The effect of M&B 22948 on methacholine- and histamine-induced contraction and inositol 1,4,5-trisphosphate levels in guinea-pig tracheal tissue

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The effect of a cyclic GMP phosphodiesterase inhibitor, M&B 22948, on methacholine- and histamine-induced contraction and inositol 1,4,5-trisphosphate (IP₃) elevation was studied in guinea-pig tracheal rings. After addition of methacholine or histamine the rise in IP₃ content was rapid and transient reaching a maximum after 5–15 s, which coincided with the maximum rate of tension development. Cyclic GMP levels of the tissue were elevated by M&B 22948 before agonist stimulation and further elevated by addition of methacholine or histamine. Cyclic AMP levels were not altered by any of these agents. M&B 22948 abolished IP₃ generation induced by methacholine or histamine, but did not alter the rate or magnitude of tension development. Thus, IP₃ generation does not appear to be responsible for the contractions induced by methacholine or histamine in this tissue.

Introduction Contractions of airway smooth muscle induced by many putative mediators of airways hyper-reactivity are believed to involve increased inositol phosphate generation (Hashimoto *et al.*, 1985; Duncan *et al.*, 1987; Hall & Hill, 1988; Langlands *et al.*, 1988). There is substantial evidence demonstrating the ability of inositol 1,4,5-trisphosphate (IP₃) to release Ca²⁺ from non-mitochondrial calcium stores such as the sarcoplasmic reticulum (Hashimoto *et al.*, 1985). For this reason, it is believed that IP₃ may be of fundamental importance in the regulation of cytosolic Ca²⁺ and the contractility of airway smooth muscle.

Recent studies have proposed that agents elevating tissue adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels may inhibit agonist induced inositol phospholipid metabolism (Hall & Hill, 1988; 1989) and tension development (Langlands *et al.*, 1989) in airway smooth muscle.

In the present study, we have used a protein binding assay system to determine levels of IP₃ during contraction of guinea-pig tracheal rings

induced by methacholine and histamine. In addition, the study examines the effects of a cyclic GMP phosphodiesterase inhibitor, M&B 22948 (Kukovetz *et al.*, 1979), on agonist-induced IP₃ formation and tension development in this tissue.

Methods Tracheal rings from male Hartley guinea-pigs (300–400 g) were suspended under an initial 20 mN resting tension in Krebs-Henseleit solution (KHS) of the following composition (mM): NaCl 118, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25 and D-glucose 11.7. Changes in isometric tension were recorded on an ink-writing curvilinear polygraph (Grass, Model 7C, Quincy, Mass). The bathing medium was maintained at 37°C and aerated with 5% CO₂ in O₂. Flurbiprofen (1 µM), a cyclo-oxygenase inhibitor, was present in the bathing medium throughout the experiment (Bryson & Rodger, 1987).

After an initial 45 min equilibration period, tissues were freeze-clamped, using tongs pre-cooled in liquid nitrogen, at pre-determined times (0, 5, 10, 15, 30, 60 s) after addition of the agonist. Where appropriate, tissues were pretreated with atropine (1 µM), mepyramine (1 µM) or M&B 22948 (10 µM) 20 min before addition of agonist. Cyclic AMP and cyclic GMP levels were determined before addition of M&B 22948, before agonist addition but in the presence of M&B 22948 and after 60 s agonist stimulation.

Inositol 1,4,5-trisphosphate levels were determined by a protein binding assay system (TRK 1000; Amersham). This assay measures levels of the 1,4,5-isomer of IP₃ without interference from other inositol phosphates. Cyclic AMP and cyclic GMP levels were determined by protein-binding and radioimmunoassay as described by Bryson & Rodger (1987).

The following drugs were used: acetyl-β-methylcholine (methacholine) and histamine acid phosphate (Sigma), atropine sulphate (Koch-Light), sodium

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flurbiprofen (Boots PLC), mepyramine sulphate (Rhône-Poulenc). All other chemicals were analar grade obtained from BDH. Cyclic AMP assay kit (TRK 432), cyclic GMP assay kit (TRK 500), guanosine 3',5'-cyclic phosphoric acid 2'-O-succinyl-3-[^{125}I]-iodotyrosine methyl ester (IM 107) and IP_3 assay kit were obtained from Amersham. M&B 22948 (2-*o*-propoxyphenyl-8-azapurin-6-one) was a gift from Rhône-Poulenc.

Results are expressed as mean $\pm$ s.e. mean ($n = 5$ in all experiments) and were analysed by the Mann-Whitney U test. Significance was accepted when $P < 0.05$.

Results Methacholine (EC_{90} ; $10\text{ }\mu\text{M}$) induced a rapid rise in the IP_3 level of the guinea-pig tracheal tissue after 10 s. Levels remained elevated at 15 s before returning to basal after 30 s (Figure 1a). Similarly, histamine (EC_{90} ; $30\text{ }\mu\text{M}$) induced a rapid rise in the IP_3 level after 5 s, which continued to rise until 10 s, before rapidly returning to basal level at 15 s (Figure 1b). The transient elevations in IP_3 content of the tissues induced by both methacholine and histamine occurred during the period of rapid tension development (Figure 1a and b) and could be abolished, together with the contractile responses, by pretreatment of the tissues with atropine ($1\text{ }\mu\text{M}$) and mepyramine ($1\text{ }\mu\text{M}$) respectively (data not shown).

After pretreatment of the tissues with M&B 22948 ($10\text{ }\mu\text{M}$), no alteration in IP_3 content was observed during stimulation with either methacholine or histamine. However, in the presence of M&B 22948 the rate and magnitude of tension development induced by both methacholine and histamine were not significantly different from control responses (Figure 1).

M&B 22948 ($10\text{ }\mu\text{M}$) produced a significant rise in the basal cyclic GMP content of the tissue after 20 min incubation (from 14.5 ± 3.2 to 34.3 ± 4.4 fmol cyclic GMP mg^{-1} tissue).

Cyclic GMP levels were further increased to 69.2 ± 17.4 and 52.3 ± 13.8 fmol cyclic GMP mg^{-1} tissue 60 s after addition of methacholine ($10\text{ }\mu\text{M}$) and histamine ($30\text{ }\mu\text{M}$) respectively. The cyclic AMP levels were not altered significantly from basal (0.35 ± 0.02 pmol cyclic AMP mg^{-1} tissue) after 20 min incubation with M&B 22948 (0.40 ± 0.04 pmol cyclic AMP mg^{-1} tissue) or after 60 s stimulation with either methacholine (0.38 ± 0.06 pmol cyclic AMP mg^{-1} tissue) or histamine (0.41 ± 0.05 pmol cyclic AMP mg^{-1} tissue).

Discussion In the present study, we have demonstrated the ability of methacholine and histamine, acting via specific receptors, to induce rapid tran-

Figure 1 The effect of (a) methacholine $10\text{ }\mu\text{M}$ and (b) histamine $30\text{ }\mu\text{M}$ alone (●) and in the presence of M&B 22948 $10\text{ }\mu\text{M}$ (○) on inositol 1,4,5-triphosphate (IP_3) levels and tension development in guinea-pig tracheal rings. Tissues were freeze-clamped at 0, 5, 10, 15, 30 and 60 s after addition of the agonist, and the IP_3 levels were determined by a protein binding assay. The contractile response is expressed as a percentage of the maximum response observed in the absence of M&B 22948. *Indicates significant difference from basal level of IP_3 (in the absence or presence of M&B 22948) $P < 0.05$, Mann-Whitney.

sient elevations in IP_3 content during contraction of the guinea-pig trachea. In addition, the level of IP_3 becomes elevated rapidly enough with both agonists to suggest that it may play an important role in the initiation of tension development by these agonists. Since only a transient elevation in IP_3 content is observed, it is likely that the formation of IP_3 is only

responsible for the initiation of tension development. The significance of the transient nature of the response is still unclear.

Due to the variety of cell types within the tissue under study, it is possible that non-smooth muscle cells contribute to the changes in IP_3 content observed during contraction. However, preliminary studies (data not shown) have demonstrated that levels of IP_3 which may be present in the cartilage are below the level of detection for this assay system. In addition, autoradiographic studies have demonstrated localization of muscarinic receptors on airway smooth muscle cells with a much poorer distribution of the receptors in epithelial and vascular smooth muscle cells (Barnes *et al.*, 1983). Thus, non-smooth muscle cells probably contribute only minimally to the levels of IP_3 determined during tissue contractions.

M&B 22948 abolished the rapid transient elevation in IP_3 which was observed during the first 60 s of stimulation with methacholine and histamine. A previous report has demonstrated inhibition of PI hydrolysis in vascular smooth muscle by agents elevating cyclic GMP content (Rapoport, 1986). The

ability of M&B 22948 to inhibit IP_3 formation induced by methacholine and histamine is consistent with this observation since basal levels of cyclic GMP were elevated by M&B 22948. Cyclic AMP was unchanged and therefore does not appear to play a role in the M&B 22948-induced inhibition of IP_3 generation. Quite surprisingly, the contractile responses to methacholine and histamine were unaltered in the presence of M&B 22948. If generation of IP_3 is necessary for release of intracellular Ca^{2+} and the initiation of airway smooth muscle contraction, we would expect the contractile response to be greatly reduced. Clearly this is not the case and it would appear that some other, as yet unknown, mechanism, independent of IP_3 generation, is responsible for the development of tension induced by both methacholine and histamine.

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