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Sympathomimetic bronchodilators and animal models for assessing their potential value in asthma

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Bronchial asthma is characterized by an increase in airways resistance which is due to three main factors: (i) contraction of the smooth muscle in the bronchioles, (ii) oedema of the bronchiolar mucosa, and (iii) increased bronchiolar secretions. For discussions of the aetiology and classification of different forms of asthma see Porter & Birch (1971) and Beall (1973). The immediate cause of asthmatic bronchospasm is probably the release of endogenous bronchoconstrictor substances such as histamine, 5-hydroxytryptamine (serotonin), slow-reacting substance of anaphylaxis, and prostaglandins of the F series. Asthmatic patients are hypersensitive to the actions of all bronchoconstrictor agents, but particularly to prostaglandin $F_{2\alpha}$ (Mathé, Hedqvist & Holmgren, 1973). Excessive parasympathetically evoked bronchoconstriction and diminished reflex sympathetic bronchodilatation may be additional factors underlying an asthmatic attack (Reed, 1974). Whatever the factors that provoke an asthmatic attack in individual cases, the symptoms rapidly revert to normal after the administration of a sympathomimetic bronchodilator drug. Responsiveness to bronchodilators is characteristic of asthma and helps to distinguish it from disease states with fixed obstruction of the airways. Although other drugs are used, the sympathomimetic bronchodilators are the main stay of asthma therapy. As a class, these drugs are illustrative of the combined attempts by medicinal chemists, pharmacologists and pharmacists to improve therapy by increased organ selectivity through specific receptor interactions and controlled bioavailability.

Adrenoceptors

Ahlquist (1948) formalized the dual adrenoceptor hypothesis and designated responses as being due to α- or β-receptor stimulation on the basis of the rank order of potency of a number of catecholamines. This classification is now generally accepted, and selective agonists and antagonists for the two types of receptor are available. Stimulation of β-adrenoceptors mediates relaxation of smooth muscles (including those of blood vessels and the bronchi), increases in rate and force of the heart beat, changes in skeletal muscle contractility, glycogenolysis and lipolysis. Sympathomimetics, acting through β-receptors, also inhibit the release of endogenous bronchoconstrictor substances, although this is probably not an important component of their bronchodilator action in asthma.

The idea that β-adrenoceptors in different tissues are not of a homogeneous type was prompted by three main observations: first, that injected noradrenaline is more effective in producing some β-receptor-mediated responses (e.g., cardiac stimulation) than others (e.g., vasodilatation); secondly that β-receptor antagonists containing alkyl substituents on the α-carbon of the ethanolamine side chain display differential blockade of cardiac and vascular responses to isoprenaline (Moran, 1966); and thirdly, that there are considerable differences in the dissociation constants found for the β-receptor antagonist pronethalol in a number of tissues (Furchgott, 1967). The subclassification of β-receptors into two types, β_1- and β_2-receptors, was first suggested by Lands, Arnold & others (1967). This division was made on the basis of studies, using catecholamines, in which two distinct orders of relative potency with respect to isoprenaline were obtained when the abilities of the compounds to produce lipolysis, cardiac stimulation, bronchodilatation and vasodepression were compared. Since the original observations of Lands and his coworkers, the range of β-receptor-mediated responses that have been subclassified has been extended (for references see Raper & McCulloch, 1971; Furchgott, 1972). In relation to the therapeutic use of β-adrenoceptor agonists in asthma, increases in the rate and force of the heart beat are important effects mediated predominantly by β_1-receptor stimulation,

and bronchodilatation, vasodilatation and effects on skeletal muscle contractility are important effects mediated mainly by β_2-receptor stimulation. Relatively selective agonists and antagonists at either β_1- or β_2-receptors are now available and the subclassification relating to the two β-receptor types has proved useful. Nevertheless, it is probably oversimplified, as evidence is accumulating that there are probably more than two types of 'isoreceptor' among β-receptors, the β_1 and β_2 classification merely representing the two extremes (Brittain, Jack & Ritchie, 1970). This is an important pharmacological concept because it provides a stimulus for the discovery of drugs with greater organ selectivity. Although a particular tissue invariably contains a predominance of one type of β-receptor, a small proportion of other isoreceptors may also be present (Åblad, Borg & others, 1975).

Structure-activity relations

Numerous studies of structure-activity relations among sympathomimetic amines have been made. Most of these have been comprehensively reviewed by Brittain & others (1970), and Patil, Miller & Trendelenbeurg (1974). Some caution is necessary in interpreting the data, since structural modifications may modify potency in different ways in different species. However, a number of broad generalizations can be made in terms of the molecular modifications to the basic phenylethanolamine nucleus that are required to impart α-, β_1- or β_2-receptor—mediated activity. The structures of some of the compounds referred to are given in Fig. 1. (i) The presence of a β-hydroxyl group in the (−)-configuration on the ethanolamine side chain is a prerequisite for high affinity for all types of adrenoceptor. (ii) In catecholamines, high potency is obtained with primary amines and with secondary amines, but tertiary amines and quaternary ammonium salts are virtually inactive. (iii) Within secondary amines, increasing the bulk of the amine substituent above methyl leads to a decrease in α- and a relative increase in β-receptor stimulant activity (e.g., isoprenaline); further increase in the bulk of the amine substituent may not affect potency but imparts relatively greater selectivity for β_2-receptors than for β_1-receptors (e.g., rimiterol). (iv) Alkyl substitution on the α-carbon of the ethanolamine side chain lowers overall potency, but usually leads to a relative enhancement of β- (especially β_2-) over α-receptor mediated activity (e.g. isotharine). (v) Possession of a ring hydroxyl group is not essential for β_2-receptor agonist activity. For example, both clorprenaline, which possesses a 2-chloro, and clenbuterol (NAB 365), which has 3,5-dichloro, 4-amino-, ring substitution, display selective β_2-receptor activity. For high potency, the groups substituted for the ring hydroxyls must have similar electron-donating and hydrogen-bonding properties.

Therapeutically-useful sympathomimetic bronchodilators

Adrenaline and ephedrine and its derivatives have long been used as bronchodilators in asthma. Drugs such as these, that stimulate α-adrenoceptors as well as β-adrenoceptors, may have the slight advantage that their vasoconstrictor action helps to relieve congestion in the upper respiratory tract. However, their pressor action is a disadvantage, and rebound vasodilatation in the upper respiratory tract, after a brief period of freedom from congestion, often produces a blockage which is more severe than before treatment. Stimulation of α-adrenoceptors may also impair ciliary movements in the upper respiratory tract. Although the smooth muscle of the airways contains predominantly β-adrenoceptors, some α-adrenoceptors that mediate bronchoconstriction are also present (Mathé, Åström & Perrson, 1971), and these may assume an abnormally greater importance in some cases of refractoriness to bronchodilator agents. It seems clear that the disadvantages of α-adrenoceptor stimulation outweigh any theoretical advantages, and selective β-adrenoceptor agonists are therefore preferable to drugs that stimulate both types of receptor.

The bronchodilator actions of isoprenaline were first reported in 1940 by Konzett, and its use in asthma by inhalation has dominated the sympathomimetic treatment of this condition until recent years. Its bronchodilator effect is powerful, rapid in onset, but short-lasting because it is rapidly inactivated by uptake into tissues (uptake 2) and by metabolism catalysed by catechol-*O*-methyltransferase (COMT) (Hertting, 1964). The drug is inactive when swallowed because it is converted to an ethereal sulphate by sulphatase enzymes in the intestine and liver, and is inactivated by liver COMT (Morgan, Sandler & others, 1969). It is effective when absorbed from the buccal cavity and it may be administered in the form of sublingual tablets. Isoprenaline is more selective than adrenaline in its actions, since it is a specific agonist for β-adrenoceptors. Nevertheless, it has disadvantages in that bronchodilatation is usually accompanied by

tachycardia and vasodilatation; in addition, especially when administered sublingually, it may give rise to muscle tremor. Between 1961 and 1966, epidemiological studies showed that there was a significant increase in the mortality rate among asthmatics, particularly in 10–14 year old children, in the U.K. and some other countries (Inman & Aldestein, 1969). There was a statistical association between the increased mortality rate and the use of bronchodilator aerosols, which at that time were mainly of isoprenaline in the countries concerned. Excessive use may lead to tolerance to and cross tolerance between sympathomimetic bronchodilators, including the endogenous adrenergic transmitter and the adrenal medullary hormones (see Benoy, El-Fellah & others, 1975). A vicious cycle may then be set up in which increasing doses are administered in an attempt to relieve the resistant bronchospasm. The actual cause of the increased mortality has not been established beyond doubt, but there is general acceptance that it was associated with the cardiovascular effects of isoprenaline. The mortality rate among asthmatics returned to its previous level in the late 1960s. This was attributed to the exercise of greater caution in the use of bronchodilator aerosols and to the development of drugs with relatively less action on the heart.

Since the development of isoprenaline, and especially in recent years, a flood of potential new bronchodilators has been synthesized and tested. Relatively few have survived beyond the stage of animal or early clinical trials. Those that have survived and which are commercially available (see Leifer & Wittig, 1975, for review) include isoetharine, orciprenaline, fenoterol, salbutamol, terbutaline, hexoprenaline, salmefamol and carbuterol. Their structures are illustrated in Fig. 1. Orciprenaline resembles isoprenaline in that it stimulates both β_1- and β_2-receptors. The remaining compounds mentioned are reported to possess a useful degree of selectivity for β_2-adrenoceptors. Compounds in which the catechol nucleus of isoprenaline has been replaced by a resorcinol nucleus (orciprenaline, terbutaline, fenoterol), or by a saligenin nucleus (salbutamol, salmefamol), or in which one ring hydroxyl has been replaced by a urea moiety (carbuterol) are not substrates for COMT and are not inactivated by uptake. Consequently, they have a longer duration of action than the catecholamines and are effective orally. In general their duration of action is in the region of 4–6 h after a single dose (compared with isoprenaline, 1·5–2 h). Fenoterol is probably the longest lasting with a duration of 8–10 h. Their onset of action is usually relatively slow (30–60 min to peak effect) compared with that of isoprenaline (2–5 min). The compound, ibuterol (KWD 2058), is the diisobutyric acid ester of terbutaline. It is a prodrug which releases the active terbutaline on hydrolysis by esterases. Its main advantage is that absorption after oral administration is greater than that of terbutaline.

Animal models for testing sympathomimetic bronchodilators

The main unwanted effects of β-receptor agonists used as bronchodilators are cardiac stimulation,

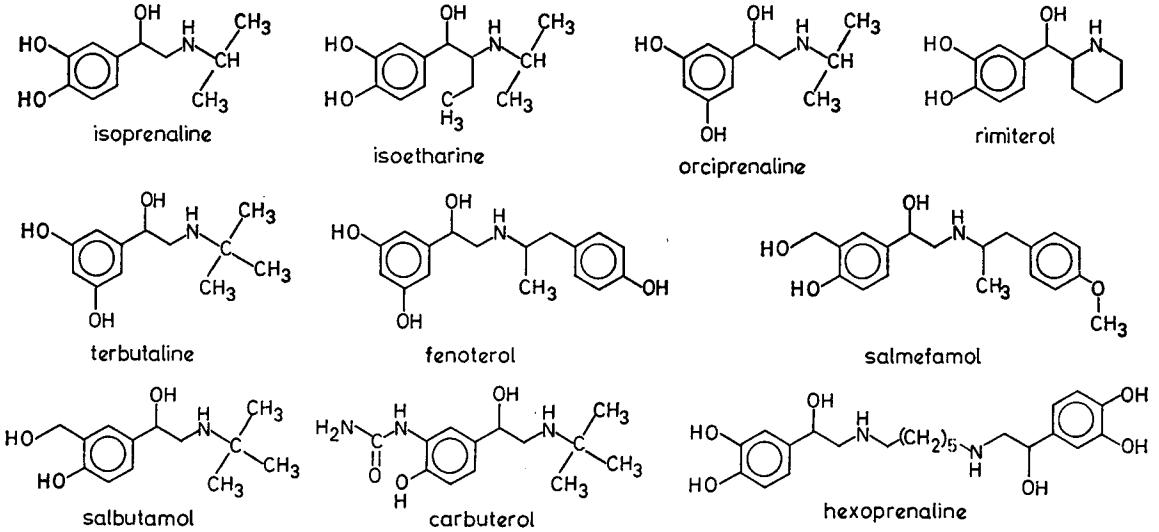

FIG. 1. Some therapeutically useful bronchodilators.

vasodepression and muscle tremor. Animal experiments are therefore designed to measure the relative extent to which a new drug produces these effects and to determine what degree of selectivity it possesses, if any, for β-receptors in bronchial smooth muscle over those in other tissues. Measurement of cardiac stimulation and of vasodepression are relatively straightforward, but animal models for assessing bronchodilatation and muscle tremor require special mention.

Bronchodilatation is usually measured as inhibition of a constant background of bronchoconstrictor tone induced by a bronchoconstrictor agent, such as histamine, 5-HT or acetylcholine. Hence the dose or concentration of a β-receptor agonist necessary to produce a given response (e.g., 50% inhibition of bronchoconstriction) is greater, the greater the degree of background tone present. This, of course, would also apply in asthma where the effective bronchodilator dose is dependent on the severity of the asthma. However, it means that bronchodilator potency cannot be measured in absolute terms, such as, for example, the dose necessary to cause 50% of the maximal bronchodilatation. For this reason, new drugs are usually compared against a standard bronchodilator, which in most cases has been isoprenaline. When the dose-response curves for isoprenaline and the drug under test are parallel, as they often are, the relative potency compared with isoprenaline is constant regardless of the background degree of bronchoconstrictor tone produced. Since it is necessary to use an internal standard (e.g., isoprenaline) in determining relative bronchodilator potency, the same internal standard is used for other parameters. Thus, a measure of the selectivity of the test compound can be gained in the form of a selectivity ratio compared with isoprenaline for each tissue (e.g., dose-ratio for heart: dose-ratio for bronchi). In terms of determining absolute selectivity the method suffers from the inherent difficulty that the standard used for comparison should itself possess no selectivity, but be equally effective in stimulating all types of β-receptor. That is to say that dose response curves over the full range, from no effect to 100% of the maximum response, should be superimposable for each tissue. In fact, results from a large number of experiments on cats suggest that isoprenaline itself shows some slight selectivity for bronchial β-receptors over those in skeletal muscle, and for the latter over those in the heart. Despite these limitations, selectivity ratios relating to isoprenaline are useful, since in practical terms the object is to find drugs with greater selectivity than isoprenaline for use in asthma. When the dose-response curves for the drug under test and for the internal standard are not parallel, potency ratios have less value, since they differ according to the level of response at which comparisons are made.

Tremor, particularly of the hands, is a common and unpleasant side-effect of sympathomimetic bronchodilators especially when they are administered orally. Salbutamol, for example, in oral doses of 2–4 mg three or four times a day produces tremor in 25–35% of patients (Kennedy & Simpson, 1969; Freedman, 1971; Legge, Gaddie & Palmer, 1971; Leifer & Wittig, 1975). Watson & Richens (1974) found that both orally administered salbutamol and terbutaline produced tremor in normal human volunteers and that there was no significant difference between the two drugs in this respect. The quantitative measurement of sympathomimetic-induced tremor in animals is difficult, since it requires that the animals be conscious and unrestrained. However, it has been proposed (Bowman & Zaimis, 1958; Bowman & Nott, 1969) that the action of sympathomimetics on the contractions of slow-contracting muscles of anaesthetized mammalian laboratory animals provides a suitable model. β-Receptor stimulants produce a decrease in the tension and an increase in the rate of relaxation of maximal twitches of the cat soleus muscle, so that the overall duration of the twitch is reduced. The decrease in the duration of the response is such that a marked decrease in fusion and in tension of incomplete tetanic contractions occurs (Bowman & Zaimis, 1958; Bowman, Goldberg & Raper, 1962; Bowman & Raper, 1962), and stimulation at a constant frequency of about 10 Hz for 1 s every 10 s provides a sensitive preparation for testing β-receptor agonists (Bowman & Nott, 1970). The effect, which is mediated by β_2-receptors (Bowman & Nott, 1970), is exerted directly on the muscle fibres and is independent of concomitant vascular changes (Bowman & Zaimis, 1958). Like other β-receptor-mediated effects, it probably involves stimulation of adenylate cyclase and increased cellular concentrations of cyclic AMP (Bowman & Nott, 1974). Similar effects are produced in the slow-contracting crureus muscle of the cat, in the soleus muscles of rabbits, rats and guinea-pigs, and in the slow-contracting plantaris muscle of the dog, which does not possess a soleus muscle (Bowman & others, 1962; Bowman & Raper, 1967). The effect is also demonstrable in the isolated soleus muscle of the guinea-pig studied *in vitro* (Tashiro, 1973). Marsden

& Meadows (1970) showed that adrenaline produces a similar effect on evoked twitches of the slow-contracting units in human muscles. In a voluntary contraction, the various muscle units within a muscle contract asynchronously and intermittently at subtetanic frequencies, but the pattern of activity is so integrated at central level that the algebraic sum of the contractions and relaxations of the units gives an almost smooth contraction from the whole muscle. A decrease produced by β-adrenoreceptor agonists in the fusion of the slow units within a muscle, like that produced on the soleus muscles of experimental animals, might be expected to produce the type of tremor that is often a side-effect of sympathomimetic bronchodilators.

Accurate assessment of true receptor selectivity demands the production of equilibrium conditions in isolated tissues after inhibition of drug biotransformation and of neuronal and extra-neuronal uptake, both of which affect biophase concentration. However, in the high concentrations necessary, inhibitors of metabolism and uptake may not be specific in their actions and might themselves affect either the drug-receptor interaction or the ability of this interaction to elicit the measured response. In any case, equilibrium conditions cannot easily be obtained, and are not applicable to the therapeutic use of the compounds. While tests *in vitro* are of value in assessing the overall pharmacological profile of a drug, the use of whole animals is more appropriate for forecasting selective actions in man. In experiments on anaesthetized animals relative selectivity of action compared with a standard drug may be assessed by simultaneously recording respiratory parameters, heart rate or force, blood pressure, and contractions of a slow-contracting skeletal muscle. Potencies of long-acting compounds are conveniently assessed by constructing cumulative dose-response curves (Nott & Raper, 1972; Rodger, 1974).

The sensitivities of bronchial and vascular smooth muscle and of slow-contracting skeletal muscles (mainly β_2-receptors) to a given bronchodilator seem to be about the same in different species, but the sensitivity of the heart (mainly β_1-receptors) differs widely according to species. In the guinea-pig heart some sympathomimetics behave as partial agonists (Brittain & others, 1970; Raper & Malta, 1973; Davey, Malta & Raper, 1974; Malta & Raper, 1974, 1976), whereas they behave as full agonists in the cat (Bowman & Rodger, 1972; Gwee, Nott & others, 1972; Davey & others, 1974; Malta & Raper, 1975). Although resistance to sympathomimetics may develop in man, there is no evidence that this arises as a result of partial agonist activity.

The selectivities for the bronchi and skeletal muscle over the heart of a range of sympathomimetic amines are much smaller in the cat (Bowman & Rodger, 1972; Gwee & others, 1972; Rodger, 1973; Davey & others, 1974; Houston & Rodger, 1974; Olsson, 1974) than they are in other laboratory animals such as the guinea-pig, the rabbit and the dog (Cullum, Farmer & others, 1969; Daly, Farmer & Levy, 1971; Laity, 1971). This suggests either that β_1- and β_2- receptors are less clearly differentiated in the cat than they are in other laboratory animals, or that the cat heart contains a more even mixture of the two types of β-receptors than do the hearts of other animal species. Whatever the explanation, the degree of organ selectivity of a particular drug clearly depends on the species, and the important question is which species most closely resembles man. In most studies in man, drugs have been administered by aerosol so that the route of administration itself imparts a high degree of bronchial muscle selectivity. In most studies on anaesthetized animals, drugs have been administered intravenously. Results from the relatively few studies on man in which drugs in a range of doses have been administered systemically (e.g., Paterson, Courtenay Evans & Prime, 1971; Legge & others, 1971; Watson & Richens, 1974; Marlin & Turner, 1975), and which are therefore directly comparable to the results from animal studies, indicate that the cat most closely resembles man in the pattern of its responses to sympathomimetic bronchodilators.

Most of the bronchodilators currently used therapeutically affect contractions of the soleus muscle in the same doses as those that produce bronchodilation. This suggests that closely similar β_2-adrenoceptors mediate the two types of response, and accounts for the observation that all of the drugs produce muscle tremor, unless selectivity of action on the bronchi is achieved by aerosol administration. Nevertheless, preliminary results with newer compounds suggest that some selectivity may be achieved even between the bronchi and skeletal muscle. For example, Kaiser, Schwartz & others (1975) found that the compound sulfonterol was more potent on the bronchi than on the soleus muscle, the heart and the blood vessels, thus encouraging the hope that bronchodilators with minimal side-effects might be developed. The cat appears to be the species of choice for preliminary animal screening.

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