Br. J. Pharmacol. (1988), **95**, 1204–1212

β-Adrenoceptor stimulation inhibits histamine-stimulated inositol phospholipid hydrolysis in bovine tracheal smooth muscle

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1 Histamine and carbachol produced concentration-related increases in the accumulation of ^{3}H-inositol phosphates in slices of bovine tracheal smooth muscle.

2 Noradrenaline alone produced a small stimulation of ^{3}H-inositol phosphate accumulation which was inhibited by the α-adrenoceptor antagonist phentolamine. In contrast, when noradrenaline (0.1 mM) was added simultaneously with histamine it significantly reduced the inositol phosphate response to high ($\geq$ 0.1 mM) concentrations of histamine. However, noradrenaline had no inhibitory effect on the carbachol-induced inositol phosphate response.

3 The non-selective β-agonist isoprenaline (IC_{50} = 0.08 μM) and the β_2-selective agonist salbutamol (IC_{50} = 0.29 μM) both produced a dose-related inhibition of the inositol phosphate response to 0.1 mM histamine. The inhibitory effect of salbutamol was antagonized by propranolol ($K_A = 2.4 \times 10^9\ M^{-1}$) and the β_2-selective adrenoceptor antagonist ICI 118551 ($K_A = 1.7 \times 10^9\ M^{-1}$).

4 The accumulation of ^{3}H-inositol phosphates induced by histamine increased steadily over a 40 min period after an initial lag period of 3–4 min. Following the simultaneous addition of histamine and salbutamol there was a further delay of 3–4 min before the appearance of the inhibitory effect of salbutamol.

5 The effect of histamine on inositol phosphate accumulation was accompanied by a stimulation of [^{3}H]-inositol incorporation into membrane phospholipids which was reduced by the presence of salbutamol. However, when histamine was used to stimulate maximally [^{3}H]-inositol incorporation during the prelabelling period, salbutamol produced a marked inhibition of histamine-stimulated ^{3}H-inositol phosphate accumulation under conditions in which there was no change in the level of incorporation.

Introduction

β_2-Adrenoceptor agonists are effective bronchodilators which are widely used in the management of asthma and which potently reverse the bronchoconstrictor response to inhalation of potential mediators of airway smooth muscle contraction such as histamine (Barnes *et al.*, 1984). The contractile response of airway smooth muscle to histamine is mediated through histamine H_1-receptor stimulation and partly involves the mobilization of calcium from intracellular stores (Kirkpatrick, 1975; Kotlikoff *et al.*, 1987). Activation of histamine H_1-receptors in a number of tissues including guinea-pig cerebral cortex (Daum *et al.*, 1984; Donaldson & Hill, 1986), vascular endothelial cells (Lo & Fan, 1987) and bovine tracheal smooth muscle (Barnes *et al.*, 1986) has been shown to stimulate the hydrolysis of inositol-containing membrane phospholipids which can lead to the formation of the two intracellular second messengers, inositol-1,4,5-trisphosphate and diacyglycerol (Berridge, 1987). There is strong evidence that inositol-1,4,5-trisphosphate which is released into the cell cytosol can mobilize calcium from intracellular stores (Hashimoto *et al.*, 1985; Berridge, 1987) while diacyglycerol, which remains within the membrane environment, is a substrate for protein kinase C (Nishizuka, 1984; Berridge, 1987). Studies in mammalian brain have suggested that the inositol phospholipid response to histamine H_1-receptor stimulation can be modulated by stimulation of other neurotransmitter or hormone receptors (Hollingsworth *et al.*, 1986; Crawford *et al.*, 1987; Hill & Kendall, 1987; Kendall & Hill, 1988).

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We now demonstrate that stimulation of β_2-adrenoceptors with salbutamol, isoprenaline or noradrenaline produces a potent inhibition of the histamine-stimulated accumulation of ^{3}H-inositol phosphates in slices of bovine tracheal smooth muscle. A preliminary account of this work has been presented to the British Pharmacological Society (Hall & Hill, 1988).

Methods

Accumulation of ^{3}H-inositol phosphates

Slices (300 × 300 μm) of bovine tracheal smooth muscle were prepared using a McIlwain tissue chopper after removing the trachea from freshly slaughtered bullocks. The slices were incubated for 30 min in a shaking water bath at 37°C under an atmosphere of 95% O_2/5% CO_2 in 100 ml of Krebs-Henseleit buffer. Slices were then incubated in a minimal volume of Krebs medium containing [^{3}H]-myo-inositol (30 μCi, final concentration 0.4 μM, total volume 8 ml) for 75 min at 37°C under an atmosphere of 95% O_2/5% CO_2. The slices were finally washed three times with 20 ml of Krebs-Henseleit buffer containing 5 mM lithium chloride and resuspended in 8 ml of medium. One hundred μl aliquots of the slice suspension were then transferred to flat-bottomed insert vials containing Krebs-Henseleit medium and 5 mM LiCl (final volume 300 μl). The tubes were gassed with 95% O_2/5% CO_2, capped and incubated for 30 min at 37°C. Antagonist drugs were added at the beginning of this incubation period. Agonists were finally added in 10 μl of medium and the incubation terminated after 45 min by the addition of 100 μl of ice-cold perchloric acid (10% w/v). Histamine and β-adrenoceptor agonist drugs were added simultaneously. Samples were neutralised with 0.75 ml KOH (0.15 M), centrifuged (1500 g, 10 min, 4°C) to precipitate $KClO_4$ and 0.75 ml aliquots of the supernatant were diluted to 3 ml with Tris buffer (50 mM, pH 7.0). Total ^{3}H-inositol phosphates were finally separated from free [^{3}H]-myo-inositol by anion-exchange chromatography as described previously (Hill & Kendall, 1987).

Incorporation of [^{3}H]-inositol

For measurement of the incorporation of [^{3}H]-inositol into inositol-containing phospholipids, 1.2 ml chloroform\methanol\10 M HCl (100:200:1, v\v\v) were added to each sample after removal of the supernatant as described above. The tubes were vortexed and left to stand for 30 min. Chloroform (0.4 ml) and water (0.4 ml) were then added and the tubes centrifuged at *circa* 1500 g for 5 min to separate the phases. Two hundred μl of the chloroform phase was then removed into a scintillation vial and dried overnight. Tritium was determined by liquid scintillation counting. In some experiments histamine (0.1 mM) was added during the last 45 min of the 75 min labelling period with [^{3}H]-inositol in order to stimulate incorporation. The slices were then washed with histamine-free Krebs medium and the experiment continued as described above for measurement of inositol phosphate accumulation.

Data analysis

Concentration-response curves from individual experiments were either drawn by inspection or fitted to a Hill equation using the programme ALLFIT (DeLean *et al.*, 1978) as described previously (Donaldson & Hill, 1985). The equation fitted was:

$$\% \text{ of maximal response} = \frac{E_{max} \times D^n}{D^n + (EC_{50})^n}$$

where D is the agonist concentration, n is the Hill coefficient, EC_{50} is the concentration of agonist giving half maximal stimulation and E_{max} is the maximal stimulation. Affinity constants (K_A) of receptor antagonists were determined from shifts in the agonist concentration-response curves using the relationship:

$$K_A = (K_2/K_1 - 1)/A$$

where A is the concentration of antagonist, K_1 is the concentration of agonist producing half-maximal response and K_2 is the concentration of agonist producing the same response in the presence of antagonist.

Statistical analysis of the data was performed by analysis of variance unless otherwise stated. Each experiment was repeated three to fifteen times. All values in the text of the results section represent mean ± s.e.mean of n separate experiments.

Chemicals

[^{3}H]-myo-inositol (16.5 Ci mmol^{-1}) was purchased from New England Nuclear. Histamine dihydrochloride and carbachol chloride were purchased from BDH. (−)-Noradrenaline bitartrate, salbutamol, (±)-propranolol hydrochloride, phentolamine hydrochloride and (±)-isoprenaline hydrochloride were obtained from Sigma. The gift of ICI 118551 hydrochloride (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol), ICI Pharmaceuticals) is gratefully acknowledged.

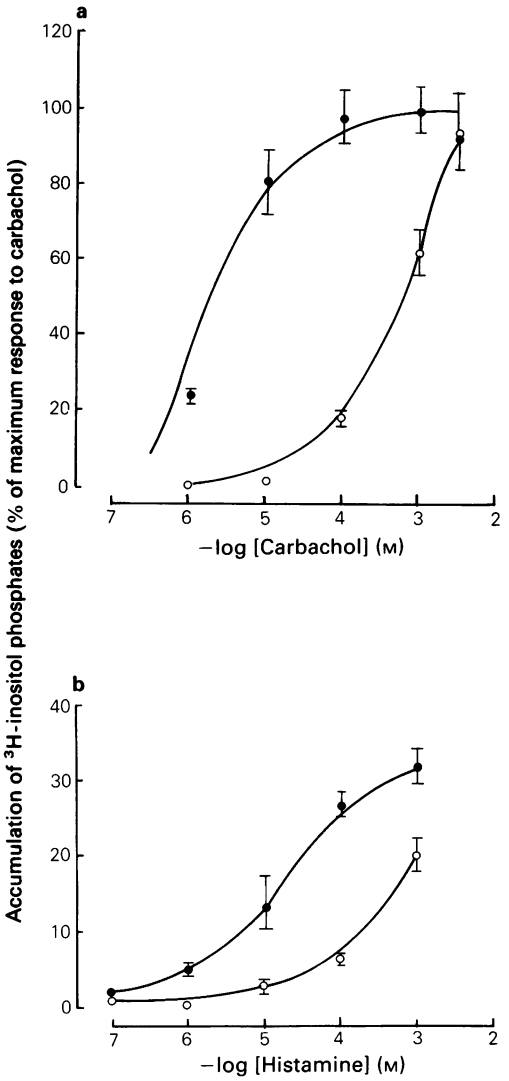

Figure 1 Concentration-response curves for (a) carbachol- and (b) histamine-stimulated accumulation of total ^{3}H-inositol phosphates in slices of bovine tracheal smooth muscle. Data were obtained in the presence (○) or absence (●) of 50 nM atropine (a) or 50 nM mepyramine (b). The ordinate scale shows the stimulation of ^{3}H-inositol phosphate accumulation expressed as a % of the maximal response to carbachol which was measured in each experiment. Values represent the combined mean of quadruplicate determinations in each of three separate experiments; vertical lines indicate s.e.mean. Curves were fitted to the data points using ALLFIT as described under Methods. The fitted parameters for the control concentration-response curves were: EC_{50}; $2.4 \pm 0.7\,\mu M$ (carbachol) and $22.7 \pm 16.3\,\mu M$ (histamine), E_{max}; $98.0 \pm 4.2\%$ (carbachol) and $35.9 \pm 5.3\%$ (histamine).

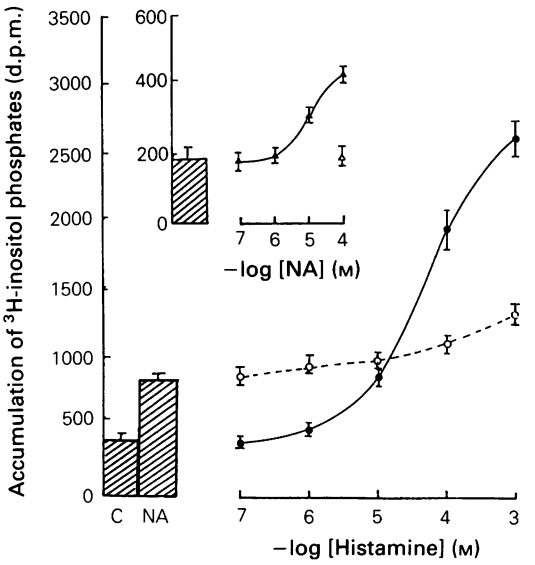

Figure 2 The effect of noradrenaline on the accumulation of total ^{3}H-inositol phosphates elicited by increasing concentrations of histamine. The inset shows the inositol phosphate response to increasing concentrations of noradrenaline (NA) alone (▲) and its inhibition by $1\,\mu M$ phentolamine (△) in a separate experiment. Concentration-response curves were obtained for histamine in the presence (○) and absence (●) of 0.1 mM noradrenaline. The basal and noradrenaline-stimulated (0.1 mM) inositol phosphate accumulations are shown by the columns marked C and NA respectively. Data represent mean of triplicate determinations in a single experiment and vertical lines indicate s.e.mean. Each experiment was repeated three times with similar results.

Results

Agonist-induced ^{3}H-inositol phosphate accumulation

Histamine and carabachol produced concentration-dependent increases in the accumulation of total ^{3}H-inositol phosphates in lithium-treated slices of bovine tracheal smooth muscle (Figure 1). The mean EC_{50} values obtained for these two agonists were $35 \pm 7\,\mu M$ ($n = 12$) and $4.0 \pm 1.1\,\mu M$ ($n = 8$), respectively.

Mepyramine (50 nM; $K_A = 3.2 \pm 0.8 \times 10^8\,M^{-1}$, $n = 3$) and atropine (50 nM; $K_A = 3.3 \pm 0.6 \times 10^9\,M^{-1}$, $n = 3$) produced parallel shifts of the concentration-response curves to histamine and carbachol respectively, consistent with competitive antagonism (Figure 1), indicating the respective involvement of histamine H_1- and muscarinic receptors. The response to 1 mM histamine in bovine tracheal slices accounted for only $32 \pm 2\%$ ($n = 5$) of

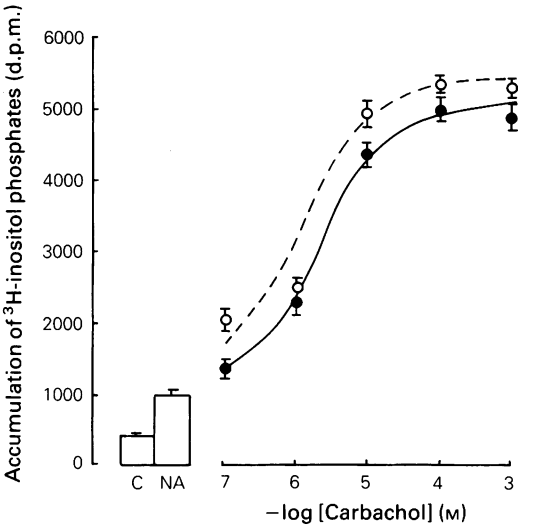

Figure 3 The effect of noradrenaline on the inositol phosphate response to carbachol. Concentration-response curves were obtained for carbachol in the presence (○) and absence (●) of 0.1 mM noradrenaline. The basal and noradrenaline-stimulated (0.1 mM) inositol phosphate accumulations are shown by the columns marked C and NA respectively. Data represent mean of triplicate determinations in a single experiment and vertical lines indicate s.e.mean. Each experiment was repeated twice more with similar results.

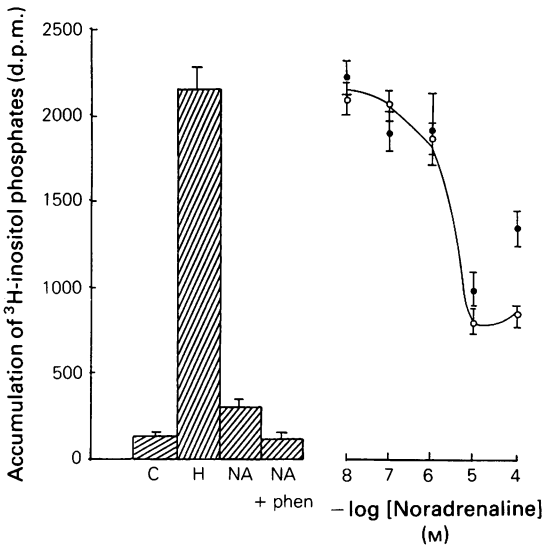

Figure 4 The effect of increasing concentrations of noradrenaline on the accumulation of total ^{3}H-inositol phosphates produced by 0.1 mM histamine. Measurements were made in the presence (○) and absence (●) of 1 μM phentolamine. The basal and histamine-stimulated (0.1 mM) accumulations of inositol phosphates are shown by the columns marked C and H, respectively. The inositol phosphate responses to 0.1 mM noradrenaline alone (NA) and its inhibition by phentolamine (1 μM; Phen) are also shown. Each data point represents the mean of triplicate determinations in a single experiment which was repeated twice more; vertical lines indicate s.e.mean.

the response to 1 mM carbachol when measured in the same experiments ($P < 0.05$, paired t test). Noradrenaline (100 μM) produced a small increase in total ^{3}H-inositol phosphates in this tissue, which was sensitive to inhibition by 1 μM phentolamine (Figure 2, inset) and which represented only 6 ± 1% of the response to 0.1 mM histamine ($n = 4$).

Inhibition of ^{3}H-inositol phosphate accumulation by noradrenaline

When noradrenaline (0.1 mM) was added simultaneously with histamine (0.1 mM), it significantly reduced ($P < 0.05$, paired t and Wilcoxon signed rank test) the inositol phosphate response to 0.1 mM and 1 mM histamine ($n = 6$, in each case) (Figure 2). At the lower concentrations of histamine used (< 0.1 mM), the accumulation of ^{3}H-inositol phosphates was similar to that due to noradrenaline (0.1 mM) alone. Noradrenaline (0.1 mM) did not, however, inhibit the inositol phosphate response to any of the concentrations of carbachol measured (0.1 to 1000 μM, Figure 3). The effect of different concentrations of noradrenaline (10^{-8} M to 10^{-4} M) on the response to 0.1 mM histamine is illustrated in Figure 4. Increasing concentrations of noradrenaline produced a marked inhibition of the inositol phosphate response to histamine which was not prevented by inclusion of phentolamine (1 μM) in the incubation medium. Phentolamine did, however, reduce the upturn in the inhibition curve (Figure 4) observed at 10 and 100 μM noradrenaline. These are the concentrations of noradrenaline at which a stimulation of ^{3}H-inositol phosphate accumulation is normally observed (Figure 2, inset). The mean IC_{50} for noradrenaline obtained in the presence of phentolamine was 0.8 ± 0.6 μM ($n = 3$).

Effect of β-adrenoceptor agonists and antagonists

The β-adrenoceptor agonist isoprenaline (0.1 mM) produced a marked decrease in the maximal inositol phosphate response to histamine without significantly altering the accumulation of ^{3}H-inositol phosphates on its own (Figure 5). The effect of increasing concentrations of isoprenaline on the accumulation of total ^{3}H-inositol phosphates elicited by 0.1 mM histamine is shown in Figure 6. It was notable that

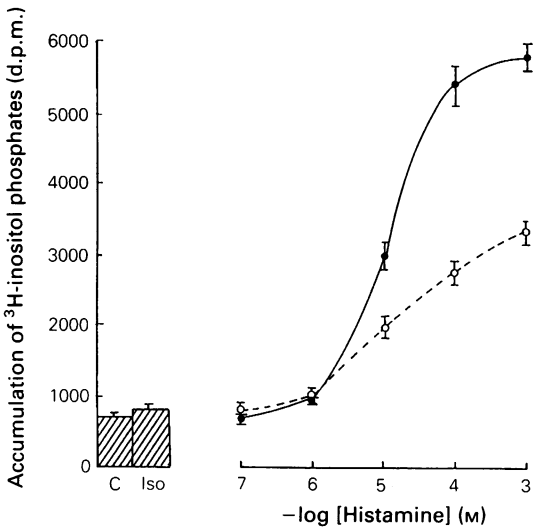

Figure 5 The effect of isoprenaline on the inositol phosphate response to increasing concentrations of histamine. Concentration-response curves were obtained for histamine in the presence (○) and absence (●) of 0.1 mM isoprenaline. The ^{3}H-inositol phosphate accumulations obtained under basal conditions or in the presence of 0.1 mM isoprenaline are shown by the columns marked C and Iso, respectively. Data represent mean of triplicate determinations in a single experiment and vertical lines indicate s.e.mean. Each experiment was repeated twice more with similar results.

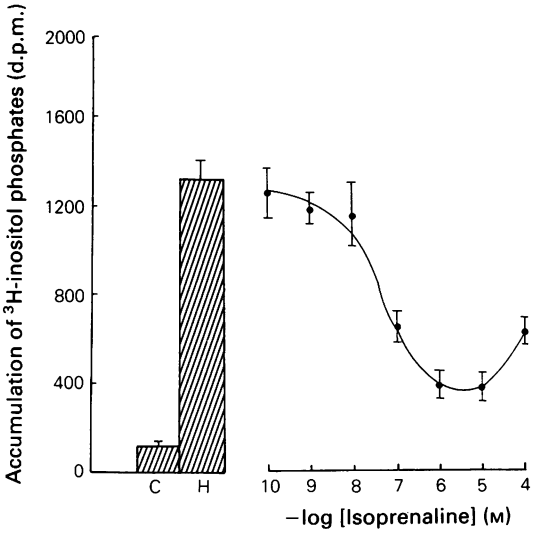

Figure 6 The effect of increasing concentrations of isoprenaline on the inositol phosphate response to 0.1 mM histamine. The basal and histamine-stimulated (0.1 mM) accumulations of inositol phosphates are shown by the columns marked C and H respectively. Values represent the means of triplicate determinations in a single experiment and vertical lines indicate s.e.mean. Similar data were obtained in four other experiments.

the inhibition curve for this agonist was characterized by a small upward deflection in the dose-response curve at high agonist concentrations (i.e. $> 1\,\mu$M, Figure 6). A similar shaped dose-response curve was obtained with the β_2-selective agonist salbutamol (Figure 7). This upward deflection seen at higher concentrations of β_2-adrenoceptor agonists did not appear to be related to the α-stimulation seen with noradrenaline since it was not attenuated by inclusion of phentolamine (1 μM) in the incubation medium ($n = 3$, data not shown). The IC_{50} values obtained for isoprenaline and salbutamol were $0.08 \pm 0.04\,\mu$M ($n = 5$) and $0.29 \pm 0.12\,\mu$M ($n = 15$), respectively. The maximum degree of inhibition of the ^{3}H-inositol phosphate accumulation produced by 0.1 mM histamine with each agent was $55 \pm 5\%$ (noradrenaline in the presence of phentolamine 1 μM, $n = 3$), $68 \pm 5\%$ (isoprenaline, $n = 5$) and $66 \pm 3\%$ (salbutamol, $n = 15$).

The salbutamol-induced inhibition of the ^{3}H-inositol phosphate accumulation produced by histamine was antagonized by both the non-selective β-antagonist propranolol and the β_2-selective antagonist ICI 118551 (Bilski *et al.*, 1983) (Figure 7). The apparent affinity constants (K_A) determined for each antagonist from the parallel shifts of the concentration-response curves for salbutamol were $2.4 \pm 0.2 \times 10^9\,M^{-1}$ and $1.7 \pm 0.4 \times 10^9\,M^{-1}$ for propranolol and ICI 118551, respectively ($n = 4$ in each case).

The time course of the salbutamol-induced inhibition of the inositol phosphate response to histamine (0.1 mM) is illustrated in Figure 8. The accumulation of total ^{3}H-inositol phosphates increased steadily over a 45 min period after a short lag period of 3–4 min following addition of histamine (0.1 mM). However, when histamine (0.1 mM) and salbutamol (1 μM) were added together, there was a further delay of 3–4 min (in addition to the delay seen with histamine alone) before the rate of formation of ^{3}H-inositol phosphates was reduced below that obtained in the presence of histamine alone (Figure 8).

Effects on the incorporation of [^{3}H]-inositol

A notable feature of the effects of histamine and carbachol on inositol phospholipid metabolism in slices of bovine tracheal smooth muscle was that the increase in ^{3}H-inositol phosphate accumulation was accompanied by an increase in the incorporation of [^{3}H]-inositol into the membrane phospholipids (Figure 9). Histamine (0.1 mM, 45 min) produced a

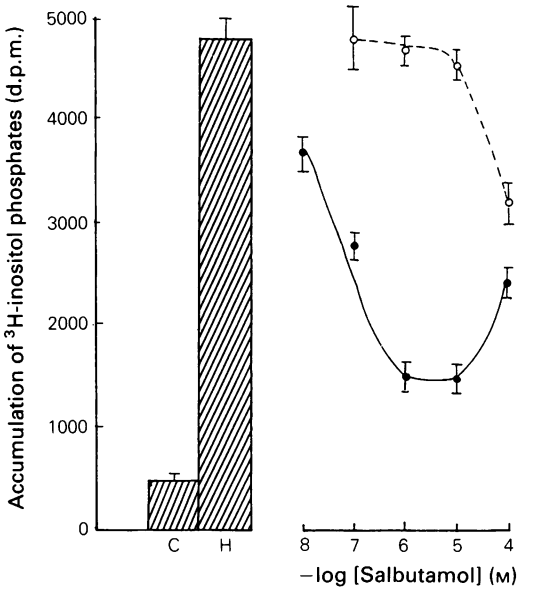

Figure 7 The effect of salbutamol on the inositol phosphate response to histamine (0.1 mM) and its inhibition by the β_2-selective antagonist ICI 118551. Concentration-response curves for the β_2-agonist salbutamol were obtained in the presence (○) or absence (●) of 1 μM ICI 118551. The basal and histamine-stimulated (0.1 mM) accumulations of inositol phosphates are shown by the columns marked C and H respectively. Values represent the means of triplicate determinations in a single experiment and vertical lines indicate s.e.mean. Similar results were obtained in three other experiments.

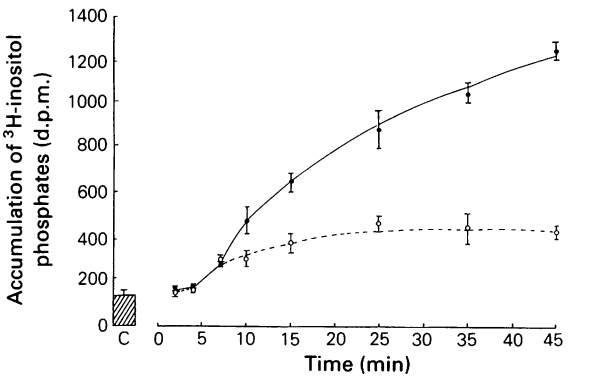

Figure 8 Time course of the accumulation of total ^{3}H-inositol phosphates in response to 0.1 mM histamine (●) or a combination of 0.1 mM histamine and 1 μM salbutamol (○). Histamine and salbutamol were added simultaneously at time zero. The basal accumulation of ^{3}H-inositol phosphates over a period of 45 min in the absence of agonists is shown by the column marked C. Each data point represents the mean of triplicate determinations and vertical lines indicate s.e.mean. The experiment was repeated twice more with similar results.

significant increase in lipid labelling (232 ± 18% of control, $n = 15$; $P < 0.001$, paired t and Wilcoxon signed rank test) after 45 min incubation (Figure 10c) which was also seen with carbachol (1 mM; 173 ± 24% compared to basal, $n = 6$, basal = 100%, $P < 0.05$). A decrease in the stimulation of [^{3}H]-inositol incorporation produced by histamine (0.1 mM) was observed in the presence of salbutamol (Figure 10c). However, inclusion of histamine (0.1 mM) during prelabelling with [^{3}H]-inositol prevented any subsequent stimulation of [^{3}H]-inositol incorporation during the incubation phase of the experiment (Figure 10d). Under these conditions, salbutamol did not modify the level of [^{3}H]-inositol in the phospholipid layer but still inhibited ^{3}H-inositol phosphate formation (Figure 10b and d).

Discussion

The present study confirms previous observations that muscarinic and histamine H_1-receptor activation leads to a potent stimulation of inositol phospholipid hydrolysis and an accumulation of inositol phosphates in slices of bovine tracheal smooth muscle (Barnes *et al.*, 1986; Grandory *et al.*, 1986;

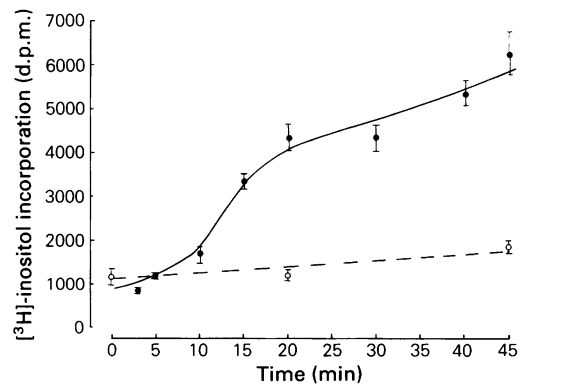

Figure 9 The effect of histamine on the incorporation of [^{3}H]-inositol into membrane phospholipids. Values represent mean of triplicate determinations in the absence (○) or presence of 0.1 mM histamine (●) in a single experiment (vertical lines indicate s.e.mean). Incorporations represent the d.p.m. present in 200 μl aliquots of the chloroform phase following extraction of membrane inositol phospholipids as described under Methods. Similar data were obtained in two further experiments.

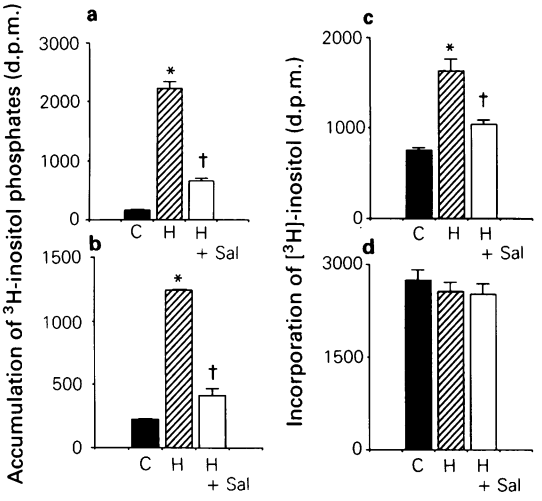

Figure 10 The effect of salbutamol on histamine-stimulated ^{3}H-inositol phosphate accumulation (a and b) and [^{3}H]-inositol incorporation into membrane phospholipids (c and d). Values represent mean (± s.e.mean, shown by bars) of eight replicate determinations under basal conditions (c, solid columns), in the presence of 0.1 mM histamine (H, hatched columns) or in response to 0.1 mM histamine + 1 μM salbutamol (Sal) (open columns). In (b) and (d) histamine (0.1 mM) was present for 45 min of the pre-labelling period with [^{3}H]-inositol in order to stimulate maximally incorporation as described under Methods. * $P < 0.001$ with respect to basal values; † $P < 0.001$ with respect to values obtained with histamine alone (Anovar). No significant differences were observed in (d). Similar data were obtained in two further experiments.

Takuwa *et al.*, 1986). The contractile responses to both histamine and carbachol in this tissue is partly mediated by the mobilization of calcium from intracellular stores (Kirkpatrick, 1975; Kotlikoff *et al.*, 1987; Takuwa *et al.*, 1987). This mobilization of intracellular calcium in response to carbachol and histamine appears to be secondary to agonist-induced inositol phospholipid hydrolysis since one of the products, inositol-1,4,5-trisphosphate, can release stored calcium from saponin-permeabilized tracheal smooth muscle cells (Hashimoto *et al.*, 1985).

Noradrenaline produced a small accumulation of total ^{3}H-inositol phosphates in slices of bovine tracheal smooth muscle with an EC_{50} of *circa* 10 μM. This response to noradrenaline was completely inhibited by the selective α-adrenoceptor antagonist phentolamine (1 μM). The most dramatic effect of noradrenaline on inositol phospholipid hydrolysis in airway smooth muscle, however, was observed when the catecholamine was added simultaneously with histamine. Under these conditions noradrenaline produced a marked inhibition (IC_{50} = 0.8 μM) of the accumulation of ^{3}H-inositol phosphate elicited by 0.1 and 1 mM histamine, which was not sensitive to inhibition by phentolamine.

The inhibitory response to noradrenaline appeared to be mediated by β_2-adrenoceptors since it could be mimicked by both isoprenaline and the β_2-selective agonist salbutamol, with IC_{50} values in the sub-micromolar range. Furthermore, the salbutamol-induced inhibition of the ^{3}H-inositol phosphate accumulation produced by histamine was antagonized by both the non-selective β-antagonist propranolol and the β_2-selective antagonist ICI 118551. The apparent affinity constants determined for each antagonist from the parallel shifts of the concentration-response curves for salbutamol were 2.4×10^9 M^{-1} and 1.7×10^9 M^{-1} for propranolol and ICI 118551, respectively. The values found for ICI 118551 at β_1- (in guinea-pig atria) and β_2- (in guinea-pig uterus) adrenoceptors are 1.5×10^7 M^{-1} and 2.0×10^9 M^{-1} respectively (Bilski *et al.*, 1983). Our data therefore suggest that the effect of β-agonists on inositol phospholipid metabolism in bovine tracheal smooth muscle is mediated via β_2-adrenoceptors.

The time course of the salbutamol-induced inhibition of the inositol phosphate response to histamine is characterized by a short delay (3–4 min), following the simultaneous addition of both agonists, before the rate of formation of ^{3}H-inositol phosphates is reduced to below that obtained in the presence of histamine alone. We have observed a similar delay of longer duration in the modulation of histamine-induced inositol phospholipid hydrolysis produced by adenosine in slices of guinea-pig and mouse cerebral cortex (Hill & Kendall, 1987; Kendall & Hill, 1988). The reason for this time lag remains to be established but it is perhaps suggestive of the need to generate another intracellular messenger, such as adenosine 3′:5′-cyclic monophosphate (cyclic AMP), which must reach a threshold concentration in order to initiate an inhibition of inositol phosphate production.

Cyclic AMP is a strong candidate for the mediator of the salbutamol-induced inhibition of histamine-induced inositol phosphate accumulation in bovine tracheal smooth muscle. Recent studies in strips of canine tracheal smooth muscle (Madison & Brown, 1988) and cultured canine tracheal myocytes (Murray *et al.*, 1988) have suggested that the adenylate cyclase activator forskolin can inhibit histamine-induced inositol phosphate formation. We have also observed a similar effect of forskolin and the cyclic AMP analogues 8-bromo-cyclic AMP and dibutyryl-cyclic AMP in slices of bovine tracheal smooth muscle (Hall & Hill, unpublished observations).

An unusual feature of the effects of histamine and carbachol on inositol phospholipid hydrolysis in

bovine tracheal smooth muscle is that there is a significant stimulation of the incorporation of [^{3}H]-inositol into membrane phospholipids in parallel with the increase in accumulation of ^{3}H-inositol phosphates. The most likely explanation for this is that turnover of phosphoinositides under basal conditions is very low and that the specific activity of ^{3}H-inositol phospholipids, which has not reached isotopic equilibrium, increases following agonist-stimulation. This would also explain the initial lag period in the accumulation of ^{3}H-inositol phosphates elicited by histamine (Figure 8). In keeping with this hypothesis, addition of salbutamol (which presumably inhibits inositol phospholipid breakdown) partially inhibited the increase in incorporation. To confirm that salbutamol was not directly inhibiting the histamine-induced stimulation of [^{3}H]-inositol incorporation, experiments were performed in which histamine was added during the normal prelabelling period to stimulate incorporation maximally. Under these conditions where no changes were observed in the level of incorporation, salbutamol was still able to inhibit the histamine-induced accumulation of ^{3}H-inositol phosphates (see Figure 10). It was notable, however, that the size of the inositol phosphate response to histamine was reduced after inclusion of histamine in the preincubation medium. This suggests that some desensitization has occurred following the prolonged exposure to histamine.

It was striking that, whilst a potent inhibition of the histamine-induced inositol phospholipid response was seen with noradrenaline, no similar effect was observed on the response to carbachol. A similar selective modulation (both inhibition and potentiation) of histamine-induced inositol phosphate accumulation has been observed with adenosine in guinea-pig and mouse cerebral cortical slices (Hollingsworth *et al.*, 1986; Hill & Kendall, 1987; Kendall & Hill, 1988). The reason underlying the differential effect on the responses to muscarinic and histamine H_1-receptor activation is unclear, although one possibility is that these two receptor systems activate different pathways of inositol phospholipid metabolism, only one of which is sensitive to inhibition by β-adrenoceptor stimulation. Circumstantial support for this proposal is provided by the marked differences in the inositol phosphate profiles produced in mouse cerebral cortex (Whitworth & Kendall, 1987), and the different calcium (Kendall & Nahorski, 1984) and temperature (Carswell *et al.*, 1987) sensitivities, of the responses to histamine and carbachol. An alternative possibility is that the effect of β-agonists on inositol phospholipid hydrolysis in airway smooth muscle is mediated via cyclic AMP production and that this is reduced by the inhibitory effect of muscarinic agonists on adenylate cyclase activity (Jacobs *et al.*, 1979; Olianas *et al.*, 1983). These possibilities are under investigation in our laboratories at present.

There have been two previous studies which have failed to demonstrate a β-adrenoceptor agonist-induced change in the inositol phospholipid response to histamine in mammalian tracheal smooth muscle (Grandory *et al.*, 1987; Fujiwara *et al.*, 1988). In the study by Fujiwara *et al.* (1988) it was shown that neither procaterol nor isoprenaline altered the hydrolysis of phosphatidyl-inositol-4,5-*bis*-phosphate during the first 60 s following application of histamine in smooth muscle cells from canine trachea. These data are, however, entirely consistent with our own observations over this time period. In the other study (Grandory *et al.*, 1987), indomethacin (1 μM) was included in the incubation medium and it was notable that the stimulation of ^{3}H-inositol phosphate accumulation produced by histamine was much less than in the present study. Furthermore, the β_2-adrenoceptor agonists were added 20 min in advance of the histamine stimulus.

The significance of the inhibitory effect of β_2-adrenoceptor agonists on inositol phospholipid metabolism with regard to the smooth muscle relaxant properties of these drugs remains to be established. *In vitro*, β_2-adrenoceptor stimulation with isoprenaline produces a rapid relaxation of precontracted tracheal smooth muscle from a variety of mammalian species (Carswell & Nahorski, 1983; Fujiwara *et al.*, 1988). Hence, in view of the observed time course, it seems unlikely that the change in inositol phosphate accumulation brought about by β_2-agonists is fundamental to the initiation of the smooth muscle relaxant effect of agents such as salbutamol. However, the high potency of β_2-agonists in causing these changes in inositol phospholipid metabolism means that they are likely to occur *in vivo* when these agents are administered to relieve bronchoconstriction. Hence, they may play an important role in the maintenance, rather than initiation, of bronchodilatation produced by agents such as salbutamol following challenge with histamine.

We thank the Asthma Research Council for financial support.

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(Received April 28, 1988
Revised July 14, 1988
Accepted July 27, 1988)