The nature of the adrenergic receptors in isolated human bronchi

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Isolated human bronchial muscle was stimulated to contract by carbachol, acetylcholine, and histamine. The effects of adrenaline and the alpha- and beta-adrenergic blocking drugs on these contractions were then observed. Phentolamine and phenoxybenzamine potentiated the relaxant effect of adrenaline. Phenoxybenzamine alone caused relaxation of bronchial muscle and inhibited the response to the various spasmogens. Propranolol alone caused a slow increase in muscle tone and antagonized the relaxant effect of adrenaline on carbachol-induced contraction. Phentolamine and phenoxybenzamine added before, with, and after propranolol failed to affect the antagonism of adrenaline by propranolol. The results suggest that the adrenergic receptors in human bronchi are of the beta type.

The effect of catecholamines and their antagonists on tracheobronchial muscle has been well studied in experimental animals. Castro de la Mata, Penna, and Aviado (1962) found that the adrenergic receptors in the bronchial muscles of dogs were mainly beta in type, but some alpha-receptors were also present because adrenaline caused bronchoconstriction after the beta-receptor sites had been blocked by dichlorisoprenaline. On the other hand, Foster (1966) found no evidence of alpha-receptors in guinea-pig tracheal muscles. There have been few reported studies of the nature of the adrenergic receptors in human bronchi, but McNeill (1964) showed that the beta-blocking drug, propranolol, could give rise to bronchoconstriction in asthmatic subjects and, later, MacDonald, Ingram, and McNeill (1967) showed that this effect was largely prevented by atropine. They concluded that bronchoconstriction following propranolol could be explained on the basis of unopposed vagal activity and that it was unnecessary to postulate the presence of alpha-receptors. However, it has recently been reported by Govindaraj and Kerr (1968) that the alpha-receptor blocking drugs, phentolamine and phenoxybenzamine, can protect asthmatic subjects from the effects of intravenous histamine, and this again raises the possibility of active alpha-receptor sites in bronchial muscle.

The present paper reports our attempts to clarify the position from work on isolated human bronchial muscle.

METHODS AND MATERIALS

The sensitivity of bronchial muscle obtained from human lungs at post-mortem examination varied with the time lag between death and the setting up of the preparation in an organ bath. In practice it was difficult to reduce this delay below six to eight hours, and although it was possible to achieve reproducible results the preparations were relatively insensitive. We later obtained bronchial muscle from the lungs of two non-asthmatic patients operated on for carcinoma, and in these instances the specimens were washed and put into Tyrode solution immediately and they provided more satisfactory material for testing. The results confirmed those obtained from cadaver material and they form the basis of this report.

The bronchi were dissected from the lungs and cleaned from connective tissue. They were cut into a spiral using a pair of scissors and trimmed into a strip 4–5 cm. long and 3–4 mm. wide. The strip was suspended in a 70-ml. organ bath filled with Tyrode solution and aerated with 5% CO₂ and 95% O₂. Drug-induced contractions were recorded isotonically on a smoked drum with a magnification of about 20 times. The protocol for the addition of drugs was as follows: a submaximal dose of a spasmogen was added to the bath and left in contact with the bronchial muscle for 2.5 to 3.5 minutes. The relaxant drug was then added and the drum was stopped after 30 seconds. Maximum relaxation was found to occur within four minutes. The bath was emptied and filled twice with Tyrode solution and the muscle was

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allowed to return to its original base-line. The cycle was repeated every 10 minutes using the same dose of spasmogen throughout the experiment but varying the dose of the relaxant drug so as to produce approximately 30% to 50% relaxation of the spasmogen-induced contraction. The alpha- and beta-adrenergic blocking drugs were added to the bath and left in contact with the bronchial muscle for 5–10 minutes. Comparisons between individual catecholamines before and after the blocking drugs were carried out on the same bronchial muscle preparation.

The drugs used were: histamine dihydrochloride, 5-hydroxytryptamine creatine sulphate, acetylcholine, carbachol, (–)-adrenaline, (±)-isoprenaline sulphate, phentolamine mesylate (Rogitine), phenoxybenzamine hydrochloride (Dibenyline), and propranolol hydrochloride. All drugs were prepared monthly in a stock solution, 1.0 mg./ml. in N/100 hydrochloric acid, and stored at 4° C. Dilutions were made in distilled water and the dose of the drug was calculated as $\mu\text{g./ml.}$ of the final dilution in the organ bath and expressed as the base, with the exception of the adrenergic blocking drugs, which were calculated as salts.

RESULTS

Preliminary experiments showed that carbachol, acetylcholine, and histamine all contracted the isolated human bronchial muscle, but carbachol was the most active. Histamine produced a dose-response curve over the range of 0.5 to 2.0 $\mu\text{g./ml.}$ The muscle continued to contract to carbachol and histamine for about 1 minute after washing out the bath. 5-Hydroxytryptamine in a concentration of 4.0 $\mu\text{g./ml.}$ failed to induce a response. Isoprenaline was more effective than adrenaline in causing relaxation of the carbachol, acetylcholine, and histamine-induced contractions. Adrenaline, however, was used in most experiments, since it is known to activate both alpha- and beta-adrenergic receptors.

EFFECT OF ALPHA-ADRENERGIC BLOCKING AGENTS
Phentolamine (2.0 to 4.0 $\mu\text{g./ml.}$) potentiated the relaxation produced by adrenaline on the various spasmogen-induced contractions. The rate of relaxation produced by adrenaline was also faster in the presence of phentolamine.

Phenoxybenzamine (0.5 $\mu\text{g./ml.}$) caused relaxation of the bronchial muscle and slightly inhibited the response of the muscle to acetylcholine and carbachol. Inhibition of the histamine response was marked and recovery took place slowly over 50 to 70 minutes. Phenoxybenzamine also potentiated the relaxant effect of adrenaline on

FIGURE. Isolated human bronchial muscle contractions. All doses are expressed as $\mu\text{g./ml.}$ of organ bath solution. 5-Hydroxytryptamine (5-HT) has no effect: adrenaline (Ad) causes relaxation of carbachol (C) and histamine (H) induced contractions: phenoxybenzamine (Px) causes direct relaxation of the muscle, inhibits the response to carbachol and histamine and potentiates the relaxant effect of adrenaline. At || the muscle was allowed to recover from the effects of phenoxybenzamine for 50 minutes. Propranolol (Pr) causes slow contraction of the muscle and antagonizes the effect of adrenaline on carbachol and histamine-induced contractions.

carbachol-induced contractions, and this effect was more prolonged than that of phentolamine.

EFFECT OF BETA-ADRENERGIC BLOCKING DRUGS
Propranolol, 1.0 $\mu\text{g./ml.}$, caused a slow contraction of the bronchial muscle. It also antagonized the relaxant effect of adrenaline on carbachol, acetylcholine, and histamine-induced contractions. The antagonism persisted for several hours despite repeated washing of the bronchial muscle. Phentolamine and phenoxybenzamine added before, with, and after propranolol had no effect on the antagonism of adrenaline.

DISCUSSION

It is well known that the bronchi of asthmatic subjects are hypersensitive to chemical mediators such as acetylcholine and histamine (Herxheimer, 1951; Tiffeneau, 1955; Makino, 1966) and this is probably an important determining factor in the production of an asthmatic attack. Investigation of the effect of catecholamines on isolated human bronchial muscle contracted by acetylcholine, carbachol, or histamine is thus of considerable practical importance, and the use of adrenergic drugs and their antagonists assists in determining the nature of the receptors.

The demonstration of a slow increase in bronchial muscle tone following propranolol is in keeping with the rise in airways resistance reported by McNeill (1964). This can be regarded either as a passive phenomenon resulting from the blockade of beta-adrenergic dilator receptors or as an active phenomenon resulting from unopposed activity of alpha-adrenergic constrictor receptors. Since the relaxant effect of adrenaline is potentiated by alpha-adrenergic blocking drugs, it is tempting to accept the latter explanation, but there are certain arguments against this.

First, the potentiation of adrenaline by phenoxybenzamine was observed after only 5 minutes, while its alpha-blockading action is a slowly developing process due to the time required for the tertiary amine to undergo cyclization to a more active intermediate form (Goodman and Gilman, 1965). Secondly, following propranolol adrenaline did not increase the carbachol-induced contraction, which suggests that no alpha-receptors were present. Alpha-adrenergic blocking drugs have effects other than their well-known blocking properties. The present work has shown that phenoxybenzamine has a direct relaxant effect on isolated bronchial muscle and inhibits its response to various spasmogens. Both phentolamine and phenoxybenzamine have been found to exert an antihistamine effect on bronchi of anaesthetized guinea-pigs (Guirgis, 1969, in preparation). These drugs have also been shown to inhibit the uptake of adrenaline into tissue stores (Iversen, 1965) and this may well explain the potentiation of the relaxant effect of adrenaline. This explanation accords with the observations of Foster (1968) on guinea-pig tracheal muscle, but an alternative explanation offered by Lish, Robbins, and Dungan

(1968) is that phentolamine releases adrenaline from the adrenal medulla and other storage sites.

The use of alpha-adrenergic blocking drugs either alone or in combination with adrenaline in the treatment of asthma clearly deserves further investigation. The antihistamine effect may be regarded as a bonus, but the systemic hypotensive effect of alpha-blocking drugs might create a serious problem. If the bronchial effect can be achieved by aerosol administration this should greatly reduce the systemic effect and obviate this difficulty.

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