Br. J. Pharmac. (1987), **91**, 385–394

Effects of sympathetic stimulation and applied catecholamines on mechanical and electrical responses to stimulation of the vagus nerve in guinea-pig isolated trachea

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1 Mechanical and electrical responses to stimulation of the vagus nerve were studied in the isolated, innervated trachea of the guinea-pig.

2 In approximately half the preparations tested, the amplitudes of mechanical constrictor responses to stimulation of the vagus were reduced substantially during a period of sympathetic stimulation. Vagal responses were unaltered in the remainder.

3 In single trachealis cells, stimulation of the vagus nerve or sympathetic stellate ganglion elicited depolarization and hyperpolarization, respectively. Vagally-mediated depolarization was decreased, unchanged or increased in amplitude after a period of sympathetic stimulation.

4 Isoprenaline almost abolished mechanical responses induced by stimulation of the vagus, and this effect was blocked by propranolol.

5 Noradrenaline attenuated markedly vagal mechanical responses also, and this effect was blocked by a combination of propranolol and phentolamine.

6 Both noradrenaline and isoprenaline hyperpolarized single trachealis cells and greatly reduced the amplitude of vagally-mediated depolarization.

7 Neither sympathetic stimulation nor applied catecholamines altered mechanical responses to applied acetylcholine, strongly suggesting that their effects on vagal responses are predominantly presynaptic.

Introduction

Airway smooth muscle possesses β-adrenoceptors which mediate bronchodilatation (Castro de la Mata *et al.*, 1962; Everitt & Cairncross, 1969; Mathé *et al.*, 1971). In some species, for example man and baboon, these receptors are probably activated primarily by circulating catecholamines, since sympathetic innervation is thought to be sparse or absent (Middendorf & Russell, 1980; Zorychta & Richardson, 1980). In other species, however, including the guinea-pig, cat and dog, the airways receive sympathetic innervation from fibres arising in the cervical sympathetic trunk, stimulation of which can elicit bronchodilatation (Diamond & O'Donnell, 1980; Yip *et al.*, 1981). In the dog, stimulation of the sympathetic nerves *in vivo* can reduce but never abolish bronchoconstriction induced by stimulation of the parasympathetic nerves, arising in the vagus (Cabezas *et al.*, 1971). It is now possible to study directly the interaction between parasympathetic excitation and sympathetic inhibition *in vitro* in the isolated, innervated trachea of the guinea-pig (Blackman & McCaig, 1983). The first aim of the present work, therefore, was to examine the effects of sympathetic stimulation on parasympathetically-induced constriction in this preparation.

The electrical correlates of parasympathetic and sympathetic stimulation in guinea-pig trachealis muscle have recently been described (McCaig, 1986a). Parasympathetic stimulation evokes one or more waves of depolarization in quiescent cells or transiently enhances slow wave amplitude in spontaneously active cells, but does not normally give rise to action potential discharge. Sympathetic stimulation, on the

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other hand, induces hyperpolarization and suppresses slow wave discharge. A second aim of the present work was to determine whether the electrical response to parasympathetic stimulation is altered after a period of sympathetic stimulation.

Exogenous noradrenaline and isoprenaline relax guinea-pig trachea and this relaxation is associated with hyperpolarization and slow wave suppression in single trachealis cells (Allen *et al.*, 1985; Honda *et al.*, 1986). In addition, it has been shown that both these catecholamines inhibit contractions evoked by field stimulation in canine trachealis muscle, and that this inhibition is associated with a marked decrease in the amplitude of the cholinergic-mediated depolarization (Ito & Tajima, 1982). The final aim of this work, therefore, was to examine the effects of exogenous noradrenaline and isoprenaline on the mechanical and electrical responses to parasympathetic nerve stimulation in the guinea-pig trachea.

Methods

Guinea-pigs (male, Dunkin Hartley, 300–600 g body weight) were killed by a blow to the head. The trachea was dissected together with the autonomic nerve supply on the right side (cervical vagus nerve and recurrent laryngeal branch, cervical sympathetic trunk and stellate ganglion) as previously described (Blackman & McCaig, 1983). The trachea was placed in a tissue bath at 37°C perfused at 4 ml min^{-1} with Krebs solution of the following composition (mM): Na^+ 143.5, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.0, Cl^- 121, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, HCO_3^- 25 and glucose 11.

Mechanical recording

The trachea was cannulated at both ends and filled with Krebs solution. One end was then closed and the other attached to a pressure transducer (Statham). Intraluminal pressure was recorded on a pen-recorder (Devices). In this closed system, increases or decreases in intraluminal pressure reflect contraction or relaxation, respectively, in the tracheal smooth muscle.

Electrical recording

The trachea was opened by cutting through the cartilaginous rings opposite the smooth muscle and pinned to the base of the chamber. Segments of the mucosal layer overlying the bands of smooth muscle were removed with watchmakers' forceps under a dissecting microscope. Single cells were impaled with glass microelectrodes filled with 0.5 M KCl and having a resistance of 60–80 MΩ. Voltage signals were fed through a unity gain amplifier (WP Instruments), displayed continuously on an oscilloscope (Tektronix) and recorded as appropriate on a pen recorder (Gould).

Responses to stimulation of the vagus nerve were recorded in a single cell before and after sympathetic stimulation or before and during exposure to drugs. When the microelectrode was dislodged from a cell during drug application, recordings were made in a neighbouring cell whose impalement was maintained where possible during washout of the drug.

Nerve stimulation

The vagus nerve (parasympathetic supply) was sucked into a suction electrode and rectangular pulses of 40 V and 1 ms duration were applied from a stimulator (Bell). In order to study mechanical responses, the vagus was stimulated with 100 pulses at 20 Hz every 90 s. This elicited consistent responses, close to the maximum for the preparation (Blackman & McCaig, 1983). When studying electrical responses, the vagus was stimulated with 8 or 16 pulses at 20 or 40 Hz. This stimulus elicits maximal depolarization in trachealis cells (McCaig, 1986a).

The stellate ganglion (sympathetic supply) was placed on a bipolar electrode and stimulated with rectangular pulses of 40 V and 1 ms duration delivered from a stimulator (Devices). Trains of pulses lasting 5 to 35 s at a frequency of 40 or 80 Hz were applied, which elicit electrical and mechanical responses close to the maximum for the tissue (Blackman & McCaig, 1983; McCaig, 1986a).

Drugs

Drugs were added to the reservoir of Krebs solution and are expressed as final concentration in the superfusing fluid. The following drugs were used: acetylcholine chloride (Sigma); isoprenaline sulphate (Sigma); neostigmine methyl-sulphate (Sigma); noradrenaline bitartrate (Sigma); phentolamine mesylate (Ciba) and propranolol HCl (I.C.I.).

Statistics

Responses were compared using t tests for independent or paired samples, as appropriate, and were deemed significantly different when $P < 0.05$.

Results

Effect of sympathetic stimulation on mechanical responses to stimulation of the vagus

Tracheal preparations had a positive resting intraluminal pressure of 3.3 ± 0.2 cmH_2O (mean ± s.e.mean, $n = 17$). Stimulation of the vagus nerve

with 100 pulses at 20 Hz evoked a transient increase in intraluminal pressure of $17.5 \pm 2.2\,cmH_2O$ ($n = 17$), and responses to vagal stimulation repeated at 90 s intervals were highly reproducible in any one preparation. Once control vagal responses had been obtained, the stellate ganglion was stimulated at 40 Hz for 35 s. This induced a gradual decrease in intraluminal pressure of $4.6 \pm 0.6\,cmH_2O$ ($n = 17$) after 25 s. At the peak of this response, the vagus nerve was stimulated and the response compared to that obtained before sympathetic stimulation. In 9/17 preparations, sympathetic stimulation had no effect on the vagal response, as shown in Figure 1a. The mean amplitudes of the vagal responses were $14.6 \pm 3.4\,cmH_2O$ and $15.0 \pm 3.6\,cmH_2O$ before and during sympathetic stimulation, respectively. In the remaining 8 preparations the amplitude of the vagal response was reduced markedly during sympathetic stimulation (mean amplitude control: $20.6 \pm 2.2\,cmH_2O$; during sympathetic stimulation: $8.8 \pm 1.5\,cmH_2O$, $P < 0.001$, by paired t test). An example of this effect is shown in Figure 1b, where it can be seen that the vagal response was considerably reduced during sympathetic stimulation but was fully restored 2 min later. This effect of sympathetic stimulation was reproducible in any preparation in this group, as also shown in Figure 1b. The amplitudes of the control vagal responses were not significantly different in the 2 groups of preparations. However, the amplitude of the decrease in intraluminal pressure, evoked by sympathetic stimulation, was significantly greater in preparations in which vagal responses were inhibited than in those which were unaffected ($6.3 \pm 0.8\,cmH_2O$ ($n = 8$) and $3.0 \pm 0.5\,cmH_2O$ ($n = 9$), respectively, $P < 0.05$). There may be a relationship, therefore, between the effects of sympathetic stimulation on resting tone and on tone induced by vagal stimulation.

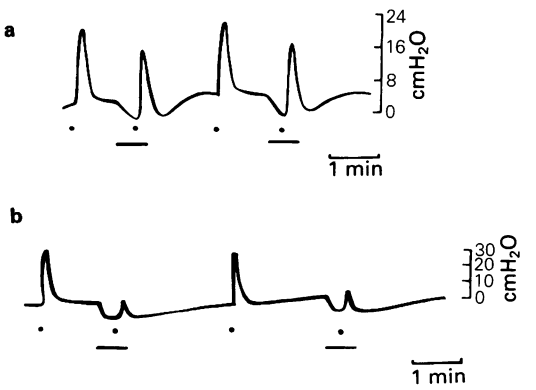

Figure 1 The effect of a period of sympathetic stimulation on the increase in intraluminal pressure induced by vagal stimulation in guinea-pig isolated trachea. The vagus was stimulated with 100 pulses at 20 Hz (indicated by dots) and the stellate ganglion at 40 Hz for 35 s (indicated by bars). During sympathetic stimulation vagal responses were unaltered (a) or attenuated markedly (b).

Effect of sympathetic stimulation on mechanical responses to exogenous acetylcholine

Acetylcholine (ACh), 10^{-4} M, evoked an increase in intraluminal pressure of $23 \pm 3\,cmH_2O$, ($n = 12$), which developed over 2 min. Responses to ACh administered at 30 min intervals were highly reproducible. Stimulation of the sympathetic nerves at 40 Hz evoked a decrease in intraluminal pressure of $7 \pm 1.6\,cmH_2O$ ($n = 5$) after 30 s. The response to ACh, 10^{-4} M, administered during continued sympathetic stimulation, was unchanged from control (control: $21.4 \pm 6.2\,cmH_2O$; during sympathetic stimulation: $26.6 \pm 6.2\,cmH_2O$, $n = 5$). Sympathetic stimulation, therefore, did not attenuate the mechanical response to exogenous ACh.

Effect of sympathetic stimulation on electrical responses to stimulation of the vagus.

The transmembrane potential of trachealis cells was -43 ± 0.5 mV ($n = 47$ cells from 10 preparations). A number of cells exhibited slow waves of amplitude 4.7 ± 0.9 mV and frequency 1.05 ± 0.03 Hz ($n = 20$). Stimulation of the vagus nerve (8 or 16 pulses at 40 Hz) elicited depolarization, often followed by a series of fluctuations in potential in quiescent cells, or a transient increase in slow wave amplitude in spontaneously active cells. The mean depolarization recorded in 14 cells from 10 preparations was 10.1 ± 1.1 mV. In each of these cells sympathetic stimulation elicited hyperpolarization (3.9 ± 0.5 mV) and reduced the amplitude of slow waves when present. The effect of a period of sympathetic stimulation on vagally-induced depolarization was examined in these 14 cells. In 8/14 cells the amplitude of the vagal depolarization was reduced (Figure 2a), with mean amplitudes before and after sympathetic stimulation of 10.9 ± 1.6 mV and 7.5 ± 1.7 mV, respectively ($P < 0.01$). In a further 3/14 cells the amplitude of the vagal response was unchanged from the control value of 11.3 ± 2.7 mV after sympathetic stimulation, as shown in Figure 2b. In the remaining 3/14 cells the amplitude of the vagal response was increased from 6.9 ± 0.5 mV to 9.2 ± 1.5 mV after sympathetic stimulation (Figure 3). The degree of hyperpolarization evoked by sympathetic stimulation did not differ in the three groups of cells, with means of 4 ± 0.7 mV, 3 ± 1.5 mV, and 5 ± 1.2 mV in cells in which the vagal response was decreased, unchanged and increased, respectively.

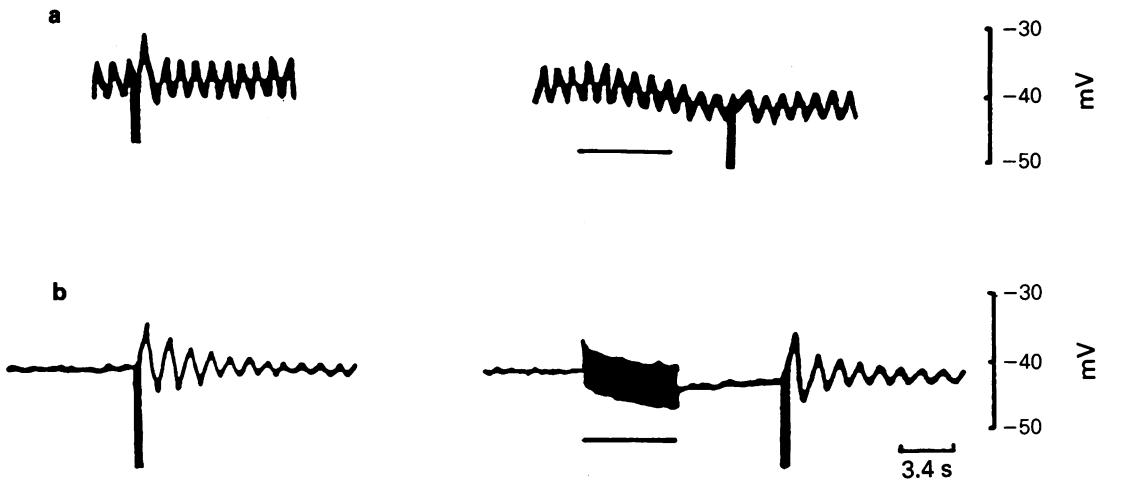

Figure 2 The effect of a period of sympathetic stimulation on the depolarizing response evoked by stimulation of the vagus in 2 guinea-pig trachealis cells, (a) and (b). Left hand record: response to stimulation of the vagus nerve with 8 pulses at 20 Hz (stimulus artefacts appear as narrow downward deflections); right hand record: response to identical vagal stimulation after sympathetic stimulation for 5 s at 40 Hz (indicated by bar below record). In (b) sympathetic stimulus artefact appears as broad black band on record but in (a) cell was further from stimulating electrode and no artefact is visible. The vagal response was attenuated in (a) but unchanged in (b).

Where more than one cell was studied in a single preparation it was found that some cells exhibited a decrease whilst others showed no change or an increase in vagal depolarization after sympathetic stimulation. In some preparations mechanical responses were examined before the electrical studies and there was no obvious relationship between the effects of sympathetic stimulation on vagal mechanical responses and on vagally-mediated depolarization.

Effects of isoprenaline and noradrenaline on mechanical responses to stimulation of the vagus

In 15 preparations the resting intraluminal pressure was $3.3 \pm 0.3\ cmH_2O$. Vagal stimulation (100 pulses at 20 Hz) elicited an increase in intraluminal pressure of $19.8 \pm 2.6\ cmH_2O$ and responses obtained at 90 s intervals were of consistent amplitude. When isoprenaline, 10^{-6} M, was added to the superfusing fluid there was a decrease in intraluminal pressure which began after 2 min and reached a maximum of $7.3 \pm 1.4\ cmH_2O$ ($n = 4$) at 5.6 ± 0.7 min ($n = 4$). Vagal responses were gradually reduced in amplitude and at the peak of this effect were profoundly inhibited (mean decrease 96%), as shown in Figures 4a and 5. The maximum inhibition of the vagal response occurred at 10.4 ± 0.7 min, significantly later ($P < 0.01$) than the peak decrease in intraluminal pressure. On washout of the isoprenaline, both resting

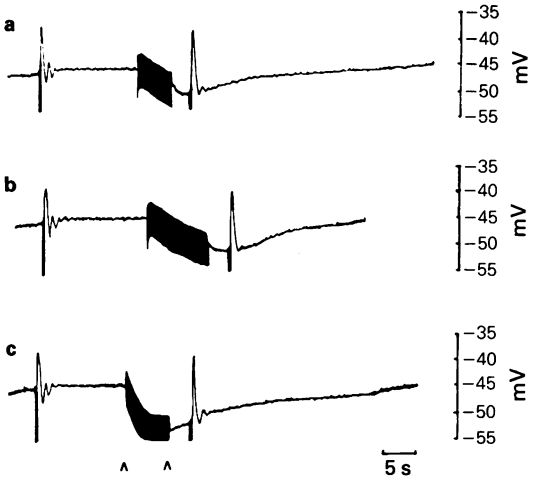

Figure 3 The effect of a period of sympathetic stimulation on the depolarizing response evoked by vagal stimulation in a single trachealis cell of the guinea-pig. The vagus was stimulated with 16 pulses at 40 Hz: (narrow downward deflections on record) before and after stimulation of the stellate ganglion (broad black bands on record) at 40 Hz for 5, 9 and 30 s in (a), (b) and (c), respectively (note change of speed of recording in (c) between arrows where horizontal calibration equals 25 s). In each case the amplitude of the vagal depolarization was increased after sympathetic stimulation.

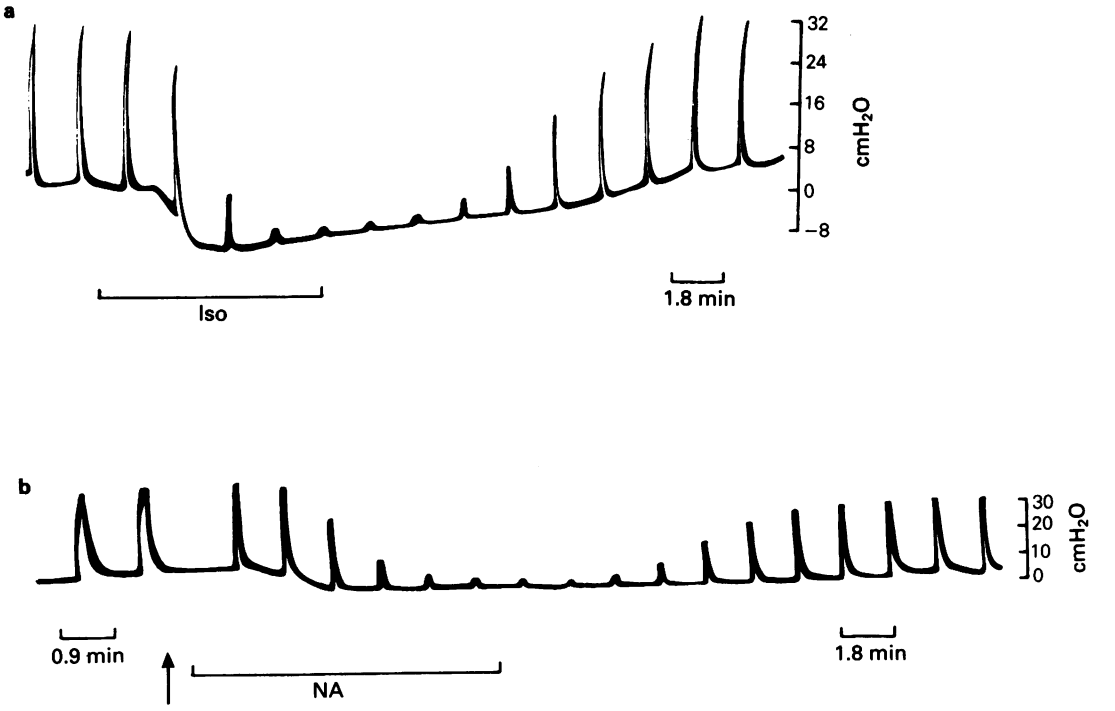

Figure 4 Effects of isoprenaline, 10^{-6} M (Iso; a) and noradrenaline, 10^{-5} M (NA; b) on increases in intraluminal pressure induced by stimulation of the vagus in 2 isolated preparations of guinea-pig trachea. The vagus was stimulated at 20 Hz for 5 s at 90 s intervals. Responses were attenuated markedly during exposure to isoprenaline or noradrenaline. (Note change in speed of recording in (b) indicated by arrow).

intraluminal pressure and vagal responses were gradually restored close to control levels over about 20 min. The decrease in intraluminal pressure and the inhibition of vagal responses by isoprenaline were completely prevented by pretreatment with propranolol (3.5×10^{-6} M, Figure 5).

Noradrenaline, at 10^{-6} M, had no effect on intraluminal pressure or on vagal responses. However, noradrenaline 10^{-5} M had effects similar to those of isoprenaline. Intraluminal pressure was decreased by 5.8 ± 1.7 cmH_2O ($n = 4$) after 7.4 ± 0.5 min and, as shown in Figures 4b and 5, vagal responses were markedly inhibited. As found with isoprenaline, peak inhibition of vagal responses occurred significantly later (at 11.9 ± 1.4 min, $n = 4$, $P < 0.05$) than the peak decrease in intraluminal pressure. Noradrenaline, when administered 20 min after propranolol (3.5×10^{-6} M), evoked an increase rather than a decrease in intraluminal pressure, of 2.3 ± 0.1 cmH_2O, which developed over 5 min and then gradually wore off. In the presence of propranolol, noradrenaline still inhibited vagal responses by a mean of 55%, as shown in Figure 5. On the addition of phentolamine, 4×10^{-6} M, vagal responses were decreased in amplitude (control: 23.3 ± 1.7 cmH_2O; phentolamine 10.7 ± 2.0 cmH_2O) as previously observed (McCaig, 1986b). Noradrenaline, given in the presence of propranolol and phentolamine, had no effect on either intraluminal pressure or vagal responses (Figure 5).

Effect of isoprenaline and noradrenaline on mechanical responses to acetylcholine

ACh, 10^{-4} M evoked an increase in intraluminal pressure of 23.9 ± 3.7 cmH_2O ($n = 7$) and responses to ACh given at 30 min intervals were of consistent amplitude. Isoprenaline, 10^{-6} M, and noradrenaline, 10^{-5} M, each reduced intraluminal pressure but had no effect on the increase in intraluminal pressure induced by ACh.

Effects of isoprenaline and noradrenaline on vagal electrical responses

The transmembrane potential of trachealis cells was -42 ± 0.5 mV ($n = 77$ cells from 15 preparations).

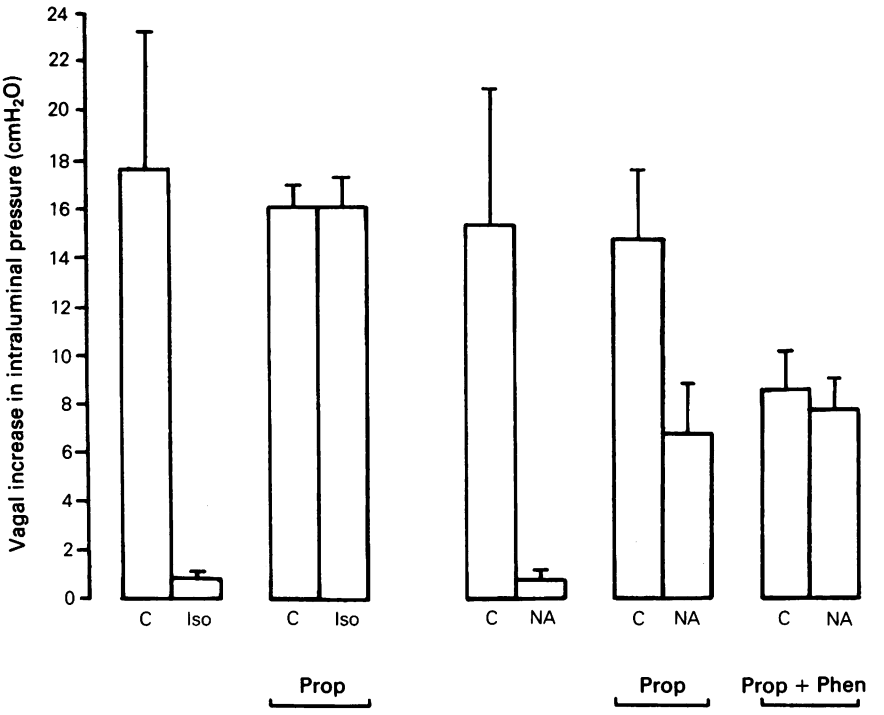

Figure 5 The effects of isoprenaline (Iso 10^{-6} M) and noradrenaline (NA; 10^{-5} M) on increases in intraluminal pressure induced by vagal stimulation in the guinea-pig isolated trachea. The left hand column of each pair represents the control (C) response to vagal stimulation and the right the response in the presence of isoprenaline or noradrenaline. Each column is the mean of 4 observations and vertical lines indicate s.e.mean. Propranolol (Prop), 3.5×10^{-6} M, and phentolamine (Phen), 4×10^{-6} M, were present where indicated.

Slow waves were recorded in some cells and had an amplitude of 4.3 ± 0.4 mV and frequency of 1.07 ± 0.13 Hz ($n = 24$). Isoprenaline, 10^{-6} M, induced hyperpolarization in 5 preparations (mean 13.8 ± 2.1 mV) but had no effect on transmembrane potential at this concentration in 2 preparations. Hyperpolarization was accompanied by the abolition of slow wave discharge after 3 to 4 min in spontaneously active preparations.

The effects of isoprenaline on vagally-mediated depolarization were examined in 5 cells and recordings made in 1 of these cells are shown in Figure 6. In this cell sympathetic stimulation evoked a hyperpolarization of 3 mV and vagal stimulation evoked a depolarization of 5.5 mV. On addition of isoprenaline, 10^{-6} M, there was a hyperpolarization beginning after 1 min and reaching a maximum of 13 mV after 3 min (dead space of system < 2 ml). Vagal depolarization was reduced in amplitude and virtually abolished after 5 min. Isoprenaline (10^{-6} M) had similar effects in all 5 cells studied and the mean amplitudes of the vagal responses before and in the presence of isoprenaline, respectively, were: 9.3 ± 2.2 mV and 1.6 ± 0.4 mV ($P < 0.05$).

It can be seen in Figure 6a that, at the peak of isoprenaline-induced hyperpolarization, sympathetic nerve stimulation was unable to evoke further hyperpolarization. When isoprenaline was washed out, the cell depolarized back to the control transmembrane potential over about 8 min and vagal depolarization increased gradually in amplitude, in this case to a final value slightly greater than control. At this point, sympathetic stimulation again elicited hyperpolarization (Figure 6b).

Isoprenaline, 10^{-6} M, was applied to 2 preparations in which vagal depolarizing responses had been enhanced by the addition of neostigmine, 5×10^{-8} M (McCaig, 1986a). Isoprenaline still induced hyperpolarization, abolished slow waves and greatly reduced vagal depolarization in the presence of neostigmine.

In 2 further preparations isoprenaline (10^{-6} M) was

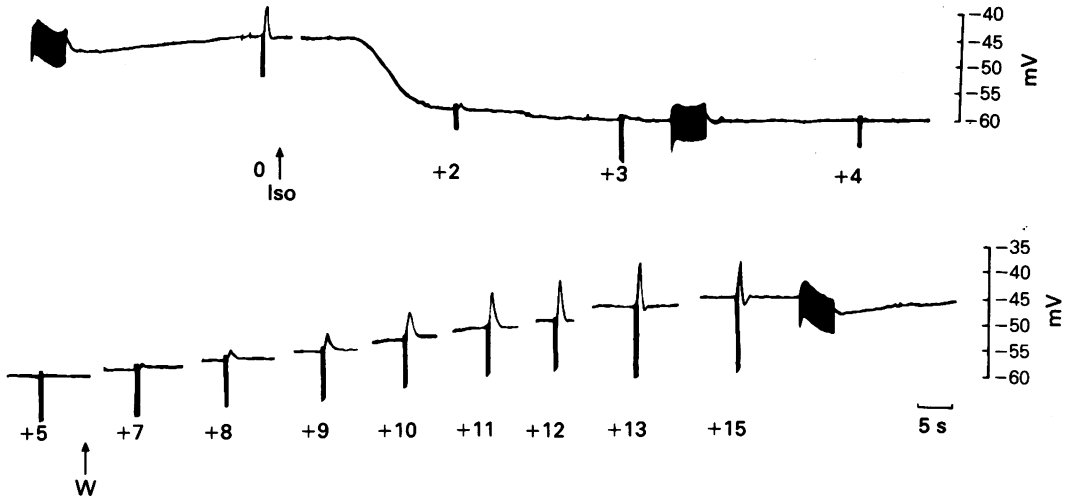

Figure 6 The effect of isoprenaline (Iso), 10^{-6} M, on transmembrane potential and responses to vagal and sympathetic stimulation in a single trachealis cell of the guinea-pig. The vagus nerve was stimulated with 16 pulses at 40 Hz (narrow downward deflections on record) and the stellate ganglion at 40 Hz for 5 s (broad black bands on record). Numbers on the records refer to the time in min after addition of isoprenaline to the reservoir of perfusing fluid, indicated by arrow in upper trace. Arrow in lower trace (W) indicates start of washout. Vagal depolarization was greatly reduced in the presence of isoprenaline but recovered quickly on washout.

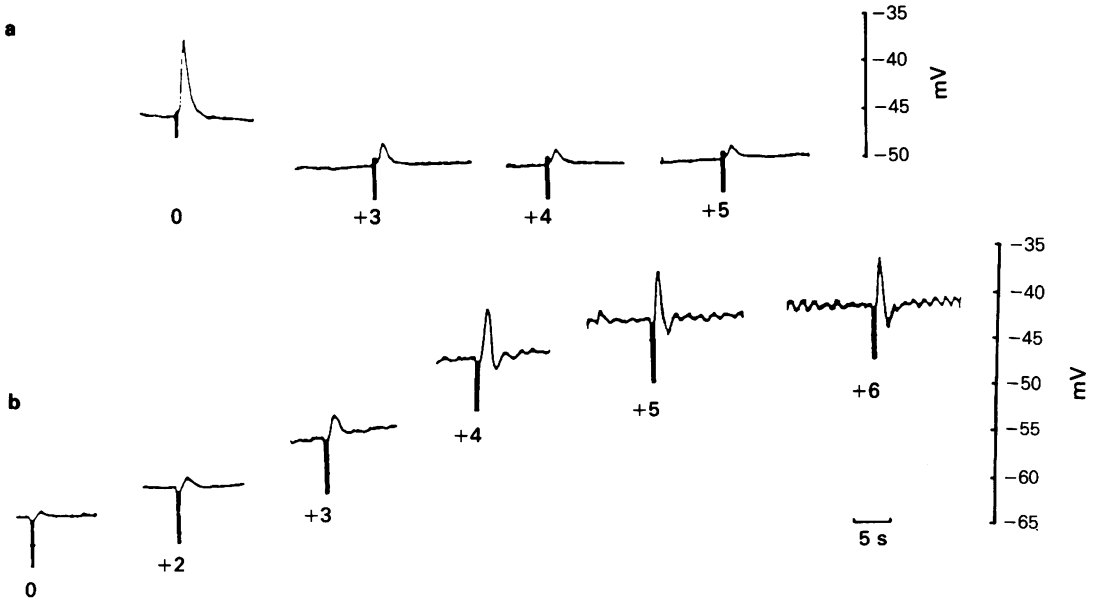

Figure 7 (a) The effect of noradrenaline, 10^{-5} M, on depolarizing responses induced by vagal stimulation in a single trachealis cell of the guinea-pig. The vagus nerve was stimulated with 16 pulses at 40 Hz (downward deflections of record). Numbers on the record refer to the time in min after addition of noradrenaline to the perfusing fluid. (b) The effect of removal of noradrenaline, 10^{-5} M, on depolarizing responses induced by vagal stimulation in a single trachealis cell. The vagus was stimulated with 16 pulses at 40 Hz and numbers on the record refer to the time in min from the start of washout of noradrenaline.

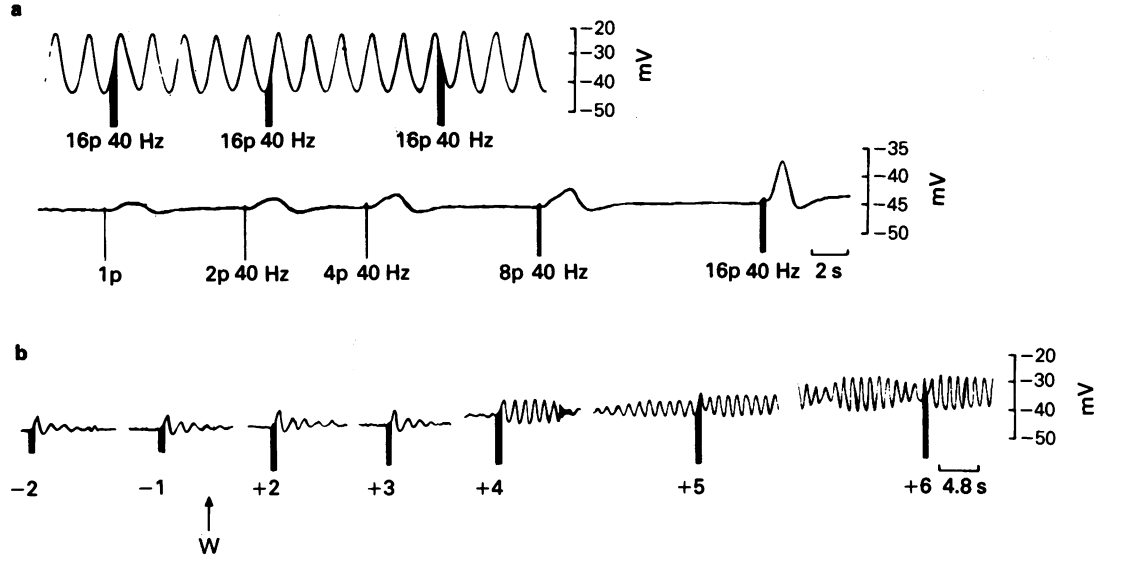

Figure 8 (a) Effects of vagal stimulation (numbers of pulses (p) and frequency (Hz) as indicated on record) in a spontaneously active trachealis cell from the guinea-pig before (upper trace) and 4 min after the addition of noradrenaline, 10^{-5} M (lower trace). In the presence of noradrenaline slow waves were suppressed and vagal depolarizing responses unmasked. (b) Effect of removal of noradrenaline (10^{-5} M), indicated by arrow (W), on depolarizing responses evoked by stimulation of the vagus nerve with 16 pulses at 40 Hz (downward deflections on record) in a single trachealis cell. In the presence of noradrenaline the cell was quiescent and vagal stimulation elicited a number of fluctuations in potential. On washout of noradrenaline, the cell gradually depolarized, slow wave discharge began and responses to vagal stimulation were masked. Numbers under the record refer to time in min from the start of washout.

administered 20 min after the addition of propranolol (3.5×10^{-6} M). In the presence of propranolol isoprenaline had no effect on transmembrane potential, slow wave discharge or vagal depolarization.

Noradrenaline, 10^{-6} M, had no effect on resting electrical properties or vagal responses in cells from 6 preparations. However, noradrenaline, 10^{-5} M evoked hyperpolarization (12.6 ± 1.4 mV, 8 cells from 8 preparations) and abolished slow wave discharge. As illustrated in Figure 7a, noradrenaline, 10^{-5} M, inhibited vagal depolarization (control: 7.8 ± 1.5 mV; in presence of noradrenaline: 3.0 ± 1.0 mV, $n = 6$; $P < 0.01$). In the cell shown in Figure 7b the effects of removal of noradrenaline are illustrated. There was a gradual depolarization and an increase in the amplitude of vagal depolarization over 6 min. Slow wave discharge commenced after 4 min in drug-free solution.

In a number of spontaneously active cells which were discharging slow waves of high amplitude, vagal stimulation was seemingly without effect, but when slow wave activity was inhibited by noradrenaline vagal responses could be discerned. An example of this effect is shown in Figure 8a. Before the application of noradrenaline, vagal stimulation had no obvious effect; during exposure to noradrenaline slow wave activity was suppressed and clear depolarizing responses were obtained to vagal stimulation. In the cell shown in Figure 8b, vagal stimulation caused a number of fluctuations in potential in a cell which was quiescent during exposure to noradrenaline. On removal of noradrenaline, the cell depolarized and began to discharge slow waves which masked the vagal response.

In 2 preparations, noradrenaline 10^{-5} M given in the presence of propranolol (3.5×10^{-6} M) and phentolamine (4×10^{-6} M) had no effect on resting electrical characteristics or vagal responses in trachealis cells.

Discussion

Stimulation of the sympathetic nerves could reduce but not abolish parasympathetic contractions in the guinea-pig isolated trachea, a finding similar to that obtained in canine airways *in vivo* (Cabezas *et al.*, 1971). A reduction in the parasympathetic response was obtained, however, in fewer than half of the preparations tested, which suggests that the degree of

sympathetic control of airway smooth muscle tone differs between guinea-pigs. A similar conclusion was reached by Douglas *et al.* (1973), who studied sympathetic modulation of responsiveness to histamine in guinea-pigs' airways *in vivo*. The airways of the guinea-pig, receive non-adrenergic, non-cholinergic (NANC) inhibitory innervation (Yip *et al.*, 1981). This pathway is present in most species studied including man (Richardson & Béland, 1976), baboon (Middendorf & Russell, 1980) and cat (Diamond & O'Donnell, 1980), but it is absent in the dog (Russell, 1980). Although NANC stimulation evokes smaller decreases in spontaneous tone than does sympathetic stimulation in guinea-pig trachea (McCaig, 1986b), the NANC system might be more effective in counteracting bronchoconstriction induced by parasympathetic stimulation. This possibility would be difficult to test directly since the NANC and parasympathetic fibres both run in the vagus nerve.

Exogenous noradrenaline and isoprenaline had a more pronounced inhibitory effect on responses to parasympathetic stimulation. Endogenous or exogenous catecholamines might inhibit cholinergic contractions through actions at one or more sites eg. (a) at the parasympathetic ganglia, to inhibit transmission, (b) at presynaptic cholinergic nerve terminals, to inhibit ACh release, (c) at the postsynaptic smooth muscle cell membrane or (d) at an intracellular site. The fact that neither endogenous (released by sympathetic nerve stimulation) nor applied noradrenaline or isoprenaline had any effect on responses to ACh strongly implies a presynaptic effect but does not distinguish between sites (a) and (b). Similarly, in canine trachea, noradrenaline and isoprenaline inhibited contractile responses to field stimulation to a far greater extent than those to applied ACh; since this inhibition was blocked by propranolol, it was suggested that cholinergic nerve terminals in the airways possess β-adrenoceptors which inhibit transmitter release (Ito & Tajima, 1982). In the guinea-pig trachea, the inhibition of vagal responses by isoprenaline was also blocked by propranolol, but inhibition by noradrenaline was blocked only by a combination of propranolol and phentolamine, suggesting the involvement of both β- and α-adrenoceptors. In keeping with this, Grundstrom *et al.* (1981) have shown that noradrenaline and adrenaline inhibit contractions to field stimulation in guinea-pig trachea (during β-adrenoceptor blockade), seemingly by acting at presynaptic α_2-adrenoceptors.

It is believed that ACh initiates contraction by three distinct mechanisms in smooth muscle (Bolton & Large, 1986): (1) by depolarizing the cell membrane, which leads to increased frequency of opening of voltage-operated Ca^{2+}-channels (VOCs); (2) by increasing frequency of opening of receptor-operated Ca^{2+}-channels (ROCs); (3) by promoting release of Ca^{2+} from intracellular stores. Noradrenaline or isoprenaline might inhibit vagally-mediated responses by interfering with one or more of these actions of ACh, but postsynaptic effects probably play a minor role in inhibition. Noradrenaline and isoprenaline apparently promote K^+-channel opening in the membrane of trachealis cells in the guinea-pig (Allen *et al.*, 1985), an effect which leads to hyperpolarization (this paper; Allen *et al.*, 1985; Honda *et al.*, 1986) and would be expected to reduce vagally-mediated depolarization and the influx of Ca^{2+} through VOCs. Hyperpolarization, however, was associated with a reduction in amplitude of vagal depolarization in some cells only, and in others it was unchanged or enhanced. This suggests that hyperpolarization and the promotion of K^+-channel opening is not of major importance in the inhibition of vagal responses. It is more likely that reductions in vagally-mediated depolarization by catecholamines can be explained in terms of presynaptic inhibition of ACh release. It has been suggested that β-adrenoceptor-mediated hyperpolarization plays a minor role in inhibition of spontaneous (as distinct from neurally-induced) tone in guinea-pig airways *in vitro* (Allen *et al.*, 1985; McCaig 1986a).

Catecholamines might inhibit vagal contractions by interfering with induced Ca^{2+} influx through either VOCs or ROCs or both. Noradrenaline and isoprenaline, for example, inhibit Ca^{2+} influx induced by exposure to high K^+-solution in rabbit aorta (Meisheri & van Breeman, 1982). In cat trachea, however, isoprenaline does not affect the influx of Ca^{2+}, but promotes sequestration of Ca^{2+} into intracellular storage sites (Ito & Itoh, 1984), an effect which might counteract ACh-induced release of stored Ca^{2+}. This suggests that there is a postsynaptic component to the inhibition of contractions induced by exogenous ACh in this tissue. However, responses to neurally-derived ACh were not studied, therefore a presynaptic effect cannot be ruled out.

Whilst exogenous noradrenaline or isoprenaline consistently inhibited vagal contractions, sympathetic stimulation inhibited vagal contractions to a lesser extent and in only half the tissues tested. Sympathetic stimulation had mixed effects also on vagally-mediated depolarization, whereas applied noradrenaline or isoprenaline consistently inhibited this. Differences in the effects of endogenous and exogenous noradrenaline could be due entirely to differences in their local concentration. Alternatively, noradrenaline released from sympathetic nerve terminals or applied to the bath may have access to different pools of adrenoceptors. Such a hypothesis has been put forward for arteriolar smooth muscle by Hurst & Nield (1980), who suggested that junctional and extrajunctional adrenoceptors are of different subtypes and effect contraction through different mechanisms. In guinea-pig trachea, activation of extrajunctional

adrenoceptors may be more effective in inhibiting vagal contractions. Since inhibition of vagal responses by noradrenaline seems to involve primarily a presynaptic effect, it is tempting to speculate that the adrenoceptors at either the parasympathetic ganglia or cholinergic nerve terminals (or both) are freely accessible to circulating noradrenaline, but in some preparations are remote from neurally-derived noradrenaline. Thus, it might be envisaged that in preparations where sympathetic stimulation effectively inhibits vagal contractions, the cholinergic and adrenergic nerve fibres are anatomically closer than in preparations in which sympathetic stimulation is ineffective.

This work was supported by a grant from the Scottish Home and Health Department.

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(Received November 1, 1986)
Revised February 2, 1987.
Accepted February 13, 1987.)