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A COMPARISON OF DRUG-INDUCED RESPONSES ON RAT TRACHEAL, BRONCHIAL AND LUNG STRIP *in vitro* PREPARATIONS

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1 A preparation of rat trachea and a new preparation of rat bronchi are described. Both preparations are fluid-filled and the intraluminal pressure is monitored.

2 A preparation of rat peripheral airways, the lung strip, is described. The preparation consists of a thin strip of lung parenchyma which is superfused, and contractions monitored isotonically.

3 The rat trachea and bronchi have no intrinsic tone but increases in pressure are elicited in response to methacholine. The preparations relax in response to isoprenaline and aminophylline in the presence of a methacholine-induced contraction. Both preparations respond weakly and show tachyphylaxis to 5-hydroxytryptamine (5-HT).

4 The lung strip contracts with equal magnitude to methacholine and 5-HT. It exhibits intrinsic tone which is inhibited by indomethacin and relaxed by isoprenaline.

Introduction

Bronchoconstriction and bronchodilatation in small animals have been generally examined by the use of isolated preparations of major airways or whole animals. The major airways used have been the tracheal tube (Farmer & Coleman, 1970) or the spirally cut trachea (Constantine, 1965). The whole animal preparations are usually variations of the technique originally described by Konzett & Rössler (1947) involving artificial respiration. None of these provide much information about the likely effect of agonists in a spontaneously breathing animal.

We have examined the effects of various agonists using preparations derived from three levels of the rat respiratory tract. First, we used an isolated tracheal tube preparation similar to that described for the guinea-pig by Farmer & Coleman (1970). Secondly, we have developed an isolated preparation of the rat major and lobar bronchi. These two large airway preparations represent the areas which contribute most to resistance or 'bronchoconstriction' changes. We have also developed an isolated lung strip preparation, similar to that from cat lung described by Sparrow & Mitchell (1976). This preparation, derived from the lung parenchyma, contains mainly small airways and alveoli which contribute to compliance changes.

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Methods

Rat tracheal tube

Male Charles River CR/CD rats (weight range 250 to 350 g) were anaesthetized with 60 mg/kg sodium pentobarbitone ('Sagatal', May & Baker Ltd.) intraperitoneally. The trachea was exposed and excised from the larynx to just above the bronchial bifurcation. The trachea was mounted on a prefabricated block similar to that described by Farmer & Coleman (1970), filled and immersed in a physiological solution at 37°C. The solution contained the following (g/l): NaCl 8.0, $NaHCO_3$ 1.0, glucose 1.0, NaH_2PO_4 0.32, $MgCl_2$ 0.42, KCl 0.2 and $CaCl_2$ 0.1.

Changes in intraluminal pressure were recorded with a Statham P23BB pressure transducer and a Vitatron flat bed recorder. Drugs were added to the bathing fluid.

Rat bronchial preparation

Male Charles River CR/CD (250 to 350 g) rats were anaesthetized as above. The thorax was opened and

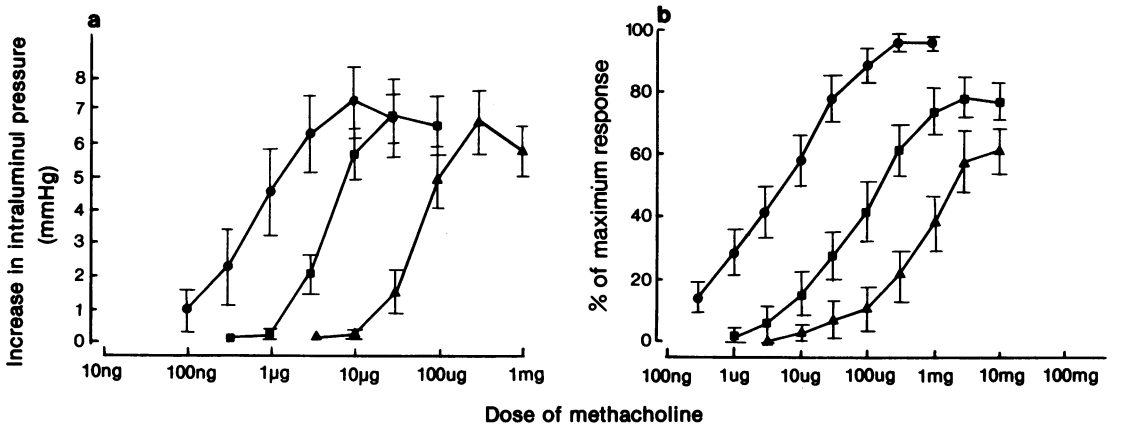

Figure 1 The effect of methacholine on (a) the rat isolated bronchial preparation, and (b) the lung strip: without atropine sulphate (●); in the presence of atropine sulphate 10 ng/ml (■) or 100 ng/ml (▲). Each point represents the mean results from 5 tissues (a) or 6 tissues (b); vertical lines show s.e. means.

the trachea, lungs and heart were dissected free. The heart, great vessels and oesophagus were carefully removed. The single lobe of the left lung was gently teased clear of the surrounding connective tissue and a ligature tied around the bronchus as it entered the parenchyma and the parenchyma removed. This procedure was repeated with the four lobes of the right lung leaving the trachea and the major and lobar bronchi. The preparation was gently massaged to fill it with physiological saline and a PP120 polyethylene cannula inserted into the trachea up to the bronchial bifurcation. A ligature was then tied around this cannula excluding the trachea from the preparation. Changes in intraluminal pressure were recorded with a Statham P23BB pressure transducer and a Vitatron flat bed recorder. The preparation was immersed in aerated physiological solution at 37°C in an overflow bath and drugs were added to the bathing fluid.

Superfused lung strip

Male Charles River CR/CD rats (250 to 350 g) were anaesthetized as above and the trachea, lungs, heart and great vessels excised as before. The left lung was removed and placed in physiological saline. The outer 2 mm of the lateral margin was cut from the lobe to form a strip. Approximately 1.5 cm of this strip was suspended in an organ bath and aerated physiological solution at 37°C was superfused at a flow rate of 2 ml/min. The tissue was placed under an initial tension of 300 mg and changes in tissue tone were recorded with a Harvard smooth muscle transducer and a Vitatron recorder. Drugs were added to the superfusate via an injection port before the peristaltic pump.

A lung strip was fixed in formal sublimate, stained with haematoxylin and eosin and cut into 5 µm sections. Microscopic examination showed that the preparation consists of alveoli, alveolar ducts, and respiratory bronchioles.

Drugs

The drugs used were aminophylline (Fisons), atropine sulphate (BDH), histamine acid phosphate (Evans Medical), indomethacin (Merck, Sharpe and Dohme), 5-hydroxytryptamine creatinine phosphate (Ralph Emanuel Limited), isoprenaline hydrochloride (Pharmax), methysergide bimaleate (Sandoz), prostaglandins E_1, E_2 and $F_{2\alpha}$ (Cambrian Chemicals), methacholine chloride (Aldrich Chemicals) and propranolol hydrochloride (ICI).

Results

Neither the isolated bronchi nor the tracheal tube possessed intrinsic tone, but the superfused lung strip started with tone and developed more during the experiment. The responses of the trachea and the bronchi were similar throughout and illustrations are shown for the bronchi only.

Methacholine

Methacholine increased the intraluminal pressure of the bronchi and the trachea. Figure 1a shows the effect on the bronchi of methacholine and its inhibition by atropine sulphate. Figure 1b shows the effect of methacholine on the lung strip. Note that atropine

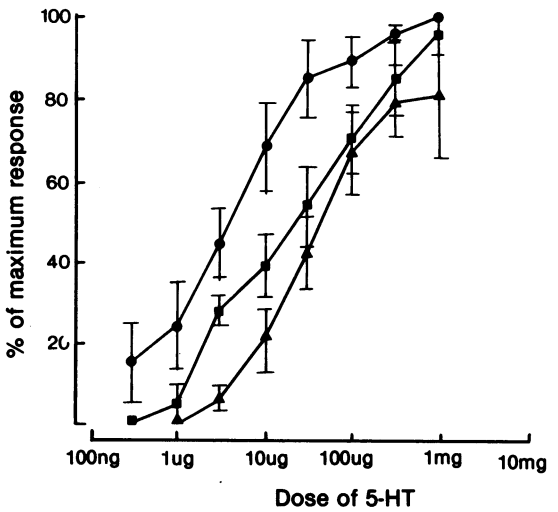

Figure 2 The effect of 5-hydroxytryptamine (5-HT) on the rat isolated lung strip without atropine sulphate (●); in the presence of atropine sulphate 10 ng/ml (■) or 100 ng/ml (▲). Each point represents the mean results from 6 tissues; vertical lines show s.e. means.

causes a dose-related reduction in the maximal response to methacholine.

5-Hydroxytryptamine (5-HT)

Both the trachea and the bronchi rapidly developed tachyphylaxis to 5-HT and a 30 min slow wash was found to be necessary to restore 5-HT's activity. However, even when using this protracted dose cycle the response to 100 μg was approximately half that of the initial response to 10 μg on the 5 tissues studied. The maximum response to 5-HT was approximately 25% of that to methacholine.

The effects of methysergide and atropine were examined on a bronchial preparation. Three consecutive doses of 10 μg/ml were given at 30 min intervals. On the third occasion, 10 ng/ml of either atropine or methysergide was present. The responses were significantly inhibited by methysergide (62% inhibition) but not altered by atropine.

5-HT produced contractions of the lung strip which were as large as those caused by methacholine on the same tissue. There was no tachyphylaxis in the superfused lung strip but it did occur when the strip was immersed in physiological solution. The responses to 5-HT seen before tachyphylaxis occurred in the bathed preparations were also as large as those to methacholine. This is a major difference between the trachea/bronchi and the lung strip. Methysergide in the superfusate at a concentration of 10 ng/ml antagonized the effect of 5-HT. Atropine at 10 ng/ml and 100 ng/ml caused a partial antagonism (Figure 2).

Isoprenaline

Neither the trachea nor the bronchi possessed intrinsic tone and isoprenaline had no direct effect on them. However, isoprenaline caused dose (1 to 10 μg/ml)-related relaxations of a methacholine-induced contraction. These relaxations were small and were antagonized by propranolol (10 ng/ml). The superfused lung strip possessed intrinsic tone as it was relaxed by isoprenaline in the dose range of 0.1 to 1 μg/ml. The relaxations were slow in onset and the tissue took about 20 min to regain its tone. Propranolol (10 ng/ml) inhibited the relaxations.

Aminophylline

Aminophylline relaxed all three tissues in the same manner as isoprenaline. Doses of 10 to 100 μg/ml were needed to produce partial relaxation of methacholine-induced tone on the trachea and bronchi, and 500 to 1000 μg were needed to relax the lung strip. Propranolol (10 ng/ml) did not antagonize the relaxations in any of the tissues.

Prostaglandins

Prostaglandins (PG) E_1, E_2 and $F_{2\alpha}$ had no effect in concentrations up to 1 μg/ml on four preparations of all three tissues under resting conditions. PGE_2 and $PGF_{2\alpha}$ produced small relaxations of a methacholine-induced contraction of the trachea and bronchi at 1 μg/ml.

Histamine

Histamine had no effect on four preparations of each tissue in concentrations as high as 1 mg/ml.

Origin of the tone in superfused lung strip

Four lung strips were superfused with physiological solution containing 1 μg/ml indomethacin. A slow relaxation resulted which could not be reversed by washing for up to one hour. The tissue responded normally to methacholine and isoprenaline during these relaxations.

Discussion

Marked differences were seen between the major airway preparations (trachea and bronchi) and the peripheral airways (lung strip). The tracheobronchial preparations represent the parts of the pulmonary

tree which contribute most to pulmonary resistance and 'bronchoconstriction' (Pedley, Schroter & Sudlow, 1970). The lung strip preparation consists of small airways and alveoli and represents the part of the lung which contributes mainly to lung compliance. It is probably also the part of the lung which responds during the so-called 'bronchoconstriction' measured in Konzett & Rössler (1947) type preparations (Widdicombe & Sterling, 1970).

The differences between the reactivity of the tracheobronchial preparations and the lung strip provide further confirmation of the heterogeneity of smooth muscle responses throughout the lung. This has been shown by Sparrow & Mitchell (1976), who demonstrated that cat peripheral airways and trachea respond differently to histamine, and by Eyre (1969) who reported that histamine will contract sheep tracheae and major bronchi, but will relax small bronchi and bronchioles. We have noted a difference in the effect of 5-HT on the tracheobronchial preparations and the lung strip. In the tracheobronchial preparation contractions to 5-HT were small and showed tachyphylaxis. This agrees with the direct observations of rat bronchi by Schuler & Iravani (1970). Tachyphylaxis was present in the bathed strips but not the superfused preparations. This could be because of reduced contact time which removes the 5-HT before it can bind irreversibly to the receptors. 5-HT could be acting indirectly by releasing another mediator which is exhausted after a large stimulation, as shown by the sequential responses of the bronchi to 10 and 100 μg of 5-HT. However, 5-HT produced contractions of the lung strip which were similar in magnitude to methacholine. This explains the observation by Church (1975) that 5-HT was a potent 'bronchoconstrictor' in the pithed rat overflow preparation, which is probably small airway based. We have also found that 5-HT is partially antagonized by atropine on the lung strip, as it was in the pithed rat overflow preparation (Church, 1975). This effect could be mediated by the M receptors described by Gaddum & Picarelli (1957).

The origin of the tone of the lung strip is a matter for speculation. The tone was inhibited by indomethacin, although PGE_1, PGE_2 and $PGF_{2\alpha}$ did not contract the tissue. The tone could be caused by a thromboxane or more likely an endoperoxide. Wasserman (1976) has demonstrated that two cyclic ether analogues of prostaglandin endoperoxide have a greater action than $PGF_{2\alpha}$ on dog pulmonary mechanics.

In all three preparations isoprenaline caused relaxation which was inhibited by propranolol. Further work on these tissues is needed to characterize the β-receptors present by the use of other agonists and antagonists as Lullich, Mitchell & Sparrow (1976) demonstrated that the cat trachea possessed predominantly β_1, while the cat lung strip β-adrenoceptors were mainly β_2.

These tissues would also provide suitable material for a comparison of the activity of sensitized tissue during challenge; if the rat mast cell contains mainly 5-HT and histamine, it would be expected that the trachea and bronchi would be relatively refractory whereas the lung strip would show a large response.

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