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Inhibitory responses to nicotine and transmural stimulation in hyoscine-treated guinea-pig isolated trachealis : an electrical and mechanical study

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1 Guinea-pig isolated trachealis muscle treated with hyoscine (1 μM) exhibited mechanical tone which could be suppressed by transmural stimulation and, in a concentration-dependent manner, by nicotine (10–1000 μM).

2 Hexamethonium (500 μM) did not itself cause tone changes, antagonized effects of nicotine but did not antagonize those of isoprenaline.

3 Tetrodotoxin (0.3 μM) did not itself cause tone changes, did not modify the action of isoprenaline but antagonized the effects of nicotine and very markedly reduced responses to transmural electrical stimulation.

4 Guanethidine (50 μM) did not itself cause tone changes, potentiated the action of isoprenaline, antagonized effects of nicotine and reduced responses to transmural electrical stimulation.

5 Propranolol (1 μM) did not itself cause tone changes, antagonized effects of both isoprenaline and nicotine and reduced responses to transmural electrical stimulation. Propranolol (10 μM) caused greater antagonism of isoprenaline but did not further antagonize nicotine or further reduce responses to electrical stimulation.

6 Intracellular electrophysiological recording from hyoscine-treated trachealis showed that 10 μM nicotine caused little or no mechanical or electrical change. Higher concentrations (100 μM and 1 mM) evoked relaxation which was often though not invariably accompanied by transient hyperpolarization and transient inhibition of electrical slow waves in the impaled cell.

7 Hexamethonium (500 μM), tetrodotoxin (0.3 μM), guanethidine (50 μM) and propranolol (1 μM) each suppressed the electrical or mechanical changes evoked by nicotine (100 μM). However, nicotine (1 mM) tested in the presence of propranolol (1 μM), caused relaxation which could be accompanied by slow wave suppression but not by change in resting membrane potential.

8 Transmural stimulation of hyoscine-treated trachea with single pulses of supramaximal voltage and 0.5 ms duration evoked neither relaxation nor membrane potential changes. Stimulation with similar pulses in trains of 5 s duration evoked relaxation which was dependent on pulse frequency. In many cells this relaxation was not accompanied by membrane potential change. In other cells suppression of slow waves occurred. At high pulse frequencies (> 16 Hz) this was generally accompanied by membrane hyperpolarization.

9 In tissue treated with hyoscine and propranolol (both 1 μM), transmural stimulation with pulse trains as decribed above always evoked relaxation but no membrane potential changes were observed.

10 It is concluded that nicotine and transmural stimulation can excite intramural noradrenergic nerves in guinea-pig trachea and thereby evoke relaxation. The membrane potential changes (slow wave suppression and hyperpolarization) are similar to those evoked by the administration of agonists at β-adrenoceptors. Nicotine and transmural stimulation also excite non-adrenergic non-cholinergic inhibitory (NANCI) nerves. The relaxation evoked by the NANCI neurotransmitter is accompanied by little, if any, membrane potential change.

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Introduction

In guinea-pig trachealis, fluorescence histochemistry has demonstrated the presence of adrenergic neurones which run parallel to the longitudinal axis of the muscle bundles. Their fluorescence is increased by pretreating animals with the monoamine oxidase inhibitor nialamide, and is lost following pretreatment with reserpine. The adrenergic neurones are more numerous at the cervical than the thoracic end of the trachea (Coburn & Tomita, 1973; O'Donnell & Saar, 1973).

These findings are confirmed by electron microscope studies which have revealed axons and axon terminals containing a predominance of small granular vesicles running between and within the trachealis muscle bundles. Such axons are presumed adrenergic and their incidence relative to other axonal types is greater in cervical than thoracic tissue (Hoyes & Barber, 1980; Jones *et al.*, 1980).

Tissue bath studies involving transmural stimulation (Coburn & Tomita, 1973; Coleman & Levy, 1974) or stimulation of extrinsic nerves supplying the trachea (McCaig, 1986a) have confirmed the existence of a functional noradrenergic inhibitory innervation to guinea-pig trachealis but have also revealed a non-adrenergic non-cholinergic inhibitory (NANCI) innervation.

In the present study we have attempted to determine the relative roles of noradrenergic and NANCI neurones in mediating inhibitory responses of the trachea to nicotine and to examine the membrane potential changes associated with both noradrenergic and NANCI neuroeffector transmission.

Methods

Guinea-pigs (350–700 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 in Krebs solution containing hyoscine (1 μM) for the isometric recording of tension changes essentially as described by Foster *et al.* (1983). However, in many experiments, the tissues were mounted between a pair of stainless steel wire electrodes (interelectrode distance 1 cm). This enabled the transmural electrical stimulation of tracheal nerves.

At the outset of each experiment tissues were subjected to imposed tension of 1 g. Approximately 20 min later, aminophylline (1 mM) 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 the relaxant drugs or the effects of transmural stimulation commenced.

The relaxant action of isoprenaline was studied by the construction of cumulative concentration-effect curves, ten fold concentration increments being made at 4 min intervals. Nicotine was studied by the construction of sequential concentration-effect curves using ten fold concentration increments. Each concentration was allowed 4 min contact before being washed from the tissue. The tissue was then challenged with the EC_{50} of isoprenaline and washed again before being exposed to the next concentration of nicotine. This procedure helped to minimize the development of tachyphylaxis to nicotine.

Transmural stimulation was with diphasic, 0.5 ms pulses of supramaximal voltage. Cumulative frequency-response curves were obtained by doubling the pulse frequency in steps from an initial 1 Hz up to 32 Hz. An increment in pulse frequency was made when it was judged that the response to a given frequency had equilibrated.

Following the construction of initial log concentration-effect curves and log frequency-response curves, 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. guanethidine, hexamethonium, propranolol, tetrodotoxin). Modifying agents were allowed at least 20 min preincubation with test tissues before the log concentration-effect curves for relaxant drugs or log frequency-response curves were reconstructed. Time-matched control tissues were treated identically but were not exposed to the modifying agent.

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 muscle 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. All experiments were carried out in Krebs solution containing hyoscine (1 μM) and, in those experiments where transmural stimulation of tracheal nerves was performed, the tissue holder was modified to carry a pair of platinum wire electrodes approximately 1 cm apart. A needle-like extension of the stimulating cathode was used to ensure activation of the tracheal nerves near (< 3 mm) the recording

microelectrode.

The effects of nicotine on spontaneous activity were studied as follows. After impalement of a trachealis cell, several minutes were allowed to elapse to check the stability of the record of electrical activity. Nicotine (10, 100 or 1000 μM) was then added to the Krebs solution. The effects of nicotine 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-nicotine activity was regained or the microelectrode became dislodged from the cell. Similar procedures were adopted when assessing responses to nicotine (100 or 1000 μM) in tissues pretreated (for at least 20 min) with hexamethonium (500 μM), tetrodotoxin (0.3 μM), guanethidine (50 μM) or propranolol (1 μM).

In experiments involving transmural stimulation of tracheal nerves, 0.5 ms pulses of supramaximal voltage were used. Pulses were delivered singly or in trains of 5 s duration. The pulse frequency within the initial train was 4 Hz. Thereafter pulse frequency was doubled in successive trains to a maximum of 64 Hz. The interval between successive trains was determined by the time required for recovery from the mechanical response to the previous pulse train.

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: guanethidine sulphate (Ciba), hexamethonium bromide (Sigma), hyoscine hydrochloride (Sigma), (−)-isoprenaline hydrochloride (Sigma), nicotine hydrogen tartrate (BDH), propranolol hydrochloride (ICI), tetrodotoxin (Sigma).

Stock solutions of isoprenaline were prepared in 0.1 M HCl, those of other agents in twice-distilled water. Dilutions of isoprenaline were prepared using distilled water containing 0.57 mM ascorbic acid as an antioxidant.

The Krebs solution used in all the experiments contained 1 μM hyoscine 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.

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

Results

Tissue bath studies of mechanical activity

Effects of hexamethonium on actions of isoprenaline and nicotine Isoprenaline (0.001–1.0 μM) and nicotine (10–1000 μM) each caused concentration-dependent suppression of the spontaneous tone of hyoscine-treated guinea-pig isolated trachealis. Nicotine (1000 μM) evoked relaxation which closely approached the maximal relaxation evoked by isoprenaline. While relaxant responses to isoprenaline were well-maintained throughout the 4 min contact time, responses to nicotine often peaked during this time and thereafter began to decline.

In these and similar experiments the use of time-matched control tissues showed that the log concentration-effect curves of both isoprenaline and nicotine altered little in shape or position when reconstructed following tissue incubation with vehicle. In the test tissues hexamethonium (500 μM) caused no change in tone, did not modify the action of isoprenaline but moved the log concentration-response curve for nicotine to the right and reduced its slope. Measurements of log concentration-ratio made at the 50% response level (Table 1) revealed 12 fold antagonism of nicotine.

Table 1 The effects of hexamethonium and propranolol on inhibitory responses of hyoscine-treated trachealis to isoprenaline, nicotine and transmural stimulation

	Control-corrected log concentration-ratio		*Greatest relaxation (% initial isoprenaline maximum) evoked by transmural stimulation*
	Isoprenaline	*Nicotine*	
Hexamethonium 500 μM	0.09 ± 0.09	1.07 ± 0.20	—
Propranolol 1 μM	1.69 ± 0.13	1.29 ± 0.09	47 ± 7
Propranolol 10 μM	2.45 ± 0.19*	1.02 ± 0.11	58 ± 6

Values of log concentration-ratio were measured at the response level corresponding to half the initial maximal response for the appropriate agonist. Data represent the means ± s.e. of at least 8 observations.
*Indicates a significant increase in the antagonism provided by propranolol when the concentration of propranolol was raised from 1 to 10 μM (single tailed, unpaired *t* test).

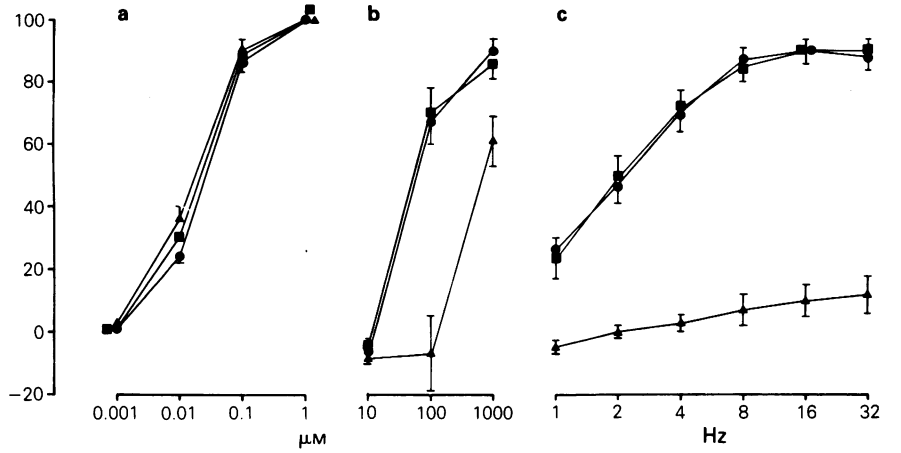

Figure 1 Hyoscine (1μM)-treated, guinea-pig isolated trachealis: the effects of tetrodotoxin (0.3 μM) on relaxant responses to isoprenaline, nicotine and transmural stimulation. The abscissae represent the concentrations of isoprenaline (a) or nicotine (b) or the pulse frequency (c) each on a log scale. The ordinate scale represents relaxation as a % of the maximal relaxation evoked by isoprenaline. (●) Pooled initial log concentration- or log frequency-response curves for test and control tissues; (■) subsequent log concentration- or log frequency-response curves constructed in control tissues after incubation in Krebs solution containing vehicle; (▲) log concentration- or log frequency-response curves constructed in test tissues following equilibration with tetrodotoxin (0.3 μM). Data indicate means of values from at least 8 tissues; s.e.mean shown by vertical bars.

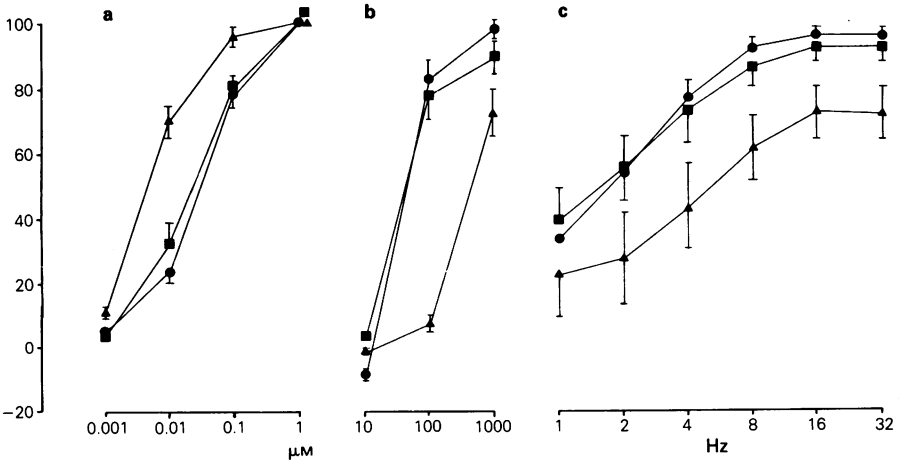

Figure 2 Hyoscine (1 μM)-treated, guinea-pig isolated trachealis: the effects of guanethidine (50 μM) on relaxant responses to isoprenaline, nicotine and transmural stimulation. The abscissae represent the concentrations of isoprenaline (a) or nicotine (b) or the pulse frequency (c) each on a log scale. The ordinate scale represents relaxation as a % of the maximal relaxation evoked by isoprenaline. (●) Pooled initial log concentration- or log frequency-response curves for test and control tissues; (■) subsequent log concentration- or log frequency-response curves constructed in control tissues after incubation in Krebs solution containing vehicle; (▲) log concentration- or log frequency-response curves constructed in test tissues following equilibration with guanethidine (50 μM). Data indicate means of values from at least 8 tissues; s.e.mean shown by vertical bars.

Effects of tetrodotoxin on actions of isoprenaline and nicotine and on responses to transmural stimulation In these experiments the relaxation evoked by transmural stimulation was frequency-dependent and reached a maximum (at 16 Hz) which closely approached the maximal relaxation evoked by isoprenaline. In these and similar experiments, the use of time-matched control tissues revealed little change in the shape or position of the log frequency-response curve for transmural stimulation following tissue incubation with vehicle. In the test tissues tetrodotoxin (0.3 μM) caused no change in tone and did not modify the action of isoprenaline. However, tetrodotoxin caused a parallel rightward shift of the log concentration-response curve for nicotine. The log concentration-ratio for nicotine was 0.94 ± 0.08 (mean ± s.e., $n = 8$) indicating approximately 9 fold antagonism. Tetrodotoxin also profoundly depressed the log frequency-response curve for transmural stimulation (Figure 1).

Effects of guanethidine on actions of isoprenaline and nicotine and on responses to transmural stimulation

In test tissues guanethidine (50 μM) evoked no change in tone but caused a parallel leftward shift of the log concentration-response curve for isoprenaline (Figure 2). The log concentration-ratio for isoprenaline was −0.78 ± 0.13 (mean ± s.e., $n = 8$) indicating approximately 6 fold potentiation. Such potentiation of isoprenaline by guanethidine is attributable to guanethidine inhibiting the extraneuronal uptake of isoprenaline (Foster, 1969). In contrast, guanethidine caused a parallel rightward shift of the log concentration-effect curve of nicotine. The log concentration-ratio for nicotine was 1.09 ± 0.03 (mean ± s.e., $n = 8$) indicating approximately 12 fold antagonism. Guanethidine also caused some depression of the log frequency-response curve to transmural stimulation (Figure 2).

Effects of propranolol on actions of isoprenaline and nicotine and on responses to transmural stimulation

In test tissues propranolol (1 μM) evoked no change in tone but caused a parallel rightward shift of the log concentration-response curves of both isoprenaline and nicotine. The appropriate log concentration-ratios are presented in Table 1 and indicated 49 and 19.5 fold antagonism of isoprenaline and nicotine

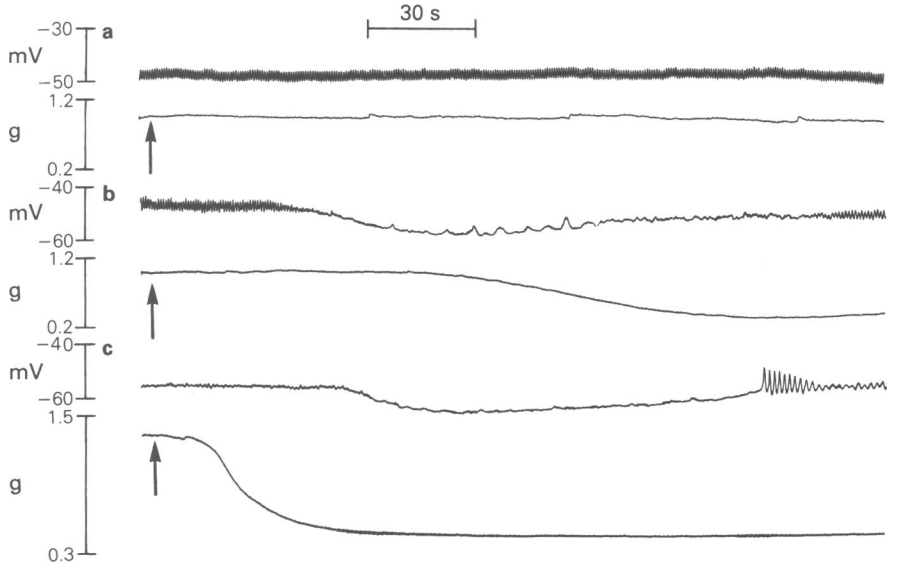

Figure 3 Effects of nicotine on the electrical and mechanical activity of guinea-pig isolated trachealis treated with hyoscine (1 μM). Each pair of traces represents results from a different preparation. In each case the upper trace represents membrane potential changes whilst the lower trace represents the mechanical activity of a contiguous segment of trachea. Nicotine 10 (a), 100 (b) or 1000 μM (c) was introduced at the arrow and was allowed to superfuse the tissues for 4 min. Note that nicotine 10 μM had little or no effect on electrical or mechanical activity. Higher concentrations of nicotine caused relaxation and transient hyperpolarization of the impaled cell. Spontaneous electrical slow waves (initially very small in (c)) were virtually abolished during the hyperpolarization. The poor correlation between the onset of relaxation and the onset of hyperpolarization in (b) and (c) may reflect differences in the rates of penetration of nicotine to the neural sites from which electrical and mechanical activity were initiated.

Table 2 The effects of tetrodotoxin, hexamethonium, guanethidine and propranolol on the relaxation and hyperpolarization of hyoscine-treated guinea-pig isolated trachealis induced by nicotine

Nicotine (μM)	*Control*		*Tetrodotoxin* (0.3 μM)		*Hexamethonium* (500 μM)		*Guanethidine* (50 μM)		*Propranolol* (1 μM)	
	mV	mg	mV	mg	mV	mg	mV	mg	mV	mg
10	−1.86 ± 1.64	−48.6 ± 26.9	–	–	–	–	–	–	–	–
100	12.93 ± 1.33	−725.7 ± 118.4	1.14* ± 0.86	−11.4* ± 51.4	−2.0* ± 0.68	−3.3* ± 3.3	−1.14* ± 0.91	−28.6* ± 21.3	−1.67* ± 0.84	−26.7* ± 13.3
1000	11.22 ± 1.52	−908.9 ± 176.7	–	–	–	–	–	–	−0.50* ± 0.82	−47.5* ± 1.60

Data (membrane potential change in mV; tension change in mg) represent the means of at least 6 observations ± s.e.mean. A positive change in membrane potential indicates hyperpolarization, a negative change depolarization. A negative change in tension indicates relaxation. *indicates a significant ($P < 0.05$) reduction in the hyperpolarization or relaxation compared with that seen in the control tissues (single tailed unpaired t test).

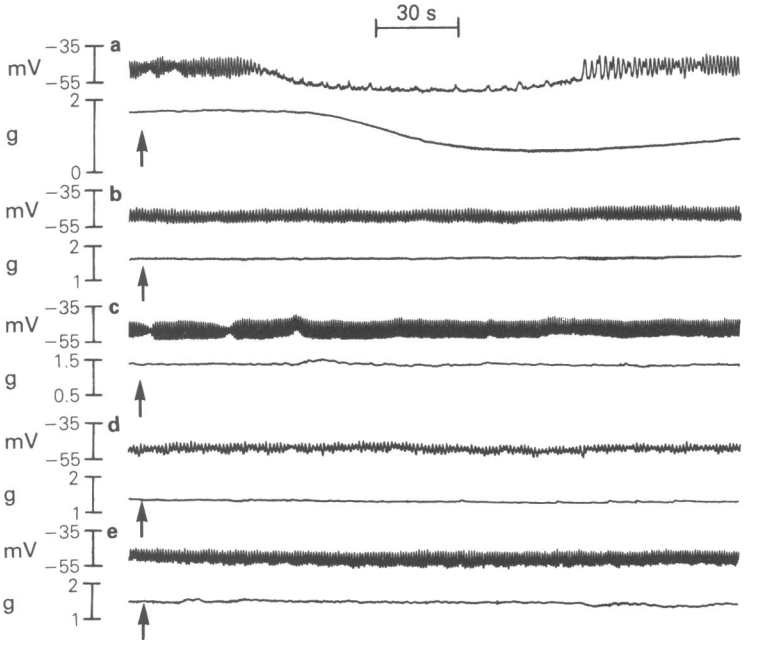

Figure 4 Effects of tetrodotoxin, hexamethonium, guanethidine and propranolol on the electrical and mechanical responses of hyoscine-treated guinea-pig isolated trachealis to nicotine (100 μM). Each pair of traces represents results from a different preparation. In each case hyoscine (1 μM) was present throughout. The upper trace represents membrane potential changes whilst the lower trace represents the mechanical activity of a contiguous segment of trachea. Nicotine (100 μM) was introduced at the arrow and was allowed to superfuse the tissues for 4 min. (a) Control; (b) tetrodotoxin (0.3 μM) present throughout; (c) hexamethonium (500 μM) present throughout; (d) guanethidine (50 μM) present throughout; (e) propranolol (1 μM) present throughout. Note that tetrodotoxin, hexamethonium, guanethidine and propranolol were each able to suppress the electrical and mechanical responses to nicotine.

respectively. The log frequency-response curve for transmural stimulation was depressed at all pulse frequencies. In a further series of experiments the concentration of propranolol was raised to 10 μM and caused a greater, parallel rightward shift of the log concentration-response curve of isoprenaline. However, this was not accompanied by a greater rightward shift in the log concentration-response curve for nicotine or by greater depression of the log frequency-response curve for transmural stimulation (Table 1).

Intracellular electrophysiological recording

Effects of nicotine In hyoscine-treated trachealis, nicotine (10–1000 μM) evoked electrical and mechanical responses which were both inhibitory and concentration-dependent (Figure 3 and Table 2). Nicotine (10 μM) had little or no effect on spontaneous electrical or mechanical activity. Higher concentrations of nicotine always caused relaxation and in most (though not all) cells this was accompanied by an inhibitory electrical response. Thus nicotine (100 μM) often caused relaxation and a transient hyperpolarization of the impaled cell. In cells exhibiting spontaneous electrical activity, the hyperpolarization was associated with a profound reduction in slow wave frequency or with slow wave abolition. Nicotine (1000 μM) caused slightly greater relaxation but did not evoke more profound or longer-lasting hyperpolarization. In cells that were electrically quiescent, recovery from nicotine-induced hyperpolarization was sometimes associated with a transient burst of slow wave activity (Figure 3c).

In some tissues the onset of hyperpolarization and relaxation were virtually coincident. In others the onset of relaxation preceded hyperpolarization or *vice-versa*. Such differences in the onset of electrical and mechanical responses probably represented differences in the rates of penetration of nicotine to the neural sites from which changes in electrical and mechanical activity were initiated.

In all experiments electrical and mechanical responses to nicotine reached peak amplitude and began to decline within the 4 min contact time. In contrast to the onset of the inhibitory responses, the decline of

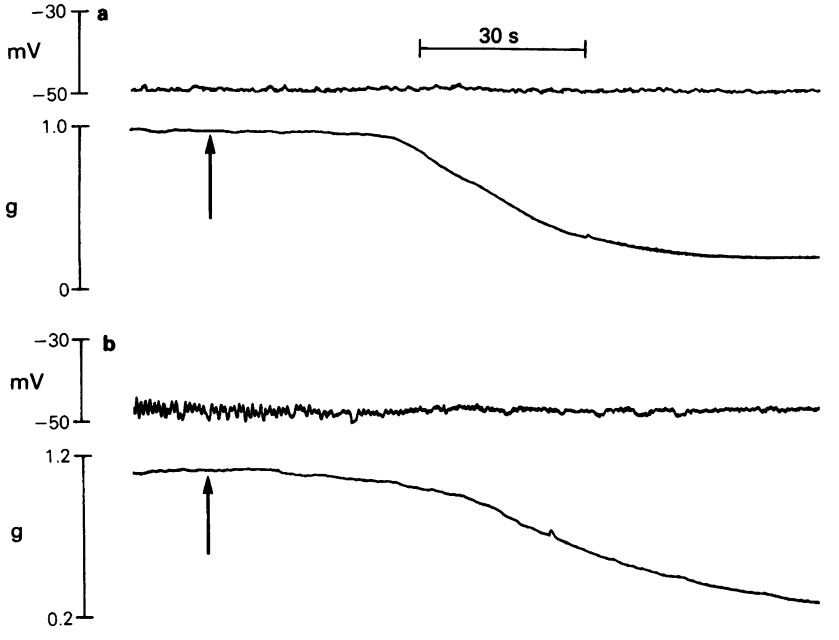

Figure 5 Effects of nicotine (1 mM) on the electrical and mechanical activity of guinea-pig isolated trachealis treated with hyoscine (1 μM) and propranolol (1 μM). Each pair of traces represents results from a different preparation. In each case the upper trace represents membrane potential changes whilst the lower trace represents the mechanical activity of a contiguous segment of trachea. Hyoscine (1 μM) and propranolol (1 μM) were present throughout. Nicotine (1 mM) was introduced at the arrows and was allowed to superfuse the tissues for 4 min. (a) Record where impaled cell was electrically quiescent. Note that relaxant action of nicotine was not accompanied by change in resting membrane potential. (b) Record where the impaled cell exhibited spontaneous slow wave activity. Note that relaxant action of nicotine was accompanied by slow wave suppression but not by change in resting membrane potential.

responses exhibited a constant difference between the electrical and mechanical components. Recovery from hyperpolarization always started before recovery from relaxation.

Trachealis cells from tissues additionally treated with hexamethonium (500 μM), tetrodotoxin (0.3 μM), guanethidine (50 μM) or propranolol (1 μM) exhibited similar electrical behaviour to those from tissues treated with hyoscine alone (Figure 4). As expected from the results of the tissue bath experiments, each of these modifying agents profoundly reduced or abolished the relaxation induced by 100 μM nicotine. The hyperpolarization and slow wave inhibition produced by this concentration of nicotine were also abolished by each of the modifying agents (Figure 4 and Table 2).

Nicotine (1 mM) was able to evoke relaxation in tissues treated with propranolol (1 μM). In spontaneously active cells, the electrical response associated with this relaxation comprised slow wave suppression. However, nicotine failed to evoke hyperpolarization either of quiescent cells or those exhibiting slow waves (Figure 5).

Effects of transmural stimulation Transmural stimulation was applied to tracheae treated with hyoscine (1 μM). Single pulses evoked no detectable relaxation. Furthermore, single pulses evoked no membrane potential changes either in cells exhibiting spontaneous slow waves or in cells which were electrically quiescent.

When pulses were delivered in trains of 5 s duration and at a pulse frequency of 4–64 Hz, relaxation was always observed and its magnitude was dependent upon the pulse frequency. The electrical phenomena accompanying the relaxation were quite variable. Irrespective of whether they were spontaneously active or electrically quiescent, some cells exhibited no inhibitory electrical changes in response to transmural stimulation (Figure 6a). Presumably these cells did not receive noradrenergic inhibitory innervation. For pulse frequencies in the range 8–16 Hz, many spontaneously-active cells exhibited slow wave suppression. At high pulse frequencies (32–64 Hz) hyperpolarization was observed both in spontaneously active cells (Figure 6b, c) and in quiescent cells. A minor proportion of cells exhibited hyperpolarization

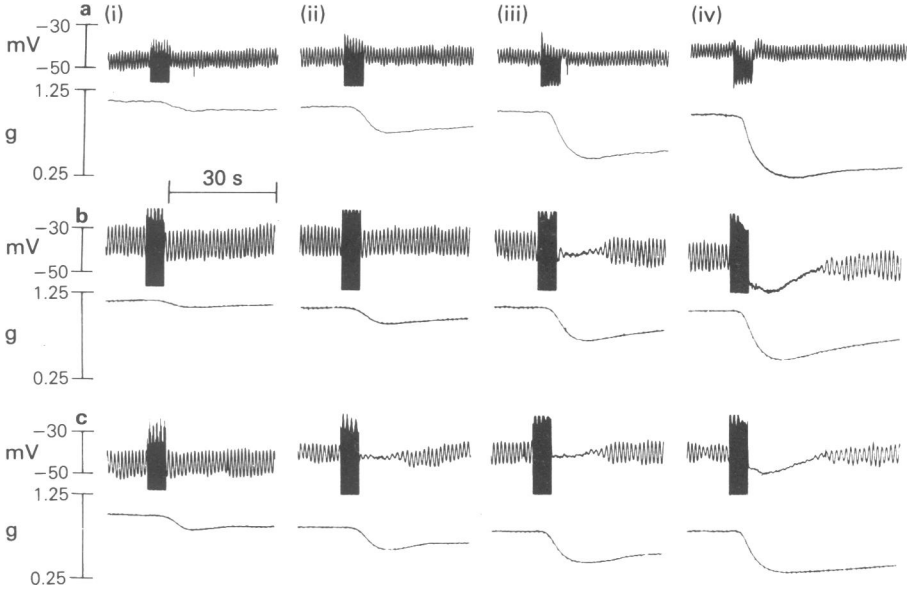

Figure 6 Effects of transmural stimulation (pulse trains) on the electrical and mechanical activity of guinea-pig isolated trachealis treated with hyoscine (1 μM). Each row of traces (a, b, c) represents results from a different preparation. In each case the upper trace represents membrane potential changes whilst the lower trace represents the mechanical activity of a contiguous segment of trachea. The columnar disturbances in the electrical record represent the stimulus artifacts to 5 s trains of transmural pulses (0.5 ms and supramaximal voltage) where pulse frequency was 4 (i), 8 (ii), 16 (iii) and 32 Hz (iv). (a) Cell presumed devoid of noradrenergic inhibitory innervation; note absence of inhibitory electrical changes despite marked relaxation; (b) and (c) cells receiving inhibitory innervation. Note that slow wave suppression was seen at pulse frequencies lower than those producing hyperpolarization of the impaled cell.

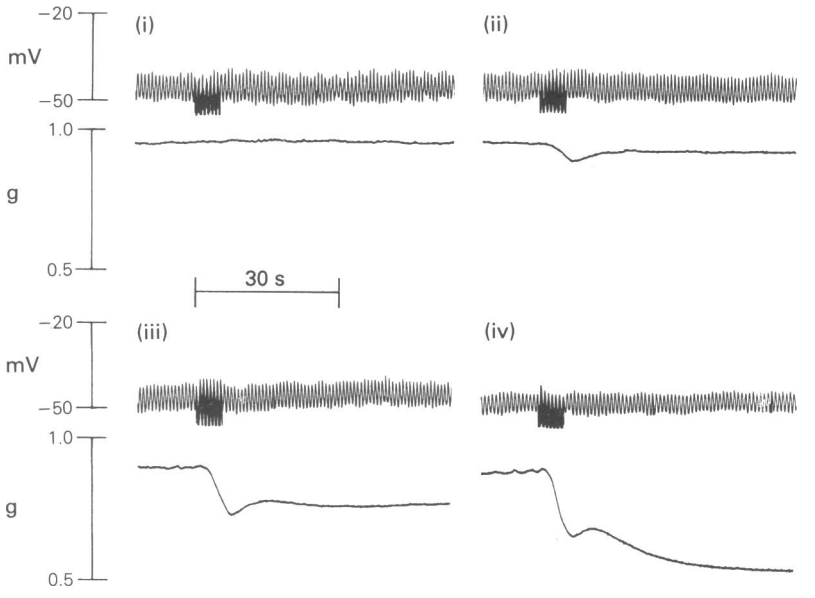

Figure 7 Effects of transmural stimulation (pulse trains) on the electrical and mechanical activity of guinea-pig isolated trachealis treated both with hyoscine (1 μM) and propranolol (1 μM). In each case the upper trace represents membrane potential changes whilst the lower trace represents the mechanical activity of a contiguous segment of trachea. All electrical records are from the same cell. The columnar disturbances in the electrical records represent the stimulus artefacts to 5 s trains of transmural pulses (0.5 ms and supramaximal voltage) where pulse frequency was 4 (i), 8 (ii), 16 (iii) and 32 Hz (iv). Note the absence of inhibitory electrical changes despite marked relaxation. Compare with traces shown in Figure 6a.

and slow wave suppression even at pulse frequencies as low as 4 Hz. In general recovery from the inhibitory electrical phenomena preceded recovery from the relaxation.

Transmural stimulation of tracheae additionally treated with propranolol (1 μM) evoked relaxation provided that trains of pulses were used. This relaxation was generally smaller than that observed in tissues treated with hyoscine alone and was never accompanied by change in the electrical behaviour of the impaled cell (Figure 7).

Discussion

Inhibitory responses to transmural stimulation

The present observation that tetrodotoxin (0.3 μM) markedly depressed relaxant responses to transmural stimulation without itself lowering tone and without antagonizing effects of isoprenaline, supports the conclusions of earlier workers (Coburn & Tomita, 1973; Coleman & Levy, 1974) that relaxation of guinea-pig trachealis induced by transmural stimulation has a neurogenic basis. Furthermore the ability of guanethidine (50 μM) and propranolol (1 and 10 μM) to reduce but not to abolish relaxant responses to transmural stimulation shown in the present experiments lends support to the suggestion (Coburn & Tomita, 1973; Coleman & Levy, 1974) that both noradrenergic and NANCI neurones contribute to the relaxant responses. One of the objectives of the present study was to determine whether the noradrenergic and NANC components of the inhibitory response to transmural stimulation could be differentiated by electrophysiological means.

When guinea-pig trachealis muscle is subjected to stimulation of its extrinsic sympathetic nerve supply at the level of the stellate ganglion, many trachealis cells do not exhibit inhibitory electrical responses. In cells which are influenced by sympathetic stimulation, hyperpolarization of quiescent cells is observed at pulse frequencies greater than 10 Hz. In spontaneously active cells, slow wave suppression occurs with or without hyperpolarization (McCaig, 1986b). Since NANC inhibitory pathways are carried in the vagus but not the sympathetic nerve trunks (Chesrown *et al.*, 1980; Yip *et al.*, 1981; McCaig, 1986a) these inhibitory electrical responses are likely to result from the action of noradrenaline released from sympathetic neurone

terminals but not from the action of the NANC inhibitory neurotransmitter.

In the present study transmural stimulation of hyoscine-treated guinea-pig trachealis evoked inhibitory electrical responses strikingly similar to those described (McCaig, 1986b) for stimulation of extrinsic sympathetic nerves. However, the present electrical responses to transmural stimulation could have resulted either from the action of noradrenaline released from sympathetic nerves or from the action of the NANCI transmitter or from both transmitters acting together.

The ability of propranolol totally to remove the inhibitory electrical responses (slow wave suppression and hyperpolarization) suggests that such responses had a purely sympathetic (noradrenergic) basis. Furthermore, since transmural stimulation in the presence of propranolol evoked relaxant responses unaccompanied by electrical change, we are led to the conclusion either that very few cells receive NANCI innervation or that the NANCI neurotransmitter acts by a mechanism which does not involve membrane potential changes.

Although the total innervation of guinea-pig trachealis is relatively sparse (Kirkpatrick, 1981), several factors prompt us to reject the former hypothesis. Firstly, the relaxation evoked by transmural stimulation in the presence of propranolol is substantial and is therefore highly unlikely to be mediated by a very small number of NANCI-innervated cells. Secondly, we impaled many cells in different preparations without once detecting electrical responses to NANCI nerve stimulation. Thirdly, stimulation of the extrinsic vagus nerve (which carries the NANCI nervous pathway) does not induce inhibitory electrical responses from atropine-treated guinea-pig trachealis (McCaig, 1986, personal communication).

The proposal that the NANCI neurotransmitter acts on airways smooth muscle by a mechanism that does not involve electrical change receives support from studies of cat trachealis (Ito & Takeda, 1982) where field stimulation in the presence of 5-hydroxytryptamine, atropine and propranolol evoked relaxation but failed to evoke changes in membrane potential or resistance. It may be that the action of the NANCI neurotransmitter is electrically silent in bovine trachealis too, but results obtained (Kirkpatrick, 1981; Cameron *et al.*, 1983) with this tissue are somewhat difficult to assess since the published records are from experiments where propranolol and (in some instances) atropine were not included in the bathing medium.

In summary, results obtained in the present study and those of other workers suggest that the inhibition of airways smooth muscle induced by the NANCI neurotransmitter may involve a potential-independent mechanism. NANCI neuroeffector transmission in airways smooth muscle therefore contrasts with that in many types of gastrointestinal smooth muscle where a marked hyperpolarization (inhibitory postjunctional potential) is often observed even in response to a single stimulus (Small & Weston, 1979).

The inhibitory action of nicotine

The ability of hexamethonium (500 μM) and tetrodotoxin (0.3 μM) each to antagonize nicotine but not to modify the relaxant action of isoprenaline, suggests that the relaxant effects of nicotine in hyoscine-treated guinea-pig trachealis are largely neurogenic in origin. These observations also suggest that the activation of neural nicotinic cholinoceptors evokes the discharge of Na^+-dependent neuronal action potentials and the subsequent release of transmitter substances from inhibitory nerve terminals.

It might be argued that the relative tetrodotoxin-resistance of the inhibitory response to 1 mM nicotine is indicative of direct effects of nicotine on the trachealis muscle. However, marked tachyphylaxis was exhibited both by concentrations of nicotine which yielded tetrodotoxin-sensitive responses and by concentrations yielding responses which were relatively resistant to tetrodotoxin. Furthermore, there have been several reports (Haeusler *et al.*, 1968; Krauss *et al.*, 1970; Pappano & Rembish, 1971) that agonists at hexamethonium-sensitive cholinoceptors can evoke transmitter release from peripheral nerves when neuronal action potentials have been abolished by tetrodotoxin. For these reasons a neurogenic basis to the responses to 1 mM nicotine need not be discounted.

From the results of experiments in which cocaine and ergotoxine were used as pharmacological tools, Hawkins & Paton (1958) concluded that nicotine-induced relaxation of guinea-pig trachea involved the stimulation of sympathetic neurones. The present work lends support to this suggestion for guanethidine (50 μM) antagonized nicotine but potentiated isoprenaline and propranolol (1 μM) antagonized both nicotine and isoprenaline.

In guinea-pig trachealis the activation of β-adrenoceptors on the smooth muscle cells by administering isoprenaline or terbutaline (Allen *et al.*, 1985; Honda *et al.*, 1986) or by electrically stimulating extrinsic sympathetic nerves (McCaig, 1986b) evokes suppression of spontaneous slow waves and cellular hyperpolarization. Since nicotine was able to mimic these electrophysiological changes (present study) its ability to stimulate tracheal noradrenergic neurones and thereby to cause relaxation seems confirmed.

The results of our present experiments with transmural stimulation were consistent with previous reports (Coburn & Tomita, 1973; Coleman & Levy, 1974; McCaig, 1986a) that guinea-pig trachealis

receives both noradrenergic and NANC inhibitory innervation. We, therefore, anticipated that nicotine might activate both types of inhibitory neurone. Several observations suggest that this, indeed, occurs. Firstly, raising the concentration of propranolol from 1 to 10 μM caused greater antagonism of isoprenaline but no further antagonism of nicotine. Hence the propranolol-resistant component of the action of nicotine is more likely to involve the release of NANCI neurotransmitter than the release of noradrenaline. Secondly, after propranolol administration, nicotine (1 mM) caused relaxation without change in resting membrane potential. As discussed above, an inhibitory response of this kind appears to be characteristic of the action of the NANCI neurotransmitter. The electrophysiological evidence therefore also suggests the involvement of NANCI neurones in the propranolol-resistant component of the relaxant action of nicotine.

Since, in propranolol-treated trachealis, the effects of transmurally stimulating the NANCI neurones did not include suppression of spontaneous slow waves, the ability of nicotine (1 mM) to suppress the slow waves under similar conditions is difficult to explain. It may be that this high concentration of nicotine liberated sufficient noradrenaline to overcome partially the β-adrenoceptor blockade produced by the propranolol. Alternatively the slow wave suppression might represent the effects of NANCI neurotransmitter at a concentration greater than that achieved by our field stimulation technique. This latter possibility may receive support from observations made in bovine trachealis (Kirkpatrick, 1981; Cameron *et al.*, 1983) which suggest that the effects of the NANCI transmitter could involve slow wave suppression. However, as pointed out above, many of the observations made on bovine trachealis were made in the absence of β-adrenoceptor blockade.

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