Br. J. Pharmac. (1985), **86**, 843–854

Electrophysiological and other aspects of the relaxant action of isoprenaline in guinea-pig isolated trachealis

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1 In guinea-pig isolated trachealis isoprenaline (0.001–0.1 μmol l^{-1}) caused concentration-dependent relaxation. Propranolol (1 μmol l^{-1}) antagonized the effects of isoprenaline by more than 100 fold but did not modify the relaxant action of sodium nitrite.

2 The tracheal relaxant actions of isoprenaline and ATP were unaffected by apamin (0.1 μmol l^{-1}) but apamin profoundly antagonized the effects of noradrenaline and ATP on guinea-pig isolated taenia caeci.

3 Tetraethylammonium (TEA; 8 mmol l^{-1}) and procaine (5 mmol l^{-1}) each evoked tracheal spasm but neither agent antagonized the isoprenaline-evoked relaxation of the trachealis.

4 Trachealis exposed to K^+-rich (120 mmol l^{-1}) Krebs solution developed near-maximal tension. Both isoprenaline and sodium nitrite relaxed the K^+-depolarized tissue though concentration-effect curves for both relaxants were moved to the right compared to those obtained in non-depolarized tissues. The maximal effect of sodium nitrite was markedly reduced.

5 Intracellular electrophysiological recording showed that isoprenaline (0.01–1 μmol l^{-1}) caused hyperpolarization and reduced or abolished slow wave discharge in trachealis muscle. These effects were accompanied by relaxation. Propranolol (1 μmol l^{-1}) virtually abolished both the electrical and mechanical responses to isoprenaline (0.1 μmol l^{-1}).

6 Apamin (0.1 μmol l^{-1}) did not alter the spontaneous electrical activity of trachealis cells or their electrical and mechanical responses to isoprenaline (0.1 μmol l^{-1}).

7 TEA (8 mmol l^{-1}) caused depolarization and often increased slow wave amplitude and induced spike discharge. Isoprenaline (0.01 μmol l^{-1}) failed to hyperpolarize TEA-treated trachealis cells. Higher concentrations of isoprenaline suppressed TEA-induced spasm, caused hyperpolarization and thereby increased slow wave or spike amplitude. Slow wave or spike frequency decreased as the hyperpolarization progressed but abolition of slow waves or spikes sometimes required more than 4 min exposure to isoprenaline.

8 Procaine (5 mmol l^{-1}) increased the amplitude of slow waves and induced spike discharge. Procaine markedly reduced the hyperpolarization induced by isoprenaline (0.1 and 1 μmol l^{-1}) but had little effect on isoprenaline-induced relaxation.

9 It is concluded that isoprenaline activates β-adrenoceptors in guinea-pig trachealis and thereby evokes relaxation and hyperpolarization of the smooth muscle. The hyperpolarization does not involve the opening of apamin-sensitive K^+-channels and it probably plays a supportive rather than a crucial role in the process by which isoprenaline-induced relaxation is achieved.

Introduction

In the trachealis of the ox and dog the inhibitory effects of adrenaline or isoprenaline are associated with hyperpolarization of the muscle cells (Suzuki *et al.*, 1976; Kirkpatrick, 1981; Ito & Tajima, 1982; Cameron *et al.*, 1983). This hyperpolarization seems to be mediated by β-adrenoceptors as it is suppressed by propranolol (Kirkpatrick, 1981; Ito & Tajima, 1982). It also seems to represent an increase in membrane conductance (presumably to K^+) because electrotonic potentials evoked by the passage of current pulses are reduced in amplitude during the hyperpolarization (Ito & Tajima, 1982; Cameron *et al.*, 1983).

Whatever the ionic basis of the hyperpolarization its role in the inhibitory process is unclear as isoprenaline

causes relaxation of canine trachealis bathed by a medium containing 120 mmol l^{-1} K^+ (Kumar, 1978). In this situation it is highly unlikely that isoprenaline can evoke hyperpolarization. It may therefore be that hyperpolarization does not play an essential role in the sequence of events by which β-adrenoceptor activation leads to relaxation of airways smooth muscle.

Guinea-pig trachealis has the advantage over bovine or canine trachealis in that it exhibits spontaneous tone *in vitro*. This facilitates studies of bronchodilator drugs. Using an extracellular recording technique, Small (1982) showed that the relaxant effects of isoprenaline in guinea-pig airways muscle were accompanied by reduced frequency of electrical slow waves or by slow wave abolition. However, the extracellular recording technique was unable to indicate a change in resting membrane potential.

The present experiments were carried out with several objectives in mind – to measure the membrane potential changes evoked by isoprenaline in guinea-pig trachealis, to investigate their underlying mechanism and their role in the inhibitory process.

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 the tone subsequently became maximal, study of the relaxant drugs commenced.

Relaxant drugs were studied by the construction of cumulative concentration-effect curves. Ten fold concentration increments were used, increments being made at intervals of 2.5 (adenosine 5′-triphosphate; ATP), 4 (isoprenaline), or 5 min (sodium nitrite). 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. apamin, procaine, propranolol or tetraethylammonium; TEA) or with K^+-rich Krebs solution (see below). Modifying agents or the K^+-rich medium were allowed at least 10 min preincubation with the tissue before the 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.

Tissue bath recording of the mechanical activity of taenia caeci

Segments of taenia caeci (2–3 cm long) were set up for isotonic recording of mechanical activity under a load of 1 g. The relaxant effects of ATP (0.1–10,000 μmol l^{-1}) and noradrenaline (0.01–10 μmol l^{-1}) were studied by constructing cumulative concentration-effect curves. Ten fold increments in agonist concentration were made at 45 s (ATP) and 1 min (noradrenaline) intervals.

In test tissues the effects of ATP and noradrenaline were studied in the absence and presence (5 min preincubation) of apamin (0.1 μmol l^{-1}). Control tissues were not exposed to apamin and were used concurrently with test preparations.

Intracellular electrophysiological recording from trachealis

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

The effects of isoprenaline 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 stabilized. Isoprenaline (0.01, 0.1 or 1 μmol l^{-1}) was then added to the Krebs solution. The effects of isoprenaline were monitored for 4 min. At the end of this period the drug was washed from the tissue and recovery of electrical and mechanical activity was monitored until the pre-isoprenaline activity was regained or the microelectrode became dislodged from the cell.

Similar procedures were adopted when assessing the electrical responses to isoprenaline in tissues pretreated with apamin (0.1 μmol l^{-1}), procaine (5 mmol l^{-1}), propranolol (1 μmol l^{-1}) or TEA (8 mmol l^{-1}). In a few instances it proved possible to maintain an impalement long enough to measure the effects of one concentration of isoprenaline in a single cell both before and after tissue exposure to a modifying agent.

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: the disodium salt of ATP

(Sigma), apamin (Sigma), (−)-isoprenaline hydrochloride (Sigma), (−)-noradrenaline bitartrate (Sigma), procaine hydrochloride (Sigma), (±)-propranolol hydrochloride (ICI), sodium nitrite (Hopkin & Williams), tetraethylammonium bromide (Sigma).

Stock solutions of isoprenaline and noradrenaline were prepared in 0.1 mol l^{-1} HCl, those of other agents in twice-distilled water. Stock solutions of apamin were kept frozen at −20°C until ready for use. Dilutions of catecholamines were prepared using 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 significance of differences between means was assessed using either a one-tailed or a two-tailed unpaired *t* test.

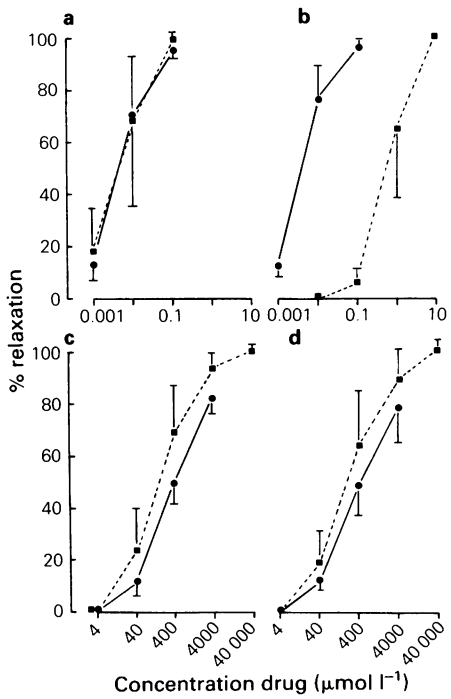

Figure 1 The effects of propranolol (1 μmol l^{-1}) on the relaxant actions of isoprenaline and sodium nitrite in guinea-pig isolated trachealis. The abscissae represent the concentrations (μmol l^{-1}) of isoprenaline (a and b) or sodium nitrite (c and d) on a log scale. The ordinates represent relaxation as a % of the maximal response to isoprenaline. Concentration-effect curves are shown for isoprenaline in control (a) and test tissues (b) and for sodium nitrite in control (c) and test tissues (d). (●) Initial log concentration-effect curve; (■) log concentration-effect curve obtained after 15 min incubation with vehicle (control tissues) or 1 μmol l^{-1} propranolol (test tissues). Data indicate the means of values from 8 tissues; s.e.mean shown by vertical lines.

Results

Tissue bath studies of mechanical activity

Antagonism of isoprenaline by propranolol Isoprenaline (0.001–0.1 μmol l^{-1}) and sodium nitrite (4–4000 μmol l^{-1}) each caused concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis. The use of time-matched control tissues showed that the log concentration-effect curve for sodium nitrite moved slightly to the left when reconstructed after tissue incubation with vehicle. The curve for isoprenaline showed no change. In the test tissues propranolol (1 μmol l^{-1}) caused no change in tone but caused more than 100 fold antagonism of isoprenaline. Propranolol did not modify the action of sodium nitrite; the log concentration-effect curve moved slightly to the left as observed in the control tissues (Figure 1).

Effects of K^+-channel inhibitors Apamin (0.1 μmol l^{-1}) caused little or no change in tracheal tone, nor did it significantly modify the relaxant actions of isoprenaline or ATP on the trachealis (Figure 2). In a single tracheal preparation the effects of apamin 1 μmol l^{-1} were examined but even at this concentration it was devoid of effect on tracheal tone or the relaxant action of ATP. As a check that our sample of apamin was able to cause K^+-channel blockade, its effects on the isolated taenia caeci were examined. Noradrenaline (0.01–10 μmol l^{-1}) and ATP (0.1 – 10,000 μmol l^{-1}) both caused concentration-dependent relaxation of the taenia. Apamin (0.1 μmol l^{-1}) was spasmogenic and caused more than 100 fold antagonism of the effects of both these relaxant agents (Table 1).

TEA (8 mmol l^{-1}) and procaine (5 mmol l^{-1}) both evoked spasm of the trachealis. The spasm was tonic initially but subsequently it often assumed a phasic pattern. Concentration-effect curves for isoprenaline were reconstructed in the presence of TEA or procaine before the onset of phasic activity. Neither TEA nor procaine caused any antagonism of the relaxant action of isoprenaline (Figure 3).

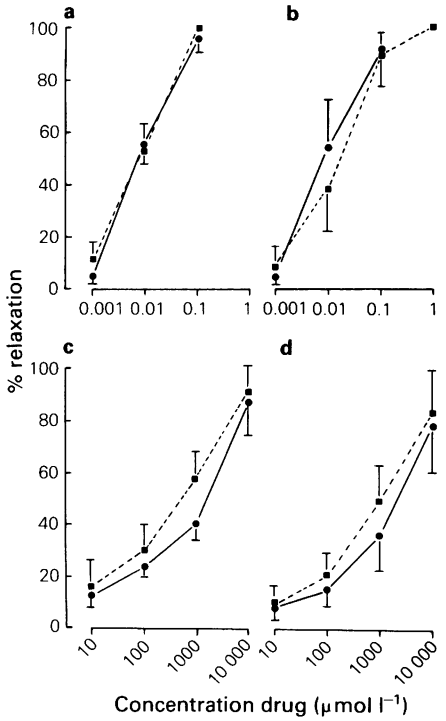

Figure 2 The effects of apamin (0.1 μmol l^{-1}) on the relaxant actions of isoprenaline and ATP in guinea-pig isolated trachealis. The abscissae represent the concentration (μmol l^{-1}) of isoprenaline or ATP on a log scale. The ordinates represent relaxation as a % of the maximal response to isoprenaline. Concentration-effect curves are shown for isoprenaline in control (a) and test tissues (b) and for ATP in control (c) and test tissues (d). (●) Initial log concentration-effect curve; (■) log concentration-effect curve obtained after a 20 min incubation with vehicle (control tissues) or with 0.1 μmol l^{-1} apamin (test tissues). Data indicate the means of values from 6 tissues; s.e.mean shown by vertical lines.

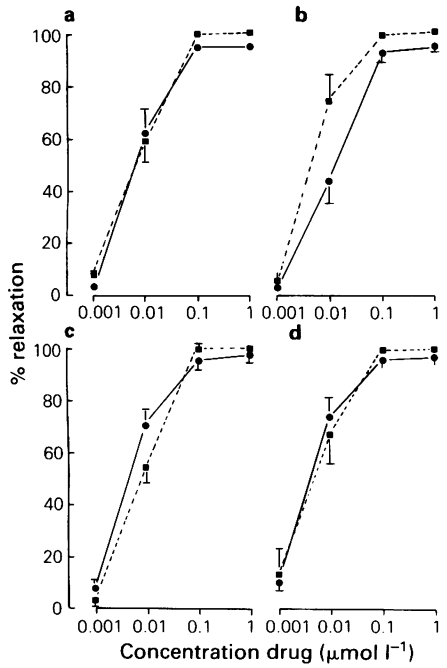

Figure 3 The effects of tetraethylammonium (TEA; 8 mmol l^{-1}) and procaine (5 mmol l^{-1}) on the relaxant action of isoprenaline in guinea-pig isolated trachealis. The abscissae represent the concentration of isoprenaline (μmol l^{-1}) on a log scale. The ordinates represent relaxation as a % of the maximal response to isoprenaline. Isoprenaline concentration-effect curves are shown for control (a) and TEA test tissues (b) and for control (c) and procaine test tissues (d). (●) Initial log concentration-effect curve; (■) log concentration-effect curve obtained after 10 min incubation with vehicle (control tissues) or with TEA 8 mmol l^{-1} or procaine 5 mmol l^{-1} (test tissues). Data indicate the means of values from 6 tissues; s.e.mean shown by vertical lines.

Effect of K^+-rich Krebs solution When test preparations of trachealis were exposed to Krebs solution containing K^+ 120 mmol l^{-1} they immediately began to develop tension. The peak tension achieved was always greater than the level of tone observed before constructing the initial log concentration-effect curves of the relaxant drugs. The spasm was tonic and well maintained.

Isoprenaline was still able to relax the K^+-depolarized tissues but its log concentration-effect curve was shifted approximately 100 fold to the right (Figure 4). Relaxant responses to isoprenaline in the K^+-depolarized tissues developed more slowly than those seen in normal Krebs solution and maximal relaxation was not generally achieved even with 100 μmol l^{-1} isoprenaline (Figure 4).

Sodium nitrite (4–4000 μmol l^{-1}) was also able to relax K^+-depolarized tissue. In the K^+-rich medium its concentration-effect curve was shifted to the right with a marked depression of the maximal response (Figure 4).

Intracellular electrophysiological recording

The effects of isoprenaline (0.01, 0.1 or 1 μmol l^{-1}) on spontaneous electrical activity were examined in at least six trachealis cells. Isoprenaline 0.01 μmol l^{-1} caused mild hyperpolarization. This was associated

Table 1 Guinea-pig isolated taenia caeci: antagonism of noradrenaline and ATP by apamin

	Mean $\log_{10}$ dose-ratio	
Agonist	*Time-matched control tissues*	*Tissues treated with apamin* ($0.1\ \mu mol\ l^{-1}$)
Noradrenaline	+ 0.25 ± 0.17	+ 2.86 ± 0.52
ATP	+ 0.35 ± 0.23	+ 2.42 ± 0.80

Data indicate mean values of $\log_{10}$ dose-ratio ± s.e.mean from 6 tissues. A positive value of mean $\log_{10}$ dose-ratio indicates a rightward shift of the agonist log concentration-effect curve.

with reduced frequency of spontaneous slow waves or, sometimes, their abolition. Relaxation of the contiguous segment of trachea also occurred (Figure 5). Higher concentrations of isoprenaline always abolished slow waves and caused more profound hyperpolarization and relaxation (Table 2 and Figures 6 and 7). These various effects of isoprenaline became maximal within the 4 min contact time.

In general the electrical and mechanical changes induced by isoprenaline were well correlated. However, relaxation sometimes preceded any detectable electrical change. This was not unexpected since the segment of tissue from which the mechanical activity was recorded was slightly upstream in the flow of Krebs solution compared with the region of electrical recording.

Provided that the microelectrode remained within the cell, recovery from the effects of isoprenaline was monitored during drug washout. Recovery from isoprenaline (0.1 or $1\ \mu mol\ l^{-1}$) was first indicated by the membrane potential commencing to fall back towards the control level. Slow wave activity generally recommenced before the control level of membrane potential was regained. Mechanical tone usually began to recover after the onset of slow wave activity (Figure 7). Sometimes recovery from the effects of isoprenaline was associated with the impaled cell adopting a lower (less negative) value of resting membrane potential than the pre-isoprenaline value (see Figures 5 and 6). This may have been a consequence of poor sealing of the microelectrode in the cell membrane rather than an effect attributable to isoprenaline.

Trachealis cells from tissues pretreated with propranolol ($1\ \mu mol\ l^{-1}$) exhibited spontaneous slow wave activity similar to that observed in untreated tissues. In the presence of propranolol isoprenaline ($0.1\ \mu mol\ l^{-1}$) caused no relaxation. The ability of isoprenaline to hyperpolarize the impaled cell was also virtually abolished (Table 2 and Figure 7).

The spontaneous electrical activity of trachealis cells was not altered by apamin ($0.1\ \mu mol\ l^{-1}$) nor did

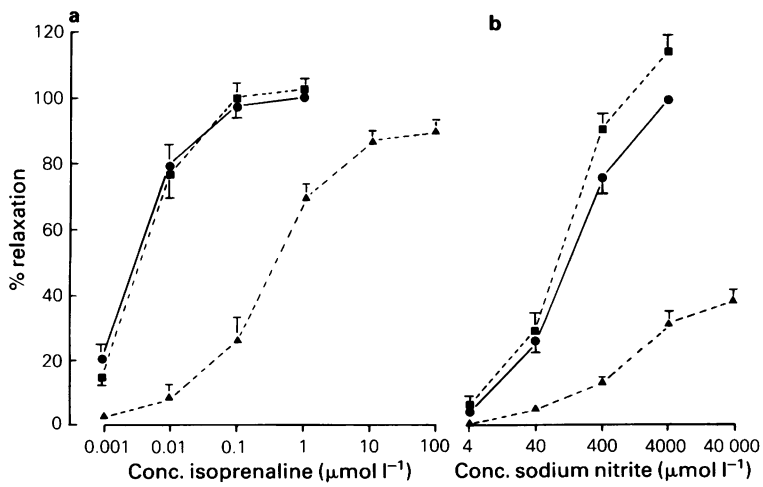

Figure 4 The effects of K^+-rich Krebs solution on the relaxant actions of isoprenaline and sodium nitrite in guinea-pig isolated trachealis. The abscissae indicate the concentration ($\mu mol\ l^{-1}$) of isoprenaline (a) or sodium nitrite (b) on a log scale. The ordinates represent relaxation as a % of the initial maximum. (●) Pooled initial log concentration-effect curves for test and control issues; (■) 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 6 tissues; s.e.mean shown by vertical lines.

Table 2 Effects of isoprenaline on the spontaneous electrical and mechanical activity of guinea-pig isolated trachealis

	Properties of cells before exposure to isoprenaline		*Measurements made 4 min after exposure to isoprenaline*			
Isoprenaline 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)
0.01	11.2 ± 1.8	1.4 ± 0.1	6.2 ± 2.5	0.7 ± 0.2	+ 4.1 ± 0.8*	− 151 ± 5.3*
0.1	9.4 ± 1.1	1.4 ± 0.1	0	0	+ 14.1 ± 1.9*	− 348 ± 7.7*
0.1†	10.9 ± 3.2	1.3 ± 0.1	10.1 ± 3.0	1.3 ± 0.1	− 1.8 ± 0.8	+ 42 ± 3.0*
1.0	6.7 ± 1.1	1.4 ± 0.1	0	0	+ 20.2 ± 2.7*	− 646 ± 1.62*

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 propranolol (1 μmol l^{-1}) present before and during isoprenaline challenge. * Indicates a significant ($P < 0.05$, two-tailed t test) change in membrane potential or mechanical tone.

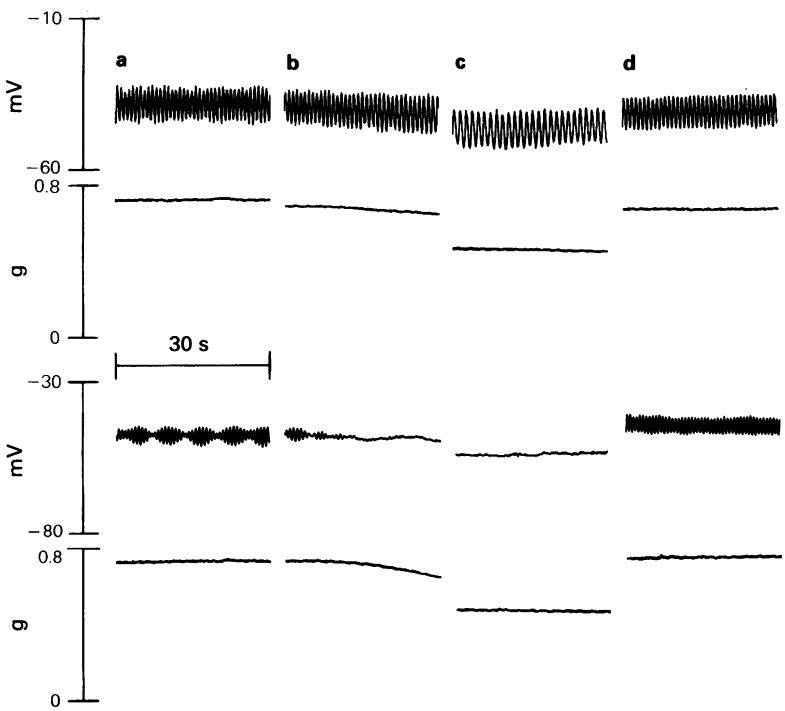

Figure 5 Effects of isoprenaline (0.01 μmol l^{-1}) on the electrical and mechanical properties of guinea-pig isolated trachealis. The upper and lower rows of the records indicate results obtained from two different cells. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. Activity was recorded (a) before (b) 1 and (c) 4 min after the addition of isoprenaline. Panel (d) shows activity recorded following washout of isoprenaline.

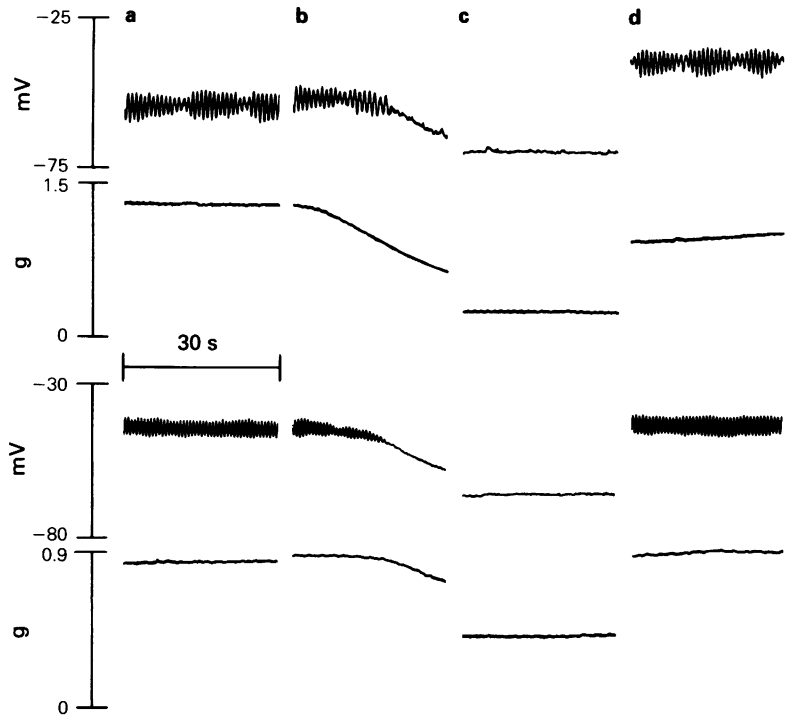

Figure 6 Effects of isoprenaline (0.1 μmol l^{-1}) on the electrical and mechanical properties of guinea-pig isolated trachealis. The upper and lower rows of the records indicate results obtained from two different cells. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. Activity was recorded (a) before (b) 1 and (c) 4 min after the addition of isoprenaline. Panel (d) shows activity recorded following washout of isoprenaline.

apamin evoke spasm. Apamin did not alter the electrical and mechanical responses of the tissue to isoprenaline (0.1 μmol l^{-1}).

In tissues treated with TEA (8 mmol l^{-1}) trachealis cells often discharged slow waves which were surmounted by a spike potential. Some TEA-treated cells discharged slow waves of low amplitude without superimposed spikes (Figure 8). Mechanical activity of the contiguous segment of trachea comprised tonic or phasic tension development as previously observed (Dixon & Small, 1983).

In TEA-treated tissues isoprenaline (0.01 μmol l^{-1}) caused no hyperpolarization and had no effect on slow wave or spike activity. In most preparations the occurrence of phasic tension waves made it difficult to determine whether there was any inhibitory effect against mechanical activity. This was particularly true when the interval between successive tension waves approached the isoprenaline contact time of 4 min.

Higher concentrations of isoprenaline (0.1 or 1 μmol l^{-1}) clearly suppressed TEA-induced spasm and caused hyperpolarization (Table 3). As the hyperpolarization developed the amplitude of slow waves

Table 3 The effects of tetraethylammonium (TEA) and procaine on the hyperpolarization of guinea-pig isolated trachealis induced by isoprenaline

	Change in resting membrane potential (mV) 4 min after exposure to isoprenaline		
Isoprenaline concentration (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})
0.01	+ 4.1 ± 0.8	− 1.1 ± 1.9*	—
0.1	+ 14.1 ± 1.9	+ 10.5 ± 2.1	+ 2.6 ± 1.9*
1.0	+ 20.2 ± 2.7	+ 20.4 ± 2.1	+ 3.7 ± 1.2*

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

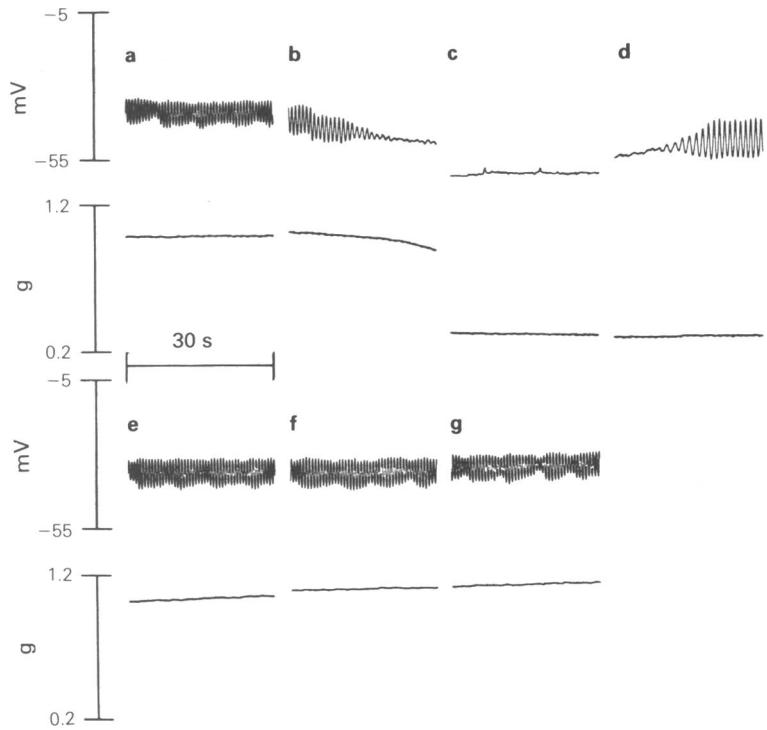

Figure 7 The effects of propranolol (1 μmol l^{-1}) on the electrical and mechanical responses of guinea-pig isolated trachealis to isoprenaline (0.1 μmol l^{-1}). In all records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. All electrical records are taken from the same cell. Activity was recorded (a) before (b) 1 and (c) 4 min after, the initial challenge with isoprenaline (0.1 μmol l^{-1}), (d) 2 and (e) 10 min after washout of isoprenaline using Krebs solution containing propranolol 1 μmol l^{-1}. Activity was also recorded (f) 1 and (g) 4 min after a second challenge with isoprenaline (0.1 μmol l^{-1}) in the presence of propranolol. Note the ability of propranolol to abolish both the electrical and mechanical responses to isoprenaline.

and spike potentials increased (Figure 8). In some cells isoprenaline (0.1 or 1 μmol l^{-1}) abolished slow wave or spike activity within the 4 min contact time (Figure 8). In other cells slow wave or spike frequency fell more slowly so that a longer exposure to isoprenaline was required to abolish the slow waves or spikes.

Procaine (5 mmol l^{-1}) induced electrical and mechanical activity similar to that evoked by TEA. In the presence of procaine the hyperpolarization induced by isoprenaline (0.1 and 1 μmol l^{-1}) was markedly reduced (Table 3 and Figure 9). Isoprenaline was still able to cause relaxation though the relaxation developed more slowly than in the absence of procaine. The time required for the suppression of slow waves or spikes was also prolonged.

Discussion

Involvement of β-adrenoceptors in isoprenaline-induced hyperpolarization

The present experiments have shown that the guinea-pig trachealis resembles that of the ox (Kirkpatrick, 1981; Cameron *et al.*, 1983) and dog (Suzuki *et al.*, 1976; Ito & Tajima, 1982) in that the inhibitory action of isoprenaline is accompanied by hyperpolarization. Since propranolol (1 μmol l^{-1}) provided selective antagonism of the relaxant effects of isoprenaline (Figure 1) and virtually abolished the hyperpolarization (Table 2 and Figure 7) there is little doubt that the hyperpolarization induced by isoprenaline in guinea-

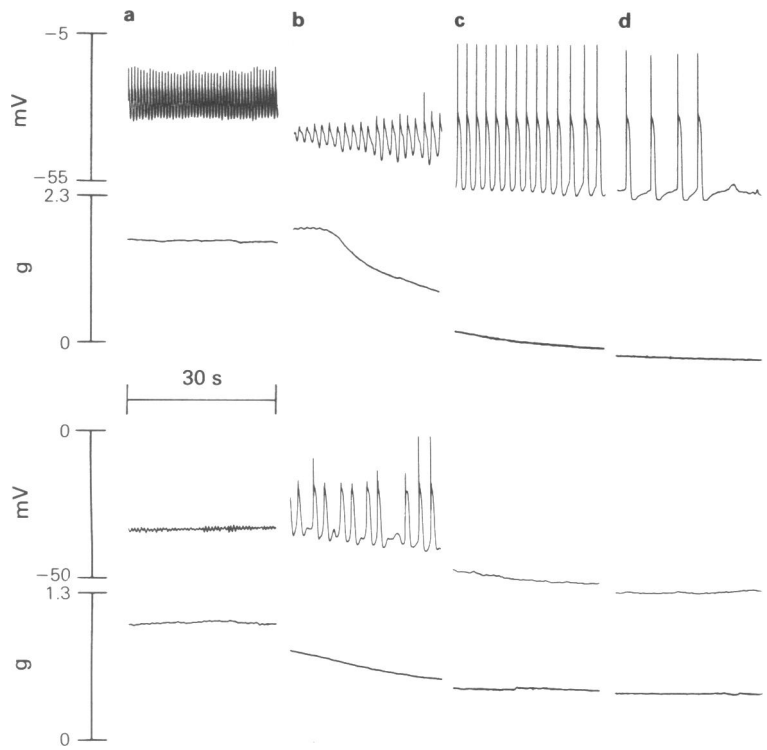

Figure 8 Effects of isoprenaline (1 μmol l^{-1}) on the electrical and mechanical activity of guinea-pig isolated trachealis treated with tetraethylammonium (TEA) 8 mmol l^{-1}. The upper and lower rows of records indicate results obtained from two different cells. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. Activity was recorded (a) before (b) 1.5 (c) 3 and (d) 4 min after, the addition of isoprenaline. Upper cell: note discharge of slow waves and spikelets at high frequency. As isoprenaline hyperpolarizes the cell, slow wave frequency declines but large spikes occur. When hyperpolarization and relaxation become maximal spike activity ceases. Lower cell: note discharge of very small slow waves. As isoprenaline hyperpolarizes the cell, slow wave frequency declines but large slow waves and spikes occur. As hyperpolarization and relaxation become maximal all oscillation of membrane potential ceases.

pig trachealis is a consequence of β-adrenoceptor activation. This confirms similar findings in studies of bovine and canine trachealis (Kirkpatrick, 1981; Ito & Tajima, 1982).

Isoprenaline-induced hyperpolarization: underlying mechanisms

Of the mechanisms by which isoprenaline might induce hyperpolarization of trachealis cells (increase in K^+ conductance, reduced Cl^- conductance or stimulation of electrogenic ion exchange), an increase in K^+ conductance is suggested by the reduced amplitude of electrotonic potentials observed in experiments with bovine or canine tissue (Ito & Tajima, 1982; Cameron *et al.*, 1983).

The ability of the K^+-channel inhibitors, TEA and procaine, to reduce isoprenaline-induced hyperpolarization (Table 3) is entirely consistent with this suggestion. Support is also gained from the fact that near-maximally effective concentrations of isoprenaline increased the resting membrane potential from its normal value of approximately − 50 mV (Allen *et al.*, 1985) to a value (− 70 mV) approaching the calculated (Kirkpatrick, 1981) K^+ equilibrium potential of trachealis muscle (− 77 mV).

Nature of K^+-channels opened by isoprenaline

The actions of apamin on smooth muscle have been

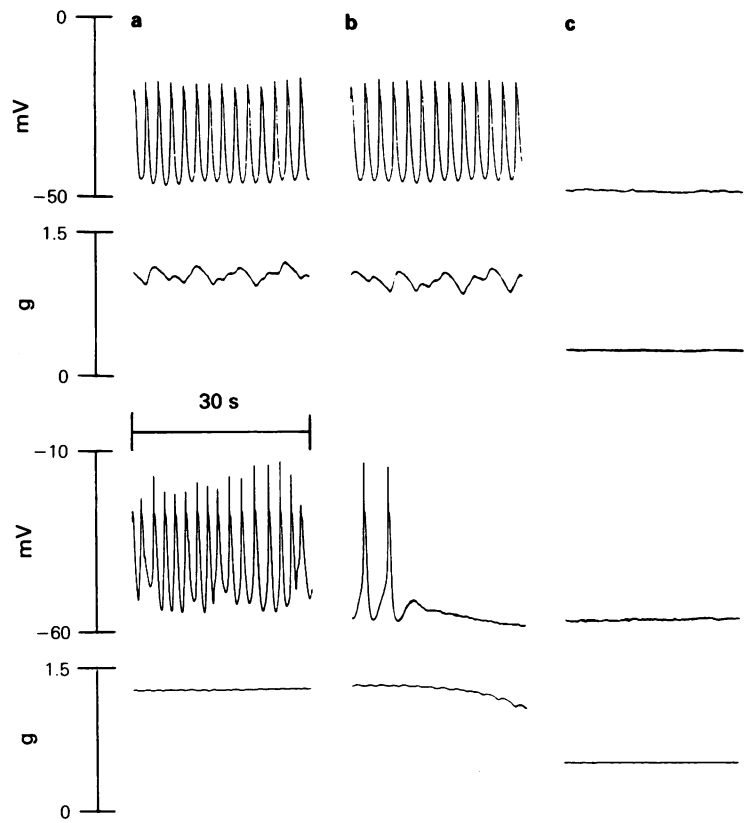

Figure 9 Effects of isoprenaline on the electrical and mechanical activity of guinea-pig isolated trachealis treated with procaine 5 mmol l^{-1}. The upper and lower rows of records indicate results obtained from two different cells. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. Activity was recorded (a) before (b) 1 and (c) 4 min after, the addition of isoprenaline 100 nmol l^{-1} (upper row) or 1 μmol l^{-1} (lower row). Note large slow waves and spike potentials induced by procaine. Note the mild hyperpolarization (compare with Figures 6 and 8) induced by isoprenaline in the presence of procaine.

reviewed by Jenkinson (1981). It seems clear that apamin antagonizes the actions of noradrenaline and ATP on guinea-pig taenia caeci by blocking K^+-channels. Our present confirmation of the effectiveness of apamin against noradrenaline and ATP in the taenia (Table 1) indicates that our sample of apamin had K^+-channel blocking activity. Hence the failure of apamin to cause spasm of the trachea or to reduce the electrical and mechanical effects of isoprenaline suggests that apamin-sensitive K^+-channels play little or no role in determining the level of tracheal tone or in the tracheal relaxation mediated by β-adrenoceptors.

Since procaine (5 mmol l^{-1}) was more effective than TEA (8 mmol l^{-1}) in antagonizing isoprenaline-induced hyperpolarization (Table 3) it could be that the K^+-channel opened by isoprenaline has a greater affinity for procaine than for TEA. However, trachealis muscle may contain more than one type of K^+-channel (Inoue *et al.*, 1983). In that event our ability to characterize the K^+-channel opened by isoprenaline is restricted by the possibility of activator (isoprenaline) and inhibitor (procaine or TEA) actions being mediated via different K^+-channels.

Role of hyperpolarization in isoprenaline-induced relaxation

We have observed (Figure 4) that isoprenaline can relax guinea-pig trachealis in a medium containing 120 mmol l^{-1} K^+. A similar observation was made by Kumar (1978) in bovine trachealis. These findings suggest that isoprenaline can relax airways smooth muscle by mechanisms not involving membrane hyperpolarization for, at such high external K^+ concen-

tration, an increase in membrane K^+ conductance has little effect on transmembrane potential (Inoue *et al.*, 1983).

The ability of "isoprenaline and other cyclic AMP-related relaxants" to inhibit K^+-depolarized smooth muscle led Kroeger (1983) to propose that membrane hyperpolarization might play a supportive rather than a crucial role in the inhibitory process. Our results with the K^+-depolarized trachea are consistent with this hypothesis but it should be noted that isoprenaline was more than 100 fold less potent in the K^+-rich medium (Figure 4).

This might imply that the isoprenaline-induced hyperpolarization seen in media of normal K^+ content acts to increase its inhibitory potency. However, TEA and procaine reduced the hyperpolarization induced by isoprenaline (Table 3) but did not modify its relaxant action (Figure 3). These findings argue against hyperpolarization acting to increase the inhibitory potency of isoprenaline.

Kumar (1978) observed that the relaxant effects of isoprenaline in depolarized bovine trachealis were dependent on the extracellular Ca^{2+} concentration. This suggests that the low potency of isoprenaline seen in the depolarizing medium results simply from a greater (K^+-stimulated) cellular influx of Ca^{2+}.

If cellular hyperpolarization plays any kind of supportive role in mediating the relaxation of guinea-pig trachealis induced by isoprenaline, in what way could this be achieved? The presently-observed ability of isoprenaline (0.01 μmol l^{-1}) to reduce the frequency of spontaneous slow waves and for higher concentrations of isoprenaline to abolish slow waves is in excellent agreement with the extracellular recordings of Small (1982). Since slow wave discharge is dependent on resting membrane potential (Kirkpatrick, 1981; Small & Foster, 1985) it is likely that slow wave suppression results from the hyperpolarization induced by isoprenaline.

Slow waves may represent nascent action potentials of trachealis muscle (Small & Foster, 1985) and it might therefore be proposed that trachealis relaxation induced by isoprenaline results from the reduced frequency or abolition of slow waves. However, organic inhibitors of Ca^{2+} influx (e.g. nifedipine and verapamil) can abolish slow waves without lowering the spontaneous tone of guinea-pig trachealis (Foster *et al.*, 1984; Ahmed *et al.*, 1985; Allen *et al.*, 1985; Small & Foster, 1985). Since the link between slow wave discharge and the maintenance of spontaneous tone is so tenuous, isoprenaline-induced slow wave suppression cannot be envisaged as playing a major role in the relaxation which follows β-adrenoceptor activation.

In summary, isoprenaline causes hyperpolarization and slow wave suppression in guinea-pig isolated trachealis. These electrical effects are mediated by β-adrenoceptors and possibly involve increased membrane conductance to K^+. The membrane potential changes evoked by isoprenaline do not result from the opening of apamin-sensitive K^+ channels and probably do not play a major role in the chain of events by which relaxation is achieved.

The financial support of the Asthma Research Council, the Mason Medical Research Foundation, the North Western Regional Health Authority and the Smith Kline (1982) Foundation is gratefully acknowledged. We thank Miss D. Gray for expert technical assistance. We thank Mrs V. Sillavan and Mrs J. Stafford for typing the manuscript.

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(Received June 25, 1985.
Accepted August 21, 1985.)