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Studies on an isolated innervated preparation of guinea-pig trachea

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1 An isolated preparation of the guinea-pig trachea with intact parasympathetic and sympathetic innervation has been devised. Responses to nerve stimulation were recorded as increases or decreases in intraluminal pressure from the fluid-filled trachea.

2 The preparation maintained a positive resting intraluminal pressure of 3–4 cmH_2O. This was unaffected by atropine, hexamethonium or propranolol.

3 Brief pressor responses, which could be completely blocked by atropine or hexamethonium, were obtained by applying short trains of stimuli to the cervical segment of the right vagus. The amplitude of responses was frequency-dependent up to a maximum at 40 Hz.

4 Depressor responses, more delayed and prolonged than the pressor responses and blocked by propranolol but not by hexamethonium, were obtained by stimulation of the right cervical sympathetic trunk or stellate ganglion in 70% of preparations. Dual pressor-depressor responses were observed in the remaining 30% of preparations. The pressor component was blocked by atropine, the depressor component by propranolol.

5 In the presence of atropine and propranolol, sustained sympathetic stimulation sometimes evoked a small, delayed pressor response which was blocked by phentolamine.

6 Under the same conditions, transmural stimulation produced a depressor response evidently due to non-adrenergic, non-cholinergic nerves.

7 Spontaneous activity was observed in some preparations under normal conditions, but could always be evoked by hypoxia. Responses to sympathetic stimulation were reduced both by hypoxia and during periods of spontaneous activity.

8 The principal advantage of this preparation is that it permits both excitatory and inhibitory responses to be elicited by stimulation of vagal and sympathetic nerves separately in the isolated trachea in the absence of agonist and antagonist drugs.

Introduction

The neural control of airway calibre is incompletely understood (Widdicombe, 1963; Staub, 1975; Nadel, 1980). Whilst a vagal excitatory input to airways smooth muscle has been clearly demonstrated, it has been more difficult to characterize a sympathetic inhibitory input; responses to sympathetic stimulation are very variable and depend not only on the species and the segment of the airways used, but also on the resting tone of the preparation (see Nadel, 1980; Russell, 1980; Zorychta & Richardson, 1980). A non-cholinergic, non-adrenergic inhibitory system has been demonstrated in a variety of species, in some of which it may represent the dominant or only inhibitory pathway (Coburn & Tomita, 1973; Richardson & Beland, 1976; Middendorf & Russell, 1978; Diamond & O'Donnell, 1980). This inhibitory mechanism can be activated *in vivo* by vagal stimulation (Chesrown, Venugopalan, Gold & Drazen, 1980; Diamond & O'Donnell, 1980; Irvin, Boileau, Tremblay, Martin & Macklem, 1980).

Responses of airway smooth muscle to autonomic stimulation are commonly studied *in vitro* with isolated airway segments in which the nerve endings are excited by transmural stimulation (Suzuki, Morita & Kiruyama, 1976; Doidge & Satchell, 1982; Ito & Takeda, 1982; Souhrada & Kivity, 1982). A disadvantage of this method is that all nerve endings within the tissue are stimulated simultaneously and different

components of the response may be distinguished only by the use of appropriate antagonist and agonist drugs.

Our aim has been to devise a preparation in which parasympathetic and sympathetic tracheal responses could be evoked separately in the absence of drugs. We describe here such a preparation of the guinea-pig trachea and the types of response which it gives. A preliminary account of the preparation has been previously published (McCaig, Wisniewski & Blackman, 1982).

Methods

For the first stages of dissection of most of the preparations described in this paper, guinea-pigs (male 400–600 g body wt) were anaesthetized with pentobarbitone (40 mg kg^{-1} i.p.). In later experiments, however, guinea-pigs were killed at the beginning with a blow to the head. The cervical trachea, with right recurrent laryngeal nerve carefully maintained intact, was exposed and freed along with the right vagal and sympathetic trunks, and cannulated below the cricoid cartilage. The chest was then opened and the trachea flushed at 10 min intervals through a cut in one bronchus with oxygenated Krebs solution at room temperature. The vagus was then freed and cut below the recurrent laryngeal branch and the sympathetic system dissected down to the stellate ganglion. Connections between the vagal and sympathetic trunks in the thorax were preserved (Figure 1). The thoracic end of the trachea was cannulated and the preparation removed and mounted horizontally at its *in vivo* length in a tissue bath perfused with Krebs solution (2 ml min^{-1}) of the following composition (mM): Na^+ 143.5, K^+ 5.9, Ca^{2+} 2.0, Mg^{2+} 1.0, Cl^- 127.2, SO_4^{2-} 1.0, $H_2PO_4^-$ 1.2, HCO_3^- 25.0, glucose 11, equilibrated in the bath with 95% O_2 and 5% CO_2 at 37°C. The lumen was filled with the same solution (replaced at 30 min intervals) and closed for recording intraluminal pressure. The time taken to complete a dissection and set up the preparation was 30–40 min. Nerves were stimulated with suction or bipolar electrodes and rectangular supramaximal pulses of 1 ms duration. Bipolar electrodes were used for transmural stimulation. Results are given as the mean ± s.e.mean.

Drugs were added to the reservoir of the perfusing solution. Drugs used were atropine sulphate (McGraw Ethical Ltd), hexamethonium methosulphate, phentolamine mesylate (Ciba) and propranolol HCl (I.C.I.).

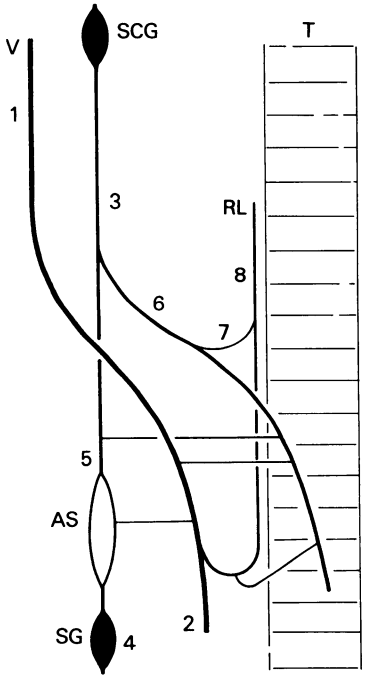

Figure 1 Schematic representation of the trachea (T) with innervation on the right side: ansa subclavia (AS), recurrent laryngeal nerve (RL), superior cervical ganglion (SCG), stellate ganglion (SG), vagus (V). Numbers show sites of stimulation referred to in text.

Results

Figure 1 shows the anatomical arrangement of the nerve pathways studied. These pathways and the connections shown are those which could be observed with ease with a dissecting microscope. Other smaller connections which were observed inconsistently, are not shown.

Intraluminal resting pressure

The resting intraluminal pressure observed 30 min after setting up the preparation was 3.4 ± 0.2 cmH_2O ($n = 30$) and was well maintained. It was readily raised or lowered by introducing or withdrawing fluid, but in many preparations the change in pressure was not sustained and the pressure gradually returned over about 30 min to the original resting value.

Atropine (6×10^{-7} M) in the bathing solution caused the resting intraluminal pressure to fall (mean change -0.9 ± 0.2 cmH_2O) in about 2 min, but within about 10 min the pressure had returned to normal. Hexamethonium (2.4×10^{-4} M) had no effect on resting intraluminal pressure and propranolol

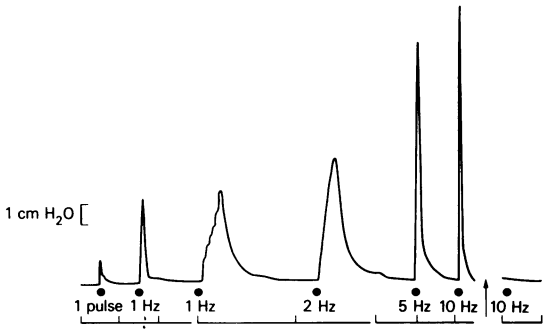

Figure 2 Typical changes in intraluminal pressure of isolated trachea evoked by stimulation of the right vagus for 5 s at the frequencies indicated. Responses were completely blocked 20 min after addition of atropine (6×10^{-7} M) at arrow. Note 3-fold increase in chart speed during recording of third and fourth responses. Time divisions 1 min.

(3.5×10^{-6} M) had no consistent effect, small increases or decreases or no change being observed.

Most preparations at some time during an experiment exhibited spontaneous fluctuations in pressure. This spontaneous activity took the form of fairly regular changes of low amplitude (1 ± 0.2 cm H_2O peak-to-peak) and of frequency about 6 min^{-1}. Occasionally, especially in the early experiments, the spontaneous activity was of sufficiently large amplitude to make it difficult to observe responses to nerve stimulation. Such activity was avoided by regularly flushing the trachea with oxygenated Krebs solution at room temperature during the period of dissection. The incidence of spontaneous activity was similar in guinea-pigs anaesthetized with pentobarbitone or killed by a blow to the head at the beginning of the dissection.

Spontaneous activity was blocked in 3 out of 6 preparations exposed to atropine (6×10^{-7} M) but was unaffected by atropine (up to 2.4×10^{-6} M) in the other 3 preparations.

Responses to stimulation of the vagus

A pure pressor response was evoked in most preparations when the right vagus at the cervical level (position 1 in Figure 1) was stimulated for a period of 5 s at any frequency from 0.2 to 80 Hz. Pressor responses were rapid in onset and of brief duration (onset time less than 0.5 s, time-to-peak 4.8 ± 0.2 s, half-width 9.3 ± 1.1 s). A single pulse elicited a measurable pressor response. Responses to the separate pulses of a train of stimuli were discernible at frequencies of 1 Hz or less whereas at higher frequencies a smooth increase in pressure occured (Figure 2).

The magnitude of pressor responses to brief (5 s) trains of stimuli increased steeply with frequency up to 20 Hz, the maximum response being reached at 40 Hz (Figure 3). Pressor responses were highly reproducible and there was no evident deterioration in amplitude or duration even when maximal responses to trains of stimuli were evoked repeatedly (e.g. 15 times) at 1.5 min intervals. Pressor responses were completely blocked by atropine (6×10^{-7} M, Figure 2), or by hexamethonium (2.4×10^{-4} M).

In fewer than 15% of preparations and only at stimulus frequencies greater than 10 Hz, the pressor response was followed by a small depressor response. This was blocked by atropine but not by propranolol (3.5×10^{-6} M).

Stimulation of the thoracic vagus (position 2 in Figure 1) evoked a pressor followed by a depressor response (dual response). Only the pressor phase of such dual responses was blocked by atropine (6×10^{-7} M); the depressor phase was blocked by propranolol (3.5×10^{-6} M). Thus responses to stimulation of the vagus in this region appear to be mediated by both cholinergic and adrenergic nerve fibres.

Sustained stimulation of the vagus

Stimulation of the cervical vagus for 2 min at frequencies of up to 10 Hz evoked sustained pressor responses (Figure 4). At higher frequencies the pressor response was not maintained at the level initially reached. In either case, when stimulation was stopped, the intraluminal pressure often fell below and then gradually returned to the initial resting level

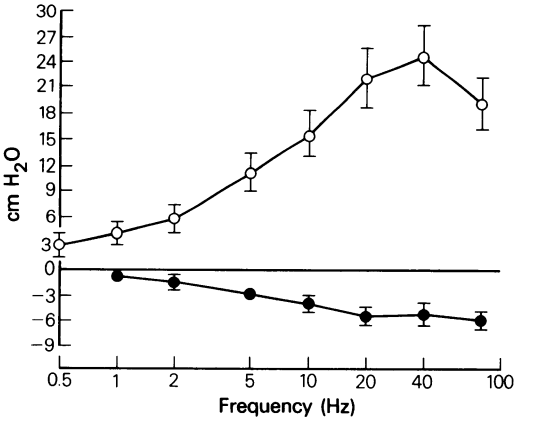

Figure 3 Frequency-dependence of vagal pressor (○) and sympathetic depressor (●) responses in guinea-pig isolated innervated trachea. In each case the duration of the stimulus train was 5 s. Values are means ± s.e.mean of 7 to 15 observations.

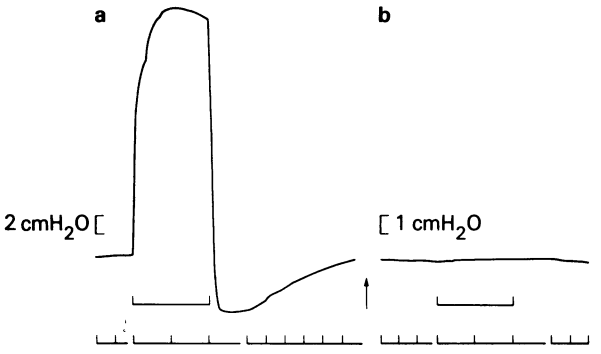

Figure 4 (a) Response of isolated trachea to stimulation of the right vagus for 2 min at 10 Hz, period of stimulation shown by horizontal line. Note depressor after-response. (b) Block of both phases of response 30 min after addition of atropine (6×10^{-7} M) at arrow. Note changes in chart speed indicated on time scale. Time divisions 1 min.

(Figure 4). Both the prolonged pressor response and the following depressor response were blocked by atropine (6×10^{-7} M) or hexamethonium (2.4×10^{-4} M). Neither was blocked by propranolol (3.5×10^{-6} M).

It appeared, therefore, that the depressor response was an 'after-response' in some way dependent on the preparation being exposed to a sustained period of raised pressure. Simple leakage of fluid out of the closed system during the period of raised pressure could explain the after-response, but the addition of dye to the luminal fluid and application of pressure 100 times normal gave no evidence of leakage. However, this observation does not exclude the possibility that fluid moves across the tracheal tissues under sustained hydrostatic pressure. Indeed, raising the intraluminal pressure, for example by placing a clamp on the tubing of the closed system, did produce a depressor after-response when the clamp was released. Stretch of compliant components of the smooth muscle and connective tissue of the trachea could equally well explain this observation. Recovery would occur as the compliant tissues returned to their original length. However, increasing pressure in this way distends the trachea and is not strictly analogous to the isovolumic change caused by nerve stimulation.

Attempts were made to see whether a negative after-response followed sustained stimulation of the vagus when changes in volume were recorded at constant pressure (4 cm H_2O). The recorded changes were small and recovery on stopping stimulation was slow and of inconstant magnitude. It was not possible to decide whether a negative after-response was present or not under these conditions.

Responses to stimulation of the sympathetic nerves

Stimulation of the sympathetic nerves (positions 3 or 4 in Figure 1) evoked either a simple depressor or a dual pressor-depressor response (Figure 5). Dual responses were generally evoked by stimulation at several other sites (positions 5, 6 and 7 in Figure 1). In early experiments, pure depressor responses were observed in fewer than half of the preparations, but with more careful separation of the vagal and sympathetic trunks under higher magnification this proportion increased to about 70%. In the remaining 30%, a pressor response, blocked by atropine, preceded the depressor response (Figure 5) and in these preparations it was not possible to avoid stimulating such cholinergic fibres by altering the site of stimulation. All the depressor responses evoked by sympathetic stimulation were blocked by propranolol (3.5×10^{-6} M) but were unaffected by hexamethonium (2.4×10^{-4} M).

Sympathetic depressor responses varied more than vagal pressor responses in magnitude and duration. In some preparations two pulses at 0.5 Hz produced a detectable response, but in others there was no depressor response to stimulation for 5 s at 10 Hz. This

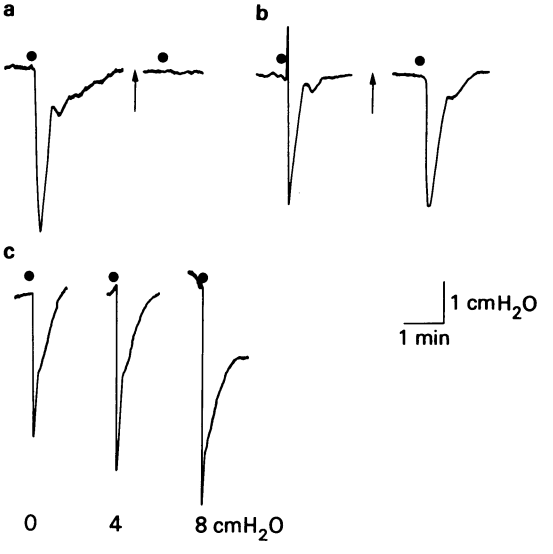

Figure 5 Typical depressor and dual pressor-depressor responses of isolated trachea evoked by stimulation (at dots) of the right sympathetic trunk for 5 s at 20 or 80 Hz. (a) Depressor response blocked 30 min after addition of propranolol (3.5×10^{-6} M) at arrow; stimulus frequency 20 Hz. (b) Pressor component of dual response blocked 20 min after addition of atropine (6×10^{-7} M) at arrow; stimulus frequency 20 Hz. (c) Depressor responses augmented by raising intraluminal pressure from 0 to 8 cm H_2O; stimulus frequency 80 Hz.

variability did not appear to be due to differences in the resting intraluminal pressure (range 2 to 4.5 cm H_2O). The onset of the response was slow, taking 3 to 4 s. Responses to stimulation for 5 s at frequencies up to 10 Hz had time-to-peak values of 13 ± 2 s and durations of 14 ± 4 s. Both time-to-peak and duration increased with the frequency of stimulation.

The amplitude of depressor responses increased with frequency to a maximum at between 20 and 80 Hz (Figure 3). The magnitude of sympathetic depressor responses could be augmented by raising the resting intraluminal pressure (Figure 5) but when the trachea was spontaneously active, depressor responses were partially or completely masked.

Sustained sympathetic stimulation

At frequencies of 10 Hz or less, sympathetic stimulation for 2 min evoked a depressor response which was sustained for the duration of stimulation (Figure 6). The pressure returned to baseline within a few minutes of ending stimulation. There was no positive after-response. Propranolol (3.5×10^{-6} M) completely blocked depressor responses to sustained sympathetic stimulation.

In preparations treated with both atropine and propranolol, sustained sympathetic stimulation sometimes evoked a small, delayed pressor response (Figure 6) which was blocked by phentolamine (4×10^{-6} M).

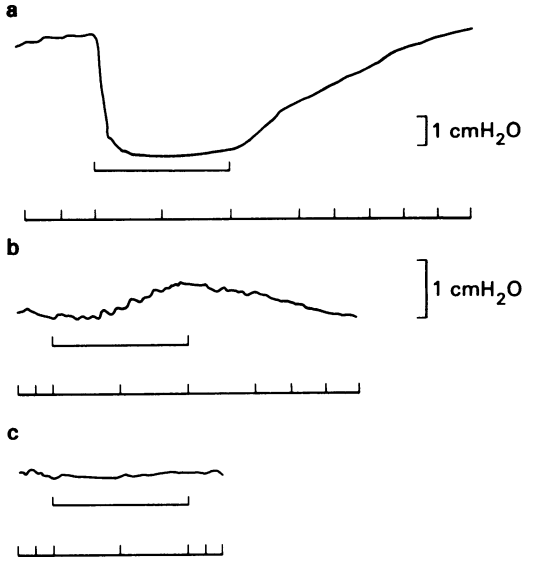

Figure 6 (a) Sustained depressor response of isolated trachea to stimulation of the stellate ganglion for 2 min at 10 Hz. (b) Delayed pressor response to stimulation of the stellate ganglion 40 min after addition of propranolol (3.5×10^{-6} M). (c) Pressor response blocked 25 min after addition of phentolamine (4×10^{-6} M). Time divisions 1 min. Note changes of chart speed.

Combined vagal and sympathetic stimulation

Interaction between vagal and sympathetic responses was examined by applying a 5 s train of stimuli first to the sympathetic trunk and then to the vagus. The interval between stimulus trains was timed so that the peak of each response would roughly coincide. Although the amplitude of the vagal pressor component of the resulting dual response was diminished, it was greater than predicted by algebraic summation of the two responses. By contrast, the pressor response resulting from simultaneous and sustained (2 min) stimulation of the vagal and sympathetic supplies was significantly smaller than predicted by algebraic summation.

Stimulation of the laryngeal nerves

Stimulation of the right laryngeal nerve (at position 8 in Figure 1) at different frequencies for 5 s evoked a dual response in each of 20 preparations. Atropine (6×10^{-7} M) blocked the pressor component and propranolol (3.5×10^{-6} M) the depressor component. The depressor phase was usually absent at low frequencies of stimulation but became apparent or was increased in magnitude at higher frequencies (> 10 Hz).

Stimulation of the left laryngeal nerve for 5 s also evoked a dual response with a large pressor component. Again, atropine blocked the pressor component. However, the depressor component, which was generally small and of long time course, was not always blocked by propranolol (2 of 5 preparations), but was blocked by atropine. It was thus similar to the after-response occasionally observed on stimulating the right vagus.

Simultaneous stimulation of the right and left laryngeal nerves evoked a large pressor response followed by a depressor response of variable magnitude. The magnitude of the pressor component evoked by combined stimulation at 40 Hz was consistently about 80% of the algebraic sum of the maximal responses evoked separately at 40 Hz, which suggests that some of the tracheal muscle fibres receive excitatory input from both laryngeal nerves.

Stimulation of the right laryngeal nerve for 2 min at frequencies of up to 10 Hz evoked a sustained pressor response. At higher frequencies the magnitude of the pressor response was not maintained and in some cases it gave way during stimulation to a depressor response. When stimulation was stopped there was a further decrease in pressure and then a gradual return to baseline.

Transmural stimulation

In a number of preparations, the response to transmural stimulation of the trachea was compared with the response to vagal and sympathetic stimulation. Transmural stimulation produced a large dual response. In the presence of concentrations of atropine (6×10^{-7} M) and propranolol (3.5×10^{-6} M) which blocked responses to vagal and sympathetic stimulation, transmural stimulation evoked a depressor response. This non-cholinergic, non-adrenergic inhibitory response represented 24% ± 4% of the total inhibitory response, a value in good agreement with that reported by Yip, Palombini & Coburn (1981) and Doidge & Satchell (1982).

Hypoxia

Spontaneous activity in the trachea appeared in early experiments to be associated with hypoxia and this was investigated. The trachea without its nerve supply was removed from the guinea-pig, set up as usual in the perfusion chamber and allowed to equilibrate for 30 min in oxygenated Krebs solution. The gas supply was then cut off. Within 5 min the intraluminal pressure fell by up to 2 cmH_2O, but then returned to baseline within 10 min. In each of 5 initially quiescent preparations, spontaneous activity began 20 min after cutting off the O_2 supply, at which time the P_{O_2} (measured with an oxygen electrode) had fallen from about 350 mmHg to about 200 mmHg. Within 5 min of restoring the gas supply, spontaneous activity ceased. In a 6th preparation which was spontaneously active during the control period, hypoxia caused a four-fold increase in the amplitude of spontaneous activity, but no significant change in frequency. Atropine (up to 2.4×10^{-6} M) had no effect on the spontaneous activity induced by hypoxia in 3 preparations. Hexamethonium (2.4×10^{-4} M) did not prevent development of spontaneous activity by hypoxia.

In 3 experiments, the nerve supplies to the trachea were kept intact and stimulated before and during hypoxia. Pressor responses to vagal stimulation were unchanged after 30 min of hypoxia. However, the magnitude of sympathetic depressor responses was reduced by 70%. Recovery of sympathetic responses was only partial after 5–10 min of restoration of the O_2 supply.

Discussion

Intraluminal pressure has been measured in the isolated trachea previously (Golla & Symes, 1913; Wellens, 1966; Coleman & Farmer, 1971). Separate stimulation of the autonomic nerves has also been achieved, but with an *in situ* preparation (Yip, Palombini & Coburn, 1981). The preparation we have described combines advantages of both techniques: it allows separate stimulation of vagal and sympathetic nerves in a completely isolated trachea. Responses are highly reproducible and easily measured. A particular advantage is that with intraluminal resting pressures normally in the range of 2 to 4 cm H_2O, inhibitory responses are readily observed without the addition of agonist drugs.

The presence of resting tone requires some explanation, for in airway smooth muscle *in vivo*, tone does not appear to be maintained without vagal input (Green & Widdicombe, 1966; Cabezas, Graf & Nadel, 1971). In our preparations, it did not seem to be due to an intrinsic release of acetylcholine as, although atropine caused a decrease in tone, this effect was transient; furthermore, hexamethonium had no effect on intraluminal pressure. An obvious alternative explanation is that the tone is due to hypoxia. Even with frequent flushing with fresh Krebs solution, the lumen of the trachea, at least, is likely to be somewhat hypoxic. However, when preparations were deliberately made hypoxic, tone did not increase; in fact, there was a transient decrease in tone. Souhrada & Kivity (1982) also reported that tone was unaffected by hypoxia in an isolated preparation of guinea-pig trachea. A small increase in tone was observed by Stephens & Kroeger (1970) in the dog trachealis muscle, but only with severe hypoxia and substrate depletion. The mechanisms controlling resting tone in airways smooth muscle are likely to be complex and may involve both extrinsic and intrinsic nerve pathways as well as the muscle itself, but for the present we can offer no explanation for its presence in our preparation. Artifactual or not, it very conveniently allows inhibitory responses to be observed in the absence of an agonist or sustained excitatory stimuli.

Intermingling of cholinergic and adrenergic neurones within nerves classified anatomically as parasympathetic or sympathetic has been described frequently (Mitchell, 1956; Pick, 1970). Our observations provide evidence of such intermingling also. Although stimulation of the vagus at the cervical level normally excited only cholinergic fibres, stimulation of the vagus near the recurrent laryngeal branch or of the recurrent laryngeal nerve itself excited both cholinergic and adrenergic fibres. In about 30% of our preparations, stimulation of the sympathetic trunk excited some cholinergic as well as adrenergic fibres (see Binger, Gaarde & Markowitz, 1931; Hebb, 1940). It was not possible to avoid such mixed responses by selecting different points of stimulation.

Although depressor responses of the trachea to sympathetic stimulation are evidently mediated by

activation of β-adrenoceptors and the smooth muscle of the trachea is endowed with β-adrenoceptors, it is not clear that the smooth muscle is directly innervated by adrenergic fibres. It has been suggested that the sympathetic adrenergic fibres terminate in the intrinsic ganglia or on blood vessels of the trachea (Staub, 1975; Nadel, 1980; Kirkpatrick, 1981). Our results offer no means of resolving this question.

Our observations support evidence for the presence of α-adrenoceptors in the guinea-pig trachea (Everitt & Cairncross, 1969; Fleisch, Maling & Brodie, 1970). Prolonged sympathetic stimulation in the presence of atropine and propranolol produced a pressor response which was blocked by phentolamine. Evidently under these conditions the response is mediated by the action of the neurotransmitter on α-adrenoceptors, although their location is not clear.

There is evidence, *in vivo* that sustained stimulation of the vagus nerve in the presence of atropine and propranolol can elicit an inhibitory response (by definition non-adrenergic, non-cholinergic) in a number of species including the guinea-pig (Chesrown *et al.*, 1980; Diamond & O'Donnell, 1980; Irvin *et al.*, 1980). Such a response was not seen in our isolated innervated tracheal preparations. However, non-adrenergic, non-cholinergic inhibition was readily obtained in the same preparations by transmural stimulation. Vagal pathways mediating non-adrenergic, non-cholinergic inhibition were either not present in our preparations or were not being stimulated.

The atropine-sensitive depressor response which was sometimes seen after stimulation of the vagus for 5 s and always after stimulation for 2 min appeared to be an after-response to a period of raised pressure. It was not clear whether this was an active relaxation due to release of ACh or whether it was a passive effect perhaps related to the distensibility of the tissue and conditioned by the magnitude and duration of the preceding response.

Our observations of the effect of combined stimulation of the vagal and sympathetic supplies to the isolated trachea suggest that the vagus is likely to have the more important influence, a conclusion which is consistent with evidence obtained *in vivo*. For example, Widdicombe (1966) has demonstrated that if the discharge rates of vagal and sympathetic efferent fibres do not exceed 10 Hz (as observed in the cat and dog), the vagus is likely to have predominant control of the trachea. Quite precise control of calibre should be achieved by small changes in vagal discharge frequency. Also, concurrent sympathetic discharge is unlikely to abolish the influence of the vagus. Indeed, in the dog, vagal responses predominate and are never completely overcome by sympathetic stimulation (Cabezas, Graf & Nadel, 1971).

Spontaneous contractile activity has been observed in the airways of guinea-pigs and other species but its physiological significance is not known (Souhrada & Dickey, 1976a; Richardson, 1977; Beinfield & Seifter, 1980; Kirkpatrick, 1981). Oscillations in muscle tone may be induced by bronchoconstrictor drugs such as acetylcholine and histamine (Kirkpatrick, 1981) or by exposure of sensitized tissues to antigen (Souhrada & Dickey, 1976b). Spontaneous activity observed in our preparations was unpredictably blocked or not blocked by atropine suggesting that it could be either neural or muscular in origin. Sympathetic stimulation did not inhibit such spontaneous activity; in fact sympathetic responses were reduced or absent during spontaneous activity. Hypoxia is an important factor for it always induced spontaneous activity in previously quiescent preparations of guinea-pig trachea. Bose & Bose (1977) have observed similar rhythmic fluctuations in tone of the dog trachea in response to hypoxia or substrate inhibition and, as Ca^{2+} was necessary for this response, they postulated that it was due to changes in membrane conductance to Na^{+} resulting from ATP depletion and a consequent alteration in Ca^{2+} binding to the cell membrane.

The fact that responses to sympathetic stimulation are reduced or abolished by hypoxia may have important implications in respiratory disease states such as bronchial asthma. Loss of the sympathetic dilator response would be to the obvious disadvantage of subjects with obstruction of their airways.

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