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Spasmogen action in guinea-pig isolated trachealis: involvement of membrane K^+-channels and the consequences of K^+-channel blockade

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1 Acetylcholine (ACh), histamine, prostaglandin E_2 and potassium chloride (KCl) each evoked concentration-dependent spasm of guinea-pig isolated trachealis treated with indomethacin (2.8 μM).

2 Neither tetraethylammonium (TEA; 0.1–10 mM) nor procaine (0.1–10 mM) potentiated these spasmogens. Indeed, procaine (10 mM) depressed the log concentration-effect curves of all the spasmogens while TEA (1–10 mM) caused some depression of the log concentration-effect curve of prostaglandin E_2.

3 Intracellular electrophysiological recording was performed in trachealis bathed by normal Krebs solution or by Krebs solution containing 2.8 μM indomethacin. In either medium the majority of trachealis cells exhibited spontaneous electrical slow waves while some cells were electrically quiescent. In either medium the spasmogenic effects of ACh (1 mM) and histamine (0.2 mM) were accompanied by depolarization and abolition of slow wave discharge. In many cases the record of membrane potential subsequently exhibited noise which incorporated fast, hyperpolarizing transients.

4 In the absence and presence of indomethacin, TEA (10 mM) and procaine (5 mM) markedly reduced the membrane noise and hyperpolarizing transients evoked by ACh or histamine without augmenting the evoked tension.

5 It is concluded that slow wave discharge does not depend on prostaglandin synthesis. The membrane noise and hyperpolarizing transients evoked by ACh and histamine represent the opening of membrane K^+-channels. While such K^+-channel opening may offset spasmogen-induced depolarization it does not moderate the evoked tension.

Introduction

Intracellular electrophysiological recording from guinea-pig trachealis *in vitro* (Ahmed *et al.*, 1984) revealed that the spasmogenic effects of acetycholine (ACh) and histamine were accompanied by a small increase in the frequency of spontaneous electrical slow waves and by depolarization. When sufficient depolarization occurred, slow wave discharge ceased. Very often the record of membrane potential subsequently became noisy and exhibited fast, hyperpolarizing transients. In the case of ACh, these hyperpolarizing transients were resistant to tetrodotoxin (3 μM) and hexamethonium (1 mM) suggesting that they did not represent inhibitory postjunctional potentials evoked by the activation of nicotinic cholinoceptors on inhibitory neurones. In the case of both ACh and histamine, the hyperpolarizing transients could be dissipated by spasmogen washout – indicating that these transients did not represent artefacts caused by blockage of the microelectrode tip or by displacement of the electrode tip from the impaled cell.

It has recently been suggested (Benham & Bolton, 1986) that the ACh- and histamine-induced hyperpolarizing transients observed by Ahmed *et al.* (1984) may have resulted from the opening of membrane K^+-channels – a process triggered by the release of Ca^{2+} from intracellular sites of sequestration. The present experiments were therefore performed to examine whether K^+-channel opening can account for the hyperpolarizing transients induced by ACh and histamine in guinea-pig trachealis and to examine whether K^+-channel opening exerts a moderating

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influence on spasmogenic responses evoked by these and other agents.

Methods

Guinea-pigs (350–550 g) of either sex were killed by stunning and bleeding. Tracheae were excised, cleaned of adhering adipose and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis.

Tissue bath studies of mechanical activity

Small segments of trachea were set up for the isometric recording of tension changes essentially as described by Foster *et al.* (1983). At the outset of each experiment tissues were subjected to imposed tension of 1 g. Since the Krebs solution contained 2.8 μM indomethacin, the tissues exhibited little spontaneous tone and any tone present at the beginning of an experiment was dissipated by several changes of bath fluid. Tissues were then allocated randomly in equal numbers to test or time-matched control groups.

The effects of a single spasmogen (ACh, histamine, KCl or prostaglandin E_2) were studied in test tissues by the construction of cumulative concentration-effect curves employing ten fold (ACh, histamine), five fold (prostaglandin E_2) or two fold (KCl) concentration increments. The tissue contact time for each concentration of spasmogen was 4 (ACh), 6 (histamine, prostaglandin E_2) or 12 (KCl) min. ACh (10 mM) was added at the summit of concentration-effect curves for histamine, prostaglandin E_2 and KCl so that the maximal effect of each spasmogen could be related to that of ACh.

Following the construction of the initial concentration-effect curve for a given spasmogen, test tissues were equilibrated for 10 min in Krebs solution containing a K^+-channel inhibitor (tetraethylammonium (TEA) or procaine) at a concentration of 0.1 mM. The spasmogen concentration-effect curve was then reconstructed in the presence of the K^+-channel inhibitor. The concentration of the K^+-channel inhibitor was subsequently increased to 1 and 10 mM. Concentration-effect curves for the spasmogen were constructed following 10 min equilibration with and in the presence of each concentration of K^+-channel inhibitor used. Time matched control tissues were treated similarly but were not exposed to the K^+-channel inhibitors.

Intracellular electrophysiological recording

Simultaneous recording of intracellular electrical activity and mechanical changes of a contiguous segment of trachea was performed using the technique and tissue holder described by Dixon & Small (1983). Accordingly, part of the trachealis was immobilized to permit long term intracellular electrical recording while mechanical activity of contiguous muscle bundles was measured under a resting tension of 1 g. The recording microelectrodes were filled with 3 M KCl and were of resistance greater than 40 MΩ.

The experiments were carried out either in normal Krebs solution or in Krebs solution containing indomethacin (2.8 μM). Interactions between receptor-activating spasmogens (ACh and histamine) and K^+-channel inhibitors (TEA and procaine) were studied as follows. After impalement of a trachealis cell, several minutes were allowed to elapse to check the stability of the record of the electrical activity. ACh (1 mM) or histamine (200 μM) was then added to the Krebs solution superfusing the tissue. These concentrations of ACh and histamine approximated to their respective EC_{90} values as regards tension development but were selected on the basis that they consistently evoked hyperpolarizing electrical transients (Ahmed *et al.*, 1984) of sufficient amplitude and frequency to permit pharmacological analysis. When (approximately 4 min) the effects of ACh or histamine were adequately developed (spasmogenic response reaching a plateau and membrane potential record exhibiting fast, hyperpolarizing transients), 10 mM TEA or 5 mM procaine was added to the Krebs solution superfusing the tissue. The interaction between the spasmogen and the K^+-channel inhibitor was then monitored for as long as the microelectrode tip remained within the impaled cell.

Studies of the interactions between the spasmogens and the K^+-channel inhibitors were controlled by a further series of experiments in which tissues were exposed to the spasmogen (ACh or histamine) but not to the K^+-channel inhibitor. In these control experiments the electrical and mechanical effects of the spasmogen were recorded for a period in excess of 8 min.

Drugs and solutions/statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. Where KCl was used as an agonist the stated concentration excludes the KCl provided by the formulation of the physiological salt solution. The following substances were used: acetylcholine chloride (BDH), histamine dihydrochloride (Sigma), hyoscine hydrochloride (Sigma), indomethacin (Sigma), potassium chloride (Hopkin and Williams), procaine hydrochloride (Sigma), prostaglandin E_2 (Sigma), tetraethylammonium bromide (Sigma). Stock solutions of acetylcholine and indomethacin were prepared in absolute ethanol, those of other drugs in twice-distilled water. The stock solution of prostaglandin E_2 was renewed daily.

The Krebs solution used in the majority of experiments contained 2.8 μM indomethacin and had the following composition (mM): Na^+ 143.5, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 127.6, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11.1. In all experiments the Krebs solution was maintained at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2.

The significance of differences between means was assessed by use of either a one-tailed or two-tailed unpaired *t* test.

Results

Tissue bath studies of mechanical activity

Effects of spasmogens ACh (10 nM–10 mM), histamine (2–200 μM), prostaglandin E_2 (0.11–14.2 μM) and KCl (5–40 mM) each caused concentration-dependent tension development in trachealis treated with 2.8 μM indomethacin. Tissues were not challenged with maximally-effective concentrations of histamine or KCl, but concentration-effect curves obtained for these agents (Figure 1–4) suggested that their maximal effects exceeded 80% of the maximal effect for ACh. In contrast, prostaglandin E_2 generally evoked a maximum response which was less than 70% of the ACh maximum.

Time matched-control experiments (Figures 1–4) showed that the concentration-effect curves for ACh, histamine and KCl exhibited a small, and often progressive, rightward shift during the course of the experiment. For each of these agonists the fourth concentration-effect curve in the series lay between 0.25 and 0.5 $\log_{10}$ units to the right of the initial curve. In the case of prostaglandin E_2, successive concentration-effect curves exhibited slightly smaller maximal responses and were of shallower slope compared with the initial curve.

Effects of TEA and its interactions with spasmogens TEA (0.1 and 1 mM) caused litle or no change in tissue tone. However, 10 mM TEA always evoked tension development. Accordingly, spasmogen (ACh, histamine, KCl, prostaglandin E_2) concentration-effect curves constructed in the presence of 10 mM TEA commenced from a greater level of baseline tone.

When test tissues were compared with the corresponding time-matched controls, it was evident that TEA (0.1–10 mM) had little or no effect on the shape or position of log concentration-effect curves to ACh, histamine or KCl. However, TEA (1–10 mM) caused some depression of the log concentration-effect curves for prostaglandin E_2 (Figures 1 and 2).

In so far that TEA failed to cause leftward shifts of the log concentration-effect curves of the various spasmogens, no evidence was obtained that TEA could potentiate responses to ACh, histamine, KCl or prostaglandin E_2.

Effects of procaine and its interactions with spasmogens Procaine (0.1 and 1 mM) caused little or no change in tissue tone. Procaine 10 mM often caused a small reduction in basal tone.

When test tissues were compared with the corresponding time-matched controls, it was evident that procaine (0.1 and 1 mM) had little or no effect on the shape or position of log concentration-effect curves of ACh, histamine, KCl or prostaglandin E_2. In contrast 10 mM procaine markedly depressed the log concentration-effect curves for each of these spasmogens (Figures 3 and 4).

In so far that procaine failed to cause leftward shifts of the log concentration-effect curves for the various spasmogens, no evidence was obtained that procaine could potentiate responses to ACh, histamine, KCl or prostaglandin E_2.

Intracellular electrophysiological recording

The apparent resting membrane potential of trachealis cells bathed by Krebs solution containing 2.8 μM indomethacin was in the range −45 to −55 mV. Some indomethacin-treated cells were electrically quiescent but the majority (Figures 5 and 6) exhibited the spontaneous discharge of slow waves. The apparent resting membrane potential and electrical behaviour of the indomethacin-treated cells was therefore similar to those observed (Allen *et al.*, 1985b; present study) in trachealis bathed by drug-free Krebs solution. Studies of the effects of the spasmogens ACh and histamine alone, or their interactions with K^+-channel inhibitors, proved very difficult to perform. In a high proportion of cases electrode displacement from the impaled cell occurred before an adequate period of observation was achieved.

Irrespective of whether the tissue was treated with indomethacin (2.8 μM) or bathed by drug-free Krebs solution, ACh (1 mM) and histamine (200 μM) each evoked a spasm which was accompanied by depolarization. When the depolarization became sufficiently intense, slow wave activity was abolished. Subsequently the record of membrane potential often became noisy and contained rapid, hyperpolarizing transients (Figures 5 and 6). These effects of ACh and histamine were very similar to those previously observed in tissue not exposed to indomethacin (Ahmed *et al.*, 1984). In control tissues the ACh- or histamine-induced tension and hyperpolarizing transients were both observed to be well-maintained during a period of 8–12 min exposure to the spasmogen.

When TEA (10 mM) was added to indomethacin-treated test tissues at a time when ACh was inducing

the hyperpolarizing transients, futher depolarization occurred and the transients ceased or were markedly reduced in amplitude or frequency (Figure 5a). TEA was never observed to increase the rate at which the tissue developed tension in the presence of ACh. The small increase in tension evident in Figure 5a after TEA administration was attributable to the fact that the ACh-induced tension rise was still slowly develop-

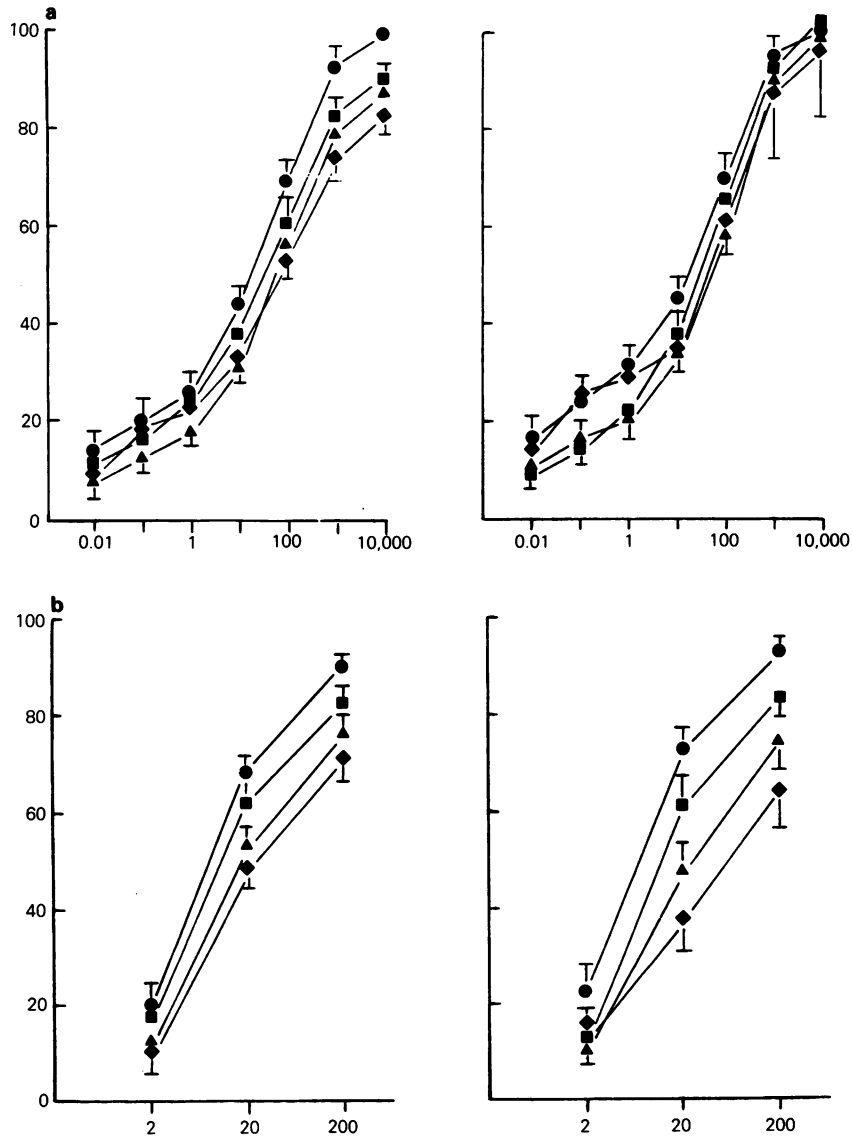

Figure 1 Indomethacin (2.8 μM)-treated, guinea-pig isolated trachealis: the effects of tetraethylammonium (TEA, 0.1–10 mM) on spasmogenic responses to acetylcholine (ACh, a) and histamine (b). The abscissae represent the concentrations of ACh or histamine on a log scale. The ordinates represent spasm as a % of the maximal spasm evoked by ACh. Right hand panels represent responses to the relevant spasmogen before (●) and after tissue equilibration with TEA 0.1 mM (■), 1 mM (▲) and 10 mM (◆). Left hand panels represent time-matched control-tissues not exposed to TEA. Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines.

ing at the time when TEA was added. Addition of procaine (5 mM) during the action of ACh also suppressed the hyperpolarising transients. This action of procaine was accompained by little further depolarization or tension development (Figure 5b).

Similar results were obtained when histamine (200 μM) was used as the spasmogen. TEA (10 mM) induced further depolarization, suppressed the transients but did not induce further tension development. Procaine (5 mM) suppressed the histamine-induced

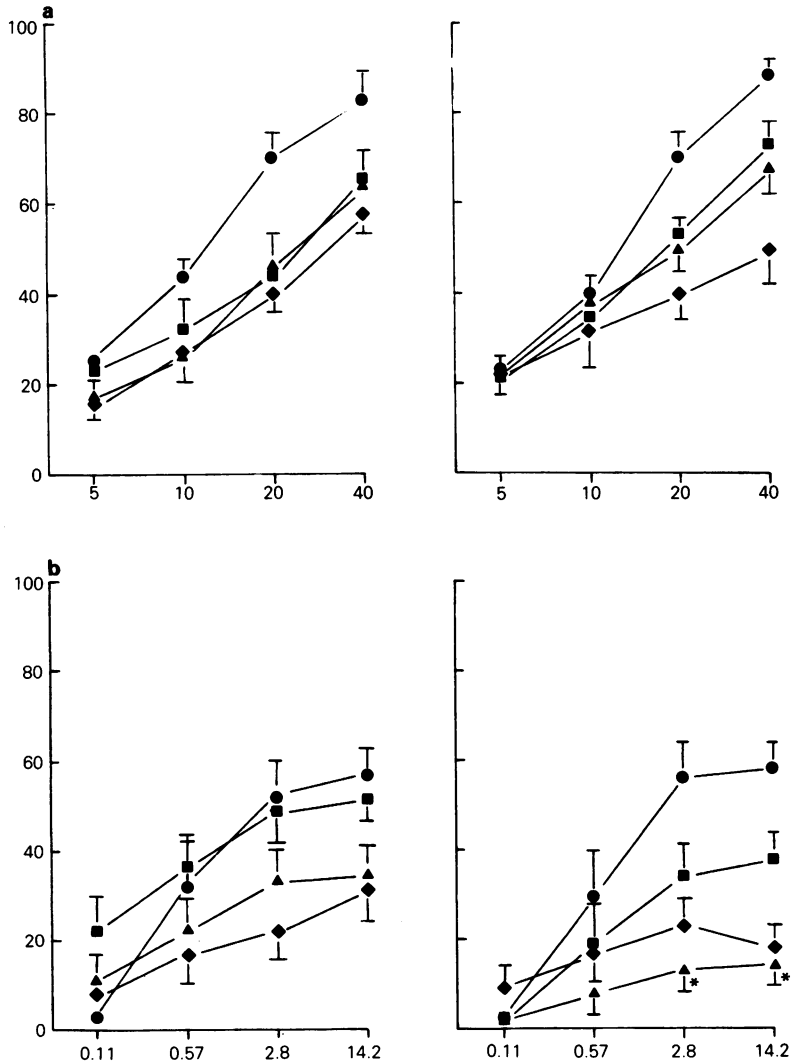

Figure 2 Indomethacin (2.8 μM)-treated, guinea-pig isolated trachealis: the effects of tetraethylammonium (TEA, 0.1–10 mM) on spasmogenic responses to KCl (a) and prostaglandin E_2 (b). The abscissae represent the concentrations of KCl or prostaglandin E_2 on a log scale. The ordinates represent spasm as a % of the maximal spasm evoked by acetylcholine (ACh). Right hand panels represent responses to the relevant spasmogen before (●) and after tissue equilibration with TEA 0.1 mM (■), 1 mM (▲) and 10 mM (◆). Left hand panels represent time-matched control tissues not exposed to TEA. Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines. *Indicates a significant ($P < 0.05$) decrease compared with the corresponding point in the time-matched control tissues.

transients without causing further depolarization or tension development.

Similar spasmogen-K^+-channel inhibitor interactins were observed in tissues not exposed to indomethacin (Figure 6). TEA again differed from procaine in that it evoked depolarization over and above that caused by the spasmogen alone (Table 1).

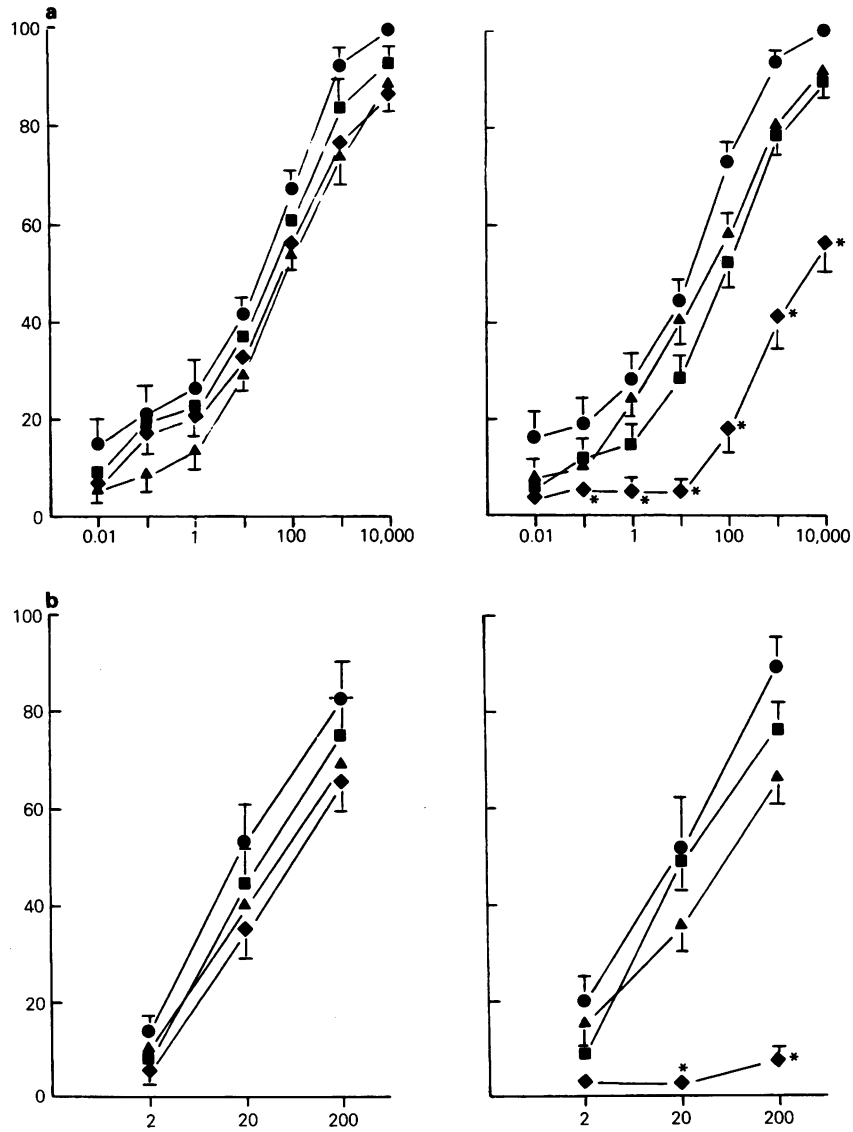

Figure 3 Indomethacin (2.8 μM)-treated, guinea-pig isolated trachealis: the effects of procaine (0.1–10 mM) on spasmogenic responses to acetylcholine (ACh, a) and histamine (b). The abscissae represent the concentrations of ACh or histamine on a log scale. The ordinates represent spasm as a % of the maximal spasm evoked by ACh. Right hand panels represent responses to the relevant spasmogen before (●) and after tissue equilibration with procaine 0.1 mM (■), 1 mM (▲) and 10 mM (◆). Left hand panels represent time-matched control tissues not exposed to procaine. Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines. *Indicates a significant ($P < 0.05$) decrease compared with the corresponding point in the time-matched control tissues.

Discussion

Slow wave discharge and prostaglandin synthesis

Indomethacin (2.8 μM) was added to the bathing medium in the tissue bath experiments to minimize the spontaneous tone of the trachealis segments and hence to minimize changes in spasmogen concentration-effect curves which may otherwise have resulted from tone changes occuring during the experimental period. Some of the electrophysiological experiments were carried out in normal Krebs solution so that direct

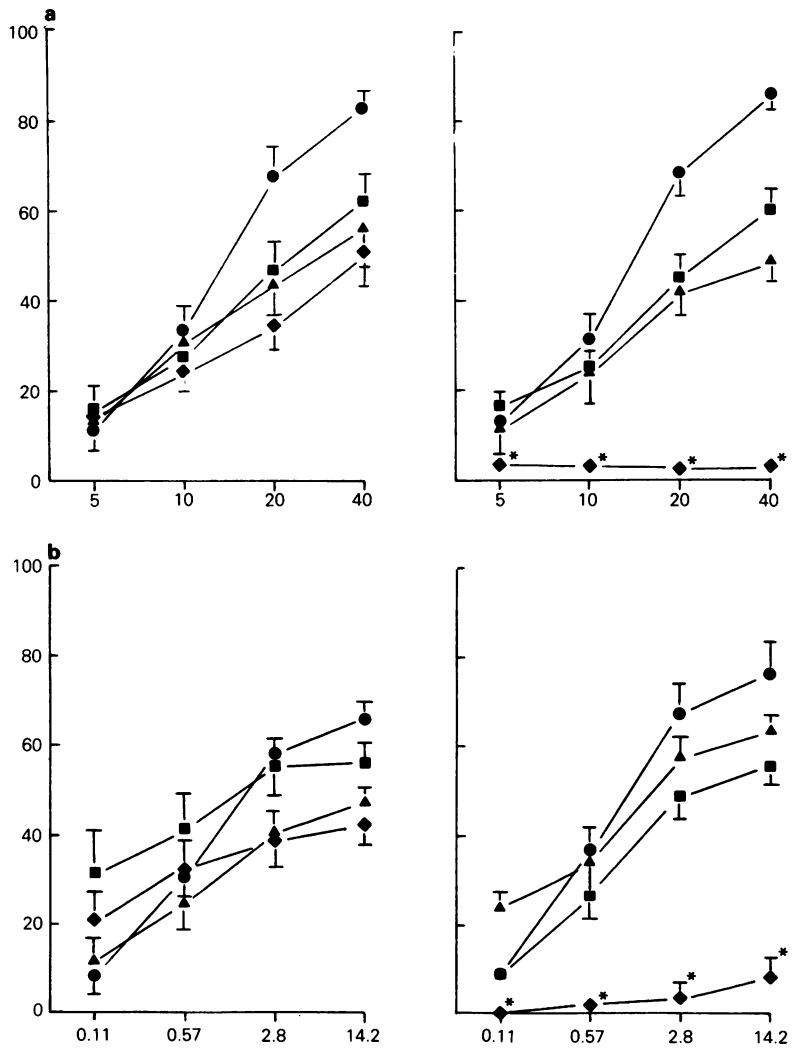

Figure 4 Indomethacin (2.8 μM)-treated, guinea-pig isolated trachealis: the effects of procaine (0.1–10 mM) on spasmogenic responses to KCl (a) and prostaglandin E_2 (b). The abscissae represent the concentrations of KCl or prostaglandin E_2 on a log scale. The ordinates represent spasm as a % of the maximal spasm evoked by acetylcholine. Right hand panels represent responses to the relevant spasmogen before (●) and after tissue equilibration with procaine 0.1 mM (■), 1 mM (▲) and 10 mM (◆). Left hand panels represent time-matched control tissues not exposed to procaine. Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines. *Indicates a significant ($P < 0.05$) decrease compared with the corresponding point in the time-matched control tissues.

comparison could be made with the results of Ahmed *et al.* (1984). The remaining electrophysiological experiments were carried out in the presence of 2.8 μM indomethacin for comparison with the present tissue bath studies.

No evidence was obtained that indomethacin modified either the spontaneous electrical behaviour of the tissue or the electrical responses of the tissue to ACh or histamine. Moreover the present failure of indomethacin to suppress spontaneous electrical slow waves is consistent with the findings of McCaig & Rodger (1986) who reported that slow wave discharge persisted in the presence of concentrations of flurbiprofen which reduced mechanical tone.

On the basis that the spontaneous mechanical tone of guinea-pig trachealis can be suppressed either by cyclo-oxygenase inhibitors or by prostaglandin antagonists, Farmer *et al.* (1974) proposed that prostaglandin synthesis by the tissue was responsible for the maintenance of mechanical tone. Since indometh-

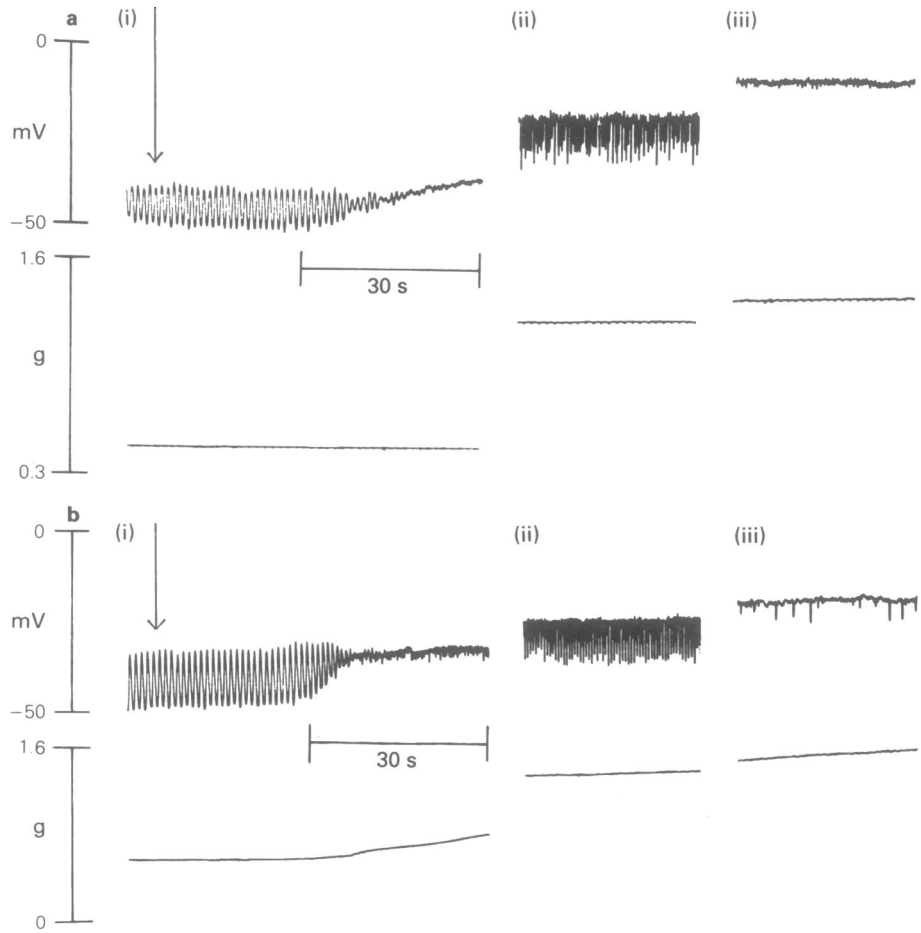

Figure 5 The effects of acetylcholine (ACh, 1 mM) on the electrical and mechanical activity of guinea-pig isolated trachealis and their modification by 10 mM tetraethylammonium (TEA, a) and 5 mM procaine (b). Indomethacin (2.8 μM) was present throughout. In each panel the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. In both (a) and (b) all electrical recordings are taken from the same cell. (i) Onset of action of ACh added at arrow; note depolarization and abolition of spontaneous slow waves. (ii) Four min after ACh addition; note spasm, membrane noise and rapid, hyperpolarizing transients. (iii) Four min after addition of 10 mM TEA (a) or 5 mM procaine (b); note reduced membrane noise and suppression of rapid, hyperpolarizing transients in each case.

acin (present study) and flurbiprofen (McCaig & Rodger, 1986) both suppressed mechanical tone without inhibiting slow wave discharge, it can therefore be suggested that slow wave discharge does not depend on prostaglandin synthesis. It more probably represents the intrinsic behaviour of the smooth muscle cell membrane.

Analysis of the membrane noise and hyperpolarizing transients induced by ACh and histamine

In an earlier study (Ahmed *et al.*, 1984) we suggested that the membrane noise and hyperpolarizing transients evoked in guinea-pig trachealis by ACh and histamine were not artefacts caused by blockade of the microelectrode tip or by displacement of the electrode from the impaled cell. This suggestion was prompted largely by our observation that, on spasmogen washout, the electrical noise and hyperpolarizing transients were dissipated, the impaled cell subsequently repolarized and slow wave discharge recommenced. The present finding that procaine and TEA were each able markedly to reduce the ACh- and histamine-induced noise and hyperpolarizing transients strength-

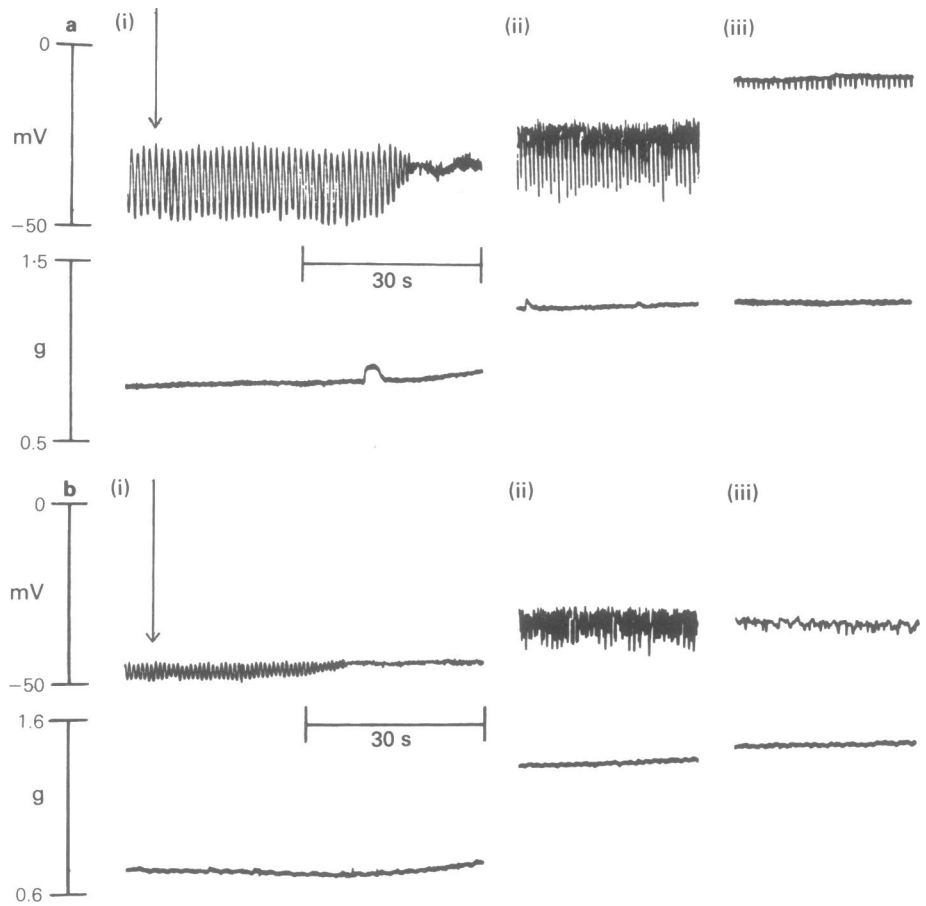

Figure 6 The effects of histamine (200 μM) on the electrical and mechanical activity of guinea-pig isolated trachealis and their modification by 10 mM tetraethylammonium (TEA, a) and 5 mM procaine (b). The illustrated experiments were conducted in indomethacin-free Krebs solution. In each panel the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. In both (a) and (b) all electrical recordings are taken from the same cell. (i) Onset of action of histamine added at arrow; note spasm, depolarization and abolition of spontaneous slow waves. (ii) Three (a) or 4 (b) min after histamine addition; note membrane noise and rapid hyperpolarizing transients. (iii) Seven min after addition of 10 mM TEA (a) or 5 min after addition of 5 mM procaine (b); note reduced membrane noise and suppression of hyperpolarizing transients.

Table 1 Membrane potential changes evoked by addition of K^+-channel inhibitors to guinea-pig trachealis exposed to acetylcholine (ACh) and histamine

Spasmogen	*K^+-channel inhibitor*	*Additional depolarisation evoked by K^+-channel inhibitor*
ACh 1 mM	TEA 10 mM	16 ± 1 mV
ACh 1 mM	Procaine 5 mM	1 ± 1 mV
Histamine 200 μM	TEA 10 mM	15 ± 1.4 mV
Histamine 200 μM	Procaine 5 mM	0.5 ± 0.5 mV

Data were collected both from tissues exposed to 2.8 μM indomethacin and from tissues not exposed to indomethacin, and represent mean ± s.e.mean of values from at least 4 tissues.

ens our conviction that these phenomena do not represent blockade or displacement of the electrode tip. Accordingly we must assume that these events represent biological responses to the actions of ACh and histamine.

What evidence have we adduced concerning the suggestion (Benham & Bolton, 1986) that the ACh- and histamine-induced noise and hyperpolarizing transients represent the opening of membrane K^+-channels? In trachealis muscle various relaxant drugs (e.g. isoprenaline, nicorandil, BRL34915) cause hyperpolarization attributable to K^+-channel opening. Such hyperpolarization can be suppressed by concentrations of procaine in the range 1–10 mM (Inoue *et al.*, 1983; Allen *et al.*, 1985a; Allen *et al.*, 1986a,b). Our present observation that procaine can suppress the ACh- and histamine-induced noise is therefore consistent with the hypothesis (Benham & Bolton, 1986) that such noise represents K^+-channel opening. However, considerations of the selectivity of the action of procaine to some extent weaken its usefulness as an analytical tool.

The present tissue bath experiments showed that, in concentrations greater than 1 mM, procaine was able to suppress concentration-effect curves of ACh, histamine, KCl and prostaglandin E_2. There is evidence (Foster *et al.*, 1984; Small & Foster, 1986; 1988) that these various spasmogens cause trachealis contraction by differing mechanisms. For example, KCl may evoke spasm principally by causing Ca^{2+} influx through voltage operated channels, while the remaining spasmogens may act principally by releasing Ca^{2+} from intracellular sites of sequestration – a process depending on their activation of specific receptors in the plasma membrane. It therefore seems likely that procaine (10 mM) interferes with spasmogen action by exerting non-specific effects at the level of the cell membrane or beyond. Hence it would be unwise to propose that procaine, as used in the present experiments, acted as a specific inhibitor of membrane K^+-channels. In this respect TEA may have proved a better analytical tool.

It has been known for more than a decade that the membrane of trachealis cells exhibits rectifying behaviour which prevents regenerative action potentials from arising spontaneously or in response to spasmogenic drugs. Several laboratories have demonstrated the ability of TEA to reduce this rectification and to allow action potential discharge in response to cathodal transmembrane current pulses (Kirkpatrick, 1975; Kroeger & Stephens, 1975; Suzuki *et al.*, 1976; Kannan *et al.*, 1983; Ito & Itoh, 1984). On the basis that TEA acts as a K^+-channel inhibitor, the rectifying behaviour of the trachealis cell membrane has been attributed to the activity of voltage-dependent K^+-channels. Recent single cell and patch clamp recordings from trachealis muscle (Klockner & Isenberg, 1985; McCann & Welsh, 1986) have confirmed the presence of such channels, their Ca^{2+}-dependency and their susceptibility to blockade by TEA.

In the present tissue bath experiments TEA (0.1–10 mM) had little or no effect on log concentration-effect curves for ACh, histamine or KCl. The depressant effects of 1–10 mM TEA against the concentration-effect curves of prostaglandin E_2 were probably a reflection of the size of the TEA-induced tone rise relative to the normal maximal effect of prostaglandin E_2, rather than the consequence of a specific pharmacological interaction between the two agents. Therefore the results of the present tissue bath experiments and the literature cited above collectively suggest that TEA provides selective inhibition of K^+-channel activity in the trachealis cell membrane. The ability of TEA (10 mM) to inhibit the membrane noise and hyperpolarizing transients induced by ACh and histamine, therefore, both supports and improves the evidence obtained using procaine that such phenomena represent the opening of membrane K^+-channels.

Mechanism of the K^+-channel opening by ACh and histamine; consequences of K^+-channel inhibition

There is substantial evidence that ACh and histamine cause contraction of guinea-pig trachealis principally by releasing Ca^{2+} from intracellular sites of sequestration (Small & Foster, 1986; 1988), inositol trisphosphate possibly acting as the messenger which links receptor occupancy to the release of Ca^{2+} from the intracellular stores (Klockner & Isenburg, 1985). The K^+-channels detected in trachealis muscle by single

cell or patch-clamp recording are Ca^{2+}-dependent (Klockner & Isenberg, 1985; McCann & Welsh, 1986) and we therefore suggest that the K^+-channel openings detected by our microelectrodes are triggered by Ca^{2+} provided by the process of receptor-operated intracellular Ca^{2+} release.

TEA, in a concentration observed to inhibit ACh- and histamine-induced K^+-channel opening (as indicated by suppression of the hyperpolarizing transients; Figures 5 and 6) also evoked additional depolarization (Table 1). Such electrical changes would be expected to increase the likelihood of the opening of plasmalemmal voltage-operated Ca^{2+} channels and hence to augment tension development. However, TEA caused no leftward shift in the log concentration-spasmogenic curves of ACh or histamine. We therefore presume that, relative to the role of Ca^{2+} released from intracellular sites of sequestration, Ca^{2+} influx through voltage-operated channels is functionally unimportant in determining the tension developed in response to several minutes exposure to ACh or histamine.

The failure of TEA to potentiate ACh- or histamine-induced responses also implies that the opening of TEA-sensitive K^+-channels does not limit the amount of tension induced by ACh or histamine. Attempts to relate K^+-channel dysfunction to hyperreactivity in airways smooth muscle (Allen *et al.*, 1986b) cannot therefore adduce potentiation of mediator action as an underlying mechanism. Nevertheless, K^+-channel inhibition by TEA raises airways smooth muscle tone and the possibility remains that K^+-channel dysfunction could lead to exaggerated spasm based on the additive effects of the raised muscle tone and the mediator action.

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