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Mechanical and electrical aspects of the relaxant action of aminophylline in guinea-pig isolated trachealis

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1 Aminophylline (1–1000 μmol l^{-1}) suppressed the spontaneous tone of guinea-pig isolated trachealis in a concentration-dependent manner.

2 In Krebs solution containing acetylcholine (1 mmol l^{-1}), histamine (200 μmol l^{-1}) or K^+ (120 mmol l^{-1}) isolated trachealis muscle developed near-maximal tension. The log concentration-effect curve for aminophylline was shifted 20 fold, 3 fold and 4 fold to the right, respectively, in the presence of these spasmogens.

3 Three K^+-channel inhibitors were tested: tetraethylammonium (TEA, 8 mmol l^{-1}) did not modify the action of aminophylline, procaine (5 mmol l^{-1}) shifted the log concentration-effect curve for aminophylline 2 fold to the left and 4-aminopyridine (5 mmol l^{-1}) shifted the curve 2.5 fold to the right.

4 Intracellular electrophysiological recording showed that aminophylline 10 μmol l^{-1} could cause relaxation in the absence of electrical changes. Higher concentrations of aminophylline (100–1000 μmol l^{-1}) suppressed spontaneous slow waves and hyperpolarized the trachealis cells.

5 In the presence of procaine (5 mmol l^{-1}) or TEA (8 mmol l^{-1}), the hyperpolarization induced by aminophylline (1000 μmol l^{-1}) was significantly reduced but its relaxant effect was unchanged.

6 In trachealis skinned of its plasma membranes, tension development induced by Ca^{2+} (20 μmol l^{-1}) was unaffected either by aminophylline (1000 μmol l^{-1}) or by isoprenaline (1 μmol l^{-1}).

7 In studies of the efflux of $^{86}Rb^+$ from muscle-rich strips of trachea, aminophylline (100–1000 μmol l^{-1}) was without effect whereas nicorandil (100 and 1000 μmol l^{-1}) increased the efflux rate constant.

8 It is concluded that aminophylline does not directly reduce the sensitivity of the contractile proteins to cytosolic Ca^{2+}. In low concentration (1–10 μmol l^{-1}) its relaxant action is not accompanied by membrane potential change but towards the upper end of its effective concentration range, aminophylline evokes hyperpolarization. This hyperpolarization may involve the opening of K^+-channels which are inhibited by procaine and (to a lesser extent) by TEA. These K^+-channels may be impermeable to $^{86}Rb^+$.

Introduction

The mechanisms that have been proposed to underlie the anti-asthmatic actions of methylxanthines have recently been reviewed by Barnes (1986). Antagonism of adenosine acting on irritant receptors, stimulation of catecholamine secretion by the adrenal medulla, inhibition of mediator release from mast cells and improved contractility of skeletal muscles involved in respiration have all been suggested to contribute to the clinical efficacy of the methylxanthines. However, concentrations of theophylline required to relax isolated airways smooth muscle (50–1000 μmol l^{-1}; Karlsson & Persson, 1981) compare favourably with the therapeutic plasma concentration range (50–100 μmol l^{-1}; Gebbie, 1983). It remains highly likely, therefore, that part of the action of the methylxanthines as anti-asthmatic agents involves their direct relaxation of airways smooth muscle.

It is commonly assumed that the methylxanthines relax airways smooth muscle by inhibiting intracellular phosphodiesterase. Inhibition of this enzyme is proposed to lead to the intracellular accumulation of adenosine 3':5'-cyclic monophosphate (cyclic AMP), which in turn initiates the sequence of biochemical changes that result in

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relaxation. However, it has been shown that concentrations of aminophylline or theophylline at the upper limit of the therapeutic plasma concentration range produce only 20–50% inhibition of the phosphodiesterase activity of human lung or the trachealis muscle of dogs, oxen or guinea-pigs (Lohmann *et al.*, 1977; Polson *et al.*, 1978, 1979; Fredholm *et al.*, 1979; Glass & Moore, 1979; Bergstrand, 1980). Since this degree of enzyme inhibition is functionally insignificant, some uncertainty exists as to the role of phosphodiesterase inhibition in mediating the smooth muscle relaxant effects of methylxanthines. Such uncertainty is increased by the observation that quaternary xanthinium derivatives which cannot penetrate cells, retain the ability to relax airways smooth muscle (Persson, 1985a,b). This observation may also suggest that the methylxanthines act at the external surface of the muscle cell membrane.

The present experiments were performed in an attempt to gain more insight into the mechanism of the muscle relaxant action of methylxanthines. To that end we have investigated some biochemical, electrophysiological and mechanical aspects of the action of aminophylline in guinea-pig isolated trachealis.

Methods

Guinea-pigs (350–700 g) of either sex were killed by stunning and bleeding. Tracheae were excised, cleaned of adhering fat and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis.

Tissue bath studies of mechanical activity of the trachealis

Small segments of trachea were set up for the isometric recording of tension changes as described by Foster *et al.* (1983). At the outset of each experiment, tissues were subjected to an imposed tension of 1 g. Approximately 20 min later aminophylline (1 mmol l^{-1}) was added in order to determine the recorder pen position at zero tone. The aminophylline was washed from the tissues and when tone subsequently became maximal, study of relaxant drugs started.

Relaxant drugs were studied by the construction of cumulative concentration-effect curves, concentration increments being made at intervals of 4 (isoprenaline) or 5 (aminophylline) min. Following the construction of initial log concentration-effect curves for the relaxant drugs, tissues were allocated randomly in equal numbers to test or time-matched control groups. Test tissues were treated with Krebs solution containing a modifying agent (e.g. acetylcholine, 4-aminopyridine, histamine, procaine or tetraethylammonium (TEA)) or with K^+-rich Krebs solution (see below). Modifying agents or the K^+-rich medium were allowed at least 10 min incubation with the tissue before log concentration-effect curves for the relaxant drugs were reconstructed. Time-matched control tissues were treated identically but were not exposed to the modifying agent or K^+-rich medium.

Experiments with trachealis skinned of its plasma membranes

Segments of trachea were prepared as described above and the trachealis muscle was skinned of its plasma membranes essentially as described by Sparrow *et al.* (1984). Tissue segments were immersed for 4 h in a skinning solution (see below) containing 1% v/v Triton X-100 and maintained at 4°C. After washing at 4°C for 15 min in the same solution but without Triton X-100, tissues were transferred to storage solution (see below) and maintained at −20°C for 10 days.

With an imposed tension of 0.5 g, segments of skinned trachea were set up for isometric recording of tension changes in 5 ml of relaxing solution (see below) maintained at 20°C. The relaxing solution did not contain added calmodulin. Tension development was induced by addition of $CaCl_2$ in an amount calculated to yield a free Ca^{2+} concentration of 20 μmol l^{-1}. When the tension became maximal (up to 25 min) it was subsequently dispelled by repeatedly washing with relaxing solution (up to 90 min).

In test tissues three such Ca^{2+} challenges were performed. Aminophylline (1000 μmol l^{-1}) or isoprenaline (1 μmol l^{-1}) was present for 5 or 4 min respectively before and throughout the second Ca^{2+} challenge. Acetylcholine (100 μmol l^{-1}) was added to the tissues once full relaxation was achieved after the third Ca^{2+} challenge. Control tissues were treated similarly, except that they were not exposed to aminophylline or isoprenaline.

Intracellular electrophysiological recording from trachealis

Simultaneous recording of intracellular electrical activity and mechanical changes of a contiguous segment of trachea was performed by the technique of Dixon & Small (1983).

The effects of aminophylline on spontaneous electrical and mechanical activity of the trachealis were studied as follows. After impalement of a trachealis cell 3 min were allowed to elapse to check that the record of electrical activity had stabilised. Aminophylline (10, 100 or 1000 μmol l^{-1}) was then added to the Krebs solution and its effects were monitored for 5 min. At the end of this period the drug was washed from the tissues and recovery of electrical and mechanical activity was monitored until the pre-aminophylline activity was regained or the microelectrode

became dislodged from the cell.

Similar procedures were adopted when assessing the electrical responses to aminophylline in tissues pretreated with procaine (5 mmol l^{-1}) or TEA (8 mmol l^{-1}).

Estimation of effects of relaxant agents on $^{86}Rb^+$ efflux

These experiments were performed on muscle-rich strips of trachea essentially as described by Allen *et al.* (1986). After pre-incubation at 37.5°C in 5 ml of Krebs solution bubbled with 95% O_2: 5% CO_2, all tissues were loaded with $^{86}Rb^+$ by incubation for 150 min with 185 MBq l^{-1} and 33 μmol l^{-1} $^{86}RbCl$ in Krebs solution. Each tissue was then transferred to the first of a series of 18 washing samples of 5 ml of Krebs solution at 37.5°C. Tissues remained in each washing sample for 4 min. In the case of test tissues the 10th and 11th washing samples contained either aminophylline (100 or 1000 μmol l^{-1}) or nicorandil (100 or 1000 μmol l^{-1}).

At the end of the efflux period, tissue digests and media were assayed for radioactivity and values of efflux rate constant were calculated as previously described (Allen *et al.*, 1986).

Drugs and solutions/statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. The following substances were used: acetylcholine chloride (Sigma), adenosine 5'-triphosphate (ATP disodium salt, Sigma), aminophylline (BDH), 4-aminopyridine (Sigma), dithioerythritol (DTE, BDH), ethyleneglycol-*bis*(β-amino-ethylether)-N,N'-tetraacetic acid (EGTA, Sigma), histamine acid phosphate (Sigma), imidazole (Sigma), (−)-isoprenaline hydrochloride (Sigma), nicorandil (Chugai, Japan), procaine hydrochloride (Sigma), sodium azide (BDH), tetraethylammonium bromide (Sigma), Triton X-100 (BDH).

Stock solutions of isoprenaline were prepared in 0.1 mol l^{-1} HCl, those of other agents in twice-distilled water. Dilutions of isoprenaline were prepared in distilled water containing 0.57 mmol l^{-1} ascorbic acid as an antioxidant.

The Krebs solution used in the majority of experiments had the following composition (mmol l^{-1}): Na^+ 143.5, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 125, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11.1. The K^+-rich Krebs solution was of identical osmolality to Krebs solution and had the following composition (mmol l^{-1}): Na^+ 26, K^+ 120, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 125, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11.1.

The solutions used in experiments with skinned trachea had the following compositions (mM): skinning solution: KCl 50, sucrose 150, DTE 0.5, EGTA 5, imidazole 20 (with or without Triton X-100 1% v/v); storage solution: $MgCl_2$ 10, ATP 7.5, sodium azide 1, DTE 0.5, EGTA 4, imidazole 20 and glycerol to 50%

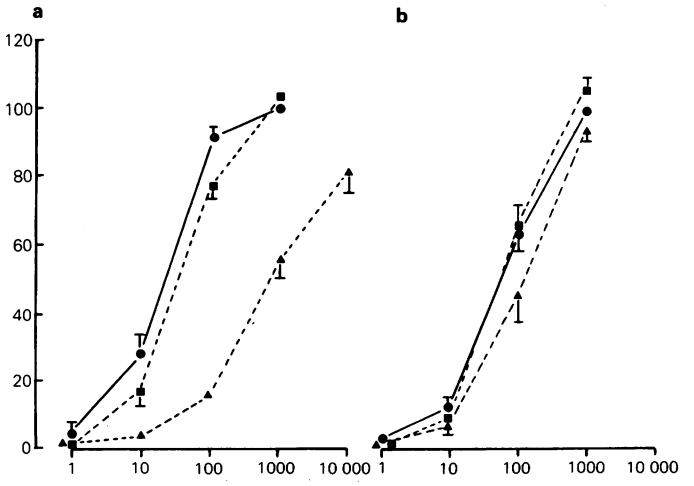

Figure 1 The effects of acetylcholine- or histamine-induced spasm on the relaxant action of aminophylline in guinea-pig isolated trachealis. Abscissa scale: concentration (μmol l^{-1}) of aminophylline on a log scale. Ordinate scale: relaxation as a % of the initial maximum. (●) = pooled initial log concentration-effect curve for test and control tissues; (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation in Krebs solution; (▲) = log concentration-effect curve constructed in test tissues after 10 min exposure to acetylcholine (1 mmol l^{-1}, a) or to histamine (200 μmol l^{-1}, b). Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines.

w/v; relaxing solution: $MgCl_2$ 10, ATP 7.5, KH_2PO_4 6, sodium azide 1, EGTA 4 and imidazole 20.

The pH of skinning, storage and relaxing solutions was adjusted to 7.4, 6.7 and 6.7 respectively with KOH.

The significance of differences between means was assessed by means of a one-tailed or a two-tailed unpaired *t* test. Larger groups were assessed by analysis of variance and the Studentized range test. A difference between means was assumed to be significant when $P < 0.05$.

Results

Tissue bath studies of mechanical activity

Effects of relaxant agents in tissues with spontaneous, acetylcholine- or histamine-induced tone Aminophylline (1–1000 μmol l^{-1}) caused concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis. The EC_{50} for aminophylline lay between 10 and 100 μmol l^{-1} and its log concentration-effect curve changed very little in shape or position when subsequently reconstructed in the same (control) tissues.

Acetylcholine (1 mmol l^{-1}) induced tonic, near-maximal and well-maintained spasm of guinea-pig trachealis. In the presence of acetylcholine, the log concentration-effect curve for aminophylline was shifted approximately 20 fold to the right and maximal relaxation was not achieved even at an aminophylline concentration of 10,000 μmol l^{-1} (Figure 1). The limited solubility of aminophylline prevented assessment of any reduction in maximum response.

Histamine (200 μmol l^{-1}) also caused near-maximal spasm of the trachea. In the presence of histamine, the log concentration-effect curve for aminophylline was shifted approximately 3 fold to the right but there was no indication that the maximal response to aminophylline had been reduced (Figure 1).

In a parallel series of experiments, isoprenaline (1–1000 μmol l^{-1}) was used as a relaxant both in the absence and presence of acetylcholine (1 mmol l^{-1}) or histamine (200 μmol l^{-1}). Acetylcholine again had the more profound effect against this relaxant, moving the log concentration-effect curve for isoprenaline approximately 100 fold to the right and depressing the maximal response. The log concentration-effect curve of isoprenaline was relatively little affected by histamine (Figure 2).

Effect of K^+-rich Krebs solution on aminophylline-induced relaxation As found previously (Allen *et al.*, 1985b; 1986) preparations of trachealis exposed to Krebs solution containing K^+ (120 mmol l^{-1}) developed tension which was tonic, near-maximal and well maintained. Aminophylline was able to relax the

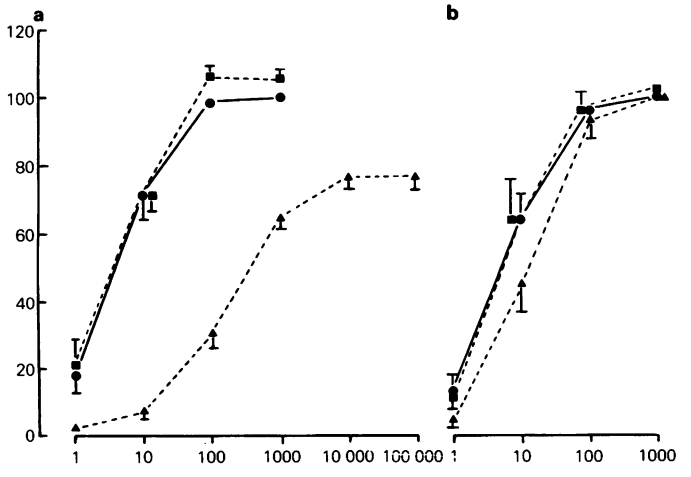

Figure 2 The effects of acetylcholine- or histamine-induced spasm on the relaxant action of isoprenaline in guinea-pig isolated trachealis. Abscissa scale: concentration (nmol l^{-1}) of isoprenaline on a log scale. Ordinate scale: relaxation as a % of the initial maximum. (●) = pooled initial log concentration-effect curve for test and control tissues; (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation in Krebs solution; (▲) = log concentration-effect curve constructed in test tissues after at least 10 min exposure to acetylcholine (1 mmol l^{-1}, a) or to histamine (200 μmol l^{-1}, b). Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines.

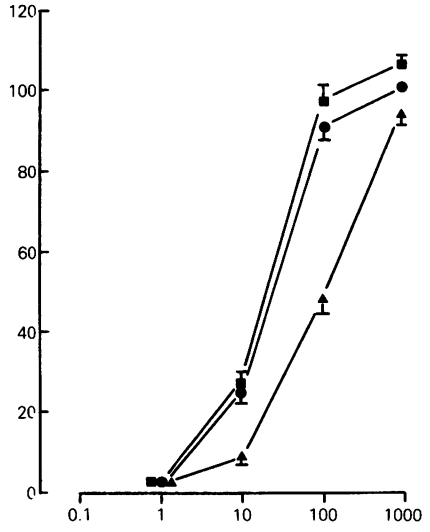

Figure 3 The effects of K^+-rich Krebs solution on the relaxant action of aminophylline in guinea-pig isolated trachealis. Abscissa scale: concentration (μmol l^{-1}) of aminophylline on a log scale. Ordinate scale: relaxation as a % of the initial maximum. (●) = pooled initial log concentration-effect curve for test and control tissues; (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation in Krebs solution; (▲) = log concentration-effect curve constructed in test tissues after 40 min exposure to K^+-rich (120 mmol l^{-1}) Krebs solution. Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines.

K^+-depolarized tissues but its log concentration-effect curve was shifted approximately 4 fold to the right. Relaxant responses to aminophylline took slightly longer to reach equilibrium in the K^+-rich medium. However, the original maximal response to aminophylline was closely approached (Figure 3).

Effects of K^+-channel inhibitors on the relaxant action of aminophylline TEA (8 mmol l^{-1}), procaine (5 mmol l^{-1}) and 4-aminopyridine (5 mmol l^{-1}) each evoked tracheal spasm. In the case of TEA and procaine the initial, tonic spasm often subsequently became phasic.

The relaxant action of aminophylline was unaffected by TEA. The other K^+-channel inhibitors had only minor effects against the action of aminophylline. In the presence of procaine the log concentration-effect curve for aminophylline was shifted 2.2 fold to the left but in the presence of 4-aminopyridine the curve was shifted 2.5 fold to the right (Figure 4). None of the K^+-channel inhibitors caused any change in the maximal response to aminophylline.

Intracellular electrophysiological recording

The tissue bath experiments described above showed that the threshold relaxant concentration of aminophylline was 1 μmol l^{-1}. This concentration of aminophylline was not tested in the electrophysiological experiments. However, at 10 μmol l^{-1} aminophylline caused relaxation without detectable change in the resting membrane potential of trachealis cells and

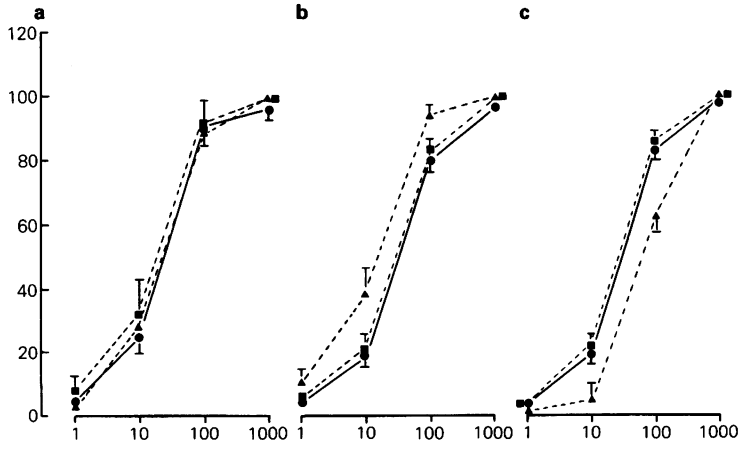

Figure 4 The effects of some K^+-channel inhibitors on the relaxant action of aminophylline in guinea-pig isolated trachealis. Abscissa scale: concentration (μmol l^{-1}) of aminophylline on a log scale. Ordinate scale: relaxation as a % of the initial maximum. (●) = pooled initial log concentration-effect curve for test and control tissues; (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation in Krebs solution; (▲) = log concentration-effect curve constructed in test tissues after at least 10 min exposure to tetraethylammonium (8 mmol l^{-1}, a) or to procaine (5 mmol l^{-1}, b) or 4-aminopyridine (5 mmol l^{-1}, c). Data indicate means of values from at least 6 tissues; s.e.mean shown by vertical lines.

without change in the discharge frequency or amplitude of spontaneous slow waves (Figure 5 and Table 1).

Clearly the curve relating log concentration of aminophylline to mechanical effects lies to the left of the corresponding curve for electrical effects, since electrical changes were first observed at an aminophylline concentration of 100 μmol l^{-1}. These electrical changes were manifest either as slow wave abolition or as a reduction in slow wave amplitude. Changes in resting membrane potential were small and variable in direction (Figure 5 and Table 1).

Aminophylline (1000 μmol l^{-1}) produced near-maximal relaxation, always abolished slow wave discharge and evoked marked hyperpolarization of the trachealis cells (Figure 6 and Table 1).

In tissues treated with TEA (8 mmol l^{-1}), trachealis muscle cells often generated large slow waves each with a superimposed spike potential. Sometimes these large slow waves and spikes were discharged continuously and sometimes in trains separated by periods during which only small slow waves were seen. Aminophylline (1000 μmol l^{-1}) was able to reduce TEA-induced mechanical activity, an action which was associated with reduced frequency or abolition (Figure 7a) of spike and slow wave activity. Complete abolition of oscillations of membrane potential often required more than 5 min exposure to aminophylline. Aminophylline also evoked hyperpolarization (Figure 7a) but the amplitude of the hyperpolarization was significantly smaller than that seen in tissues not exposed to TEA (Table 2).

Procaine (5 mmol l^{-1}) induced electrical and mechanical activity similar to that evoked by TEA except that spike and slow wave discharge occurred at a lower frequency (Figure 7b). Aminophylline (1000 μmol l^{-1}) suppressed procaine-induced mechanical activity and almost always abolished spike dis-

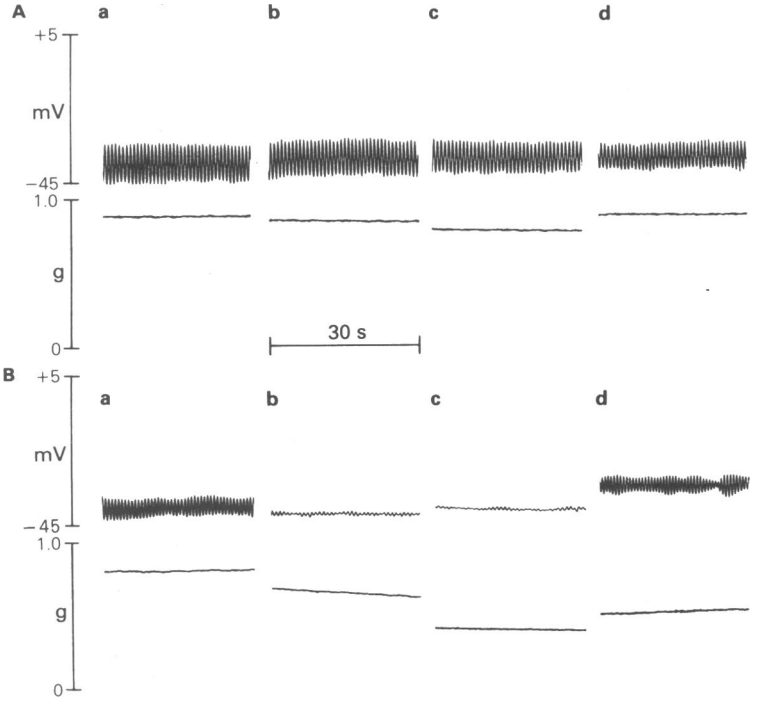

Figure 5 Effects of aminophylline (10 and 100 μmol l^{-1}) on the electrical and mechanical activity of guinea-pig isolated trachealis. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. In each row all electrical recordings are taken from the same cell. (A) Activity was recorded before (a) and 2.5 (b) and 5 (c) min after addition of aminophylline (10 μmol l^{-}). Panel (d) shows activity recorded 6 min after drug washout. Note the ability of aminophylline (10 μmol l^{-1}) to evoke relaxation with negligible change in electrical activity. (B) Activity was recorded before (a) and 2.5 (b) and 5 (c) min after addition of aminophylline (100 μmol l^{-1}). Panel (d) shows activity recorded 6 min after drug washout. Note the ability of aminophylline (100 μmol l^{-1}) to evoke relaxation accompanied by a reduction in slow wave amplitude and frequency.

Table 1 Effects of aminophylline on the spontaneous electrical and mechanical activity of guinea-pig isolated trachealis

	Properties of cells before exposure to aminophylline		*Measurements made 5 min after exposure to aminophylline*			
Aminophylline concentration (μmol l^{-1})	*Maximal amplitude of slow waves* (mV)	*Slow wave frequency* (Hz)	*Maximal amplitude of slow waves* (mV)	*Slow wave frequency* (Hz)	*Change in resting membrane potential* (mV)	*Change in mechanical tone* (mg)
10	9.5 ± 1.8	1.5 ± 0.1	8.2 ± 2.0	1.4 ± 0.1	−0.8 ± 0.8	−120 ± 29*
100	5.8 ± 1.4	1.5 ± 0.2	1.1 ± 0.5	0.7 ± 0.2	1.8 ± 2.1	−637 ± 138*
1000	6.2 ± 1.2	1.5 ± 0.1	0	0	23 ± 1.4*	−1188 ± 253*

Data indicate mean ± s.e.mean of observations from at least 6 cells. A positive change in membrane potential indicates hyperpolarization. A negative change in mechanical tone indicates relaxation. *indicates a significant ($P < 0.05$, two-tailed t test) change in membrane potential or mechanical tone.

charge within 5 min. In the presence of procaine, aminophylline caused only minor hyperpolarization (Figure 7b and Table 2).

Experiments with skinned trachea

Control experiments showed that skinned trachea responded to an initial Ca^{2+} (20 μmol l^{-1}) challenge by generating tension which reached a peak value (140–550 mg) within 25 min. Responses to subsequent Ca^{2+} challenges became progressively smaller (Figure 8). Acetylcholine (100 μmol l^{-1}) never evoked spasm of the skinned preparation.

When test tissues were compared with their appropriate time-matched controls it was evident that neither aminophylline (1 mmol l^{-1}) nor isoprenaline (1 μmol l^{-1}) reduced the spasm evoked by Ca^{2+} in skinned trachea.

$^{86}Rb^{+}$ efflux studies

After an initial very high value, the efflux rate constant quickly settled to a low and consistent value, as reported previously (Allen *et al.*, 1986). Test-tissues were exposed to nicorandil or aminophylline for the period between 36 and 44 min after the start of

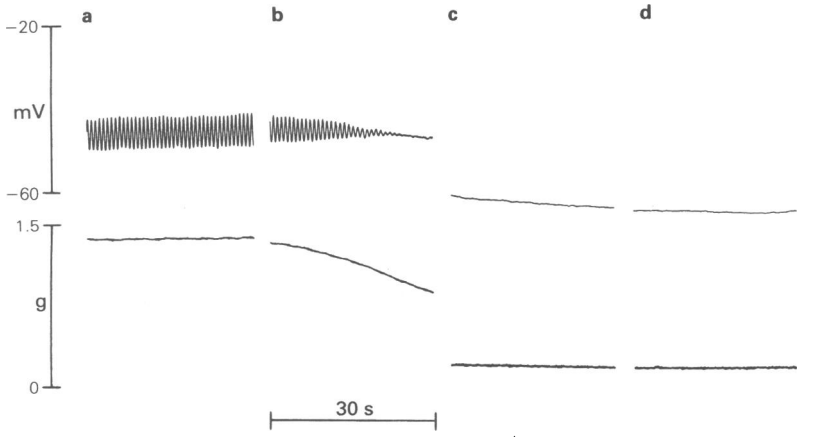

Figure 6 Effects of aminophylline (1000 μmol l^{-1}) on the electrical and mechanical activity of guinea-pig isolated trachealis. In each case the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. All electrical recordings are taken from the same cell. Activity was recorded before (a) and 1.5 (b) and 3 (c) and 4.5 (d) min after addition of aminophylline (1000 μmol l^{-1}). Note the abolition of slow waves and the marked hyperpolarization which accompany the aminophylline-induced relaxation.

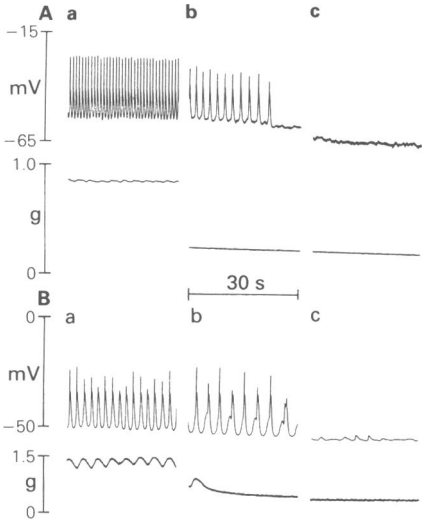

Figure 7 Effects of aminophylline (1000 μmol l^{-1}) on the electrical and mechanical activity of guinea-pig isolated trachealis treated with tetraethylammonium (TEA, 8 mmol l^{-1}) or procaine (5 mmol l^{-1}). In each case the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. In each row all electrical recordings are taken from the same cell. (A) Tissue treated with TEA (8 mmol l^{-1}). Activity was recorded before (a) and 3(b) and 5 (c) min after addition of aminophylline (1000 μmol l^{-1}). (B) Tissue treated with procaine (5 mmol l^{-1}). Activity was recorded before (a) and 2 (b) and 5 (c) min after addition of aminophylline (1000 μmol l^{-1}).

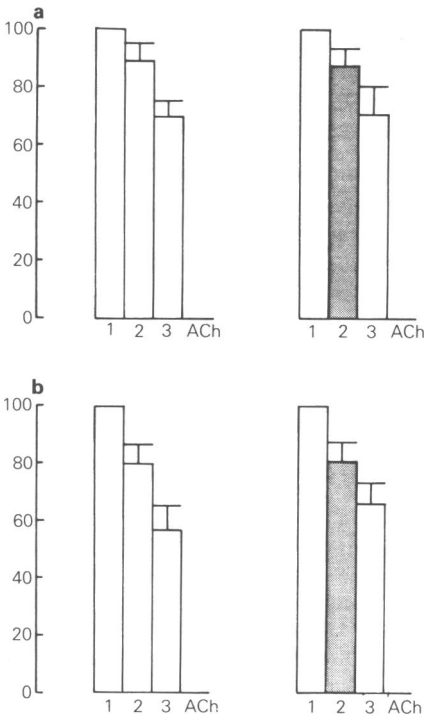

Figure 8 The effects of aminophylline and isoprenaline on responses of skinned trachealis to Ca^{2+}. The abscissa scales indicate the first, second and third (1,2,3) challenge with 20 μmol l^{-1} Ca^{2+} or with 100 μmol l^{-1} acetylcholine (ACh). Left hand panels: time-matched control tissues; right hand panels: test tissues. Shaded columns indicate the presence of aminophylline 1 mmol l^{-1} (a) or isoprenaline 1 μmol l^{-1} (b). Column heights represent the means of at least 6 experimental values and vertical lines indicate the s.e.mean. There was no significant difference ($P < 0.05$) between column 2 means either in (a) or (b) (two-tailed unpaired t test).

Table 2 The effects of tetraethylammonium (TEA) and procaine on the hyperpolarization of guinea-pig isolated trachealis induced by aminophylline (1000 μmol l^{-1})

Change in resting membrane potential (mV) 5 min after exposure to aminophylline (1000 μmol l^{-1})		
Cells bathed by normal Krebs solution	Cells treated with TEA (8 mmol l^{-1})	Cells treated with procaine (5 mmol l^{-1})
+ 23 ± 1.4	+ 15.0 ± 2.3*	+ 5.9 ± 2.4*

Data represent the means of at least six observations ± s.e.mean. A positive value indicates hyperpolarization. *indicates a significant ($P < 0.05$) change in the hyperpolarization compared with that seen in control cells bathed by normal Krebs solution (two-tailed unpaired t test).

efflux. Figure 9 shows that nicorandil (100 and 1000 μmol l^{-1}) caused enhancement of ^{86}Rb efflux. Concentration-dependency of this effect is indicated by the lower concentration of nicorandil producing significant promotion of efflux only at the second of the two time points corresponding with the period of drug contact.

In contrast, aminophylline (100 and 1000 μmol l^{-1}) failed to promote ^{86}Rb efflux (Figure 9) despite use in concentrations known to cause marked relaxation and hyperpolarization of the trachealis cells (Table 1).

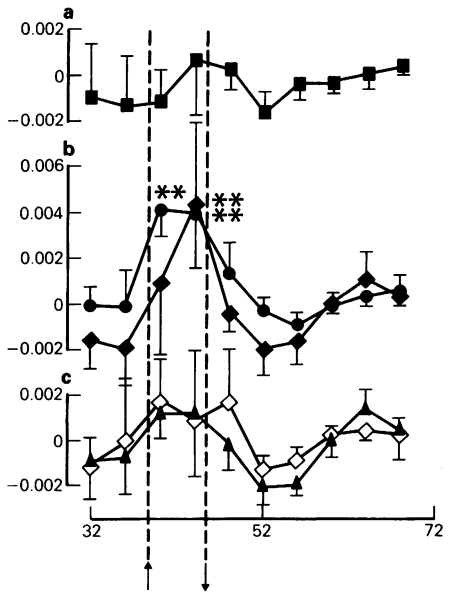

Figure 9 The effects of aminophylline and nicorandil on the efflux of ^{86}Rb from muscle-rich strips of guinea-pig isolated trachea. The abscissa scale indicates time (min) and the ordinates represent the relative efflux rate constant (min^{-1}). Drug (test tissues only) was present during the period between the arrows. (a) Time matched control tissues (■); (b) test tissues treated with nicorandil 100 $\mu mol\,l^{-1}$ (◆) or 1000 $\mu mol\,l^{-1}$ (●); (c) test tissues treated with aminophylline 100 $\mu mol\,l^{-1}$ (◇) or 1000 $\mu mol\,l^{-1}$ (▲). Data indicate means of values from 8 tissues: vertical bars indicate s.e.mean. **indicates a significant ($P<0.01$) difference from the corresponding point in the time-matched control tissues.

Discussion

Aminophylline-induced hyperpolarization and underlying mechanisms

We have recently reviewed and contributed to experimental evidence that the relaxant action of nicorandil on airways smooth muscle is accompanied by the opening of K^+-channels in the cell membrane. In guinea-pig trachealis for example (Allen *et al.*, 1986) such evidence includes the observation that nicorandil (10–1000 $\mu mol\,l^{-1}$) evokes concentration-dependent hyperpolarization and that nicorandil (1000 $\mu mol\,l^{-1}$) stimulates the efflux of $^{86}Rb^+$ from the tissue. The present results confirm the ability of nicorandil to stimulate $^{86}Rb^+$ efflux and demonstrate that this action is concentration-dependent and observable only at concentrations known (Allen *et al.*, 1986) to cause marked (> 15 mV) hyperpolarization.

The concentration-dependent hyperpolarization induced by aminophylline in the present study could result from stimulation of electrogenic ion exchange, a decrease of membrane Cl^- conductance or the opening of membrane K^+-channels. Since aminophylline was capable of increasing the membrane potential to a value approaching the calculated (−77 mV; Kirkpatrick, 1981) K^+-equilibrium potential of trachealis muscle, an increase in membrane K^+ conductance seems the most likely explanation of the hyperpolarization. The ability of the K^+-channel blocking agents procaine and TEA to reduce aminophylline-induced hyperpolarization, observed in the present experiments, is consistent with this suggestion.

The failure of aminophylline to promote $^{86}Rb^+$ efflux despite causing marked hyperpolarization suggests that, if aminophylline opens membrane K^+-channels, then such channels are different from those opened by nicorandil and are impermeable to $^{86}Rb^+$. In this respect, the action of aminophylline more closely resembles the action of isoprenaline than nicorandil (Allen *et al.*, 1985b, 1986).

Role of hyperpolarization in aminophylline-induced relaxation

In a K^+-rich (120 $mmol\,l^{-1}$) medium, the opening of trachealis K^+-channels cannot evoke cellular hyperpolarization because the K^+ equilibrium potential approaches the transmembrane potential. The relatively minor rightward shift of the aminophylline log concentration-effect curve seen in the K^+-rich medium contrasts with the large corresponding shift observed for isoprenaline (Allen *et al.*, 1985b) and suggests that the relaxant effects of aminophylline are largely independent of membrane potential changes. This conclusion is supported by two further observations made in the present study. In normal Krebs solution low concentrations of aminophylline (1–10 $\mu mol\,l^{-1}$) were observed to cause relaxation in the absence of membrane potential changes. Furthermore, the K^+-channel inhibitors TEA and procaine each significantly reduced aminophylline-induced hyperpolarization but did not reduce the relaxant action of aminophylline.

Aminophylline is therefore able to exert relaxant effects even in the absence of membrane hyperpolarization. An action of this kind in guinea-pig trachealis is shared both by isoprenaline (Allen *et al.*, 1985b) and by nicorandil (Allen *et al.*, 1986).

Fundamental mechanism of the inhibitory action of aminophylline

Working with feline trachea chemically skinned of its plasma membranes, Ito & Itoh (1984) used the failure of acetylcholine to evoke spasm as a criterion that their

skinning of trachealis muscle was complete. Since acetylcholine (100 μmol l^{-1}) never evoked spasm from our preparations of guinea-pig tissue we may assume that our skinning process, too, was functionally complete.

Ito & Itoh (1984) also showed that isoprenaline did not directly modify the sensitivity of the intracellular contractile machinery to Ca^{2+}. The present study has extended this observation with respect to isoprenaline acting on the guinea-pig trachea and has shown that aminophylline is similarly without effect. In the intact smooth muscle cell, however, it is possible that aminophylline (and other methylxanthines) could act on the plasma membrane and thereby reduce the Ca^{2+} sensitivity of the contractile machinery by an indirect mechanism. Alternatively we must presume that methylxanthines act to lower the cytosolic concentration of free Ca^{2+}.

If methylxanthines indeed lower the cytosolic concentration of Ca^{2+}, what can be deduced about such an action? In guinea-pig trachealis there is evidence that KCl and TEA evoke contraction by opening voltage-operated channels and hence permitting the influx of Ca^{2+} from the extracellular fluid. On the other hand, acetylcholine and histamine evoke spasm principally by releasing Ca^{2+} from intracellular sites of sequestration (Allen *et al.*, 1985a; Small & Foster, 1986). The ability of aminophylline and theophylline to suppress spasm induced by KCl, TEA, muscarinic agonists or histamine (Karlsson & Persson, 1981; present study) might therefore suggest that methylxanthines act both to close voltage-operated Ca^{2+} channels and to interrupt the linkage between receptor (muscarinic and histamine H_1) activation and intracellular Ca^{2+} release. A simpler explanation is that aminophylline and other methylxanthines act to reduce the cytoplasmic concentration of free Ca^{2+} by a single mechanism which does not discriminate between extra- or intracellular Ca^{2+} sources.

Whatever the precise mechanism of action may be, it seems to operate less effectively against spasm induced by muscarinic agonists than spasm induced by histamine, for the aminophylline log concentration-effect curve was moved further to the right by acetylcholine than by an approximately equispasmogenic concentration of histamine (Figure 1). Since similar results have been obtained when isoprenaline was used as the relaxant (Spilker & Minatoya, 1975; present study, Figure 2) this may imply that muscarinic agonists induce a greater rise in cytosolic free Ca^{2+} than histamine or that the two spasmogens release Ca^{2+} from intracellular stores by different mechanisms.

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