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REDUCTION OF ANTIGEN-INDUCED CONTRACTION OF SENSITIZED HUMAN BRONCHUS *in vitro* BY INDOMETHACIN

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1 Contraction of passively sensitized human bronchus induced by antigen challenge *in vitro*, was reduced by the prostaglandin $F_{2\alpha}$ synthetase inhibitor, indomethacin.

2 Prostaglandin $F_{2\alpha}$ was released during challenge and this release was inhibited by indomethacin.

3 There was a significant correlation between prostaglandin $F_{2\alpha}$ release and antigen challenge-induced contraction, suggesting that this substance may contribute to the bronchoconstrictor response of sensitized human bronchus to antigen.

Introduction

Ever since the discovery of prostaglandins in human lung (Änggärd, 1965; Karim, Sandler & Williams, 1967) and of the potent bronchoconstrictor effects of prostaglandin $F_{2\alpha}$ in man (Mathé, Hedquist, Holmgren & Svanborg, 1973) there has been speculation about a possible role for prostaglandins in the pathogenesis of asthma. The release of prostaglandins by antigen-challenged human lung *in vitro* (Piper & Walker, 1973) and the presence of a prostaglandin $F_{2\alpha}$ metabolite in the blood of asthmatic patients on inhalation of antigen (Gréen, Hedquist & Svanborg, 1974) gave support to the hypothesis that prostaglandin $F_{2\alpha}$ is a mediator of bronchoconstriction in anaphylaxis. The effect of indomethacin, an inhibitor of prostaglandin $F_{2\alpha}$ synthetase (Vane, 1971), was investigated to establish whether this prostaglandin participates in the contraction of sensitized human bronchus when exposed to antigen *in vitro*.

Methods

Human bronchus 2-3 mm in diameter was dissected free of macroscopically normal lung tissue removed at thoracotomy for bronchial carcinoma, and passively sensitized by overnight storage at 4° C in undiluted pooled serum obtained from patients with asthma due to house dust mite allergy. Sensitized bronchi were cut into spiral strips and one pair from any one lobectomy specimen was suspended in 10 ml organ baths, under a load of 250 mg, perfused with Krebs solution at 37° C and gassed with 95% O_2 and 5% CO_2. Changes in muscle length were measured with an isotonic transducer (SRI) and recorded on moving paper (Rikadenki-Kyogo). Change in the responsiveness of the tissues were detected by parallel control experiments. After about one hour, when the muscle tone was steady maximum responses of test and control preparations to acetylcholine (180-360 μg/ml for 3 min) were obtained and the responses to a standard dose of arachidonic acid (2-10 μg/ml for 5 min) were measured. Indomethacin (3.6 μg/ml) was added to the organ bath and when a steady state of tone had been regained, the test doses of acetylcholine and arachidonic acid were repeated. Finally, each preparation and its paired control was challenged with diluted freeze dried house dust mite antigen (*D. pteronyssius*, 10 μg/ml). Responses to the drugs were expressed as millimetres of pen deflection. The antigen challenge responses were expressed as a percentage of the maximum acetylcholine response (MAR) recorded immediately prior to challenge; in this way results from different tissues could be compared.

The remaining bronchial muscle spirals were used for radioimmunoassay of the prostaglandin $F_{2\alpha}$ released. Parallel controls were carried out, half the preparations being incubated in Krebs solution alone and the remainder in the presence of indomethacin (3.6 μg/ml) throughout the experiment. The spirals were placed in tubes containing 5 ml of gassed Krebs solution at 37° C and were washed three times. They were then incubated for 12 min and the bathing fluid from the tubes was acidified to pH 1-2. extracted with ether and the prostaglandin $F_{2\alpha}$ content measured

using the radioimmunoassay method described by Hennam, Johnson, Newton & Collins (1974) giving the basal level of prostaglandin $F_{2\alpha}$. Finally, the bronchial muscle spirals were placed in tubes containing house dust mite antigen (10 μg/ml) and following challenge the prostaglandin $F_{2\alpha}$ was measured as before. Each preparation was dried and weighed and the results expressed as picograms (pg) prostaglandin $F_{2\alpha}$ per mg of bronchial tissue.

In a similar series of experiments the amounts of prostaglandin $F_{2\alpha}$ released during a maximal contraction induced by acetylcholine (360 μg/ml) was measured and this was compared with the amount released by the same tissues on challenge.

Results

All sensitized bronchial spiral preparations contracted on exposure to acetylcholine and arachidonic acid. Indomethacin caused a slow relaxation of the preparations but on attaining a new steady state of tone the response to acetylcholine was unaffected ($P > 0.05$, Student's t-test) while the effect of arachidonic acid was reduced ($P < 0.001$) from 22 ± 6 mm (mean ± s.e. mean) to 3 ± 1 mm. The responses to both acetylcholine and arachidonic acid were unchanged in the parallel untreated controls ($P > 0.05$). Antigen challenge resulted in contraction of the bronchial spirals, but the mean response of the indomethacin-treated tissues (74.2 ± 10.3% MAR) was significantly ($P < 0.01$) reduced compared with the untreated controls (119.5 ± 11% MAR).

Radioimmunoassay of prostaglandin $F_{2\alpha}$ revealed slightly higher basal synthesis in indomethacin-treated spirals than in untreated controls ($P > 0.05$). On antigen challenge the mean concentration of prostaglandin $F_{2\alpha}$ in the bathing fluid (± s.e. mean) expressed as pg/mg dry weight of bronchial tissue rose from 25.4 ± 7.3 to 148.1 ± 57.7 (15 experiments, $P < 0.05$). In the indomethacin-treated tissues the amount of prostaglandin $F_{2\alpha}$ decreased on antigen challenge from 51.1 ± 14.6 to 47.9 ± 10.6 pg/mg ($P > 0.05$). There was a significant correlation between percentage change in organ bath prostaglandin $F_{2\alpha}$ concentrations on antigen challenge and contraction of the paired tissues in the organ bath ($r = 0.61$, $P < 0.05$, method of least squares).

A dose of acetylcholine which induced a maximal contraction of the bronchial spirals in the organ bath caused a slight but not significant increase in the amount of prostaglandin $F_{2\alpha}$ released, from 27.0 ± 8.2 to 3.7 ± 8.1 pg/mg ($P > 0.05$). In the same preparations antigen challenge caused a mean increase from 29.4 ± 6.1 to 117.4 ± 16 pg/mg prostaglandin $F_{2\alpha}$ ($P < 0.001$).

Discussion

The results of the experiments reported in this paper provide further evidence in favour of the hypothesis that prostaglandin $F_{2\alpha}$ synthesis contributes to anaphylactic bronchoconstriction. Organ bath concentrations of indomethacin, sufficient to inhibit contractions elicited by the prostaglandin precursor arachidonic acid, reduced the antigen-induced contraction of human bronchi by 40% compared with paired controls. Parallel experiments indicated that the synthesis of prostaglandin $F_{2\alpha}$ increased during antigen challenge and that there was a significant correlation between response to antigen challenge and percentage change in prostaglandin $F_{2\alpha}$ organ bath concentrations. Complete inhibition of prostaglandin $F_{2\alpha}$ synthesis by indomethacin did not completely abolish antigen-induced bronchial muscle contraction, indicating that it only contributed to the antigen response.

Release of prostaglandin $F_{2\alpha}$ during bronchospasm could be a secondary event following muscle contraction such as occurs in the guinea-pig trachea (Orehek, Douglas, Lewis & Bouhuys, 1973) but since there was no significant increase in the amount of prostaglandin $F_{2\alpha}$ synthesized by human bronchus during contractions induced by acetylcholine of the same order as those induced by antigen this explanation seems unlikely. Alternatively, prostaglandin $F_{2\alpha}$ released during anaphylaxis could act by sensitizing bronchial muscle to the other mediators released such as histamine and slow reacting substance (SRS)-A, but definite exclusion of these possibilities awaits results of experiments in progress. Although its mode of action remains in doubt, it appears that prostaglandin $F_{2\alpha}$ synthesis does contribute to the antigen-induced contractile response of sensitized human bronchus *in vitro*.

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