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Autonomic responses of the isolated, innervated trachea of the guinea-pig: interaction with autonomic drugs, histamine and 5-hydroxytryptamine

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1 Intraluminal pressure was measured in the isolated, fluid-filled trachea of the guinea pig, with autonomic innervation on the right side intact. Increases or decreases in intraluminal pressure reflected excitatory or inhibitory responses respectively, in the tracheal smooth muscle.

2 Stimulation of the cervical vagus nerve evoked a cholinergic excitatory response. After cholinergic blockade with atropine, a non-adrenergic, non-cholinergic inhibitory response was obtained.

3 Stimulation of the cervical sympathetic trunk, or stellate ganglion, evoked β-adrenergic inhibitory responses. In the presence of propranolol, sympathetic stimulation evoked α-adrenergic excitatory responses which were of low amplitude ($< 5\%$ of cholinergic excitatory responses). In the presence of phentolamine but not prazosin, β-adrenergic inhibitory responses were potentiated.

4 Neostigmine potentiated responses to vagal stimulation, increasing the amplitude and duration of response. At higher concentrations neostigmine (i) raised intraluminal pressure, a response blocked by atropine, and (ii) attenuated sympathetic inhibitory responses, an effect largely blocked by atropine.

5 Histamine increased intraluminal pressure and this response was attenuated by atropine. In the presence of histamine, vagal excitatory responses were attenuated. Sympathetic inhibitory responses at low frequencies of stimulation (up to 10 Hz) were inhibited by histamine.

6 5-Hydroxytryptamine (5-HT) increased intraluminal pressure also, an effect partially blocked by atropine. 5-HT had no effect on vagal excitatory responses. Like histamine, 5-HT attenuated sympathetic inhibitory responses at lower frequencies of stimulation.

Introduction

Autonomic control of the airways is achieved through at least three neural mechanisms, a cholinergic excitatory system and adrenergic and non-adrenergic, non-cholinergic (NANC) inhibitory systems (Widdicombe, 1963; Nadel, 1980; 1983). Blackman & McCaig (1983) recently described an innervated preparation of guinea-pig trachea in which mechanical responses to separate stimulation of parasympathetic and sympathetic nerves could be obtained in the completely isolated trachea in the absence of drugs. Whilst stimulation of the vagus nerve *in vivo* in the guinea-pig evokes NANC responses, during cholinergic blockade (Chesrown *et al.*, 1980), we were unable to elicit such responses *in vitro* other than by transmural stimulation. Subsequently, however, it was found that by increasing the intensity of stimulation, vagally-mediated NANC responses may be obtained in the isolated trachea. I describe here further studies on the autonomic responses of the trachea and the effects of drugs that modify autonomic function. The effects of histamine and 5-hydroxytryptamine (5-HT) have been examined, since both these drugs are known to induce airway hyperresponsiveness to vagal stimulation in the dog *in vivo* (Benson & Graf, 1977; Hahn *et al.*, 1978; Loring *et al.*, 1978; Sheller *et al.*, 1982; Kikuchi *et al.*, 1984).

Methods

Fifty-four guinea-pigs (male, approximately 400 g body weight) were killed by a blow to the head. The trachea was dissected together with the right vagus nerve and recurrent laryngeal branch and right cervical sympathetic trunk and stellate ganglion, as previously described (Blackman & McCaig, 1983).

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The preparation was mounted horizontally at its *in vivo* length in a tissue bath perfused with Krebs solution (2 ml min^{-1}), at 37°C equilibrated in the bath with 95% O_2: 5% CO_2 (Po_2 300 mmHg; Pco_2 33 mmHg; pH 7.36). The Krebs solution was of the following composition (mM): Na^+ 143.5, K^+ 5.9; Ca^{2+} 2.5, Mg^{2+} 1.0, Cl^- 121.2, SO_4^{2-} 1.0, $H_2PO_4^-$ 1.2, HCO_3^- 25.0 and glucose 11. The trachea was filled with Krebs solution which was replaced at approximately 30 min intervals. One end of the trachea was closed and the other connected to a pressure transducer (Statham) for recording intraluminal pressure (ILP). Increases or decreases in intraluminal pressure reflected smooth muscle contraction or relaxation, respectively.

The stellate ganglion was stimulated through a bipolar electrode placed at the rostral end of the gangion and located 1.5 cm from the trachea. Approximately 3 cm of the vagus nerve or sympathetic trunk was maintained and the terminal 0.5–1 cm drawn into a suction electrode the tip of which was 2 cm from the trachea. Trains of rectangular pulses of 40 V and 1 ms duration were applied to the nerves for 5 s from a stimulator (Grass, Model SD9, output impedence < 1 kohm). In order to evoke vagally-mediated NANC responses, pulse duration was increased to 2–4 ms and trains of pulses were applied for 30 s to 2 min. It is unlikely that these responses were due to intramural stimulation of NANC nerve endings since, in the absence of the vagal stump, no response could be elicited from the preparation, even when the electrode was placed in close apposition to the tissue. Effective transmural stimulation of intramural nerves could be achieved with a bipolar electrode with one pole running inside, the other outside, the tracheal lumen.

Drugs were added to the reservoir of Krebs solution and are expressed as final concentration in perfusing fluid. The following drugs were used: atropine sulphate (McGraw Ethical Ltd.); histamine acid phosphate (B.D.H.); 5-hydroxytryptamine creatinine sulphate (B.D.H.); neostigmine methylsulphate (SIGMA); phentolamine mesylate (CIBA); prazosin HCl (Pfizer) and propranolol HCl (I.C.I.).

Results

Tracheal responses

Four types of neurally-mediated responses could be recorded from the fluid-filled trachea and the frequency:response relationship for each is shown in Figure 1.

(i) *Cholinergic excitatory response* Stimulation of the cervical vagus nerve evoked increases in ILP. A single pulse elicited a measurable response and responses to 5 s train of 1 ms pulses increased in amplitude as

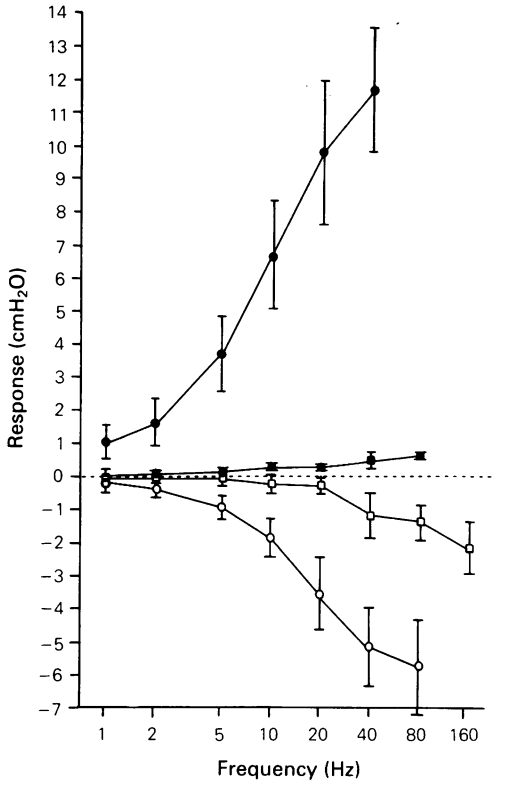

Figure 1 Frequency-response relationships for 4 types of response recorded from isolated, innervated trachea of guinea-pig. Results are means from 6 preparations with s.e.mean shown by vertical lines. (●) Stimulation of the cervical vagus nerve for 5 s with 1 ms pulses at frequencies indicated (no drugs); (○) stimulation of the stellate ganglion for 5 s with 1 ms pulses (no drugs); (■) stimulation of the stellate ganglion for 30 s with 1 ms pulses in the presence of propranolol (3.5×10^{-6}M); (□) stimulation of the cervical vagus for 30 s with 2–4 ms pulses in the presence of atropine (6×10^{-7}M) and propranolol (3.5×10^{-6}M). Resting intraluminal pressure 3.4 ± 0.3 cmH_2O.

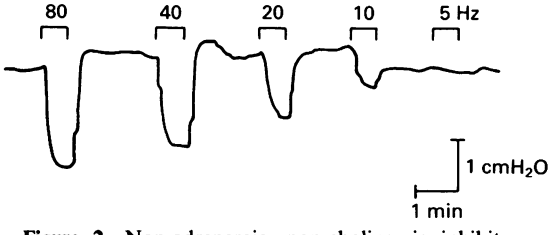

Figure 2 Non-adrenergic, non-cholinergic inhibitory responses recorded from guinea-pig trachea. Stimulation (indicated by bars) of vagus nerve with pulses of 40 V and 4 ms duration in the presence of atropine (6×10^{-7}M) and propranolol (3.5×10^{-6}M).

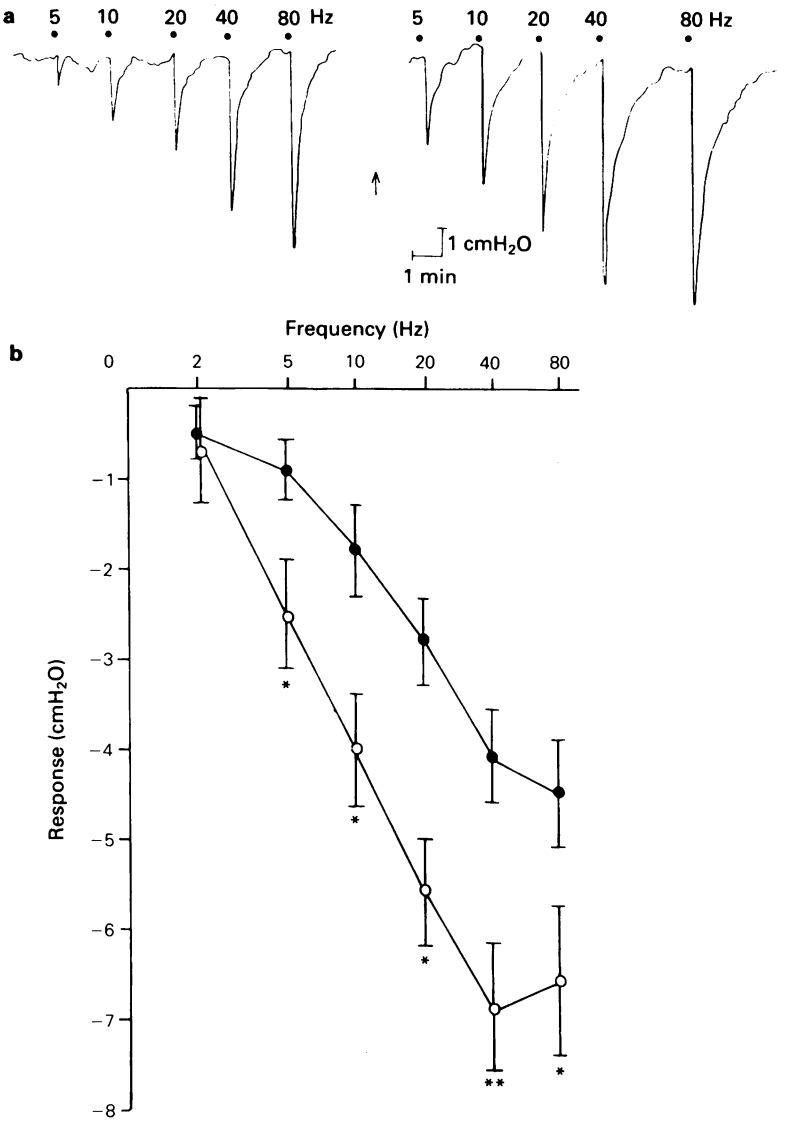

Figure 3 Effect of phentolamine, 4×10^{-6}M, on sympathetic inhibitory responses in guinea-pig isolated trachea. (a) Typical responses to stimulation of the stellate ganglion for 5 s with 1 ms pulses at frequencies indicated, before and 30 min after phentolamine (addition indicated by arrow). (b) Frequency-response relationship before (●) and 30 min after (○) phentolamine. Values are means of 6 experiments with s.e.mean shown by vertical lines. Asterisks denote values significantly different from controls (as gauged by paired t test) at $^{*}P < 0.05$ or $^{**}P < 0.02$.

frequency was increased, to a maximum at 40 Hz. Responses were blocked completely by atropine (6×10^{-7}M).

(ii) *β-Adrenergic inhibitory response* Stimulation of the cervical sympathetic trunk or stellate ganglion evoked decreases in ILP. In some preparations 2 pulses at 0.5 Hz elicited a response but often 5 pulses at 1 Hz were required to obtain a response. In a few preparations as many as 50 pulses at 10 Hz were required. Maximal responses were obtained with 5 s trains of pulses at 40–80 Hz. Responses were blocked completely by propranolol (3.5×10^{-6}M).

(iii) *α-Adrenergic excitatory response* Stimulation of the cervical sympathetic trunk or stellate ganglion

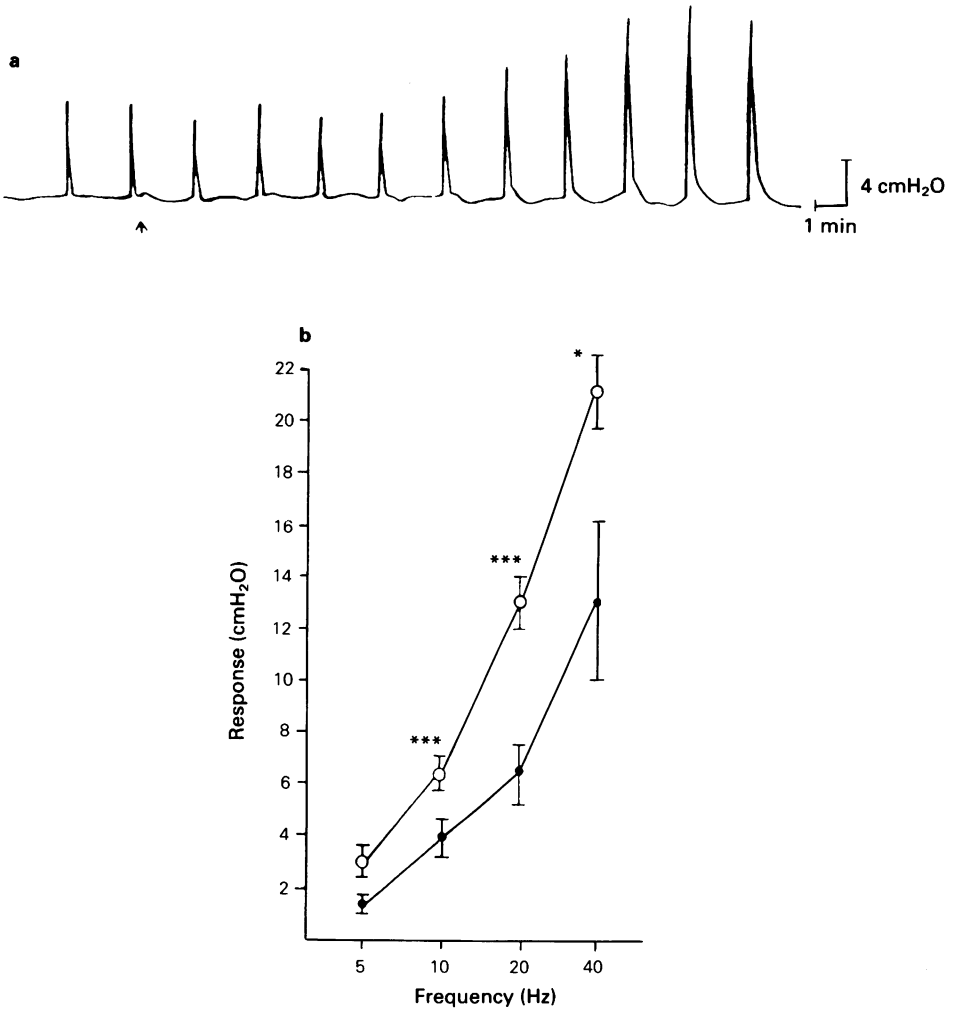

Figure 4 Effect of neostigmine, 5×10^{-8}M on vagal excitatory responses of guinea-pig isolated trachea. (a) Responses to vagal stimulation (for 5 s with 1 ms pulses at 20 Hz, every 2 min) before and after the addition of neostigmine (added to reservoir of Krebs solution at arrow). (b) Frequency-response relationship before (●) and 30 min after the addition of neostigmine (○). Values are means of 5 experiments with s.e.mean shown by vertical lines. Asterisks denote values significantly different from controls (as gauged by paired t test) at $^{*}P < 0.05$ or $^{***}P < 0.01$.

during β-adrenergic blockade with propranolol (3.5×10^{-6}M) evoked small excitatory responses. Sustained stimulation was required to elicit these responses. In some preparations a 30 s train of 1 ms pulses at 2 Hz was sufficient whilst in others a 30 s train at up to 20 Hz was needed to produce a response. Responses were blocked completely by phentolamine (4×10^{-6}M).

(iv) *Non-adrenergic, non-cholinergic inhibitory response* Stimulation of the cervical vagus nerve during cholinergic blockade with atropine, 6×10^{-7}M, evoked small decreases in ILP. Sustained stimulation with pulses of 2–4 ms duration were required for these responses. Responses were rarely discernible at stimulation frequencies below 5 Hz (for 30 s) and reached a maximum at 160 Hz (Figures 1 and 2). Responses were unaffected by propranolol at a concentration (3.5×10^{-6}M) which blocked sympathetic inhibitory responses, demonstrating that adrenergic

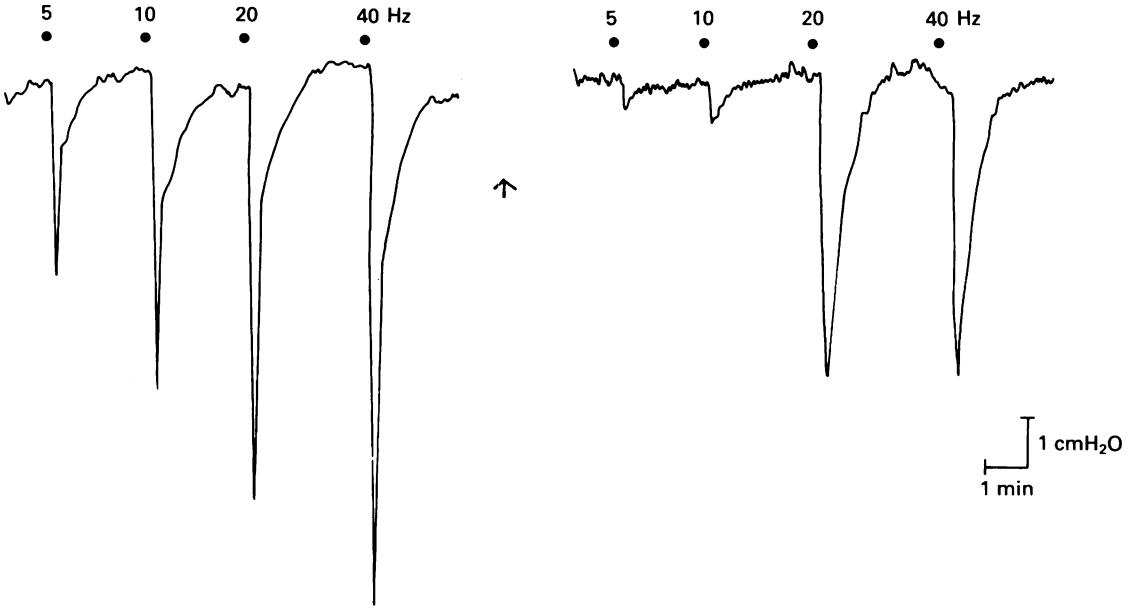

Figure 5 Sympathetic depressor responses obtained before and 40 min after addition of neostigmine 3×10^{-6}M (indicated by arrow) in the guinea-pig trachea. Base line intraluminal pressures pre- and post-neostigmine were 3.9 and 10.1 cmH_2O respectively. Stimulation of stellate ganglion indicated by dots.

neurones are not excited by cervical vagal stimulation.

Effect of cholinergic and adrenergic antagonists on tracheal responses

Preparations had a positive resting ILP (3.5 ± 0.2 cmH_2O, $n = 54$). Atropine (6×10^{-7}M) caused a slight, transient decrease in ILP, as previously described (Blackman & McCaig, 1983). Propranolol (3.5×10^{-6}M), phentolamine (4×10^{-6}M) and prazosin (10^{-6}M) had no effect on ILP.

Vagal excitatory responses were blocked by atropine (6×10^{-7}M) and were unaffected by propranolol (3.5×10^{-6}M) or prazosin (10^{-6}). Responses were reduced, however, in the presence of phentolamine (4×10^{-6}M, mean reduction $-58 \pm 4\%$ at frequencies of 5–40 Hz, $n = 6$).

Sympathetic inhibitory responses were blocked by propranolol (3.5×10^{-6}M) and unaffected by atropine (6×10^{-7}M). During α-adrenergic blockade with phentolamine (4×10^{-6}M) sympathetic inhibitory responses were potentiated (Figure 3) at frequencies of stimulation above 2 Hz. When specific α_1-adrenoceptor blockade was induced by prazosin (10^{-6}M), however, sympathetic inhibitory responses were unchanged from control (responses at 5, 10, 20 Hz respectively; control -1.7 ± 0.5; -3.1 ± 0.8; -4.1 ± 1.0 cmH_2O; prazosin -1.7 ± 0.5; -3.4 ± 0.9; -4.1 ± 1.0 cmH_2O (mean ± s.e.mean, $n = 6$).

Neostigmine

Neostigmine evoked concentration-related increases in ILP, with the threshhold for response between 1 and 3×10^{-7}M. The response developed slowly, peaking at 11 ± 1.8 min ($n = 8$) and then gradually subsiding. The increase in ILP was blocked by atropine (6×10^{-7}M, $n = 3$).

Neostigmine at a concentration of 5×10^{-8}M had no effect on ILP but potentiated vagal excitatory responses (Figure 4). At higher concentrations (3×10^{-7} to 3×10^{-6}M), neostigmine caused an increase in ILP but increased the amplitude of vagal responses only in some preparations whilst markedly increasing response duration.

At lower concentrations, neostigmine had no effect on sympathetic inhibitory responses, but at high concentrations (e.g. 3×10^{-6}M) these responses were substantially attenuated at all frequencies of stimulation (Figure 5) with a mean reduction of $-65 \pm 5\%$ ($n = 7$). At this concentration neostigmine raised ILP by 12.5 ± 3.2 cmH_2O ($n = 7$). Inhibition of sympathetic responses was reduced considerably when neostigmine was given in the presence of atropine (6×10^{-7}M), with a mean decrease of $-25 \pm 8\%$ ($n = 3$).

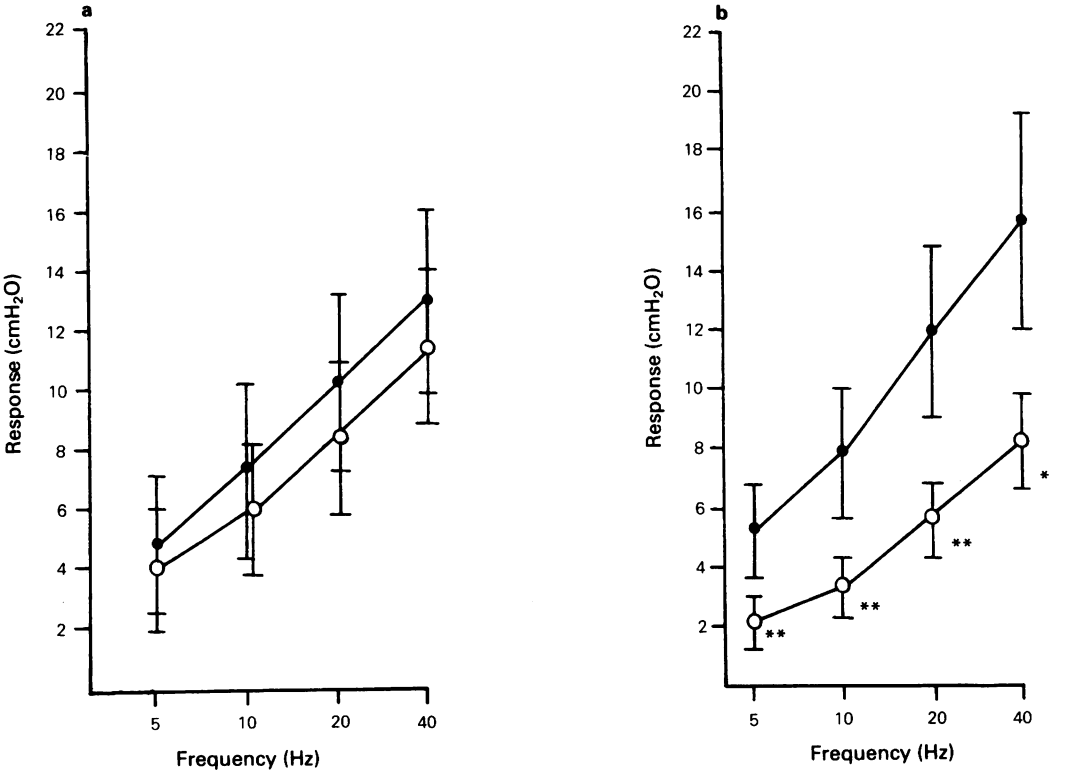

Figure 6 Effect of histamine on vagal excitatory responses in guinea-pig isolated trachea. (a) Responses before (●) and in the presence of histamine 10^{-6}M (○). Values are means of 5 experiments with s.e.mean shown by vertical lines. Baseline intraluminal pressure: control 4.4 ± 0.6 cmH_2O; histamine 5.9 ± 0.6 cmH_2O. (b) Responses before (●) and in the presence of histamine 2×10^{-5}M (○). Values are means of 6 experiments with s.e.mean shown by vertical lines. Asterisks denote values significantly different from controls (as gauged by paired t test) at $*P < 0.05$ or $**P < 0.02$. Baseline intraluminal pressure: control 3.8 ± 0.7 cmH_2O; histamine 17.3 ± 2.8 cmH_2O.

Histamine

Histamine caused concentration-dependent increases in ILP with threshold at approximately 10^{-6}M. At low concentrations responses peaked in 5 min then there was a gradual return to baseline by 15 min. At higher concentrations (e.g. 2×10^{-5}M) the peak response was reached sooner (2 min) and the response lasted longer (response 60–70% of maximum at 40 min, histamine present throughout). The increase in ILP induced by histamine could be partly blocked by atropine (6×10^{-7}M (−31 ± 10%, $n = 5$).

Two concentrations of histamine, 10^{-6}M and 2×10^{-5}M, were selected to study the interactions between the drug and autonomic responses. In the presence of histamine, 10^{-6}M, there was no significant difference in vagally-induced increases in ILP (Figure 6a). In the presence of histamine, 2×10^{-5}M, however, vagal excitatory responses were significantly smaller than controls at each frequency of stimulation (5–40 Hz, Figure 6b). At this concentration, histamine increased ILP by + 13.5 ± 2.7 cmH_2O ($n = 6$).

Sympathetic inhibitory responses were unaltered in the presence of histamine, 10^{-6}M. At the higher concentration (2×10^{-5}M) histamine had a dual effect on responses, depending on the frequency of sympathetic stimulation. The response to stimulation at 5 Hz was reduced substantially in each of 8 preparations, at 10 Hz responses were reduced in 5/9, unchanged in 2/9 and increased in 2/9 preparations and at 20–80 Hz responses were increased in all preparations (Figure 7a and 8a).

5-Hydroxytryptamine

5-HT induced concentration-dependent increases in

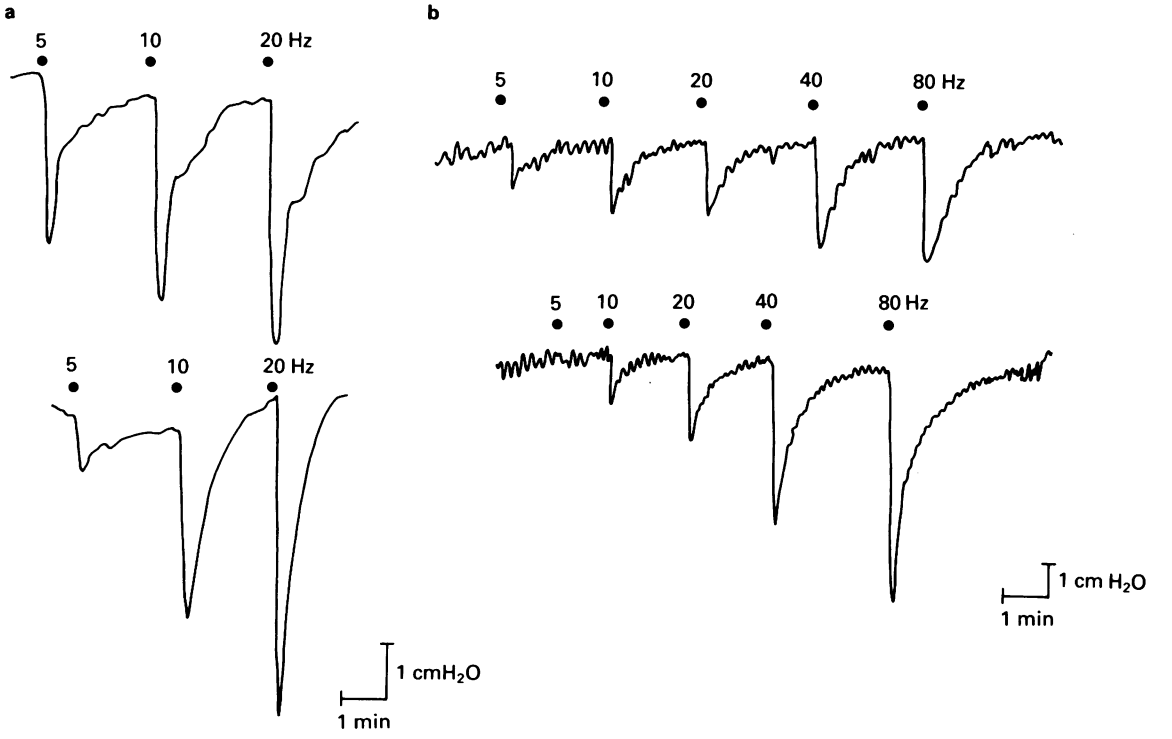

Figure 7 Responses to stimulation of the stellate ganglion (indicated by dots): (a) before (upper trace) and 30 min after (lower trace) histamine 2×10^{-5}M. Baseline intraluminal pressure: control 4.4 cmH_2O; after histamine 12.4 cmH_2O: (b) before (upper trace) and 30 min after (lower trace) 5-hydroxytryptamine (5-HT) 2×10^{-6}M. Baseline intraluminal pressure: control 2.9 cmH_2O; after 5-HT, 2.9 cmH_2O.

ILP. The threshold for this effect was approximately 10^{-7}M. The response developed over 3 min but was not maintained in the continued presence of the drug and ILP was restored close to control levels within 15 min. In 4/6 preparations the response was reduced in the presence of atropine (6×10^{-7}M) by a mean of $59 \pm 13\%$ but in the other two preparations the response was unaltered.

Vagal excitatory responses were unaltered in the presence of 5-HT (10^{-7} to 5×10^{-6}M); 15 min after the addition of 5-HT, baseline ILP was changed by small amounts only, and the amplitudes of vagal responses were unchanged from control.

5-HT, at a concentration of 10^{-6}M, had no effect on sympathetic inhibitory responses but at 2×10^{-6}M, effects similar to those of histamine were observed (Figure 7b and 8b). Sympathetic responses to stimulation at 5 Hz were attenuated, at 10 Hz responses were reduced in 5/9, unchanged in 1/9 and increased in 3/9 preparations and at 20–80 Hz responses were increased in all preparations.

Discussion

Cholinergic excitatory and adrenergic inhibitory responses are readily obtained in this preparation by stimulation of the cervical vagus or sympathetic nerves, respectively. During cholinergic blockade, NANC inhibitory responses can be elicited by vagal stimulation and direct comparison of adrenergic and non-adrenergic inhibition in the same preparation can be made. It is not possible, however, to stimulate only NANC fibres in the vagus, hence NANC responses cannot be evoked selectively in the absence of cholinergic blockade. The relative importance of adrenergic and non-adrenergic inhibitory systems in the regulation of airway smooth muscle has yet to be established. Non-adrenergic inhibition has been demonstrated in most species studied including guinea-pig (Coburn & Tomita, 1973); cat (Irvine *et al.*, 1980); baboon (Middendorf & Russell, 1978) and man (Richardson & Beland, 1976) but with the notable exception of the dog (Russell, 1980).

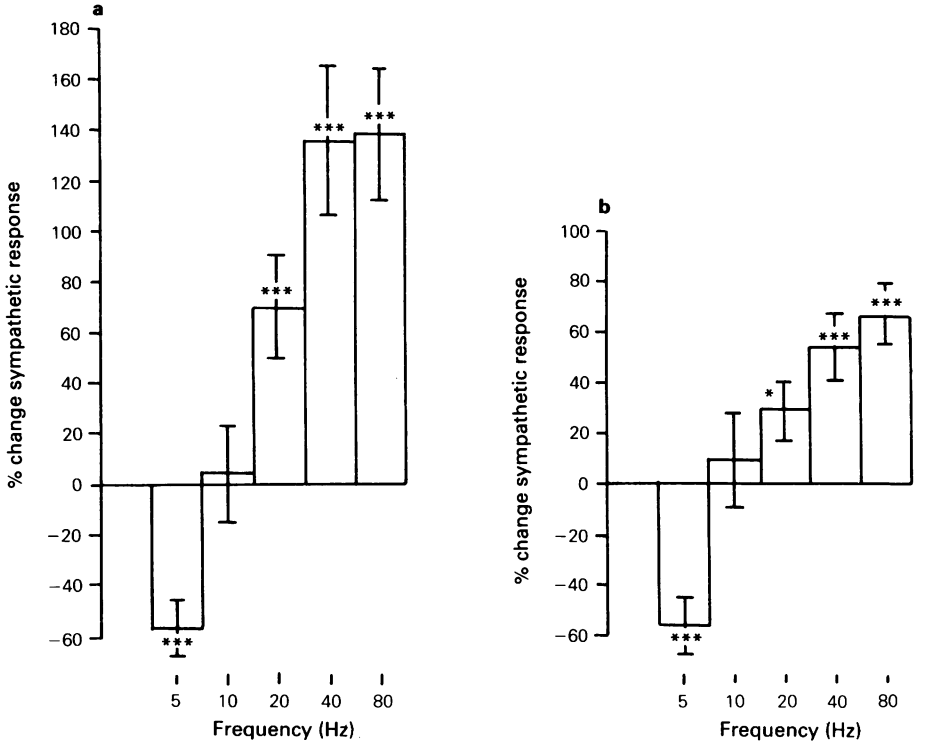

Figure 8 Changes in sympathetic inhibitory responses induced by histamine or 5-hydroxytryptamine (5-HT) in guinea-pig isolated trachea: (a) % change in sympathetic responses 30 min after histamine 2×10^{-5}M. Values are means of 7–9 observations with s.e.mean shown by vertical lines. Asterisks denote statistically significant changes at $*P < 0.05$ or $***P < 0.01$ (as gauged by t test). (b) % change in sympathetic responses 30 min after 5-HT 2×10^{-6}M. Values are means of 6–9 observations with s.e.mean shown by vertical lines. Statistical significance as in (a).

Vagal excitatory responses in the trachea were potentiated by neostigmine, as predicted from its anticholinesterase activity. At concentrations above those required for potentiation, neostigmine evoked an excitatory response which was blocked by atropine, and was due evidently to activation of cholinergic muscarinic receptors. It has been shown that this bronchoconstrictor action of neostigmine is attributable to release of acetylcholine (ACh), an effect independent of its anticholinesterase properties (Carlyle, 1963; Kirkpatrick & Rooney, 1979). Sympathetic inhibitory responses were attenuated in the presence of neostigmine in concentrations sufficient to induce an increase in intraluminal pressure. It is likely that inhibition of sympathetic responses was due to the release of ACh and its accumulation during cholinesterase inhibition. In keeping with this view, inhibition of sympathetic responses by neostigmine was much reduced during cholinoceptor blockade with atropine. ACh, both endogenous and exogenous has been shown to reduce the efflux of noradrenaline during electrical stimulation of airways of the rabbit or dog (Mathé *et al.*, 1977; Russell & Bartlett, 1981) and thus may inhibit sympathetic responses through both pre- and postsynaptic mechanisms.

β-Adrenergic depressor responses were potentiated during α-adrenoceptor blockade with phentolamine. This could be due to removal of the opposing effects of excitatory α-adrenoceptors on the smooth muscle or removal of feedback inhibition of noradrenaline release by blockade of presynaptic α-adrenoceptors (Westfall, 1977). α-Adrenergic excitatory responses could be obtained by sympathetic stimulation during β-adrenoceptor blockade with propranolol but responses were very small, suggesting that the population of α-receptors on the smooth muscle is small, or that noradrenaline released from adrenergic nerve terminals has restricted access to α-receptors. It seems more likely that potentiation is due to the removal of feedback inhibition. In keeping with this, potentiation showed frequency-dependence, occurring at stimulation at 5 Hz or above, but not at 2 Hz. In addition,

there was no potentiation of β-adrenergic responses after treatment with the specific α_1-adrenoceptor antagonist, prazosin. The reduction in vagal excitatory responses by phentolamine was an unexpected result which is difficult to explain and may be independent of the α-adrenoceptor blocking action of the drug.

The bronchoconstrictor action of drugs such as histamine and 5-HT is complex. Constriction may be elicited by a direct action on the airway smooth muscle, whilst stimulation of irritant receptors in the airways may lead to reflex bronchoconstriction, mediated by the vagus (see Nadel, 1980). In addition, both histamine and 5-HT facilitate ganglionic transmission (Trendelenburg, 1956; Wallis & Woodward, 1974) so may have effects on the tracheal ganglia. When exposure to histamine or 5-HT is combined with vagal stimulation in the dog, the resulting bronchoconstriction is supra-additive (Benson & Graf, 1977; Loring *et al.*, 1978; Hahn *et al.*, 1978; Sheller *et al.*, 1982; Kikuchi *et al.*, 1984). This may be a purely mechanical effect of changing the resting tone of the airways (Benson & Graf, 1977; Loring *et al.*, 1978). Alternatively, potentiation could be due to interactions between the drugs and cholinergic mechanisms, at either the pre- or postsynaptic level. In the dog, 5-HT did not potentiate responses to exogenous ACh, suggesting an effect on pre-synaptic vagal pathways (Sheller *et al.*, 1982). It has been reported that histamine potentiated responses to vagal stimulation in guinea-pig trachea (Douglas *et al.*, 1972) but responses to methacholine were potentiated also, indicating a postsynaptic effect. In the present experiments there was no evidence of potentiation of vagal responses by histamine or 5-HT in the guinea-pig trachea. In fact, vagal responses were attenuated in the presence of histamine. It is difficult to compare these results with those of Douglas *et al.* (1972), since details of their experiments were not reported. They used a subthreshold dose of histamine, but in the present experiments there was no potentiation with sub- or suprathreshold doses of histamine.

Histamine and 5-HT had dual effects on sympathetic depressor responses, attenuating responses at lower frequencies and potentiating responses at higher frequencies of sympathetic stimulation. Potentiation could be due simply to the increase in tone of the preparation by the drugs, but inhibition is harder to explain. Inhibition was apparent at frequencies of sympathetic stimulation (up to 10 Hz) that are close to rates of firing found *in vivo* in efferent sympathetic fibres in the airways of the dog or cat (Widdicombe, 1966). It has been shown that histamine can inhibit sympathetic contractions of dog saphenous vein and tibial artery (McGrath & Shepherd, 1976), seemingly by reducing the output of noradrenaline from sympathetic nerve terminals during electrical stimulation. There may be a similar effect at sympathetic nerve terminals in the guinea-pig trachea.

Sympathetic responses seem to be rather sensitive to external influences. The present findings show inhibition in the presence of neostigmine and during exposure to histamine or 5-HT. Previously, it was shown that sympathetic responses were reduced in the presence of spontaneous mechanical activity in the trachea, or during hypoxia (Blackman & McCaig, 1983). Modulation of sympathetic responsiveness by one or more of these factors could result in a reduced capacity to counteract bronchospasm, for example during acute asthmatic episodes.

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Reference

BENSON, M.K. & GRAF, P.D. (1977). Bronchial reactivity: interaction between vagal stimulation and inhaled histamine. *J. appl. Physiol.*, **43**, 643–647.

BLACKMAN, J.G. & McCAIG, D.J. (1983). Studies on an isolated innervated preparation of guinea-pig trachea. *Br. J. Pharmac.*, **80**, 703–710.

CARLYLE, R.F. (1963). The mode of action of neostigmine and physostigmine on the guinea-pig trachealis muscle. *Br. J. Pharmac.*, **21**, 137–149.

CHESROWN, S.E., VENUGOPALAN, C.S., GOLD, W.M. & DRAZEN, J.M. (1980). *In vivo* demonstration of non-adrenergic inhibitory innervation of the guinea-pig trachea. *J. clin. Invest.*, **65**, 314–320.

COBURN, R.F. & TOMITA, T. (1973). Evidence for non-adrenergic inhibitory nerves in the guinea-pig trachealis muscle. *Am. J. Physiol.*, **224**, 1072–1080.

DOUGLAS, J.S., HELGERSON, R.B. & BOUHUYS, A. (1972). Interaction of humoral agents and regulation of airway smooth muscle responses. *Fedn Proc.*, **31**, 336.

HAHN, H.L., WILSON, A.G., GRAF, P.D., FISCHER, S.P. & NADEL, J.A. (1978). Interaction between serotonin and efferent vagus nerves in dog lungs. *J. appl. Physiol.*, **44**, 144–149.

IRVINE, C.G., BOILEAU, R., TREMBLAY, J., MARTIN, R.R. & MACKLEM, P.T. (1980). Bronchodilatation: non-cholinergic, non-adrenergic mediation demonstrated *in vivo* in the cat. *Science*, **207**, 791–792.

KIKUCHI, Y., OKAYAMA, H., OKAYAMA, M., SASAKI, H. & TAKISHIMA, T. (1984). Interaction between histamine and vagal stimulation on tracheal smooth muscle in dogs. *J. appl. Physiol.*, **56**, 590–595.

KIRKPATRICK, C.T. & ROONEY, P.J. (1979). The contracture produced in tracheal smooth muscle by anticholinesterases. *Br. J. Pharmac.*, **67**, 464P.

LORING, S.H., DRAZEN, J.M., SNAPPER, J.R. & INGRAM, R.H. (1978). Vagal and aerosol histamine interactions on airway responses in dogs. *J. appl. Physiol.*, **45,** 40–44.

MATHÉ, A.A., TONG, E.Y. & TISHER, P.W. (1977). Norepinephrine release from the lung by sympathetic nerve stimulation inhibition by vagus and methacholine. *Life Sci.*, **20,** 1425–1430.

McGRATH, M.A. & SHEPHERD, J.T. (1976). Inhibition of adrenergic neurotransmission in canine vascular smooth muscle by histamine. *Circulation Res.*, **39,** 566–573.

MIDDENDORF, W.F. & RUSSELL, J.A. (1978). Innervation of tracheal smooth muscle in baboons. *Fedn Proc.*, **37,** 553.

NADEL, J.A. (1980). Autonomic regulation of airway smooth muscle. In *Physiology and Pharmacology of the Airways.* ed. Nadel, J.A. pp. 217–257. New York: Marcel Dekker.

NADEL, J.A. (1983). Bronchial reactivity. *Adv. Int. Med.*, **28,** 207–223.

RICHARDSON, J. & BELAND, J. (1976). Non-adrenergic inhibitory nervous system in human airways. *J. appl. Physiol.*, **41,** 764–771.

RUSSELL, J.A. (1980). Innervation of airway smooth muscle in the dog. *Bull. Eur. Physiopath. Resp.*, **16,** 671–692.

RUSSELL, J.A. & BARTLETT, S. (1981). Adrenergic neurotransmission in airways: inhibition by acetylcholine. *J. appl. Physiol.*, **51,** 376–383.

SHELLER, J.R., HOLTZMAN, M.J., SKOOGH, B. & NADEL, J.A. (1982). Interaction of serotonin with vagal- and ACh-induced bronchoconstriction in canine lungs. *J. appl. Physiol.*, **52,** 964–966.

TRENDELENBURG, U. (1956). Modification of transmission through the superior cervical ganglion of the cat. *J. Physiol.*, **132,** 529–541.

WALLIS, D.I. & WOODWARD, B. (1974). The facilitatory actions of 5-hydroxytryptamine and bradykinin in the superior cervical ganglion of the rabbit. *Br. J. Pharmac.*, **51,** 521–531.

WESTFALL, T.C. (1977). Local regulation of adrenergic neurotransmission. *Physiol. Rev.*, **57,** 659–728.

WIDDICOMBE, J.G. (1963). Regulation of tracheobronchial smooth muscle. *Physiol. Rev.*, **43,** 1–37.

WIDDICOMBE, J.G. (1966). Action potentials in parasympathetic and sympathetic efferent fibres to the trachea and lungs of dogs and cats. *J. Physiol.*, **186,** 56–88.

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