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HISTAMINE ACTIVATES Cl^- AND K^+ CURRENTS IN GUINEA-PIG TRACHEAL MYOCYTES: CONVERGENCE WITH MUSCARINIC SIGNALLING PATHWAY

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SUMMARY

1. We investigated the effects of histamine on membrane currents and contractile state of isolated guinea-pig tracheal myocytes using perforated patch and whole-cell recording techniques. The effects of histamine were compared to those of acetylcholine (ACh) and caffeine.

2. During voltage clamp ($V_{hold} = -60$ mV), histamine elicited contraction and an inward current (I_{hist}) which was often followed by current oscillations. I_{hist} had a reversal potential (V_{rev}) of -9 ± 3 mV.

3. I_{hist} was dependent on the Cl^- gradient and was antagonized by the Cl^- channel blocker niflumic acid. V_{rev} was more positive ($+2 \pm 1$ mV) when K^+-selective currents were blocked by Cs^+ and TEA. When all external Na^+ was replaced with *N*-methyl-D-glucamine, there was a small reduction in the amplitude of I_{hist}.

4. The histamine-induced current was similar to that elicited by ACh and by caffeine with respect to time course, amplitude, and current–voltage relationship. Responses to histamine and to ACh were non-additive, consistent with a convergence of histaminergic and cholinergic signalling pathways. I_{hist} was antagonized by the H_1 histaminergic receptor antagonist astemizole, but not by atropine.

5. When recorded using the perforated patch configuration, I_{hist} could be elicited repeatedly for more than 30 min. When cells were studied in the whole-cell configuration using a pipette solution containing 0·025 mM EGTA, the amplitude of I_{hist} was initially the same as that obtained using perforated patch but then decreased; the time required for the responses to decrease to 50% ($t_{\frac{1}{2}}$) was $8·2 \pm 1·0$ min. When 1 mM EGTA was included in the pipette solution (whole-cell configuration), the initial response to histamine was significantly decreased in size and $t_{\frac{1}{2}}$ was reduced to $3·3 \pm 0·7$ min.

6. The characteristics of the signalling pathway were examined in cells studied using the whole-cell configuration with 0·025 mM EGTA in the recording pipette. Heparin significantly reduced $t_{\frac{1}{2}}$ to $4·3 \pm 0·8$ min. GTPγS elicited inward current and oscillations; both effects were enhanced by histamine. GTPγS also reduced $t_{\frac{1}{2}}$ to $1·4 \pm 0·1$ min. Pertussis toxin did not alter the amplitude or time course of I_{hist}.

7. We conclude that in guinea-pig tracheal myocytes, binding of histamine to H_1 receptors leads to release of Ca^{2+} from intracellular stores and subsequent activation

of Cl^- and K^+ conductances as well as contraction. Furthermore, we demonstrate that ACh elicits similar physiological responses due to a convergence of the histaminergic and muscarinic signalling pathways.

INTRODUCTION

Histamine has long been known to cause membrane depolarization and contraction of mammalian airway smooth muscle (Ahmed, Foster, Small & Weston, 1984; Finney, Karlsson & Persson, 1985). The excitatory effects of this inflammatory mediator are mediated through H_1 receptors (Duncan *et al.* 1980) and involve accumulation of inositol phosphates and subsequent release of Ca^{2+} from internal stores (Abdel-Latif, 1986; Kotlikoff, Murray & Reynolds, 1987; Madison & Brown, 1988; Chilvers, Barnes & Nahorski, 1989; Hall, Donaldson & Hill, 1989; Murray, Bennett, Fluharty & Kotlikoff, 1989; Murray & Kotlikoff, 1991). Histamine is released from mast cells and may play an important role in initiating pathological constriction of the airways.

The ionic mechanisms underlying the histamine-induced membrane depolarization of airway smooth muscle are unclear (Hill, 1990). In cultured human airway smooth muscle cells, histamine causes an influx of Ca^{2+} which, on the basis of fluorescent dye studies, has been suggested to involve neither voltage-dependent calcium conductances nor non-selective cation conductances (Murray & Kotlikoff, 1991). In other smooth muscle cell types, histamine suppresses spontaneous transient outward K^+ currents (Désilets, Driska & Baumgarten, 1989; Neliat, Masson & Gargouil, 1989), activates a non-selective cation conductance (Komori, Kawai, Takewaki & Ohashi, 1992) and augments a voltage-dependent Ca^{2+} conductance (Oike, Kitamura & Kuriyama, 1992).

Electrophysiological studies of multicellular smooth muscle tissues have revealed that the actions of histamine are similar in many respects to those of acetylcholine (ACh), leading to the proposal that histaminergic and cholinergic signalling pathways converge (Bolton, Clark, Kitamura & Lang, 1981; Benham & Bolton, 1983; Ahmed *et al.* 1984). ACh-induced membrane conductance changes have been characterized in a wide range of smooth muscle types (for example: Sims, Walsh & Singer, 1986; Clapp, Vivaudou, Walsh & Singer, 1987; Cole & Sanders, 1989; Inoue & Isenberg, 1990; Komori & Bolton, 1990; Pacaud & Bolton, 1991; Kume & Kotlikoff, 1991; Janssen & Sims, 1992; Komori *et al.* 1992; Sims, 1992). Convergence of the histaminergic and cholinergic signalling pathways may occur at the level of the second messengers, as cholinergic agonist-mediated contractions and depolarization have also been shown to involve an accumulation of inositol phosphates and release of Ca^{2+} from internal stores in various smooth muscle tissues (Hashimoto, Hirata & Ito, 1985; Madison & Brown, 1988; Chilvers *et al.* 1989; Kobayashi, Kitazawa, Somlyo & Somlyo, 1989; Pacaud & Bolton, 1991).

The goal of this study was to characterize the effects of histamine on membrane currents of myocytes freshly dispersed from guinea-pig trachealis. We also examined the receptor–channel coupling mechanisms underlying these responses and compared the conductance changes elicited by histamine to those elicited by acetylcholine. We demonstrate that stimulation of histaminergic H_1 receptors causes activation of Cl^- and K^+ conductances. In addition, histamine caused cells to contract, even when

held under voltage clamp. We have studied the responses to histamine and ACh in the same cells, and provide evidence for convergence of histaminergic and muscarinic signalling pathways.

METHODS

Procedures used for dissociation of and electrophysiological recording from smooth muscle cells of guinea-pig trachealis have been described in detail elsewhere (Janssen & Sims, 1992). Briefly, guinea-pigs (250–300 g; either sex) were stunned and bled and tracheae were removed. Strips of trachealis were excised and incubated in digestion solution (60 min; see below for composition) containing collagenase (400 units/ml; type I), papain (30 units/ml; type IV), bovine serum albumin (1 mg/ml), and (−)-1,4-dithio-L-threitol (DTT; 1 mM). Individual myocytes were obtained by gentle trituration, were allowed to settle and adhere to the bottom of a recording chamber (1 ml bath volume perfused at a rate of 2–3 ml/min), were maintained at 25 °C, and were studied within 6 h. Electrophysiological responses were tested in cells that were phase dense, appeared relaxed, and which contracted upon stimulation with histamine. These contractile responses to histamine could be repeated several times in each cell. Membrane currents were recorded using conventional whole-cell (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) and nystatin perforated patch configurations. Pipette tip resistances ranged from 2–5 MΩ. Access resistance was always less than 40 MΩ and 70–80% series resistance compensation was employed.

To investigate the voltage dependence of agonist-activated ionic conductances, the membrane was depolarized using ramp commands from −100 to +10 mV (110 mV/s), repeated at 6 s intervals (e.g. see Janssen & Sims, 1992). Current–voltage (I–V) relationships of the agonist-evoked currents were obtained by digital subtraction of the membrane currents recorded before and during agonist application. The reversal potential was defined as the potential at which the difference current crossed the zero-current axis. Liquid junction potentials between recording pipette and bathing solution were −2, −6, and −8 mV for pipette solutions containing 140, 40, and 20 mM Cl^-, respectively (determined using 3 M KCl–agar bridges). Measurements of reversal potentials provided in the text have been corrected for these errors.

Unless indicated otherwise, all recordings were performed in physiological saline solution (PSS) containing the following (mM): Na^+, 130; K^+, 5; Ca^{2+}, 1; Mg^{2+}, 1; Cl^-, 139; Hepes, 20; D-glucose, 10; pH 7·4. Sodium-free solution contained 160 mM *N*-methyl-D-glucamine (NMG; free base, titrated to pH 7·4 with HCl), and had the same osmolality as the standard solution (280–290 mosmol/kg H_2O). Low-Cl^- solution was prepared by replacing a fraction of the NaCl with sodium aspartate. Digestion solution contained the following (mM): Na^+, 125; K^+, 4·7; Ca^{2+}, 1; Mg^{2+}, 1; Cl^-, 134; Hepes, 10; EDTA, 0·25; D-glucose, 10; taurine, 10; pH 7·0.

Standard pipette solution contained the following (mM): K^+, 140; Cl^-, 143; Ca^{2+}, 0·4; Mg^{2+}, 1; EGTA, 1; Hepes, 20; pH 7·2; free [Ca^{2+}] was estimated to be 100 nM. In the perforated patch configuration, this pipette solution was supplemented with nystatin (final concentration of 300 units/ml). For studies in which K^+ channels were blocked, the pipette solution contained the following (mM): Cs^+, 130; TEA^+, 10; Mg^{2+}, 1; Cl^-, 142; Hepes, 20; EGTA, 5; pH 7·2. A weakly Ca^{2+}-buffering pipette solution was prepared by decreasing the concentration of EGTA to 0·025 mM and omitting Ca^{2+}. In some experiments, heparin (0·2 mM), GTPγS (0·5 mM), or pertussis toxin (0·1 μg/ml) were added to this pipette solution. Pertussis toxin was activated by incubating for 60 min at 37 °C in 20 mM DTT (Ribeiro-Neto *et al.* 1985); the final concentration of DTT in the pipette solution was 2 mM. Low-chloride pipette solutions were prepared by replacing a fraction of the KCl with potassium aspartate.

All chemicals were obtained from Sigma Chemical Co, or BDH Ltd except for astemizole (Janssen Pharmaceutica Inc., Mississauga, Ontario, Canada) and GTPγS (Boehringer Mannheim, Laval, Quebec, Canada). ACh, histamine, and caffeine were prepared as aqueous stock solutions, while niflumic acid was prepared in absolute ethanol. These stock solutions were then diluted 100- to 1000-fold with bathing solution and applied by pressure ejection (Picospritzer II; General Valve Corp., Fairfield, NJ, USA) from micropipettes placed within 100 μm of the cells. Control applications evoked no membrane current (e.g. see Fig. 6). Atropine and astemizole were prepared as aqueous solutions, then added directly to the bathing medium.

Responses are reported as means ± S.E.M. Comparisons between means were done using a two-tailed unpaired Student's t test or ANOVA. Differences were considered significant when $P < 0·05$.

RESULTS

Histamine causes excitation of guinea-pig tracheal smooth muscle cells

We first investigated the effects of histamine on membrane current with the cells held at −60 mV. Histamine (125 μM in application pipette) elicited inward current (I_{hist}) and contraction. I_{hist} had a latency of ≈ 1 s, reached a peak within 1–2 s, and then decreased to baseline levels even with continued application of histamine (Fig. 1). The magnitude of I_{hist} recorded in 42 cells using standard pipette solution ranged

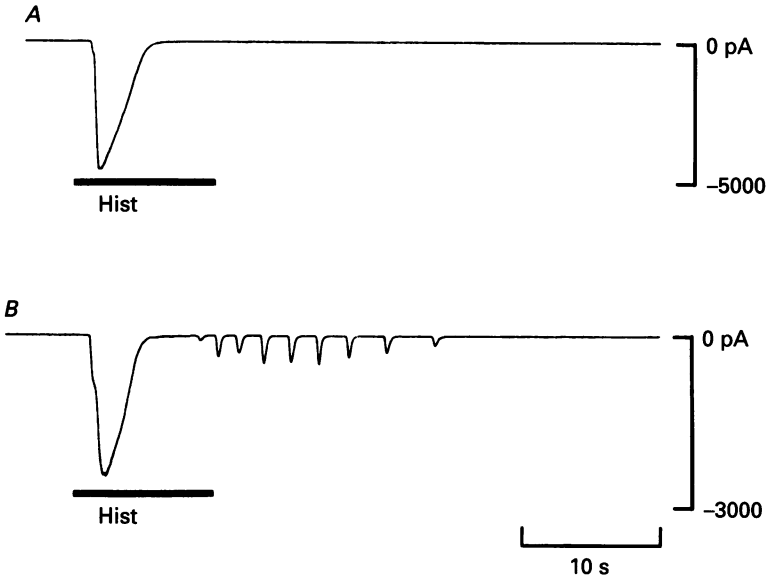

Fig. 1. Inward currents elicited by histamine in guinea-pig tracheal smooth muscle cell. Under voltage clamp (V_{hold} = −60 mV), histamine (125 μM in application pipette) elicited large transient inward current with a latency of ≈ 1 s and which decayed during continued application of histamine. These responses appeared as single events (*A*) or as an initial inward current followed by inward current oscillations (*B*).

from 120 to 4550 pA, with a mean amplitude of 1471 ± 165 pA. In 58 of a total of 117 cells studied using various pipette solutions and recording configurations, this initial inward current was followed by a series of brief inward currents which recurred for several seconds even after application of histamine had ended (Fig. 1*B*). We will refer to these repetitive inward currents as current oscillations. Close inspection reveals that the onset of I_{hist} has a complicated time course, which may reflect the contribution of multiple conductances to I_{hist} and/or a complex activation of the current. Consistent with this activation of inward current, histamine caused depolarization accompanied by a membrane conductance increase in cells studied in current clamp mode (not shown).

Histamine activates Cl^- and K^+ conductances

We investigated the contribution of Na^+ to I_{hist} by replacement with the impermeant cation *N*-methyl-D-glucamine. In cells initially bathed in Na^+-free medium, histamine could still evoke a large inward current (Fig. 2, left; representative of 7 cells), indicating that a large proportion of the histamine-

activated conductance was selective for some ion(s) other than Na^+. Re-introduction of Na^+ caused a small enhancement of I_{hist} (Fig. 2, right). Conversely, for cells initially bathed in Na^+-containing solution, removal of Na^+ caused a reduction of I_{hist} to $83 \pm 9\%$ of the control response ($n = 7$). These findings suggest there may be a relatively small contribution of Na^+ to I_{hist}.

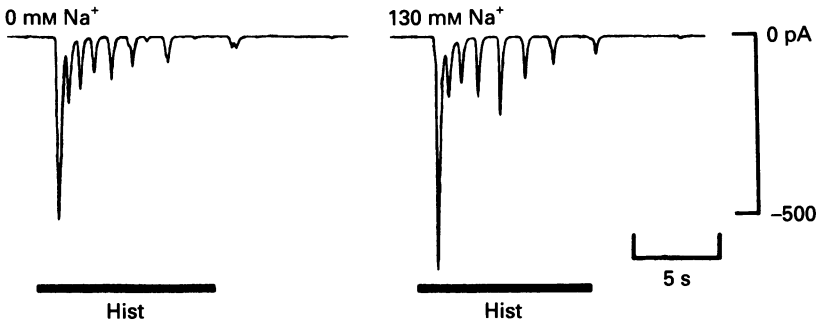

Fig. 2. Histamine evokes large inward current in the absence of Na^+. Stimulation with histamine in a cell bathed in Na^+-free solution elicited inward current followed by prominent current oscillations. Introduction of Na^+ resulted in an increase in the magnitude of I_{hist}.

We used ramp depolarizations to characterize the I–V relationship of I_{hist} (see Methods). The difference current for I_{hist} was inward and linear at negative potentials and showed outward rectification at positive potentials (Fig. 3*A*). The mean reversal potential for I_{hist} in nineteen cells was -9 ± 3 mV (Fig. 3*B*). This reversal potential does not correspond to the equilibrium potential for a single ion species, indicating that histamine activated a mixed conductance. Blockade of K^+ conductances (by replacing K^+ in the pipette with 130 mM Cs^+ and 10 mM TEA) eliminated the outwardly rectifying nature of the current, leaving a nearly linear current which reversed at $+2 \pm 1$ mV ($n = 4$; Fig. 3*A* and *B*).

We investigated the contribution of Cl^- to I_{hist} by replacing external or internal Cl^- with aspartate, thus altering the Cl^- gradient. The results of these ion substitution experiments are summarized in Fig. 3*B*. In cells studied using the Cs^+-containing pipette solution, reduction of external Cl^- from 139 to 49 mM (thereby displacing chloride equilibrium potential (E_{Cl}) from +1 to +26 mV) caused a displacement of V_{rev} from +1 (above) to $+24 \pm 4$ mV ($n = 4$). When internal [Cl^-] was reduced to 40 or 20 mM (keeping external [Cl^-] at 139 mM, thereby displacing E_{Cl} to -32 or -49 mV, respectively), V_{rev} was -32 ± 3 mV ($n = 7$) and -50 ± 8 mV ($n = 3$), respectively. The close correspondence between V_{rev} and calculated E_{Cl} is illustrated in Fig. 3*B*. Disregarding the data obtained when the contribution of outward K^+ was large (i.e. the 19 points obtained using the 140 mM KCl-containing pipette solution), the slope of the best-fit line derived from linear regression of V_{rev} *versus* E_{Cl} was $1{\cdot}0 \pm 0{\cdot}1$ ($r^2 = 0{\cdot}96$), consistent with activation of a Cl^- conductance by histamine. Further support for this conclusion was obtained when we used the Cl^- channel antagonist niflumic acid (100 μM in application pipette, applied for 30 s immediately prior to application of histamine). Niflumic acid decreased I_{hist} by $93 \pm 2\%$ ($n = 3$; Fig. 4) but did not affect the contractile response to histamine. Following wash-out of niflumic acid (3–4 min), I_{hist} recovered to $84 \pm 20\%$ of control (Fig. 4).

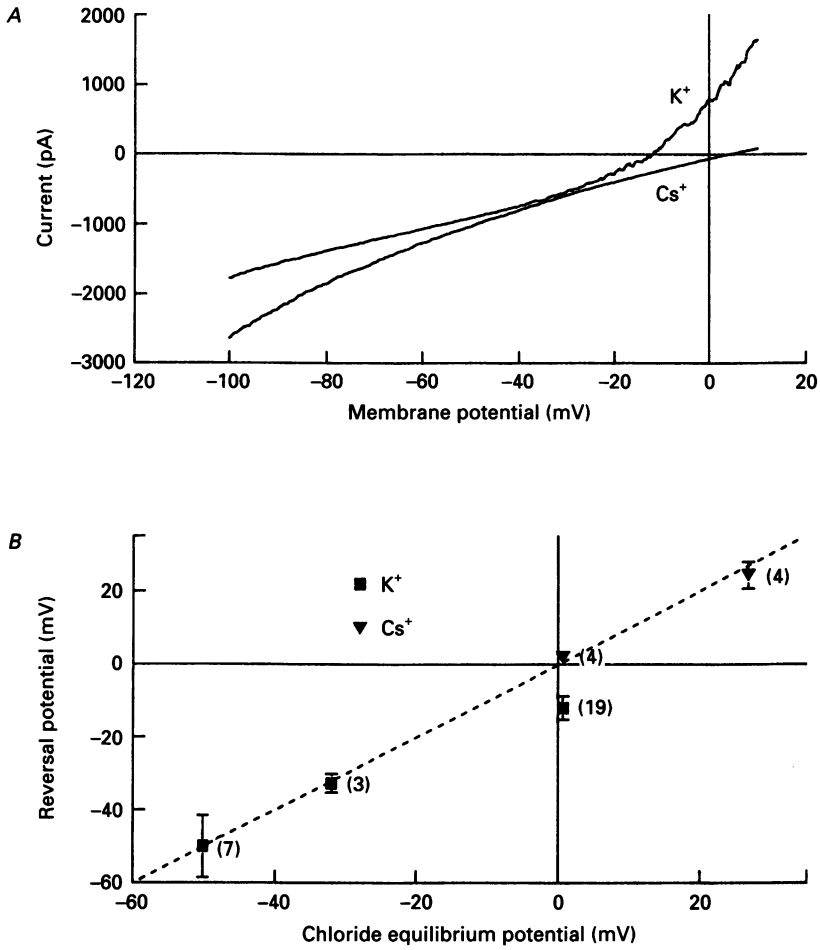

Fig. 3. Current–voltage relationship of histamine-evoked current. Ramp depolarization (−100 to +10 mV at 110 mV/s; V_{hold} = −60 mV) elicited outwardly rectifying currents. Digital subtraction of the ramp-elicited membrane current responses recorded before and during application of histamine yielded difference currents for I_{hist}. *A*, difference currents obtained using 140 mM K^+-containing pipette solution or one in which K^+ was replaced with 130 mM Cs^+ and 10 mM TEA. Cells were bathed in physiological saline solution. *B*, reversal potentials of I_{hist} (obtained using ramps, as in *A*) were largely dependent on the Cl^- gradient. E_{Cl} was displaced in the negative direction by decreasing [Cl^-] in the pipette solution (replacing with aspartate) and perfusing the cells with standard PSS (data indicated by ■). E_{Cl} was also displaced in the positive direction by replacing external Cl^- with aspartate and recording using the Cs^+-containing pipette solution (data indicated by ▼). Numerals in brackets indicate number of cells. Dashed line indicates predicted results if I_{hist} was a pure Cl^- current. Excluding the data obtained when outward K^+ was large (i.e., experiments using 140 mM KCl-containing pipette solution), linear regression gave a value for the slope of V_{rev} *versus* E_{Cl} of 1·0±0·1 (r^2 = 0·96, n = 18).

Convergence of histaminergic and cholinergic signalling pathways: role of Ca^{2+} release from stores

The results presented above provide evidence that histamine evoked Cl^- and K^+ currents, which we will show below are Ca^{2+}-activated. In our previous study, we have shown that ACh activates a membrane conductance which is similar to I_{hist}

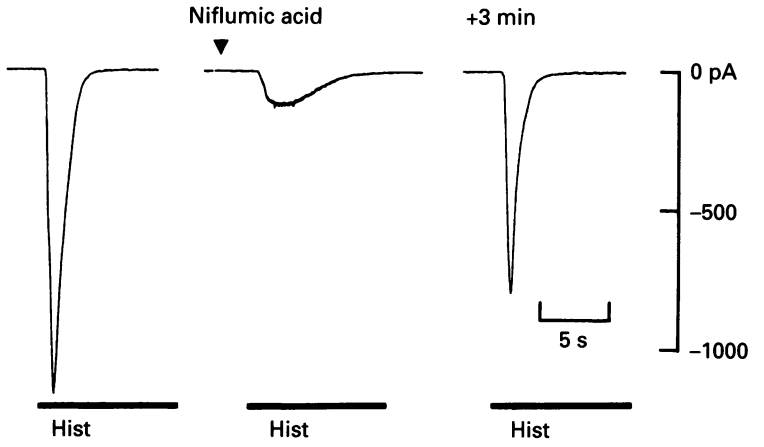

Fig. 4. Chloride channel blocker reduced the histamine-induced current. Inward currents were elicited by histamine at 3–4 min intervals ($V_{hold} = -60$ mV). Niflumic acid (100 μM in application pipette) was applied for 30 s prior to second application of histamine, causing a marked reduction in the magnitude of I_{hist}. Recovery from the inhibitory effect of niflumic acid is shown at right after 3 min.

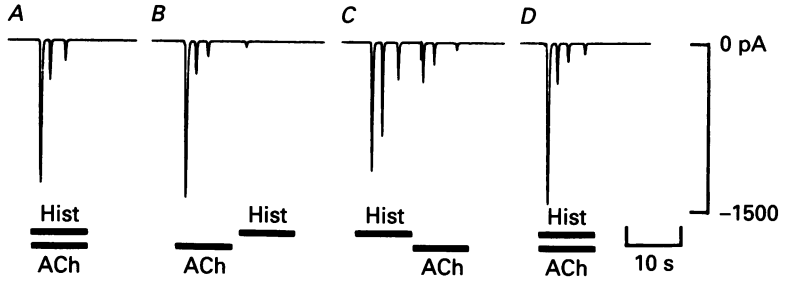

Fig. 5. Histamine and ACh activate the same ionic conductances. During voltage clamp ($V_{hold} = -60$ mV) of a single cell, the responses to sequential or simultaneous applications of histamine and ACh (125 and 20 μM, respectively, in application pipettes) were investigated. Approximately 3 min elapsed between applications to allow for recovery. *A*, simultaneous application of the two agonists elicited a large inward current with current oscillations. *B*, application of ACh elicited a response with similar amplitude and time course, whereas histamine applied immediately after ACh elicited no response. *C*, histamine also elicited an inward current with similar magnitude and time course, whereas ACh applied immediately after histamine elicited a very small response. *D*, simultaneous application of both agonists again elicited a response similar to those shown in *A–C*.

with respect to time course and *I–V* relationship (Janssen & Sims, 1992). To test whether histamine and ACh activated the same conductances, we examined the interactions between these two agents. Figure 5 illustrates the protocol used, in which histamine and ACh (125 and 20 μM in application pipettes, respectively) were applied simultaneously or sequentially at 3 min intervals. Simultaneous application elicited a large inward current followed by oscillations (Fig. 5*A*). ACh alone elicited inward current with a similar time course, amplitude and sensitivity to Cl^- channel blockers (Fig. 5*B*; Janssen & Sims, 1992). Application of histamine immediately after ACh had no additional effect on membrane current (Fig. 5*B*). Three minutes later, however, histamine evoked current similar to that elicited by ACh; subsequent

application of ACh immediately after histamine elicited only small membrane current (Fig. 5*C*). Finally, histamine and ACh were again applied simultaneously (Fig. 5*D*), eliciting a similar response as before (Fig. 5*A*), indicating that there was no 'run-down' of these responses. In six cells tested in this way, there was no

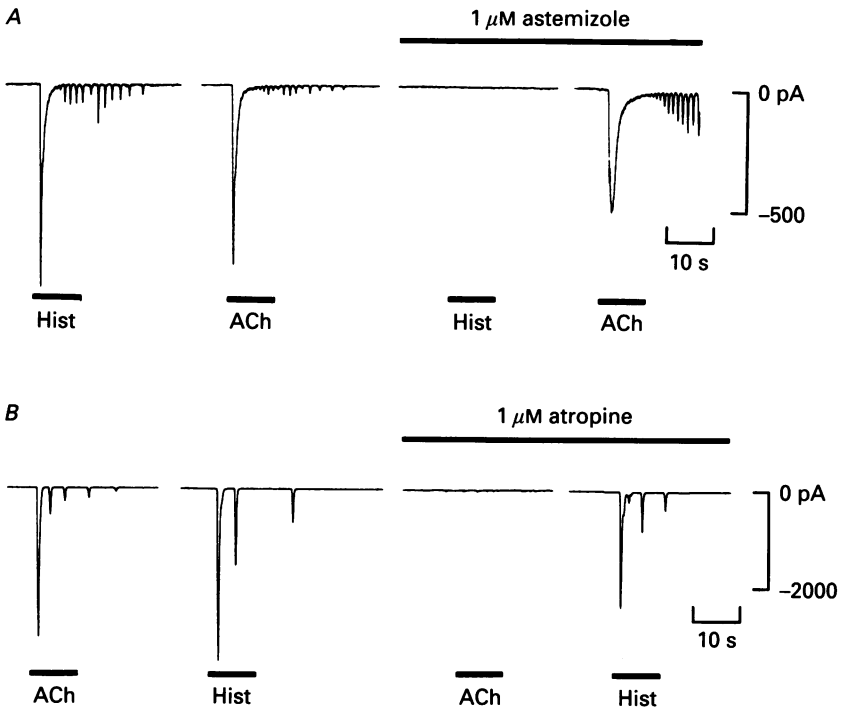

Fig. 6. Histamine and ACh act through distinct receptors. Inward currents were elicited by histamine or ACh (125 and 20 μM, respectively, in application pipettes) in alternating fashion at 3–4 min intervals ($V_{hold} = -60$ mV). Control responses were obtained, then 1 μM astemizole (*A*) or 1 μM atropine (*B*) were added to the bath solution. The H_1-selective antagonist astemizole blocked the histaminergic response but not the cholinergic response, whereas atropine blocked only the muscarinic response.

significant difference between the mean responses to histamine alone (1512 ± 369 pA), ACh alone (1483 ± 301 pA), and ACh plus histamine (1244 ± 366 pA).

Evidence that histamine and ACh were acting through different receptors was obtained using the H_1 histaminergic receptor antagonist astemizole (Hill, 1990) and the muscarinic receptor antagonist atropine. Following control responses to histamine and ACh, astemizole or atropine were added to the bath (final concentration of 1 μM in both cases). Responses to histamine were completely blocked by astemizole, but responses to ACh could still be elicited (Fig. 6*A*; $n = 3$). Conversely, responses to ACh were completely antagonized by atropine, but histamine still evoked inward current (Fig. 6*B*; $n = 3$).

The results presented above are consistent with histamine and ACh both activating Cl^- and K^+ conductances. Further support for this conclusion was obtained by comparing the reversal potentials of the currents evoked by these agonists. Since the effects of these agonists are thought to involve release of Ca^{2+}

from intracellular stores (Hashimoto *et al.* 1985; Abdel-Latif, 1986; Kotlikoff *et al.* 1987; Kobayashi *et al.* 1989; Murray & Kotlikoff, 1991; Pacaud & Bolton, 1991; Janssen & Sims, 1992; Sims, 1992), we also tested caffeine (5 mM in application pipette). In six cells, histamine, ACh and caffeine were applied in sequence, giving inward currents with similar time courses and amplitudes, with reversal potentials of -11 ± 3, -9 ± 3, and -14 ± 3 mV, respectively ($n = 6$). These values of V_{rev} were not significantly different from each other, nor did they differ from the values reported above.

Thus, release of Ca^{2+} from internal stores by ACh, histamine, or caffeine led to activation of Cl^- and K^+ conductances. Our earlier study showed that ACh also activates a non-selective cation conductance, whereas these studies suggest that histamine does so poorly or not at all. This distinct effect of ACh may account for the small inward current evoked by ACh immediately after application of histamine (Fig. 5*C*), and may suggest that the non-selective cation conductance is not Ca^{2+}-activated. In support of this, we found that the caffeine-evoked current (measured using Cs^+-containing pipette solution) did not change in amplitude upon removal of external Na^+ (mean amplitude in absence of Na^+ was $101 \pm 5\%$ of that in the presence of Na^+; $n = 3$).

We directly examined a role for Ca^{2+} in activating membrane currents using the conventional whole-cell recording configuration to buffer internal [Ca^{2+}]. We assessed the amplitude of the current responses to histamine as well as their persistence under different internal Ca^{2+}-buffering conditions. Persistence of I_{hist} was quantified by determining the time required for the successive responses to decrease by 50% (referred to as $t_{\frac{1}{2}}$). $t = 0$ was the time of the first histamine application; in whole-cell configuration, less than 1 min elapsed between break-in and first application of histamine. Representative current traces are presented in Fig. 7*A–C*, and the mean responses are given in Fig. 7*D*. When studied using the perforated patch configuration, histamine typically elicited currents for greater than 30 min; the mean amplitude of the initial responses was 1635 ± 330 pA ($n = 17$). When studied using the whole-cell configuration with 1 mM EGTA in the pipette solution, the size of the initial response to histamine was significantly reduced to 209 ± 110 pA ($n = 8$) and I_{hist} diminished rapidly ($t_{\frac{1}{2}} = 3{\cdot}3 \pm 0{\cdot}7$ min). As a control, we decreased the concentration of EGTA to 0·025 mM. In this case, the magnitude of the initial current responses to histamine (1251 ± 261 pA; $n = 19$) was not significantly different ($P > 0{\cdot}05$) from that recorded using the perforated patch configuration and $t_{\frac{1}{2}}$ was $8{\cdot}2 \pm 1{\cdot}0$ min. Thus, based on the changes in the magnitude of the initial current and of $t_{\frac{1}{2}}$, increased buffering of Ca^{2+} prevents activation of I_{hist}.

Histamine acts through G proteins and IP_3

Agonist-induced release of Ca^{2+} from intracellular stores in many cell types, including smooth muscle, involves G protein-mediated activation of phospholipase C and generation of inositol 1,4,5-trisphosphate (IP_3) (reviewed by Berridge & Irvine, 1989). To investigate whether these histaminergic responses involve such a mechanism, we tested the effects on I_{hist} of various modulators of second messenger pathways added to the pipette solution containing 0·025 mM EGTA (whole-cell configuration). The effects of these agents on I_{hist} are summarized in Table 1.

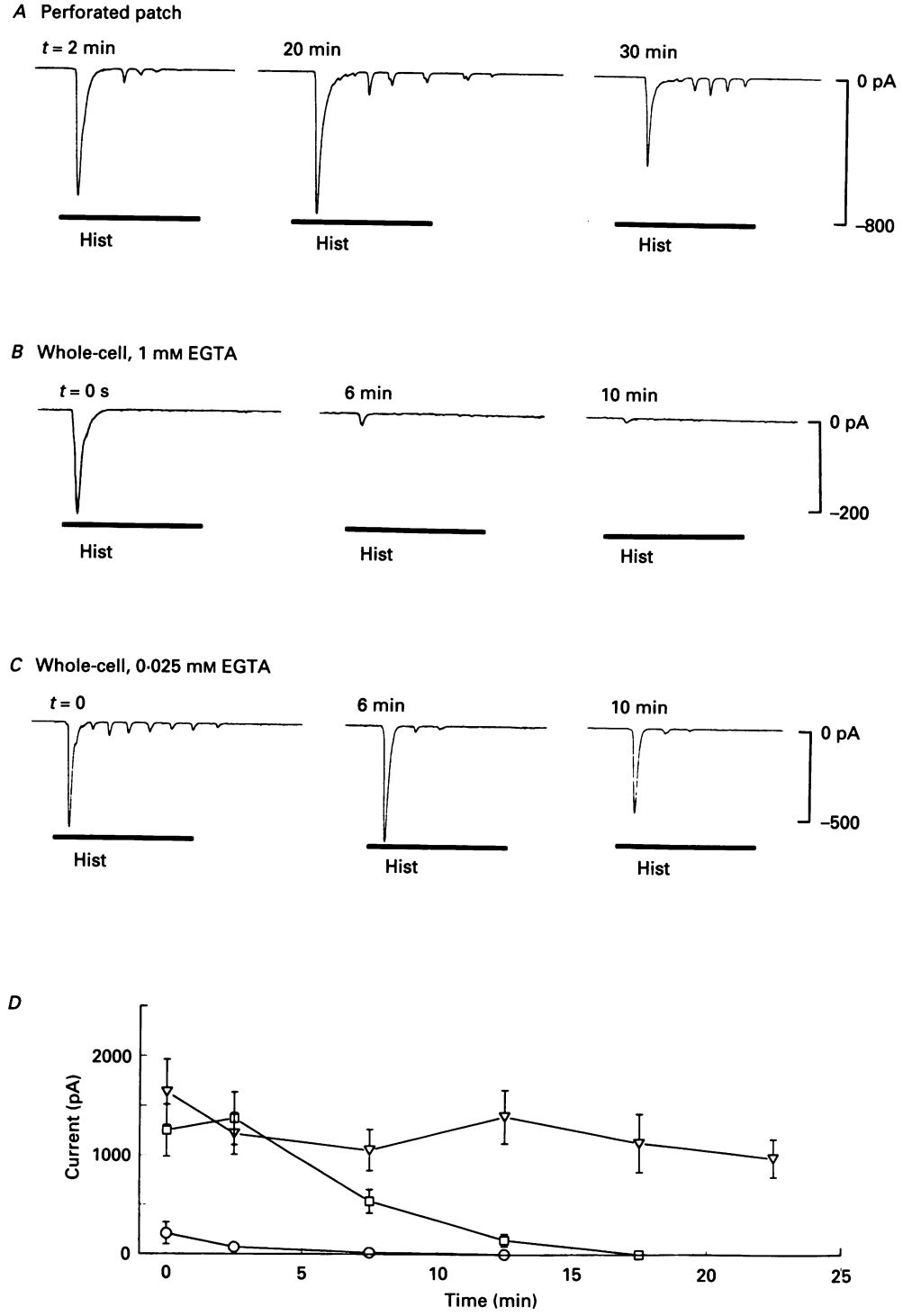

Fig. 7. Time course of histamine-induced current using different recording configurations. Histamine was applied at 2 min intervals in cells studied using perforated patch or whole-cell configurations ($V_{hold} = -60$ mV). $t = 0$ was defined as the time when the first response to histamine was recorded; in whole-cell configuration, less than 1 min elapsed between

GTPγS is a non-hydrolysable analogue of GTP which irreversibly activates G proteins and is therefore used to elicit responses normally evoked by activation of G protein-coupled receptors (Murray *et al.* 1989; Inoue & Isenberg, 1990; Komori & Bolton, 1990; Komori *et al.* 1992; Oike *et al.* 1992). GTPγS (0·5 mM) alone activated a tonic inward current and recurring inward current oscillations, both of which

TABLE 1. Effects on I_{hist}* of modulators of second messenger pathways introduced via recording pipette

	Mean $t_{\frac{1}{2}}$† (min)	Number of cells
Control	8·2±1·0	19
1 mM EGTA	3·3±0·7‡	8
+0·5 mM GTPγS	1·4±0·1‡	5
+0·5 mM GTP	9·2±2·4	5
+0·2 mM heparin	4·3±0·8‡	7
+0·1 μg/ml PTX	7·3±1·0	7
+2 mM DTT	7·3±0·6	5

* I_{hist} was recorded using whole-cell configuration with 0·025 mM EGTA in pipette solution.
† Mean time (±S.E.M.) required for elicited responses to decrease in magnitude by 50%.
‡ Significantly different from control ($P < 0·05$).

persisted for several minutes ($n = 4$; Fig. 8*A*). In five other cells, histamine was applied soon after establishing whole-cell recording, accelerating the development of the persistent inward current and the current oscillations (Fig. 8*B*). Subsequent challenge with histamine did not result in additional current (Fig. 8*B*); as a result, $t_{\frac{1}{2}}$ was significantly reduced in these cells compared to control (Table 1). In control experiments, GTP (0·5 mM) did not significantly alter $t_{\frac{1}{2}}$ (Table 1) nor did it elicit inward current or current oscillations ($n = 5$; not shown).

Pertussis toxin (PTX) ribosylates certain subtypes of G proteins, thereby irreversibly inactivating them (Ribeiro-Neto *et al.* 1985). Since the reducing agent DTT used to activate the toxin was also present in the pipette solution, we also performed control experiments using DTT in the recording pipette. PTX did not alter $t_{\frac{1}{2}}$ ($n = 7$; Table 1). DTT alone also had no effect ($n = 5$; Table 1).

Heparin blocks the IP_3 receptors which gate Ca^{2+} channels on the sarcoplasmic reticulum (Ehrlich & Watras, 1988) and has therefore been used to antagonize

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break-in and first application of histamine. *A*, the magnitude of I_{hist} recorded at the indicated times using the perforated patch configuration. *B*, when studied using the whole-cell configuration and when the pipette solution contained 1·0 mM EGTA, the initial response to histamine was reduced and no currents were elicited after 6 min. *C*, when the pipette solution contained 0·025 mM EGTA (whole-cell configuration), the initial response was comparable in magnitude to that obtained using the perforated patch configuration, and there was little run-down over the course of 10 min. *D*, quantification of time course of histamine-induced currents. Data represent mean±S.E.M. of the initial responses ($t = 0$) or of the responses recorded over each subsequent 5 min interval. I_{hist} in cells studied using the perforated patch configuration (▽) persisted for up to 25 min. When studied using the whole-cell configuration, however, histamine-induced currents were less persistent: I_{hist} decreased relatively quickly with 0·025 mM EGTA in the recording pipette (□) and was abolished in less than 10 min with 1 mM EGTA in the recording pipette (○).

receptor-evoked responses in smooth muscle (Kobayashi *et al.* 1989; Komori & Bolton, 1990; Pacaud & Bolton, 1991). Addition of heparin (0·2 mM) to the pipette solution caused a significant reduction in $t_{\frac{1}{2}}$ to 4·3 ± 0·8 min ($n = 7$; Table 1).

These observations are consistent with a mechanism in which histamine receptor–channel coupling involves PTX-insensitive G proteins and IP_3-induced release of Ca^{2+} from internal stores.

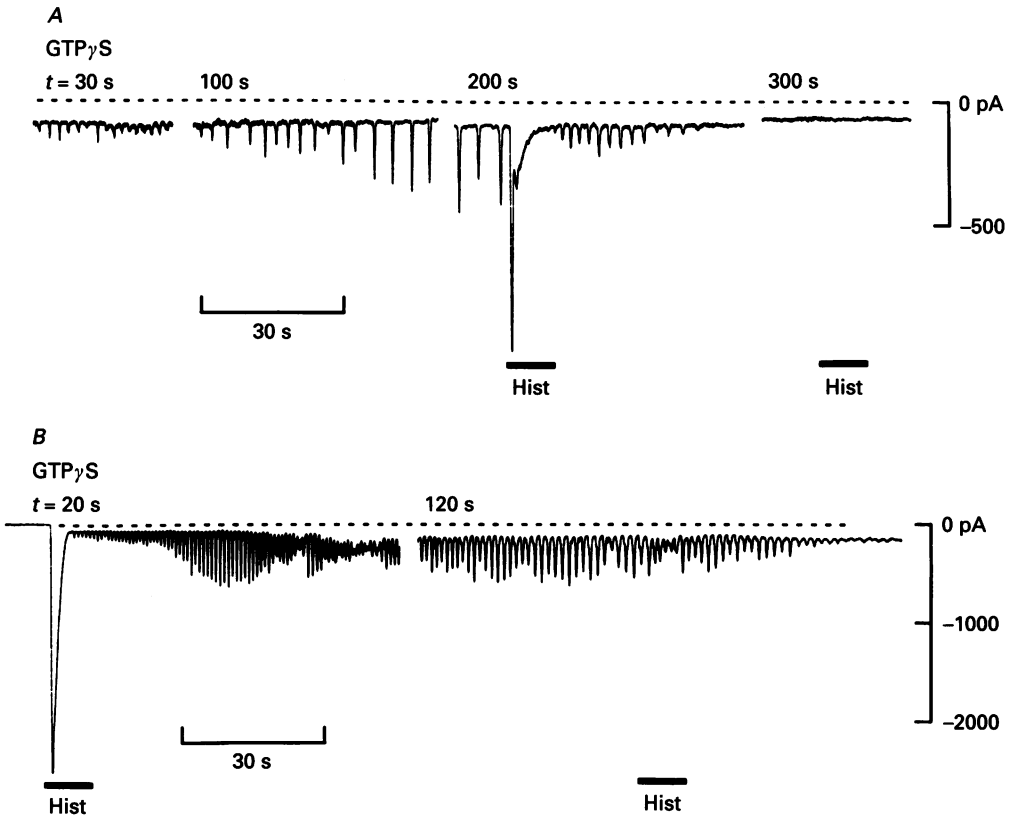

Fig. 8. GTPγS elicited sustained inward current and current oscillations. Cells were held at −60 mV in whole-cell configuration; pipette solution contained 0·025 mM EGTA and 0·5 mM GTPγS. *A*, GTPγS elicited sustained inward current and current oscillations. Histamine elicited inward current in this cell at 200 s but not when applied approximately 2 min later. *B*, when histamine was applied within 20 s of establishing whole-cell recording conditions in another cell, a typical histaminergic response was elicited followed by a sustained inward current and current oscillations. A second application of histamine 2 min later had no effect. Dashed lines indicate zero current level.

DISCUSSION

Histamine-elicited conductance changes

We have investigated the effects of histamine on ion conductances and signalling pathways in guinea-pig tracheal smooth muscle cells. Acting on H_1 receptors, histamine caused activation of Cl^- and K^+ conductances based upon several results. The evidence that histamine activated a Cl^- conductance includes the observation that the reversal potential was largely dependent on the Cl^- equilibrium potential

(Fig. 3) and that I_{hist} could be reversibly antagonized by the Cl^- channel blocker niflumic acid (Fig. 4). Histamine also activated a K^+ conductance, since blockade of K^+-selective conductances using Cs^+ and TEA resulted in a significant displacement of V_{rev} from -9 to $+2$ mV (i.e. to E_{Cl}) and eliminated the outwardly rectifying nature of I_{hist} (Fig. 3). When all external Na^+ was removed, there was only slight decrease in the amplitude of I_{hist} (Fig. 2), suggesting there was little or no contribution of a non-selective cation conductance to I_{hist}.

At resting membrane potentials, the contribution of the K^+ current to I_{hist} would seem to be minimal, since we found the Cs^+/TEA-sensitive component of I_{hist} was negligible at potentials negative to ≈ -30 mV (Fig. 4). Thus, at membrane potentials in the physiological range, I_{hist} is largely a Cl^- current. Since E_{Cl} in smooth muscle ranges from -33 to -6 mV (Aickin, 1990), activation of the Cl^- current can account for the depolarization elicited by histamine.

Histamine acts by releasing Ca^{2+} from intracellular stores

In addition to inducing inward current, histamine elicited contraction of the cells. Since contractions in smooth muscle serve as an indirect assay of $[Ca^{2+}]_i$, these contractions under voltage clamp suggest histamine caused an elevation in $[Ca^{2+}]_i$ that was independent of voltage-gated Ca^{2+} channels. Our finding that I_{hist} was reduced by 1 mM EGTA in the recording pipette (whole-cell mode; Fig. 7) can also be explained by elevation of $[Ca^{2+}]_i$ being responsible for mediating the effects of histamine on membrane conductances.

The source of this Ca^{2+} may be the sarcoplasmic reticulum, since histamine has been shown to evoke release of Ca^{2+} from intracellular stores in many cell types, including airway smooth muscle (Abdel-Latif, 1986; Kotlikoff *et al.* 1987; Pacaud & Bolton, 1991; Murray & Kotlikoff, 1991; Komori *et al.* 1992). Other observations support a role for histamine in releasing Ca^{2+} from internal stores. First, caffeine evoked a current response with similar latency, duration, amplitude, and *I–V* relationship as I_{hist}. Second, I_{hist} was antagonized by heparin (Table 1), which antagonizes IP_3-mediated release of Ca^{2+} from the sarcoplasmic reticulum (Ehrlich & Watras, 1988). Third, histamine evoked current oscillations (Fig. 1*B*); oscillations in membrane current have been identified in other cell types and have been attributed to oscillations in cytosolic Ca^{2+} initiated by emptying of the intracellular stores (Désilets *et al.* 1989; Petersen & Wakui, 1990).

G proteins mediate the responses to histamine

G proteins have been shown to mediate the conductance changes evoked by cholinergic (Cole & Sanders, 1989; Inoue & Isenberg, 1990; Komori & Bolton, 1990; Kume & Kotlikoff, 1991; Komori *et al.* 1992) and histaminergic (Komori *et al.* 1992; Oike *et al.* 1992) stimulation of some types of smooth muscle cells. In the tracheal myocytes studied here, GTPγS elicited persistent inward current and current oscillations (Fig. 8), providing evidence for the involvement of G proteins in regulating ionic conductances. Histamine accelerated the development of inward current and current oscillations (Fig. 8). This is consistent with histamine activating G proteins, since dissociation of GDP from the G proteins (which must occur before GTPγS can bind to and activate the G proteins) occurs at a low basal rate but is

greatly accelerated by binding of agonists to their receptors. The G protein(s) mediating the responses to histamine were PTX insensitive in our experiments. Oike *et al.* (1992) have shown that histamine augments Ca^{2+} currents in rabbit saphenous arterial smooth muscle myocytes, an effect also mediated by PTX-insensitive G proteins. However, in this case, the responses were reported to be due to H_3 receptors. In guinea-pig ileal smooth muscle (Komori *et al.* 1992), histamine activates PTX-insensitive G proteins, leading to release of Ca^{2+} from internal stores and activation of a Ca^{2+}-dependent K^+ conductance. In addition, histamine is also proposed to act via PTX-sensitive G proteins which lead to activation of a cationic current (Komori *et al.* 1992).

Convergence of excitatory signalling pathways

Based on electrophysiological studies of intact smooth muscle tissues, it has been suggested that histaminergic and cholinergic receptors mediate excitation by acting on the same ion channels (Bolton *et al.* 1981; Benham & Bolton, 1983). The ionic conductances regulated by ACh have been examined in single cells from a variety of smooth muscle types, where ACh has been found to suppress K^+ currents (Sims *et al.* 1986; Cole & Sanders, 1989; Kume & Kotlikoff, 1991), augment voltage-dependent Ca^{2+} conductances (Clapp *et al.* 1987), and activate chloride (Janssen & Sims, 1992) and non-selective cation conductances (Inoue & Isenberg, 1990; Komori & Bolton, 1990; Pacaud & Bolton, 1991; Janssen & Sims, 1992; Komori *et al.* 1992; Sims, 1992). Like ACh, histamine also suppresses K^+ conductances (Désilets *et al.* 1989; Neliat *et al.* 1989), activates a non-selective cation conductance (Komori *et al.* 1992), and augments a voltage-dependent Ca^{2+} conductance (Oike *et al.* 1992). Therefore, in general, histamine and ACh do seem to act in a similar manner.

We have investigated the effects of both histamine and ACh in guinea-pig tracheal myocytes, and have found that these agents elicit membrane conductance changes which are similar in their latency, amplitude, duration, and sensitivity to Cl^- channel blockers. These results suggest that histamine and ACh activate similar membrane conductances. Furthermore, the effects of histamine and of ACh were not additive and both agonists produced short-term heterologous desensitization (Fig. 5), also consistent with a convergence of the signalling pathways utilized by these agonists.

Thus, excitatory input from two distinct signalling pathways converges through a common coupling mechanism (i.e. release of Ca^{2+} from internal stores). At what level does this convergence occur? It is clear that convergence occurs 'downstream' from the level of the receptors, as revealed by the selective action of receptor blockers (Fig. 6). However, the fact that caffeine, histamine and ACh evoked similar effects suggests convergence occurs at a level 'upstream' from the sarcoplasmic reticulum.

The histaminergic and muscarinic signalling pathways may converge at the G protein level. In these experiments, I_{hist} was unaltered by PTX (Table 1), suggesting that the G proteins activated by histamine in guinea-pig tracheal myocytes are PTX insensitive, as also described for rabbit saphenous artery (Oike *et al.* 1992). In contrast, some cholinergic responses in smooth muscle are mediated by PTX-sensitive G proteins (Cole & Sanders, 1989; Inoue & Isenberg, 1990; Kume & Kotlikoff, 1991). Thus, it may be that the histaminergic and muscarinic receptors are coupled to phospholipase C via different G proteins (PTX-insensitive and PTX-

sensitive, respectively), as has been shown previously (Ashkenazi, Peralta, Winslow, Ramachandran & Capon, 1989).

Alternatively, convergence may occur at the level of phospholipase C, with the two classes of receptors being coupled to a common phospholipase via different G protein subtypes. This could account for the different sensitivities of histaminergic and cholinergic responses to PTX. Such a model of convergence has been demonstrated in neuronal tissue, in which the K^+ conductance underlying after-hyperpolarization is suppressed by two different G protein-mediated mechanisms (Nicoll, 1988). Histamine, noradrenaline, and corticotropin-releasing factor suppress this conductance by a mechanism involving stimulation of adenylate cyclase (i.e. a G_s-mediated mechanism). ACh also suppresses this conductance, but is known to suppress adenylate cyclase and is believed to be exerting its effect via generation of diacylglycerol (the muscarinic receptors would therefore be coupled to G_i and/or G_p).

It is also possible that distinct types of phospholipase C give rise to a common pool of IP_3. However, Chilvers *et al.* (1989) found that histamine- and carbachol-induced accumulation of inositol phosphates were non-additive in bovine tracheal smooth muscle, which suggests that convergence occurs at a higher level.

Convergence of different neurotransmitter signalling pathways seems to be a widespread phenomenon. For example, a K^+ conductance in neuronal tissue has been shown to be suppressed by noradrenaline, histamine, ACh, serotonin, and corticotropin-releasing factor, while a second K^+ conductance is activated by GABA, serotonin, and adenosine (Nicoll, 1988). In smooth muscle, substance P and ACh both suppress the same K^+ current in gastric cells of *Bufo marinus* (Sims *et al.* 1986), while histamine, noradrenaline, and angiotensin II all augment a voltage-dependent Ca^{2+} conductance in rabbit saphenous arterial cells (Oike *et al.* 1992).

Other agonists evoke contractions in guinea-pig airway smooth muscle, including substance P, prostanoids, serotonin, angiotensin II, bombesin, and neurotensin (Mizrahi, Couture, Caranikas & Regoli, 1982). It is possible that the responses to these agonists are also mediated through the same convergent pathway utilized by histamine and ACh. If this is true of airway smooth muscle in general, the most useful agents for the treatment of asthma and related breathing disorders may be those which interfere with the receptor–effector coupling mechanisms rather than agents which block the spasmogens at the receptor level.

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