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AN *in vitro* STUDY OF VARIOUS DRUGS ON CENTRAL AND PERIPHERAL AIRWAYS OF THE RAT: A COMPARISON WITH HUMAN AIRWAYS

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1 The effect of histamine and other drugs on the central and peripheral airways of the rat was studied by applying them directly to isolated tracheal and lung strip preparations. These effects were then compared with those observed on human isolated bronchial muscle preparations.

2 Acetylcholine and 5-hydroxytryptamine (5-HT) both contracted the lung strip and trachea of the rat, and both were more potent on the trachea than the lung strip.

3 Histamine and prostaglandins E_2 (PGE_2) or F_2, (PGF_2,) produced no effect on either the lung strip or trachea of the rat.

4 On the human isolated bronchial preparation, in contrast to the rat airways, both histamine and PGF_2, produced marked concentration-dependent contractions and 5-HT either produced no response or a slight relaxation.

5 In view of these results, the use of anaphylactic bronchoconstriction in the rat as a model for the study of asthma in man is questioned.

Introduction

Even though the intestine is the primary shock organ in the rat (Farmer, Richards, Sheard & Woods, 1975), Church, Collier & James (1972) have characterized anaphylactic bronchoconstriction in this species, and Farmer *et al.* (1975) have found that histamine, and other mediators of anaphylaxis are released from isolated sensitized rat lungs after anaphylactic challenge.

In the guinea-pig, IgG, rather than IgE, is the primary antibody responsible for mediating anaphylactic bronchoconstriction, and furthermore in this species anaphylactic bronchoconstriction is not inhibited by disodium cromoglycate (Church, 1975). However, in the rat, as in man, allergic bronchoconstriction is mediated primarily through antigen combining with IgE antibody, and allergic bronchoconstriction is inhibited by disodium cromoglycate (Church *et al.*, 1972). It has therefore been proposed that anaphylactic bronchoconstriction produced in the rat is a better model for human asthma, than that occurring in the guinea-pig (Church *et al.*, 1972; Church, 1975).

Akcasu (1952) found that the isolated rat trachea was unresponsive to histamine, but contracted markedly to acetylcholine. However, it has been demonstrated in the cat that even though histamine does not contract the isolated trachea, it markedly contracts peripherally located airways smooth muscle (Lulich, Mitchell & Sparrow, 1976; Persson & Ekman, 1976). Therefore when documenting the properties of airways smooth muscle *in vitro*, it is advisable to characterize both the central and peripheral airways.

In the present study, we have used the isolated lung strip and trachea of the rat to characterize the responses of the peripheral and central airways of this species to histamine and other drugs *in vitro*. In addition we have applied these drugs to human isolated bronchial muscle strips so that the drug-induced responses on the airways in rat could be compared with those in man.

Methods

Preparation of rat tracheal, rat lung strip, and human bronchial, preparations

Male Wistar rats (300 to 350 g) were stunned and bled. The trachea, lungs and heart were then dissected out and placed in modified Krebs solution (Lulich *et al.*, 1976) aerated with 95% O_2 and 5% CO_2, at room temperature. The isolated lung strip and single open tracheal ring were then prepared as described by Lulich *et al.* (1976); in the rat, the approximate

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dimension of the lung strip was 12 × 2 × 2 mm, and the open tracheal ring was approximately 1 mm in width.

Human bronchi were obtained from macroscopically normal parts of human lung, removed because of carcinoma. The isolated bronchi, approximately 3 to 5 mm in diameter, were then cut spirally into strips of muscle approximately 15 mm long and 1.5 mm wide.

After dissection, the isolated preparations were placed in 10 ml organ baths containing modified, aerated Krebs solution at 37°C. While the isolated lung strip of the rat had a load of 0.9 g tension applied, both the rat tracheal and human bronchial preparations had a tension of 0.5 g applied. The tissues were allowed to equilibrate for 60 to 90 min, with the solution changed at 15 min intervals. Consistent responses could be obtained for at least 4 h, from preparations kept at 37°C.

Changes in tension, produced by the tracheal and bronchial preparations, were measured isometrically with a Grass FTO3C force displacement transducer. The maximal contraction produced by the rat lung strip was in the range 21 to 71 mg, so a FTA ± 1 g myographic force displacement transducer connected to a 4 channel Hewlett Packard 7404 oscillographic recorder (17403A carrier preamplifier) was used. This system reliably measured the changes in tension of the lung strip preparation produced by drugs.

Calculations

On both the isolated trachea and lung strip of the rat, concentration-response relationships were determined with a cumulative dose schedule (Van Rossum, 1963), after which the organ bath was flushed, and the tension allowed to return to its original value. On the human bronchial strip the preparation was allowed to recover fully after the application of each concentration of drug. For comparative purposes all contractions were expressed as a percentage of the maximal contraction to acetylcholine in the same preparation.

On both the isolated lung strip and trachea, the potencies of the drugs were expressed in the form of an EC_{50} value. The EC_{50} value was defined as the molar concentration of drug required to elicit 50% of the maximal contraction produced by that drug, and presented as a geometric mean with the 95% confidence limits in parentheses. The relative maximal contraction (r.m.c.) produced by a drug, on either the lung strip or trachea was expressed as a percentage (mean ± s.e. mean) of the maximal contraction to acetylcholine. Statistical comparisons were made by an unpaired Student's t test; differences were considered significant when $P < 0.05$.

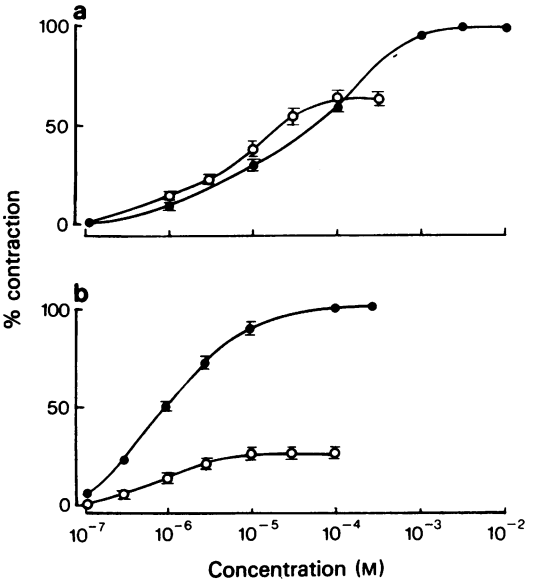

Figure 1 Mean concentration-response curves to acetylcholine (●) and 5-hydroxytryptamine (5-HT, ○) on the isolated lung strip (a) and trachea (b) of the rat. The contractions are expressed as a percentage (mean) of the maximal contraction produced by acetylcholine; vertical bars indicate s.e. mean. For acetylcholine each point is the mean of 6 experiments, and for 5-HT each point is the mean of 12 experiments. Standard error bars are shown when they exceed the dimensions of the symbols.

Drugs

The following drugs were used: acetylcholine chloride, histamine diphosphate, 5-hydroxytryptamine (creatinine sulphate complex), (±)-isoprenaline hydrochloride, (Sigma), and prostaglandins E_2 and $F_{2\alpha}$ (Upjohn).

Results

Isolated rat lung strip and trachea

Isoprenaline (10^{-4} M) did not relax the lung strip ($n = 6$) or the tracheal ring ($n = 6$); therefore neither of these isolated preparations exhibited an intrinsic tone under the defined experimental conditions. Histamine (10^{-7} to 10^{-3} M) did not contract the tracheal ring ($n = 12$) or the isolated lung strip ($n = 12$). Neither prostaglandin E_2 nor $F_{2\alpha}$, (10^{-7} to 10^{-5} M) contracted the tracheal ring ($n = 6$), or the isolated lung strip ($n = 6$).

Acetylcholine and 5-hydroxytryptamine (5-HT) contracted both the lung strip and trachea of the rat (Figure 1, Table 1). Acetylcholine was 48 times more

potent on the isolated trachea than on the lung strip. Although the relative maximal contraction produced by 5-HT on the lung strip (64.2 ± 3.6%) was significantly greater than 26.5 ± 2.9%, the relative maximal 5-HT-induced contraction on the isolated trachea, 5-HT was 7 times more potent on the trachea. While 5-HT was 7 times more potent than acetylcholine on the lung strip ($P < 0.001$), acetylcholine and 5-HT were equipotent on the trachea.

Human isolated bronchus

Histamine, $PGF_{2\gamma}$, 5-HT, and acetylcholine, were applied to 5 spirally cut bronchial muscle preparations; each was obtained from the lung of a different patient. While histamine (10^{-6} to 10^{-4} M), $PGF_{2\gamma}$ (10^{-6} to 10^{-4} M), and acetylcholine (10^{-6} to 10^{-3} M), markedly contracted the human bronchial preparation in a concentration-dependent manner, 5-HT (10^{-6} to 10^{-4} M) produced either no response or a slight relaxation.

Discussion

Using the isolated lung strip to represent peripheral airways, and the isolated trachea to represent central airways, we have demonstrated that 5-HT and acetylcholine contract both central and peripheral airways smooth muscle in the rat. Both acetylcholine and 5-HT were found to be more potent on the central than on the peripheral airways. Similarly Burns & Doe (1978) found that methacholine was more potent in increasing the intraluminal pressure of the isolated bronchial preparation than in contracting the isolated lung strip of the rat.

On the isolated trachea, 5-HT and acetylcholine were equipotent; however, on the lung strip 5-HT was 7 times as potent as acetylcholine. This compares with the *in vivo* finding by Church (1975) that 5-HT was 3 times more potent than acetylcholine in producing bronchoconstriction. On both the lung strip and trachea, acetylcholine produced a greater maximal contraction than 5-HT. However, *in vivo* 5-HT produces a greater maximal bronchoconstriction than acetylcholine (Church, 1975). Since 5-HT has been reported to stimulate vagal fibres (Garattini & Valzelli, 1965), the discrepancy between our *in vitro* and the *in vivo* results obtained by Church could be attributed to stimulation of the vagus by 5-HT in the intact animal.

In agreement with Burns & Doe (1978), we found that histamine and PGE_2 and $PGF_{2\alpha}$ did not contract either the lung strip or the trachea of the rat. Similarly Church (1975) showed that histamine, $PGF_{2\gamma}$, and slow reacting substance of anaphylaxis (SRS-A) were ineffective on the airways of the rat *in vivo*, except at extremely high doses.

Acetylcholine produced a marked concentration-dependent contraction on the isolated bronchial preparation of man as it did in the rat airways. However, while 5-HT produced a marked contraction on rat airways, it produced either no response or a relaxation on the human bronchial strip. Conversely, even though high concentrations of $PGF_{2\gamma}$ and histamine did not contract isolated airway smooth muscle of the rat, both these drugs produced marked concentration-dependent contractions of the isolated bronchial strip of man. Thus the responses of the airways of these two species to 5-HT, histamine, and $PGF_{2\gamma}$, are completely different.

Farmer *et al.* (1975) have demonstrated that challenge of sensitized rat lung with antigen releases 5-HT, histamine, SRS-A and prostaglandins. However our *in vitro* results, and those of Burns & Doe (1978), and the *in vivo* findings obtained by Church (1975) suggest that of these substances only 5-HT significantly contracts rat airways. This is consistent with reports that the major mediator of anaphylactic bron-

Table 1 A comparison of the potency (EC_{50}), and relative maximal contraction (r.m.c.) induced by acetylcholine, and 5-hydroxytryptamine (5-HT) in the lung strip and trachea of the rat

	Lung strip		*Trachea*	
Drug	*EC_{50}(M)	†r.m.c.	EC_{50}(M)	r.m.c.
Acetylcholine	5.2×10^{-5} (3.6×10^{-5}–7.6×10^{-5}) ($n = 6$)	100%	1.1×10^{-6} (9.5×10^{-7}–1.2×10^{-6}) ($n = 6$)	100%
5-HT	7.3×10^{-6} (4.8×10^{-6}–1.1×10^{-5}) ($n = 12$)	64.2 ± 3.6%	1.1×10^{-6} (7.7×10^{-7}–1.5×10^{-6}) ($n = 12$)	26.5 ± 2.9%

* The EC_{50} values are expressed as geometric means with the 95% confidence limits in brackets.
† The relative maximal contraction (r.m.c.) is expressed as a percentage (mean ± s.e. mean) of the maximal contraction to acetylcholine.
The number of preparations examined is represented by *n*.

choconstriction in the rat is 5-HT (Church *et al.*, 1972; Church, 1975).

In both human asthma and anaphylactic bronchoconstriction in the guinea-pig, histamine and SRS-A are considered to be important primary spasmogens, while 5-HT is considered insignificant (Brocklehurst 1971, Austen & Orange, 1975). Prostaglandins have been demonstrated to be released from guinea-pig and human isolated lung (Piper & Vane, 1969; Piper & Walker, 1973), and a large body of evidence indicates that they may play a role in asthma (Hedqvist & Mathé, 1977). However because asthmatics are seldom helped by drugs which inhibit prostaglandin synthesis, such as indomethacin or aspirin, it is considered that prostaglandins do not play a principal role in this disease (Piper, 1977).

Although anaphylactic bronchoconstriction in the rat is a convenient model for the determination of anti-allergic activity of drugs, our study demonstrates that the airways of this species respond differently to chemical mediators from human airways. Therefore the suitability of inducing anaphylactic bronchoconstriction in the rat to simulate asthma in man is questioned.

As in man, histamine, $PGF_{2\alpha}$ and SRS-A contract guinea-pig airways (Drazen & Austen, 1974), but anaphylactic bronchoconstriction in the guinea-pig is not favoured as a model for human asthma because in this species it is primarily IgG-mediated and disodium cromoglycate is ineffective as a prophylactic agent (Church, 1975). Recently Carney (1976) has described a method for producing IgE-mediated anaphylactic bronchoconstriction in the guinea-pig, which is significantly inhibited by disodium cromoglycate. We suggest IgE-mediated anaphylactic bronchoconstriction in the guinea-pig should be further explored, as it may then prove to be a more suitable animal model for the study of asthma in man.

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