J. Pharm. Pharmac., 1971, **23**, 905–910 *Received May* 21, 1971

Some bronchoconstricting and bronchodilating responses of human isolated bronchi: evidence for the existence of α-adrenoceptors

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Isolated strips of human bronchi obtained during thoracic surgery exhibited pharmacological responses very similar to those of other species (e.g. guinea-pig). Analysis of the action of some sympathomimetic amines indicated that the human bronchi also possess a sparse population of α-adrenoceptors. Propranolol (>1 μg/ml) had a direct bronchoconstricting action, whereas another β-adrenoceptor blocking agent, alprenolol, produced bronchodilatation. Phentolamine (>4 μg/ml) also produced a bronchodilatation of its own. Theophylline and dibutyryl cyclic AMP produced dose-dependent relaxations. The strips were effectively contracted by acetylcholine, histamine, prostaglandin $F_2\alpha$ ($PGF_2\alpha$), slow reacting substance (SRS) and bradykinin. Bradykinin, regardless of the dose, produced bronchoconstriction in some preparations and dilatation in others. 5-Hydroxytryptamine produced bronchodilatation but at very high concentrations a constriction was obtained.

The pharmacological responses of bronchial smooth muscle preparations from human lungs removed during surgical procedures have been investigated for some time in our laboratory (Mathé & Strandberg, 1971; Mathé, Strandberg & Åström, 1971). The present study describes the action of a number of sympathomimetic amines and some β-adrenoceptor blockers thought to be of particular interest since propranolol has been found to produce bronchoconstriction in animal experiments (Herxheimer & Langer, 1967) as well as in man (McNeill, 1964; Zaid & Beall, 1966; MacDonald, Ingram & McNeill, 1967). Furthermore, bronchoconstricting agents including prostaglandin $F_2\alpha$ ($PGF_2\alpha$), slow reacting substance (SRS) and bradykinin, and also 5-hydroxytryptamine were studied. The action of these substances seemed to be of interest in view of their possible involvement in bronchial asthma (Brocklehurst, 1962; Austen, 1965; Piper & Vane, 1969).

MATERIALS AND METHODS

Bronchi were obtained from macroscopically normal parts of human lungs which had been removed because of carcinoma. A total of 89 preparations from 33 different lungs were immediately dissected from the lung tissue and kept in Tyrode solution. Helical strips were prepared from approximately 3–5 mm wide bronchi cut spirally into about 3 cm long and 3 mm wide strips and suspended in a 25 ml bath of Tyrode solution of the following composition g/litre: NaCl 8, KCl 0·2, $CaCl_2$ 0·2, $MgCl_2$ 0·2, $NaHCO_3$ 1, NaH_2PO_4 0·05 and glucose 1. The solution was kept at 37°, pH 7·3 and aerated with a mixture of 6·5% carbon dioxide in oxygen. The strips were allowed to equilibrate under a tension of 0·5 g for at least 1 h. Changes in active tone were measured isometrically with a Grass force-displacement transducer

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(model FT 03) and recorded on a Grass polygraph. Three to four preparations were usually run simultaneously. Thus tests with various agents and doses could be performed in differing order and with adequate controls. After each test and washing of the bath, a 15 min rest was allowed. Before each new test the tension was checked and, if required, adjusted to the original 0·5 g. Good responses could as a rule be obtained for about 8 h after the preparation of the strips.

Drugs. Acetylcholine chloride, (—)-adrenaline bitartrate, alprenolol (Aptin, Hässle), atropine hydrochloride, bradykinin (BRS 640, Sandoz), histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate, (—)-isoprenaline sulphate, methysergide (Sansert, Sandoz), phenylephrine (Neosynephrine, Winthrop), (—)-noradrenaline bitartrate, phentolamine (Regitin, Ciba), propranolol (Inderal, ICI), prostaglandin $F_2\alpha$, mepyramine, theophylline (Teofyllamin, ACO Läkemedel AB), cyclic N^6-2′O-dibutyryl-adenosine-3,5-monophosphate (cyclic AMP, Mannheim-Boerhinger), purified slow reacting substance not containing any prostaglandins (Strandberg, 1969) and polyphloretin phosphate (PPP). Doses of adrenaline, noradrenaline, isoprenaline, acetylcholine and histamine refer to the free base. Other drugs were calculated as salts.

RESULTS

The bronchodilating effect of adrenaline was readily abolished by β-adrenoceptor blockade (propranolol 0·4–5 μg/ml, Fig. 1; alprenolol 1–10 μg/ml, Fig. 2). After β-adrenoceptor blockade adrenaline elicited bronchoconstriction. The dose required for the constrictor effect was usually ten times higher than that used to produce an effective relaxation before the β-adrenoceptor blockade. This constriction could be abolished by phentolamine (Figs 1 and 2). Results similar to those with adrenaline were obtained with noradrenaline and phenylephrine. The constriction elicited by sympathomimetic amines after blockade of the β-adrenoceptors was never more than 10–20% of the maximum response elicited by acetylcholine or histamine. The bronchodilating action of noradrenaline and phenylephrine was approximately only 1/10 and 1/300 that of adrenaline. Isoprenaline on the other hand was about 3 times as potent a bronchodilator as adrenaline. Its action was completely abolished after β-adrenoceptor blockade. In contradistinction to the other sympathomimetic amines studied, no reversal of the effect could be observed. The ED50 values in μg/ml for the different sympathomimetic amines were: adrenaline 0·1 ± 0·01 (n = 7), noradrenaline 1·1 ± 0·2 (n = 5), phenylephrine 31 ± 13 (n = 7) and isoprenaline 0·03 ± 0·003 (n = 5).

The adrenoceptor blocking agents propranolol, alphrenolol and phenotalmine, in most experiments, produced an action *per se*. The bronchoconstriction observed

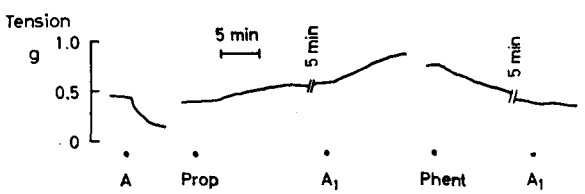

FIG. 1. Isolated strip of human bronchus. Dilating action of adrenaline (A 0·4 μg/ml, control) and constricting effect of a larger dose of A (A_1 4 μg/ml) after propranolol (Prop 4 μg/ml). The constriction caused by adrenaline (A_1 4 μg/ml) was abolished after phentolamine (Phent 16 μg/ml). Propranolol *per se* induced constriction and phentolamine relaxation.

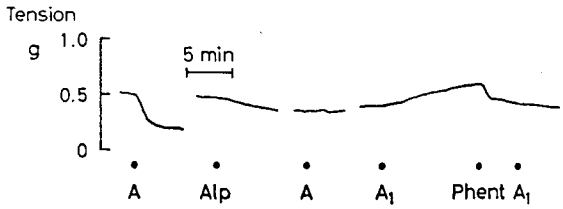

FIG. 2. Isolated strip of human bronchus. Dilating action of A (0·4 μg/ml, control). Alprenolol (Alp 4 μg/ml) in itself produced a dilatation and also abolished the dilating effect of the same dose of A (0·4 μg/ml). Ten times higher dose of A (A_1 4 μg/ml) elicited a constricting effect which was abolished after phentolamine (Phent 20 μg/ml).

with 1–5 μg/ml of propranolol was slow in onset and required up to 15 min to reach its maximum (Fig. 1). In contrast to propranolol, the β-adrenoceptor blocker alprenolol (1–10 μg/ml) produced a bronchodilatation (Fig. 2) confirming its previously reported β-adrenoceptor stimulating property (Åblad, Brogård & Ek, 1967). Phentolamine (5–20 μg/ml) elicited a bronchodilatation (Figs 1 and 2).

The bronchoconstricting effects of acetylcholine and histamine on the isolated bronchi were not modified by α-adrenoceptor blockade. The constriction produced by these agonists was readily abolished by atropine and an antihistamine (mepyramine). The ED50 value for acetylcholine was 0·4 ± 0·1 μg/ml (n = 24) and for histamine 0·5 ± 0·1 μg/ml (n = 12).

$PGF_2\alpha$, but not PGE_2, produced a dose-dependent bronchoconstriction as did SRS. The ED50 value for $PGF_2\alpha$ was 0·3 ± 0·1 μg/ml (n = 12) and for SRS 12 ± 3 units/ml (n = 9). In contrast to acetylcholine and histamine, the bronchoconstricting effect of $PGF_2\alpha$ and SRS took a longer time to reach its peak and lasted up to 30 min in spite of repeated washings with Tyrode solution. As reported elsewhere, polyphloretin phosphate (Diczfalusy, Fernö & others, 1953) antagonized the bronchoconstricting action of both $PGF_2\alpha$ and SRS (Mathé & others, 1971; Mathé & Strandberg, 1971).

Bradykinin (0·1–4 μg/ml) induced a bronchodilatation in 10 of 31 experiments (ED50 = 0·9 ± 0·3 μg/ml, n = 10) and a clear constriction in the other experiments. In no case were bronchoconstriction and bronchodilatation observed in the same preparation. Both types of action were dose dependent (Fig. 3). In a few instances the bronchoconstricting effect was diminished on repeated administration. The dilating effect was not antagonized by β-adrenoceptor blockade nor was the constriction modified by α-adrenoceptor blockade or by atropine.

5-Hydroxytryptamine (5-HT) in doses 2–10 μg/ml induced a bronchodilatation. This effect decreased with increasing dose, and at a concentration exceeding 20 μg/ml the response had either decreased to zero or been converted into a constricting effect. Methysergide (10–200 μg/ml) antagonized both types of action of 5-HT.

Dibutyryl cyclic AMP produced a dose dependent bronchodilatation. Although this compound is considered to reach its site of action more readily than the cyclic AMP, the doses required were high (50–500 μg/ml) and comparable with those used by Moore, Iorio & McManus (1968). The effect was gradual in onset and it often took 30 min or more for the preparation to return to the control level of tension in spite of repeated washings of the bath. In similarity with theophylline (Fig. 3B) the action of cyclic AMP was not modified by β-adrenoceptor blockade (Fig. 4).

The dose-dependent and readily reproducible bronchodilatation produced by

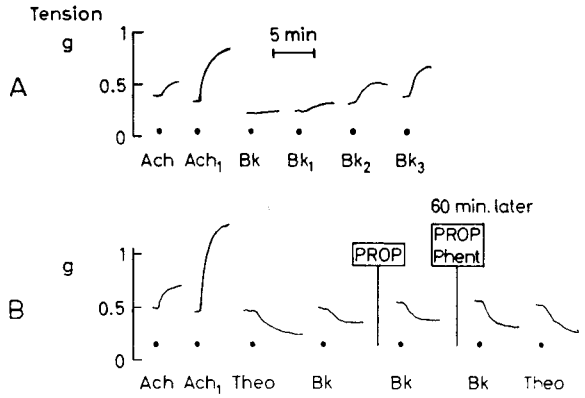

FIG. 3. Isolated strips of human bronchi from two different lungs. A. Bronchoconstricting responses produced by acetylcholine (Ach 0·04 and Ach_1 0·4 μg/ml) and different concentrations of bradykinin (Bk 0·005, Bk_{1-3} 0·02, 0·04, 0·4 μg/ml). B. Bronchoconstricting effect of acetylcholine (Ach 0·04 and Ach_1 0·4 μg/ml) and bronchodilating action of bradykinin (Bk 0·4 μg/ml). The bronchodilatation elicited by bradykinin was unchanged after β-adrenoceptor blockade (Prop 0·4 μg/ml). One h later, when the responses to both α- and β-adrenoceptors had been blocked, the bronchodilating response of bradykinin remained unchanged. The action of theophylline (Theo 40 μg/ml), used for comparison, was also unaffected by the α- and β-adrenoceptor blockade.

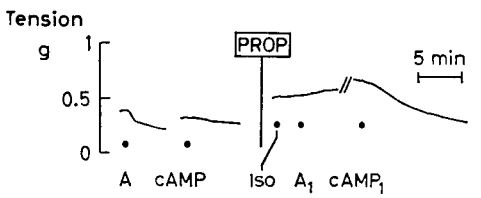

FIG. 4. Isolated strip of human bronchus. Dilating action of adrenaline (A 0·4 μg/ml) and dibutyryl cyclic AMP (cAMP 200 μg/ml). After β-adrenoceptor blockade (Prop 0·8 μg/ml) the dilating effect of isoprenaline (Iso 0·04 μg/ml) and adrenaline (A_1 4·0 μg/ml) was abolished but dibutyryl cyclic AMP ($cAMP_1$ 500 μg/ml) still elicited dilatation.

theophylline (10–100 μg/ml) was fully effective also in strips contracted with acetylcholine, histamine, $PGF_2\alpha$, SRS and bradykinin. The action of theophylline was not modified by β-adrenoceptor blockade (Fig. 3B).

DISCUSSION

The results obtained show that human bronchi respond in principally the same manner as those of guinea-pig, rabbit and dog. The observation that the relaxation normally produced by adrenaline, noradrenaline and phenylephrine, but not that by isoprenaline, could be converted into a constriction after β-adrenoceptor blockade is also in harmony with results obtained in animals (Castro de la Mata, Penna & Aviado, 1962; Persson & Johnsson, 1970; Fleisch, Maling & Brodie, 1970). On the basis of the results presented here it may be concluded that the human bronchi also possess α-adrenoceptors. However, the α-adrenoceptors do not seem to be prominent in the human bronchi, at least as studied in this investigation. Even with noradrenaline and phenylephrine the response at all dose levels tested was dilatation before β-adrenoceptor blockade.

The finding that the dilating effect of isoprenaline could not be converted into a constricting action by a β-adrenoceptor blocker supports the concept that this amine is a pure β-adrenoceptor stimulating agent, whereas phenylephrine seems to be

less pure in its α-stimulating property, since it regularly produced bronchodilatation before β-adrenoceptor blockade.

The bronchodilating effect of phentolamine *per se* has previously been demonstrated in the guinea-pig (Lish, Robbins & Dungan, 1968). It was suggested that this action resulted from a liberation of catecholamines. In our study, however, the bronchodilating action of phentolamine could be demonstrated also after β-adrenoceptor blockade.

Propranolol augments the response of guinea-pig lung *in vivo* to histamine, acetylcholine and $PGF_2\alpha$ (Collier & James, 1967; James, 1969). However, such a potentiation was not observed in our *in vitro* preparations. This discrepancy would seem to support the concept that constrictor responses of the bronchi *in vivo* are counteracted by an opposite action elicited reflexly via the sympathetic system as proposed by McCulloch, Proctor & Rand, (1967).

It has been observed clinically that propranolol causes an increased airway resistance in healthy subjects and even more so in asthmatic patients (McNeill & Ingram, 1966 MacDonald & others, 1967). In the former this effect of propranolol has been explained as being the result of a blockade of a normally existing β-adrenergic tone in the bronchial smooth muscle leading to a shift of the autonomic balance in the parasympathetic direction, whereas in the latter a hypothesis of a pre-existing partial β-adrenoceptor blockade in the lung has been proposed (Szentivanyi, 1968). The finding in this study that propranolol produces a bronchoconstriction also in isolated preparations, suggests that the bronchoconstriction *in vivo* may, at least in part, be elicited by a direct action on the bronchial smooth muscle. Whether this action is identical with the membrane-stabilizing property which is independent of the β-adrenoceptor effect (Fitzgerald, 1969) can not be answered by our study. It should be noted, however, that alprenolol, *per se*, causing a bronchodilatation, also possesses such a property. Hyper-reactivity of asthmatic bronchial smooth muscle to a variety of bronchoconstricting stimuli has been well documented (Tiffeneau, 1958). Thus the greater sensitivity of asthmatic patients to propranolol could alternatively be partially explained as an exaggerated response of the hyper-reactive bronchial smooth muscle to the bronchoconstricting effect of propranolol *per se*.

Bradykinin has been found to increase airway resistance to flow in the guinea-pig (Collier, Holgate & others, 1960; James, 1969). In this investigation bradykinin produced dilatation in some and a constriction in other preparations. In both cases the bronchi responded with constriction in the usual way when acetylcholine or histamine were added to the bath. It has been reported that bradykinin, administered as aerosol, elicits bronchoconstriction in patients with bronchial asthma but not in healthy subjects (Douglas, 1965). However, strips reacting with constriction in our experiments were not obtained from patients with bronchial asthma. The reason why some preparations reacted with constriction and others with dilatation therefore remains unknown.

The action of 5-HT on the bronchi seems to vary from one species to another. Guinea pig lung *in vivo* and also isolated trachea react with constriction (James, 1969). In a recent study (Booij-Noord, Orie & De Vries, 1969) it was found that about 25% of patients with chronic, nonspecific lung disease reacted to 5-HT inhalation with bronchoconstriction. However, the role of 5-HT, as well as that of bradykinin, in anaphylaxis is unclear (Austen, 1965; Piper & Vane, 1969). In the human isolated bronchi, 5-HT produced dilatation whereas constriction was obtained only at very

high concentrations (20–200 μg/ml).

As in experiments on the guinea-pig isolated trachea (Moore & others, 1968), the dibutyryl form of the cyclic AMP had to be used in order to elicit bronchodilatation. As expected, dibutyryl cyclic AMP produced a dose-dependent relaxation independent of β-receptor blockade.

The observation that theophylline regularly elicited bronchodilatation also in strips contracted with acetylcholine, histamine, $PGF_2\alpha$, SRS and bradykinin gives good pharmacological support to the clinical experience that it is effective in cases of bronchoconstriction of various etiology. The result that the dilatation was produced also after β-adrenoceptor blockade is in line with the current opinion that its action is exerted at a stage beyond that of the adrenoceptor.

Acknowledgements

We wish to thank Drs. V. O. Björk and D. L. E. Rodriquez, Dept. of Thoracic Surgery, Karolinska Hospital, for supplying the lungs and to Dr. B. Högberg, AB Leo, Hälsingborg, Sweden, for supplying the PPP. Purified SRS was kindly put at our disposal by Dr. K. Strandberg, Dept. of Pharmacology, Karolinska Institutet.

This investigation was supported in part by NIMH fellowship no. 1-FO3-MH-43516-01(PS).

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