, it is possible to explain the results above. The use of a closely related series varying only in the aromatic end could give rise to

one classification, here β_1, whilst another group of compounds varying in the side chains could produce a different effect, here β_2. Hence when attempting to classify a receptor in a tissue, several factors must be considered. Firstly, it is essential to use agonists and antagonists. Secondly, these should belong to β_1 and β_2 selective groups. Finally, careful consideration must be given to the chemical structures, so that close analogues are avoided to ensure that any selectivity detected is real and not an artifact of the ligands used.

The observations reported above suggest that β stimulants might be useful antiallergic agents in man since they inhibit the anaphylactic release of histamine. However, as the characterisation of the β-adrenoceptor has proven to be difficult and there is species specificity, model systems to select potential antiallergic compounds must be chosen with care.

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