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THE INTERACTION BETWEEN INDOMETHACIN AND CONTRACTILE AGENTS ON HUMAN ISOLATED AIRWAY MUSCLE

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1 Concentration-effect curves to acetylcholine and histamine were produced in fresh human bronchial muscle (2 to 4 h after removal from the patients) and in preparations previously stored at 4°C for 12 h.

2 Sensitivities of fresh human airway muscle preparations to acetylcholine (pD_2 value, 5.89 ± 0.03); $n = 4$) and histamine (pD_2 value, 5.41 ± 0.03; $n = 13$) were similar. There was no significant difference in the sensitivities of stored preparations (acetylcholine: pD_2 value, 5.70 ± 0.06; $n = 23$ and histamine: pD_2 value, 5.44 ± 0.07; $n = 16$) when compared to the fresh preparations.

3 Indomethacin did not significantly change the basal tone in preparations of either fresh or stored human airway muscle.

4 A low concentration of indomethacin (0.17 μM) significantly reduced responsiveness and sensitivity to histamine in stored bronchi but not in fresh bronchi. The acetylcholine concentration-effect curves were unaltered by exposure to this concentration of indomethacin in either fresh or stored tissues. High concentrations (1.7 μM and 17 μM) depressed the maximal responsiveness of the bronchi to both agonists.

5 These results suggest indirectly that the regulatory role of prostaglandins in human airway muscle may be different from that in other species.

Introduction

Human lungs can form prostaglandins, their precursors and their metabolites (Ängärd, 1965; Karim, Sandler & Williams, 1967). The quantities of these compounds released from lung tissue when compared to the amounts stored in tissues suggest that a release of prostaglandins probably involves new synthesis from precursors such as arachidonic acid (Piper & Vane, 1971; Grodzinska, Panczenko & Gryglewski, 1975; Yen, Mathe & Duggan, 1976). The role of these substances is not clear but the findings of Orehek, Douglas, Lewis & Bouhuys (1973) suggest that products of the arachidonic acid cascade may act as local hormones and contribute to the control of normal and/or abnormal airway muscle tone. In guinea-pig tracheal preparations, indomethacin reduces muscle tone and affects the responsiveness of the tissue to several agonists (Farmer, Farrar & Wilson, 1974; Orehek, Douglas & Bouhuys, 1975). Orehek *et al.* (1975) demonstrated that when the synthesis of prostaglandins is inhibited by indomethacin, responses to low concentrations of agonists are decreased while responses to high concentrations are potentiated. It has been suggested that local production of prostaglandins may result from the mechanical change of the cellular membranes of the airway tissue (mucosa, submucosa and/or muscle).

We therefore examined the effects of indomethacin on human airway muscle tone and on the sensitivity to histamine and acetylcholine.

Methods

Human bronchial tissue was obtained from patients undergoing surgery for bronchial carcinoma or tuberculosis. Subsequent to resection of a lung or a lobe, part of a bronchus was dissected free from apparently normal lung tissue and placed in Tyrode solution. The tissues were either set up in 10 ml organ baths 2 to 4 h after excision (fresh tissue) or were first stored in Tyrode solution at 4°C for 12 h (stored tissue). Care was taken to prepare the bronchial spirals of approximately the same length from portions of about

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the same internal diameter (4 to 6 mm). The bronchial spirals were set up for isometric measurements under a load of 3 g and equilibrated for a period of 1.5 h in Tyrode solution at 37°C (gassed with 5% CO_2 in O_2).

Bronchial spirals were examined as follows: (a) Concentration-effect curves were produced in fresh bronchial tissues subsequently stored at 4°C and re-examined after 12 h; (b) Bronchial spirals were cut into two equal portions, one being examined fresh and the other examined after 12 h of storage at 4°C; (c) From other pairs of fresh bronchial spirals one portion was incubated with indomethacin (see (d)) while the other was exposed to Tyrode solution (control); (d) Concentration-effect curves were established in stored tissues which were then incubated with Tyrode solution alone or containing indomethacin (0.17 μM; 1.7 μM or 17 μM) for 30 min. The tissues were then washed with fresh Tyrode solution and concentration-effect curves were determined in the absence of indomethacin. After each experiment the tissues were dried in an oven (65°C) for 12 h and weighed. Sixty two spiral preparations from 28 different individuals were examined.

Concentration-effect curves were produced by adding graded concentrations of acetylcholine or histamine, in a volume of less than 0.5 ml, in random order, to the tissue bath. When the response to an agonist reached a plateau, the bath fluid was exchanged for fresh Tyrode solution, every 5 min with acetylcholine and every 3 min with histamine. The preparations returned passively to their resting tone. Contractions produced by drugs were expressed as a percentage either of the maximal control response or of the maximal force (g/mg dry wt.). The latter results are presented here as both methods gave similar assessments. Mean concentration-effect curves were calculated from responses to fixed concentrations of agonists and pD_2 values ($-\log_{10} EC_{50}$) for acetylcholine and histamine were interpolated from each curve. In addition, average concentration-effect curves were determined from the mean concentrations which induced 20, 40, 50, 60 and 80% of maximal contraction.

The change in basal tone of each preparation was analyzed from the records by calculating the resting tone (g/mg tissue dry weight) at the beginning of the experiment, the end of the equilibration period, then before the incubation period and at the end of the experiment. We also compared the basal tone in the experiments with indomethacin. The time taken for the tissue to relax after drug wash out was compared for each concentration of agonist, and the recovery ratio, that is, the recovery period after Tyrode solution or indomethacin (0.17 μM) compared to the recovery before drug incubation, was calculated.

The composition of the Tyrode solution was (mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5; pH, 7.3. The initial load ensured that when the experiments began the basal tone was approximately 2.5 g. Under these conditions responses were maximal and reproducible. The responses were recorded with an Apelab 05.7004 isometric strain gauge and an Apelab I recorder. The drugs used were acetylcholine chloride, histamine dihydrochloride (Merck Laboratories) and indomethacin (Sigma Chemical Company). The results are shown as means ± s.e. mean; Student's t test for paired or unpaired variates was used for statistical evaluation.

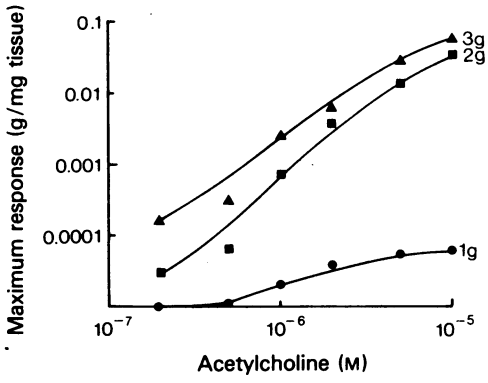

Figure 1 Effects of initial load on the responses of human bronchial spiral preparations. Each point represents the average of two bronchi from different individuals when the same preparations were equilibrated under different loads.

Results

A 3 g initial load ensured that after equilibration, responsiveness to acetylcholine was optimal (Figure 1); higher initial loads (4 to 6 g) in the same preparations (not shown) decreased the responses. There was no significant difference in either histamine responsiveness or sensitivity in fresh or stored bronchial muscle (Figure 2). Acetylcholine concentration-effect curves in fresh bronchial spirals ($n = 4$) gave a pD_2 value of 5.89 ± 0.03 and a maximal responsiveness of 0.03 ± 0.09 g/mg dry wt. These data were not significantly different ($P > 0.05$) from those with stored preparations (Figure 3). In paired samples of fresh bronchi from the same lung the pD_2 values to histamine seemed unaffected by indomethacin treatment (Tyrode solution, pD_2 value of 5.41 ± 0.08; indomethacin (1.7 μM), 5.61 ± 0.09; $n = 3$; $P > 0.05$). The lower concentrations of indomethacin (1.7 and 0.17 μM) did not significantly affect the maximal response to histamine but indomethacin (17 μM) decreased the maximal force developed (Tyrode solu-

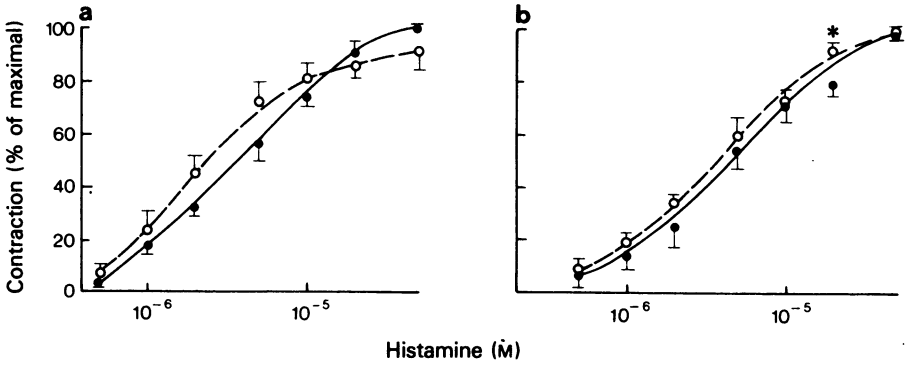

Figure 2 Histamine concentration-effect curves produced in bronchial muscle tissue from different individuals were examined immediately and following storage. (a) Tissues from different individuals were examined immediately (●) and after 12 h storage at 4°C (○). The pD_2 values (see Methods) were not significantly different in fresh and stored tissues (5.40 ± 0.06; 5.60 ± 0.07; respectively, $n = 6$). There was no significant difference in the maximal force developed by these tissues (fresh, 0.02 ± 0.01 g/mg tissue; stored, 0.02 ± 0.01 g/mg tissue). (b) Concentration-effect curves to histamine in two pieces of bronchus from the same individual. One spiral was used fresh (●) and the other was stored at 4°C for 12 h. Subsequent to storage, histamine concentration-effect curves (○) were produced. pD_2 values were not significantly different in fresh and stored tissues (5.41 ± 0.07; 5.45 ± 0.11; respectively, $n = 7$). The maximal force developed by these preparations was the same (fresh, 0.02 ± 0.04 g/mg tissue; stored, 0.02 ± 0.01 g/mg tissue). Values presented are mean ± s.e. mean; * $P < 0.05$.

tion, 0.02 g/mg tissue; indomethacin 0.004 g/mg tissue, $n = 1$). Acetylcholine concentration-effect curves in fresh bronchi showed no change in pD_2 values (Tyrode solution, 5.79 ± 0.04; indomethacin (17 μM), 5.72 ± 0.09; $n = 3$; $P > 0.05$). The maximal responsiveness of these three preparations was not significantly different (Tyrode solution, 0.03 ± 0.04 g/mg dry wt.; indomethacin (17 μM), 0.03 ± 0.08; $P > 0.05$).

Basal tone fell significantly during the experiments (Table 1), and the fall with indomethacin was not significantly different from that in Tyrode solution for 30 min. In the presence of (0.17 μM) indomethacin the concentration-effect curves for acetylcholine and histamine were always reproducible, but in (1.7 μM) indomethacin only the acetylcholine concentration-effect curves were reproducible; with 17 μM indomethacin responses to both agonists were non-reproducible and depressed (Table 2).

We examined the time interval between the moment of peak response of stored bronchi for each

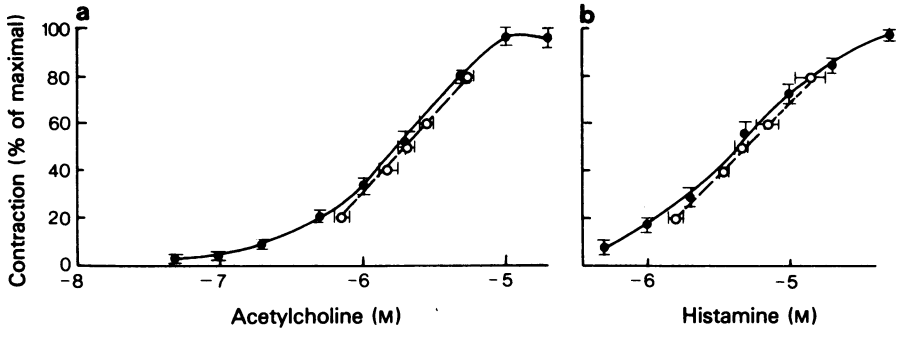

Figure 3 Concentration-effect curves produced in stored human muscle preparations. (a) Acetylcholine concentration-effect curves in bronchial spirals. Data were analyzed by determining mean responses to selected concentrations (●) or by determining the mean concentrations which induced a fixed response (○). Bars represent s.e. mean. The pD_2 value (mean ± s.e. mean) for acetylcholine was 5.70 ± 0.06 and the maximal response developed was 0.03 ± 0.03 in 23 experiments. (b) Histamine concentration-effect curves in stored human muscle preparations. Data analysis was as described in (a). The pD_2 value was 5.44 ± 0.07 (mean ± s.e. mean) and the maximal response was 0.02 ± 0.01 in 16 experiments.

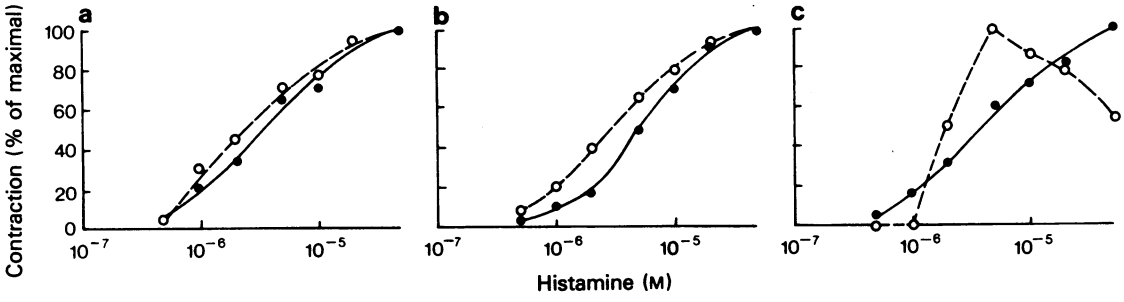

Figure 4 The effect of indomethacin on histamine concentration-effect curves in fresh human bronchial tissues. Each panel represents a pair of bronchi from the same individual incubated with Tyrode solution (●) or indomethacin (○): 0.17 μM (a); 1.7 μM (b); 17 μM (c). The pD_2 values to histamine after incubation with Tyrode solution were 5.48; 5.30 and 5.40 respectively. After incubation with indomethacin the values were similar 5.60; 5.52 and 5.70 respectively. The maximal responsiveness after incubation in Tyrode solution were 0.02 g/mg tissue; 0.03 g/mg tissue and 0.02 g/mg tissue, respectively. After indomethacin the maximal responses were 0.02 g/mg tissues; 0.02 g/mg tissue and 0.004 g/mg tissue respectively.

Table 1 The effects of various treatments on the basal tone of stored human airway smooth muscle *in vitro*

Treatment (*In vitro*)	*Number of Preparations*	*Basal tone* (g/mg tissue) *Initial* (A)	*Post equilibration* (B)	*Pre incubation* (C)	*Post incubation*	*End of experiment* (D)
Tyrode solution	5	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01*
Indomethacin						
17 μM	3	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	0.07 ± 0.01†
1.7 μM	7	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01*
0.17 μM	11	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	0.02 ± 0.01*

Values represent mean ± s.e. mean.
(A) Time zero (beginning of experiment); (B) 1.5 h (end of equilibration period); (C) 4.07 ± 0.13 h (post equilibration period); (D) 8.09 ± 0.29 h (post equilibration period).
* $P < 0.05$ decreased tone; † $P < 0.05$ increased tone.

Table 2 A comparison of the responsiveness and sensitivity of stored human bronchial spiral preparations to histamine and acetylcholine before and after a 30 min incubation with either Tyrode solution or indomethacin

			Histamine *Maximum response*	*Histamine* pD_2-*value*	*Acetylcholine* *Maximum response*	*Acetylcholine* pD_2-*value*
Tyrode Solution		Before	0.03 ± 0.01	5.46 ± 0.11	0.03 ± 0.01	5.79 ± 0.06
		After	0.03 ± 0.01	5.40 ± 0.11	0.03 ± 0.01	5.80 ± 0.05
Indomethacin	17 μM	Before	0.02 ± 0.08‡	5.32 ± 0.12‡	0.04 ± 0.01‡	5.69 ± 0.10‡
		After	NR	NR	NR	NR
	1.7 μM	Before	0.03 ± 0.10‡	5.46 ± 0.08‡	0.03 ± 0.02‡	5.77 ± 0.06‡
		After	NR	NR	0.02 ± 0.01	5.60 ± 0.11
	0.17 μM	Before	0.02 ± 0.01	5.38 ± 0.18	0.02 ± 0.01	5.56 ± 0.09
		After	0.01 ± 0.01*	4.58 ± 0.10*	0.01 ± 0.01*	5.33 ± 0.14

Maximum response is the maximal force developed g/mg dry weight of tissue. Values are mean ± s.e. mean. Number of preparations is at least 5 except ‡ $n = 3$. NR = not reproducible.
* $P < 0.05$.

concentration of either agonist and the moment when the preparation returned passively with washing (see Methods) to its prechallenged value. The recovery period ratio (see Methods) for control preparations of stored bronchi in Tyrode solution, was approximately one ($n = 5$). Preparations exposed to low concentrations of histamine gave recovery ratios of one whereas with high concentrations (5, 2 and 1 μM) the ratios were lower (0.58 ± 0.13; 0.60 ± 0.12 and 0.55 ± 0.04, respectively $P < 0.05$). In five preparations the recovery ratios for all concentrations of acetylcholine (1.0, 0.5, 0.2, 0.1, 0.05, and 0.02 μM) were 1.05 ± 0.07; 1.06 ± 0.06; 1.04 ± 0.05; 0.93 ± 0.05; 0.93 ± 0.09 and 1.07 ± 0.09 respectively. There was no significant difference ($P > 0.05$) in the ratios for each concentration when compared with controls.

Discussion

Histamine and acetylcholine concentration-effect curves in human isolated airway muscle show no significant change in sensitivity (pD_2 values) or responsiveness (g/mg tissue) after storage at 4°C for 12 h. The initial load of the preparation is important for the maximal isometric tissues responsiveness (Figure 1). When muscle preparations are stretched to an optimal length (L_0), i.e., the muscle fibres are at their l_{max}, the preparations respond reproducibly (Stephens, 1970). Although the initial load employed here differs from that used by other workers (Gardiner, 1975; Avner, Noland & Jenne, 1977) we used isometric rather than isotonic measurements. The type of bronchial preparation used may affect the optimal initial load (Kneussl & Richardson, 1978).

Previous work (Douglas, Ridgway & Brink, 1977) demonstrated only a small variability in the sensitivity of guinea-pig tracheal spirals to histamine. These authors obtained a pD_2 value of 5.33 ± 0.03 compared with our values of 5.44 ± 0.07 and 5.41 ± 0.03 in stored and fresh preparations respectively. The acetylcholine concentration-effect curves for human isolated airway muscle also approximate those found for the guinea-pig (Orehek *et al.*, 1975).

Indomethacin did not significantly lower the basal tone of either fresh or stored human airway muscle. Dunlop & Smith (1975) described a slow relaxation by indomethacin in sensitized human bronchi but it was not clear whether this relaxation was significantly different from that in unsensitized bronchi or in control sensitized bronchi.

Previous work (Orehek *et al.*, 1975) has indicated that locally released prostaglandins may affect muscle tone and modulate contractions to various agents. The absence of a significant effect of indomethacin on the resting tone of human bronchial muscle indicates either that the production of prostanoids for muscle tone is unimportant, or that the prostanoids formed have opposing actions. These results are in good agreement with those obtained with anti-inflammatory drugs on airway muscle *in vivo* where, in normal individuals, indomethacin neither altered baseline pulmonary mechanics nor affected airway sensitivity to bronchoconstrictor agents (Ogilvy, Douglas, Tabatabai & DuBois, 1978).

Orehek *et al.* (1975) observed that very high concentrations of indomethacin depressed both maximal responsiveness and sensitivity to histamine of guinea-pig tracheal preparations. We observed a significant decrease in maximal responsiveness of fresh bronchial preparations to histamine with a high concentration of indomethacin (17 μM). The decreased histamine responsiveness of stored tissue following the indomethacin treatment may also be explained, in part, by an altered influx of calcium (Northover 1973), and/or internal calcium sequestration, a process essential for contraction of airway smooth muscle (Schild, 1967; Somlyo & Somlyo, 1969).

Several investigators have already demonstrated the generation and release of prostaglandins and/or their precursors from contracted preparations of isolated lung tissues (Piper & Vane, 1971; Yen *et al.*, 1976; Gryglewski, Dembinska-Kiec & Grodzinska, 1977). Contracted guinea-pig trachea produces prostaglandins E and F (Orehek *et al.*, 1975), while thromboxane A_2 is the main product released from histamine-contracted lung parenchymal preparations (Gryglewski *et al.*, 1977). These observations suggest that lung tissues produce substances which can either relax or contract the airways. The significant decrease in the recovery period following contractions to high concentrations of histamine in preparations treated with indomethacin may indicate that stored tissues may liberate a prostanoid which hinders muscle relaxation, but it is not clear why this occurs only with histamine.

Our results with indomethacin indirectly suggest that the regulatory role of prostanoids in human airway muscle may be less than in other species. Although prostaglandins can affect human isolated bronchial muscle (Sweatman & Collier, 1968; Gardiner, 1975) their role *in vivo* is not clear. Prostaglandin E_1 can inhibit the contractile response of human bronchi to histamine (Sheard, 1967), but it is not known whether a modification of arachidonic acid metabolism in man affects the responsiveness to contractile agents.

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