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THE ROLE OF HISTAMINE H_1- AND H_2-RECEPTORS IN THE GENERATION OF THROMBOXANE A_2 IN PERFUSED GUINEA-PIG LUNGS

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1 When isolated perfused lungs from normal and ovalbumin sensitized guinea-pigs were challenged with histamine and 2-methylhistamine (agonists for H_1-receptor), a release of thromboxane A_2-like substance was observed. The effect of histamine on production of thromboxane A_2 (TXA_2) in sensitized lungs, was more pronounced than in normal lungs ($P < 0.01$).

2 Specific activation of histamine H_2-receptors in normal lungs with large doses (100 μg) of dimaprit and 4-methylhistamine, does not produce thromboxane-like or prostaglandin-like substances.

3 Perfusion of the lungs with pyrilamine (10 μg/ml) inhibited the release of arachidonate metabolites induced by histamine H_1-receptor stimulation, whereas cimetidine (5 μg/ml) was ineffective.

4 It is concluded that only the stimulation of histamine H_1-receptors appears to be responsible for generation of thromboxane A_2 and other prostaglandin-like substances in normal guinea-pig lungs. In sensitized lungs, an increased ability of histamine to release TXA_2 could be due to a possible interconversion of H_2 into H_1-receptors.

Introduction

Two types of histamine receptors, termed H_1 and H_2, are responsible for various physiological and pathological actions of histamine (Black, Duncan, Durant, Ganellin & Parsons, 1972). The availability of H_2-receptor agonists and antagonists allowed the elucidation of some of the actions of these two distinct histamine receptors; however their function in the lungs and in airways smooth muscle, is still a much debated question. The role played by the prostaglandins and unstable metabolites of arachidonic acid that are reported to be released upon histamine stimulation (Bakhle & Smith, 1972), is not completely clear, particularly in connection with anaphylactic reactions.

Yen, Mathé & Dougan (1976), found that in sensitized guinea-pig lungs, the stimulation of H_1-receptors is predominantly associated with a generation of prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), whereas activation of H_2-receptors evokes the synthesis of prostaglandin E_2(PGE_2). These findings may suggest a 'protective function' of the histamine H_2-receptor in the airways system, by promoting the formation of bronchodilator PGE_2. PGE_2 might also inhibit histamine release from mast cells by activation of an adenylate cyclase in these cells (Rosenthale, Dervinis & Strike, 1976). Thromboxane A_2 (TXA_2) is the main product released by histamine from the lungs (Gryglewski, 1977). The specificity of histamine receptors involved in its generation has not been studied.

Although the pathophysiological significance of TXA_2 production by the lungs is not clear, it has already been established that this unstable compound displays a more potent contractile activity on smooth muscle of vascular and respiratory system than $PGF_{2\alpha}$, (Hamberg, Svensson, Hedqvist, Strandberg & Samuelsson, 1976; Bunting, Moncada & Vane, 1976).

In order to verify the physiological importance of the two histamine receptors on thromboxane release, experiments were performed on guinea-pig isolated perfused lungs using specific agonists and antagonists of the histamine receptors.

Methods

Lungs were removed from male guinea-pigs (300 to 400 g) and perfused through the pulmonary artery with a Krebs-bicarbonate solution at a flow rate of 10 ml/min, as previously described by Piper & Vane (1969). The pulmonary outflow was used to superfuse a set of four isolated tissues. These included two rabbit aortae which are contracted by prostaglandin endoperoxides and thromboxane A_2 but not by stable prostaglandins and prostacyclin (Bunting *et al.*, 1976),

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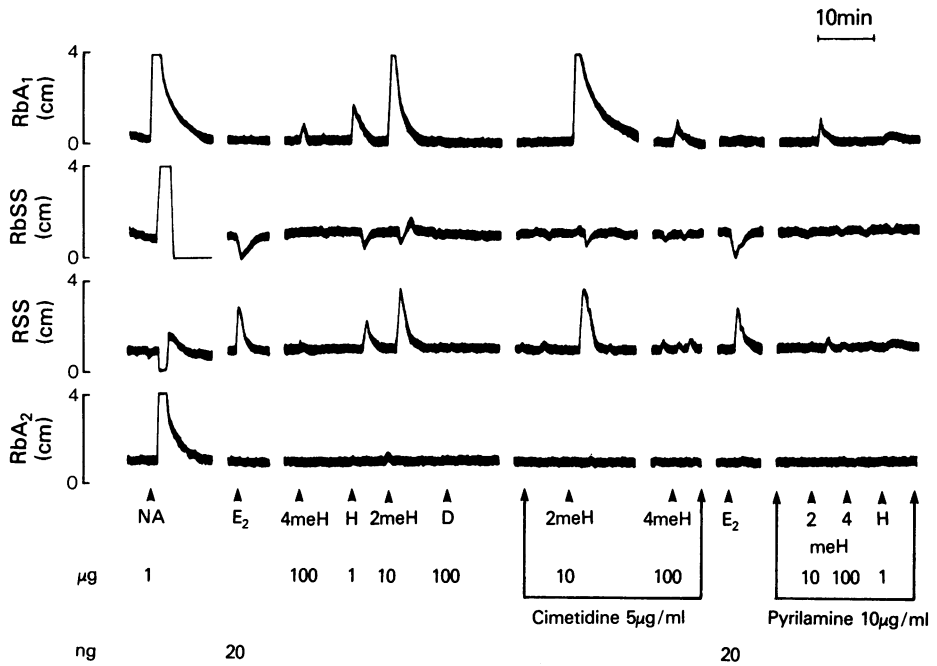

Figure 1 Prostaglandin-like material and thromboxane A_2 (TXA_2) generation by specific activation of H_1 and H_2-receptors on isolated lungs of normal guinea-pigs. The assay tissues were rabbit aortic strips (RbA_1 and RbA_2), rabbit stomach strip (RbSS) and rat stomach strip (RSS). Sensitivity of the bioassay tissues was checked with noradrenaline (NA) before superfusion with the antagonist mixture. Between the first rabbit aortic strip and the other bioassay tissues a delay coil (120 s) was inserted in order to cause inactivation of TXA_2 (half life of TXA_2 in aqueous medium at 37°C = 32.5 s). The agonists, histamine (H), 2-methylhistamine (2meH), 4-methylhistamine (4meH) and dimaprit (D) were injected as a bolus through the lungs. The antagonists cimetidine and pyrilamine were continuously infused through the lungs as indicated in the figure. Contractions of the tissues in cascade were matched by prostaglandin E_2 (E_2).

a rabbit stomach strip (circular fibres) which is contracted by prostacyclin and $PGF_{2\alpha}$ and relaxed by PGE_2 (Moncada, Mugridge & Whittle, 1977) and a rat stomach strip which is contracted by prostaglandins and by unstable archidonic acid metabolites.

The bioassay tissues were treated with a mixture of antagonists according to Gilmore, Vane & Willie (1968) and indomethacin (1 μg/ml) in order to prevent endogenous prostaglandin generation. Between two rabbit aortae, a delay coil was inserted in order to allow degeneration of TXA_2 activity in the effluent fluid. Changes in tone of the assay tissues were recorded with an isometric transducer (Hewlett-Packard model FTA-10-1).

Sensitized guinea-pigs were obtained following the procedure described by Engineer, Piper & Sirois (1976). The animals were injected subcutaneously and intraperitoneally with two equal doses of ovalbumin (Sigma grade II); the lungs were removed three weeks later. TXA_2 was identified through thin layer chromatography of products isolated after perfusion of [1-^{14}C]-arachidonic acid (30 μg) through the lungs, according to Hamberg, Svensson & Samuelsson, (1975).

Quantitative evaluation of TXA_2 formation was carried out by a radioimmunoassay technique as described by Granström, Kindahl & Samuelsson, (1976). Fifteen seconds after challenging the lungs with the different agonists, the generated TXA_2 was immediately converted into a mono-*O*-methyl TXB_2 derivative by treatment of the perfusates with a large volume of methanol. Collection of the perfusates lasted 2 min, and during this time, maximal biological activity was monitored.

Experimental data were analysed following the method of analysis of variance (one way classification) for completely randomized design after the homogeneity of variance was checked by the Bartlett (1937) test. Finally multiple comparison according to Duncan (1955) was performed.

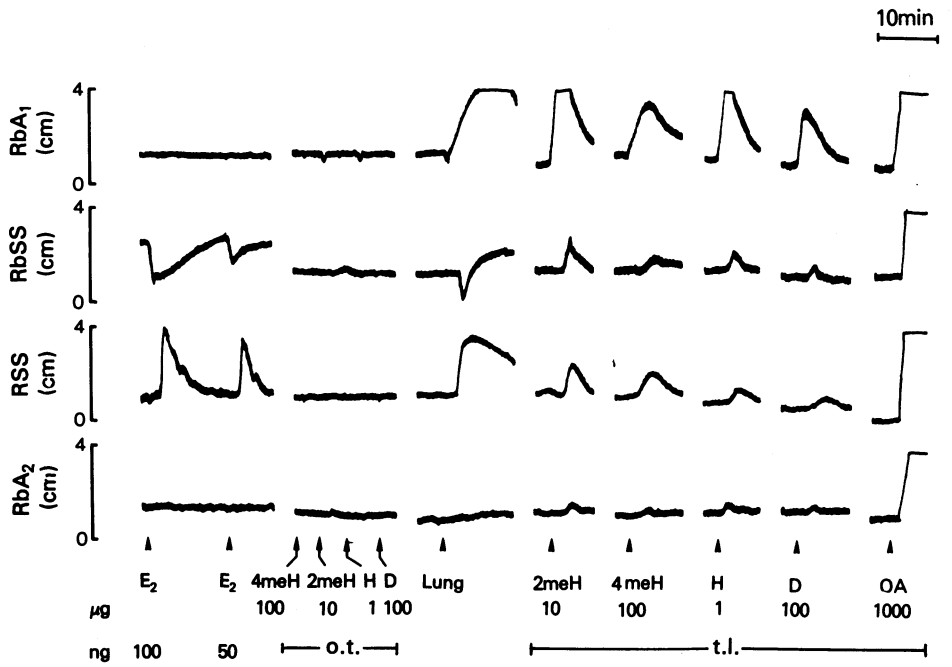

Figure 2 Thromboxane A_2 generation in response to activation of H_1 receptors by histamine (H) and by 2-methylhistamine (2meH) in the perfused lungs of a sensitized guinea-pig. The bank of tissues in cascade was set up as described in Figure 1. The agonists under examination, histamine, 2-methylhistamine, dimaprit (D), and 4-methylhistamine (4meH) were administered as a single injection through the lungs (t.l.). The agonists had no direct activity when injected over the tissues (o.t.) in cascade. At the end of the experiment ovalbumin (OA) was given through the lungs in order to verify the sensitization of the tissue.

Drugs

The following compounds were used: histamine dihydrochloride (Carlo Erba), 2-methylhistamine dihydrochloride (SKF), 4-methylhistamine dihydrochloride (SKF), dimaprit dihydrochloride (SKF), pyrilamine dihydrochloride (M & B), cimetidine (SKF), prostaglandins E_2 and $F_{2\alpha}$ (Upjohn), [1-^{14}C]arachidonic acid (56.2 mCi/nmol) from the Radiochemical Centre, Amersham, propranolol hydrochloride (ICI), phenoxybenzamine hydrochloride (SKF), methysergide hydrogen maleate (Sandoz), hyoscine hydrobromide (B.D.H.) and indomethacin (M.S.D.).

Results

When histamine (1 μg) or 2-methylhistamine (10 μg), was injected through the pulmonary artery of the isolated lungs of normal guinea-pigs, TXA_2-like activity appeared in the effluent, as detected by a contraction of the upper rabbit aortic strip in the bioassay cascade (Figure 1). The small contractions of the rat stomach strip indicated that little prostaglandin-like activity was released. (Figure 1). During perfusion of the lungs with the H_1-receptor antagonist pyrilamine (10 μg ml^{-1} min^{-1}), histamine and 2-methylhistamine almost completely lost their ability to generate TXA_2-like activity from the lungs. In contrast, during perfusion of the lungs with the selective histamine H_2-receptor antagonist cimetidine (5 μg ml^{-1} min^{-1}), 2-methylhistamine fully retained its capacity to stimulate the generation of a TXA_2-like substance.

When a specific agonist for the histamine H_2-receptor, such as 4-methylhistamine or dimaprit, was administered as a bolus injection (100 μg) through the isolated lungs, neither prostaglandin-like material nor TXA_2 was released as shown by the tissues in the cascade (Figure 1).

In other experiments carried out with ovalbumin-sensitized guinea-pigs, the isolated lungs were significantly more sensitive to histamine H_1-receptor activation and consequently a marked generation of thromboxane A_2 and possibly some prostaglandins occurred, as revealed by the bioassay tissues (Figure 2). In the lungs of sensitized guinea-pigs, 4-methylhistamine and dimaprit (100 μg single injection of each)

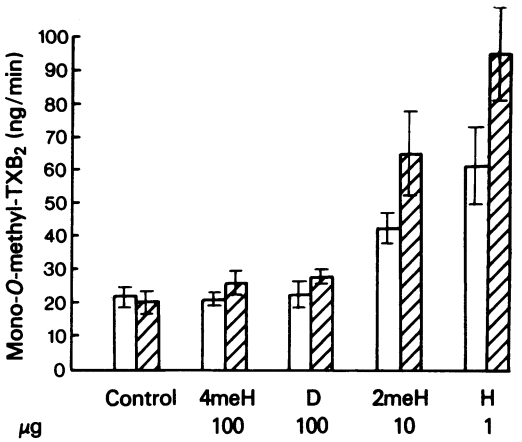

Figure 3 Thromboxane A_2 (TXA_2) measured as mono-*O*-methyl-TXB_2, generation by normal (open columns) and sensitized (hatched columns) guinea-pig lungs induced by bolus injection of histamine (H), 2-methylhistamine (2meH), 4-methylhistamine (4meH) and dimaprit (D). TXA_2 expressed as ng/min, refers to the total amount present in the effluent from individual lungs collected for 2 min after drug injection. Each column represents the mean of 4 experiments. Vertical bars show s.e. mean.

Duncan's test carried out on the experimental data gives the following results of the comparison between each two means: control vs 4-methylhistamine = NS ($P > 0.05$); control vs dimaprit = NS ($P > 0.05$); control vs 2-methylhistamine = highly significant ($P < 0.01$); control vs histamine = highly significant ($P < 0.01$); normal 2-methylhistamine vs sensitized-2-methylhistamine = NS ($P > 0.05$); normal histamine vs sensitized-histamine = significant ($0.01 < P < 0.05$).

caused a modest generation of TXA_2-like material (Figure 2). This effect was not specific for histamine H_2-receptor activation, since it was antagonized by pyrilamine.

In order to confirm the results obtained using the cascade system, TXA_2 present in the pulmonary outflow was identified by thin layer chromatography and quantitatively evaluated by a specific radioimmunoassay technique.

The results obtained in these experiments, shown in Figure 3, indicated that stimulation of H_2-receptors by dimaprit and 4-methylhistamine did not increase TXA_2 production in normal guinea-pig lungs. Stimulation of the receptors either by histamine (1 μg) or by 2-methylhistamine (10 μg) caused an increased generation of TXA_2. In the lungs from sensitized guinea-pigs both histamine and 2-methylhistamine caused a greater (150%) release of TXA_2 although only the histamine effect was significant ($P < 0.05$). The H_2-agonists were ineffective.

Studies with other agonists, e.g. carbachol and physiological antagonists, e.g. salbutamol, are in progress to prove a direct link between histamine and TXA_2 production not associated with, or secondary to, any change in muscle tone.

Discussion

Thromboxane A_2 is the main product among arachidonate metabolites which are released by histamine from guinea-pig lungs (Gryglewski, 1977). However, whether this unstable compound is involved in bronchomotor and pulmonary vascular tone regulation or in the pathogenesis of lung anaphylaxis has yet to be clarified.

The presence of histamine H_1- and H_2-receptors, particularly in the vascular bed, claimed by Türker (1973) and by Tucker, Weir, Reeves & Grover (1975), raises the question of the type of histamine receptors involved in the generation and release of thromboxane from this organ. The present results indicate that only H_1-receptor activation is responsible for TXA_2 formation, and therefore our data cast some doubt on the functional significance of the H_2-receptor in guinea-pig lungs.

According to Gryglewski (1977), thromboxane A_2 seems to be generated by the lung parenchyma and not by airways smooth muscle. On the other hand, histamine-contracted respiratory smooth muscle forms PGE_2 which antagonizes bronchoconstriction induced by TXA_2. Nonetheless a relationship between H_2-receptor activation in respiratory smooth muscle and PGE_2 generation has not been proved.

We have shown that stimulation of H_2-receptors by dimaprit and 4-methylhistamine does not induce the generation of TXA_2 in the perfused lungs of normal guinea-pigs. A small release of TXA_2 during stimulation of H_2-receptors in perfused lungs of sensitized guinea-pigs is blocked by pyrilamine.

Our preliminary results obtained by analysis of the pulmonary effluent during histamine-H_2-receptor stimulation, did not reveal PGE_2 and $PGF_{2\alpha}$ formation, whereas specific H_1-receptor activation, caused a substantial production of the two natural prostaglandins along with the generation of TXA_2.

These findings conflict with those obtained by Yen *et al.* (1976): these investigators propose a correlation between stimulation of histamine H_2-receptor and increased biosynthesis of PGE_2, although they were not able to demonstrate any increase of PGE_2 formation after challenge of the perfused lungs with histamine in the presence of pyrilamine.

It is concluded that there is no direct relationship between H_2-receptor activation and TXA_2 formation in the perfused guinea-pig lungs, although a modulat-

ing role of H_2-receptors on thromboxane formation cannot be ruled out.

Furthermore the increased ability of histamine (and 2-methylhistamine) to generate TXA_2 in sensitized lungs, and the slight effect of H_2-agonists not seen in normal lung but blocked by H_1-antagonists, could be explained not only by an increased sensitivity of the histamine H_1-receptors, but also by the conversion of H_2-receptors to the H_1-type during hypersensitization. In this respect, Chand & Eyre (1977), working with sensitized cat trachea, have already suggested an altered tissue reactivity to histamine during the process of sensitization.

Undoubtedly, in order to clarify the mechanism by which histamine produces its effects on the lungs and on bronchial smooth muscle tone, a possible transmutation of drug receptors in relation to development of hypersensitivity, should be considered.

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