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Comparison of autonomic responses in the trachea isolated from normal and albumin-sensitive guinea-pigs

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1 Mechanical and electrical responses to stimulation of the parasympathetic and sympathetic nerves were compared in the trachea isolated from normal guinea-pigs and from guinea-pigs sensitized to albumin and exposed repeatedly to inhaled albumin (a model of bronchial asthma).

2 Sensitized trachealis exhibited mechanical hyper-responsiveness to vagal stimulation characterized by a shift to the left of the frequency-response relationship and a 71% increase in the maximum response.

3 Transmembrane potential was significantly less negative in sensitized trachealis cells.

4 The amplitude of the depolarization evoked by vagal stimulation (4 or more pulses) was significantly greater in sensitized tissues. Vagally-mediated depolarization was associated with the appearance of regenerative electrical activity (spikes) in sensitized but not in control tissues.

5 Spontaneous discharge of slow waves occurred in cells from both control and sensitized trachea but the proportion of spontaneously active cells was higher in sensitized tissues. Spontaneous depolarization, like nerve-mediated depolarization, gave rise to abortive spikes only in sensitized trachealis.

6 Inhibitory responses to stimulation of the sympathetic stellate ganglion, mediated by β-adrenoceptors, were unaltered in sensitized trachealis.

7 Possible explanations for the hyper-responsiveness to vagal input in sensitized trachealis are discussed.

Introduction

Airway hyper-reactivity is a prominent feature of bronchial asthma characterized by exaggerated responses to bronchoconstrictor stimuli (Dautreband & Philippot, 1941; Curry, 1946; Empey, 1982). Many possible causes of airway hyper-reactivity have been examined. It has been proposed, for example, that in asthma there is increased activity in afferent nerve fibres arising from irritant receptors in the lungs which gives rise to reflex bronchoconstriction mediated by the vagus nerve (see Nadel, 1983). The involvement of vagal efferent pathways has been demonstrated clearly by the attenuation of irritant-induced bronchoconstriction by cholinergic blockade or by cooling of the vagus nerve (Nadel & Widdicombe, 1962; Nadel *et al.*, 1965; Gold, 1975). However, the possibility that the airways are hyper-responsive to vagal input does not appear to have been considered. The first aim of this work, therefore, was to examine responsiveness to vagal stimulation in the isolated trachealis muscle in a guinea-pig model of asthma.

An alternative view of airway hyper-reactivity was proposed by Szentivanyi (1968) in his 'β-theory of asthma'. According to this theory there is a deficiency of β-adrenoceptors in the airways, and other tissues, of asthmatics, which leads to impaired β-adrenoceptor-induced relaxation and a reduced capacity to counteract bronchoconstriction. A second aim of this work, therefore, was to study adrenergic relaxation, also in the guinea-pig model of asthma. The isolated, innervated trachea was used since this allows discrete stimulation of the parasympathetic and sympathetic nerve supplies (Blackman & McCaig, 1983). Mechanical responses to stimulation of the extrinsic nerves were compared in tracheae from control guinea-pigs and guinea-pigs sensitized to egg albumin and repeatedly challenged with albumin in an aerosol form, which exhibit significant airway hyper-reactivity *in vivo* (Stewart & Fennessy, 1983; Daly *et al.*, 1984).

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The electrical events associated with neuroeffector transmission in guinea-pig trachealis have recently been described (McCaig, 1986). As a final aim of this study, it was decided to examine the electrophysiological properties of single cells in 'sensitized' trachealis to determine whether changes at this level might be involved in airway hyper-reactivity.

Methods

Animals

Twenty guinea-pigs (male, Dunkin-Hartley) were divided randomly into 2 groups of 10. Animals of group 1 served as controls and were untreated. Animals of group 2 were sensitized to albumin (Sigma) by injecting 100 mg i.p. and 100 mg s.c. on day 1 and a further 10 mg i.p. on day 8. From day 14 sensitized animals were exposed to an aerosol of 4% albumin for 4 min daily for 18 ± 1 days. The aerosol was administered in a closed chamber, dimensions 30 × 17 × 11 cm. Group 2 animals were used for experiments 1 day after the last exposure to albumin. All animals were killed by a blow to the head.

Mechanical experiments

The 2 cartilaginous rings of the trachea immediately below the cricoid cartilage were removed and suspended under 500 mg tension in an organ bath containing Krebs solution at 37°C (composition (mM): Na^+ 127, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 121, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, HCO_3^- 25, glucose 11) and tension was recorded with an isometric transducer (Devices). Albumin was added to the bath to check for sensitization. Tracheal segements from sensitized guinea-pigs (group 2) all contracted on exposure to 0.1% albumin, whereas none of the control segments responded.

The remainder of the trachea was dissected with the right extrinsic innervation intact (vagus nerve plus recurrent laryngeal branch and sympathetic trunk and stellate ganglion; Blackman & McCaig, 1983). The preparation was placed in a chamber through which Krebs solution (37°C) flowed at a rate of 4 ml min^{-1}.

The trachea was filled with Krebs solution, then one end was closed and the other connected to a pressure transducer (Statham) for recording intraluminal pressure on a pen recorder (Devices). Resting intraluminal pressure was set at 3 cmH_2O. The vagus nerve was stimulated through a suction electrode with rectangular pulses of 40 V and 1 ms duration and responses were recorded as increases in intraluminal pressure. The sympathetic stellate ganglion was stimulated similarly through a bipolar electrode placed at the rostral end and responses were recorded as decreases in intraluminal pressure.

Electrophysiological experiments

The innervated trachea was subsequently opened by cutting through the cartilaginous rings on the ventral surface and pinned to the base of the chamber. Segments of the mucosal layer overlying the smooth muscle bands were removed using watchmaker's forceps. Single trachealis cells were impaled with glass microelectrodes filled with 0.5 M KCl (resistance 70–80 MΩ). Electrical signals were fed through a unity gain amplifier (WPI), displayed on an oscilloscope (Tektronix) and recorded on a pen recorder (Gould). Measurements were made of the transmembrane potential, spontaneous eletrical activity and responses to nerve stimulation.

Results

Mechanical responses to stimulation of the vagus

In control tissues vagal stimulation for 5 s at frequencies of 1–80 Hz evoked rapid, brief increases in intraluminal pressure (Figure 1), the amplitude of the

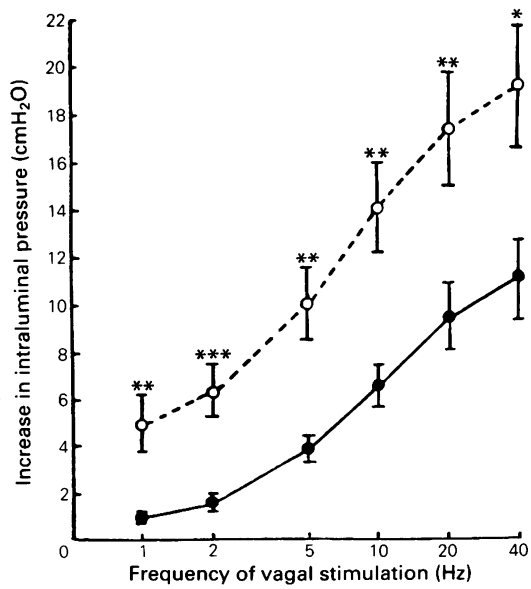


Figure 1 The relationship between increase in intraluminal pressure (ordinate scale) and frequency of stimulation of the vagus nerve (abscissa scale) in the isolated, fluid-filled guinea-pig trachea. The vagus nerve was stimulated for 5 s through a suction electrode with rectangular pulses of 40 V and 1 ms duration. Responses in control (●) and sensitized (○) trachea, are shown. Each point is the mean of 10 observations and vertical lines indicate s.e.mean. Asterisks denote values significantly different from the corresponding control: $*P<0.02$; $**P<0.01$; $***P<0.001$.

response increasing with frequency of stimulation to a maximum at 40 Hz, as previously described (Blackman & McCaig, 1983). In sensitized tissues there was a shift upward and to the left of the frequency-response relationship. Thus the increase in intraluminal pressure elicited at any given frequency of vagal stimulation was significantly greater in sensitized tissues than in controls (Figure 1). There was also a substantial increase in the maximum response (mean 71%) in sensitized tissues (Figure 1). Therefore, the trachea isolated from sensitized guinea-pigs exhibits clear mechanical hyper-responsiveness to vagal input. In 6 preparations from each of the control and sensitized groups atropine (6×10^{-7} M) had no effect on resting tone but blocked completely the responses to vagal stimulation. Thus there was no evidence of a non-cholinergic component in the response.

Mechanical responses to stimulation of the stellate ganglion

In control tissues stimulation of the stellate ganglion for 5 s evoked frequency-dependent decreases in intraluminal pressure reaching a maximum at 40 Hz, as previously described (Blackman & McCaig, 1983). These inhibitory responses were slower in onset, of smaller amplitude and of longer duration than vagal excitatory responses. Responses obtained in sensitized trachealis were not significantly different from control at any frequency of sympathetic stimulation (range 1–40 Hz, Figure 2). Responses were blocked completely by propranolol (3.5×10^{-6} M) in both control and sensitized trachea ($n = 6$). Thus there was no evidence of reduced β-adrenoceptor-mediated relaxation elicited by sympathetic nerve stimulation in sensitized guinea-pig trachea.

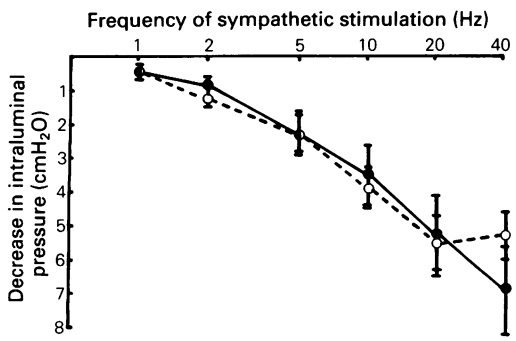


Figure 2 The relationship between decrease in intraluminal pressure (ordinate scale) and frequency of stimulation of the stellate ganglion (abscissa scale) in the isolated, fluid-filled guinea-pig trachea. The stellate ganglion was stimulated for 5 s through a bipolar electrode with rectangular pulses of 40 V and 1 ms duration. Responses in control (●) and sensitized (○) trachea are shown. Each point is the mean of 9 or 10 obervations and vertical lines indicate s.e.mean. Values in sensitized trachealis were not significantly different from control at any frequency of stimulation.

Electrophysiological properties of trachealis cells

Trachealis cells from control tissues had a transmembrane potential of -43 ± 0.3 mV (mean ± s.e.mean, $n = 156$). The transmembrane potential was 10 mV less negative in trachealis cells from sensitized tissues (-33 ± 0.6 mV, $n = 91$, $P < 0.001$; t test).

In control cells stimulation of the vagus elicited a transient depolarization (or excitatory junction potential (e.j.p.)) which increased in amplitude as the number of stimuli was increased from 1 to 16 pulses, all at 40 Hz (Figures 3a and 4), as described previously (McCaig, 1986). The initial depolarization was smooth but was often followed by one or more smaller fluctuations in potential. It was much more difficult to maintain impalements in sensitized trachealis cells during vagal stimulation, presumably as a result of the larger contractions occurring in these tissues and responses to each of 5 different stimuli (1, 2, 4, 8 and 16 pulses, all at 40 Hz) were obtained in 5 cells only. As shown in Figures 3b and 4 the amplitude of the e.j.p. evoked by 4 or more pulses was significantly greater in cells from sensitized trachealis than in controls. Responses to 1 or 2 pulses were not significantly different in the two groups. In many cells only a few responses were obtained before the microelectrode became dislodged but the same trend was apparent in these cells. At 16 pulses, for example, the mean e.j.p. amplitude in 30 control cells was 8.7 ± 0.7 mV, while in 23 cells from sensitized trachea the mean amplitude was 14.3 ± 0.9 mV ($P < 0.001$). In control cells maximum depolarization was obtained at 16 (Figure 5a) or occasionally 32 stimuli. However, in cells from sensitized trachea, depolarization was often maximal at fewer stimuli (e.g. 8 pulses in the cell shown in Figure 5b).

In some cells from sensitized tissues vagal stimulation elicited a smooth depolarization, similar in appearance to that obtained in control cells. In many cells, however, depolarization was associated with the generation of abortive spikes or with a series of fluctuations in potential at the height of the response (Figures 3b and 5b). Such activity was never seen in control cells.

Some cells from both control and sensitized trachea exhibited spontaneous discharge of slow waves. The characteristics of slow wave activity are summarized in Table 1, from which it can be seen that the amplitude and frequency of discharge of slow waves did not differ between the 2 groups. The proportion of cells showing high amplitude slow waves (> 10 mV) was also similar

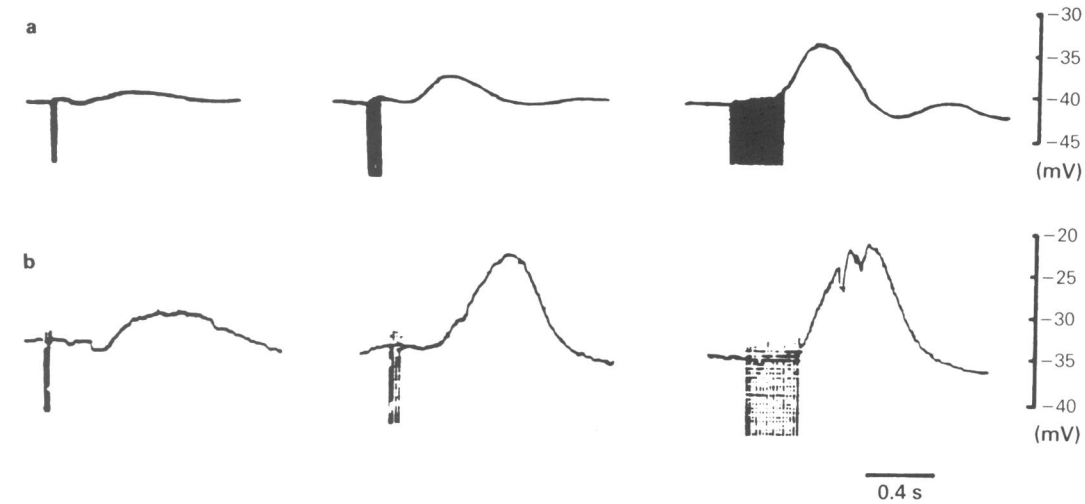
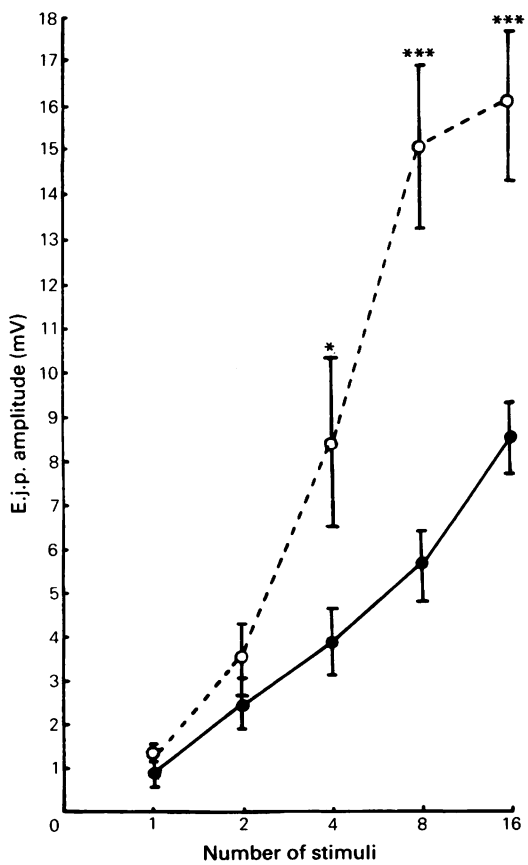


Figure 3 Intracellular recordings of responses to stimulation of the vagus nerve (2, 4 and 16 pulses at 40 Hz, each pulse of 40 V and 1 ms duration) in trachealis cells from control (a) and sensitized (b) guinea-pigs. The amplitude of depolarization was increased in the sensitized cell and at 16 pulses there were oscillations in potential at the height of depolarization.



in both groups. However, there were two clear differences between the groups. Firstly, a higher percentage of cells was spontaneously active in sensitized trachealis. Secondly, spontaneous depolarization was associated with the discharge of small spikes (Figure 5c), or with fluctuations in potential at the height of depolarization only in cells from sensitized tissues. Thus, both spontaneous and neurally-evoked depolarization can reach the threshold for initiation of regenerative activity in sensitized, but not in control trachealis cells.

Electrical responses to sympathetic stimulation were encountered rarely in control cells, in keeping with the previous findings (McCaig, 1986). Responses, when present, consisted of a small hyperpolarization (1–4 mV) and/or suppression of slow wave activity. In the first 3 sensitized preparations studied, no cells were found to respond to sympathetic stimulation, therefore no further attempts were made to characterize the electrophysiological response in sensitized tissues.

Figure 4 The relationship between excitatory junction potential (e.j.p.) amplitude recorded in single trachealis cells (ordinate scale) and number of stimuli (all at 40 Hz) applied to the vagus nerve (abscissa scale) in the trachealis muscle isolated from control (●) and sensitized (○) guinea-pigs. Each point is the mean of 12 and 5 observations in control and sensitized tissues, respectively and vertical lines indicate s.e.mean. Asterisks denote values significantly different from the corresponding control: $*P<0.02$; $***P<0.001$.

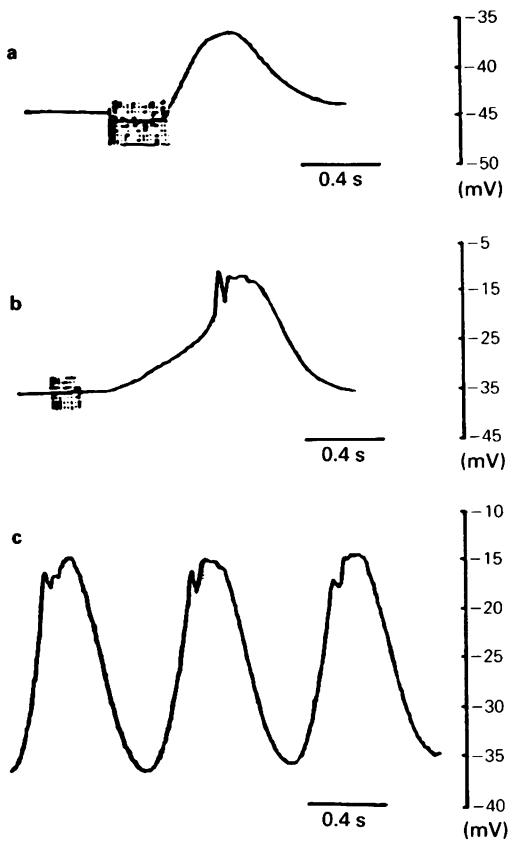


Figure 5 Intracellular recordings of responses to stimulation of the vagus nerve in single trachealis cells from control (a) and sensitized (b) guinea-pig trachea. Maximum depolarization in the sensitized cell was obtained with fewer stimuli (8 pulses, cf. 16 pulses in control), was of greater amplitude and had a spike component. (c) Spontaneous depolarizations in a single trachealis cell from a sensitized guinea-pig which also had a small spike component.

Discussion

These results show that the trachealis muscle isolated from sensitized guinea-pigs is hyper-responsive to vagal stimulation. Thus an identical stimulus applied to the vagus evokes a larger contraction in sensitized as compared to normal trachea. This increased sensitivity to vagal input could play a role in airway hyper-reactivity. It has been suggested that in asthma the lung irritant receptors are exposed to higher concentrations of irritants as a result of increased penetration to the receptors through a damaged epithelial layer (Nadel, 1983). This causes an increased rate of firing of the afferent fibres arising from the irritant receptors which act on central pathways inducing increased activity in vagal efferent pathways. An increased responsiveness to vagal input *per se* would exacerbate this effect, leading to even more bronchoconstriction than expected from an increase in vagal traffic alone.

Electrically it was found that sensitized trachealis, in the resting state, was depolarized by about 10 mV, as compared to control muscle, a result in good agreement with previous work (McCaig & Souhrada, 1980; Souhrada & Souhrada, 1981). Depolarization could arise from changes in the properties of the smooth muscle cell membrane. It has been shown, for example, that electrogenic Na^+ pumping contributes to the resting membrane potential in guinea-pig trachealis (Souhrada *et al.*, 1981). Thus inhibition of Na^+-pumping by exposure to ouabain, or lowering the temperature to 21°C causes depolarization of around 10 mV (Souhrada *et al.*, 1981; McCaig, 1986). Inhibition of the electrogenic Na^+pump could account for the depolarization seen in sensitized tissues. Other possible changes in the trachealis cell membrane would include a reduction in the number or in the frequency of opening of the K^+-channels; these have been demonstrated in canine trachealis with the patch-clamp technique (McCann & Welsh, 1986). Alternatively, depolarization could originate prejunc-

Table 1 Characteristics of spontaneous activity (SA) recorded in single trachealis cells from control (group 1) and sensitized (group 2) guinea-pigs

	Amplitude (mV)	*Frequency* (Hz)	*% of cells with SA*	*% of SA cells with amplitude > 10 mV*	*% of SA cells with spikes*
Group 1 control ($n = 156$)	6.7 ± 0.6	0.97 ± 0.04	40	22	0
Group 2, sensitized ($n = 91$)	6.5 ± 0.5	0.98 ± 0.02	74	21	39

The number of cells in each group is shown in parentheses. Values for amplitude and frequency are the mean ± s.e.mean of 63 and 67 observations, respectively.

tionally through spontaneous release of acetylcholine (ACh) from cholinergic nerve terminals. However, it has been shown that atropine does not affect resting tone or membrane potential in control trachealis (Blackman & McCaig, 1983; McCaig, 1986), suggesting that basal release of ACh is not significant. In the present work atropine had no effect on resting tone in control or sensitized tissues.

The amplitude of the e.j.p. evoked, by vagal stimulation, with 4 or more pulses, was significantly greater in sensitized trachealis than in controls. Such a change in e.j.p. could arise pre- or post-junctionally. The amount of ACh released during vagal stimulation could be increased if, for example, negative feedback inhibition of ACh release were compromised in sensitized tissues. Prejunctional inhibitory muscarinic receptors have been demonstrated in the cholinergic nerves supplying the airways in the guinea-pig (Faulkner *et al.*, 1986) and cat (Blaber *et al.*, 1985). The fact that the e.j.p. amplitude in sensitized trachealis was significantly increased only in response to 4 or more stimuli applied to the vagus, lends some support to the idea that reduced feedback inhibition of ACh release might be involved. Thus it is likely that at low numbers of pulses or frequency of stimulation there is normally no inhibition of ACh release anyway, so that removal of inhibition would have no effect on e.j.p. amplitude. An increase in e.j.p. amplitude would only become apparent at higher numbers of pulses or frequency of stimulation.

It may be argued that the resting depolarization of the treachealis cell membrane could of itself account for the increase in e.j.p. amplitude. ACh is thought to have 3 distinct excitatory actions in smooth muscle, namely promotion of opening of both receptor-operated Ca^{2+}-channels (ROCs) and voltage-operated Ca^{2+}-channels (VOCs) and the release of intracellularly-stored Ca^{2+} (Bolton & Large, 1986). The threshold potential for opening VOCs is thought to be around -45 mV (Bolton *et al.*, 1984). This suggests that even in normal guinea-pig trachealis at a resting potential of -43 mV there would be significant VOC opening. This is supported by the presence of resting tone in the tissue (Blackman & McCaig, 1983). It would be anticipated that at the depolarized resting potential of -33 mV there would be increased opening of VOCs and significantly more resting tone (not assessed in these experiments). In addition the same degree of depolarization induced by ACh might result in more VOCs opening in sensitized tissues. This is supported by the appearance of abortive spikes and other more variable fluctuations in potential during depolarization in sensitized, but not normal, trachealis. Such spikes in other smooth muscles are thought to result from Ca^{2+} influx through VOCs (see Bolton & Large, 1986). In the absence of spikes, however, the recorded e.j.p. may still represent a combination of non-specific increases in ionic conductances induced by ACh and Ca^{2+} influx through VOCs. This would be true not only in sensitized tissues but also in normal tissues since the resting potential in the latter is above the proposed threshold for VOC opening, and depolarization of only a few millivolts results in an increase in tension (Ito & Itoh, 1984). It might be possible to distinguish the components of the e.j.p. by treating the tissues with Ca^{2+} antagonists which are thought to block VOCs (Spedding, 1987).

In airway smooth muscle it is not normally possible to induce regenerative electrical activity (Suzuki *et al.*, 1976; Kirkpatrick, 1981; McCaig, 1986). Depolarization seems to be limited during excitation by the efflux of K^+ ions through Ca^{2+}-dependent K^+-channels (Bolton & Large, 1986) and so does not reach the firing threshold. When K^+-channels are blocked, for example by tetraethylammonium, depolarization is associated with the appearance of spikes (McCaig & Souhrada, 1980; Kirkpatrick, 1981). A partial blockade or inactivation of K^+-channels might, therefore, account for the changes observed in sensitized muscle. Thus reduced K^+ conductance at rest would cause depolarization and a smaller increase in K^+ conductance during excitation would reduce the capacity to counteract depolarization, resulting in an increase in e.j.p. amplitude and the initiation of spike discharge. The resultant increase in Ca^{2+} influx would in turn lead to a larger contraction. Whether or not there is abnormal K^+-channel behaviour in sensitized tissues, drugs which selectively promote K^+-channel opening, such as BRL 34915, might be useful in asthma therapy, as suggested by Allen *et al.* (1985).

Changes in the electrophysiological characteristics of airway smooth muscle would be expected to modulate sensitivity not only to vagal input but also to bronchoconstrictor mediators, since these are thought to act, at least partly, through potential-dependent mechanisms. It is difficult to assess this possibility as yet, since studies of airway smooth muscle responsiveness in animal models of asthma have yielded conflicting results. Hyper-reponsiveness to a number of agents has been demonstrated in sensitized canine trachealis and guinea-pig lung parenchymal strips (Antonissen *et al.*, 1980; Rubinfeld *et al.*, 1982; Morcillo *et al.*, 1984). In sensitized guinea-pig trachealis responsiveness to histamine has been found to be unchanged (Stewart & Fennessy, 1983) or reduced (Souhrada & Souhrada, 1981), and the degree of depolarization induced by a high concentration of histamine was also reduced (McCaig & Souhrada, 1980, Souhrada & Souhrada, 1981).

It has been suggested that a paucity of β-adrenoceptors in the airways of asthmatics might play a role in airway hyper-reactivity (Szentivanyi, 1968). In the present work β-adrenoceptor-mediated inhibitory responses, elicited by sympthetic nerve stimulation were

unimpaired in sensitized guinea-pig trachealis *in vitro*. This is in contrast to the results of Souhrada *et al.* (1980) who found reduced β-adrenoceptor-mediated relaxation in their guinea-pig model of asthma. During sensitization, however, their animals received, in addition to albumin, *pertussis* vaccine which itself has been shown to induce partial β-adrenoceptor blockade (Fishel & Szentivanyi, 1963). Norris (1983) demonstrated that animals treated with albumin alone do not exihibit impaired β-adrenoceptor-mediated responses, but that reduced responses occur when an adjuvant is included in the treatment. However, it should be noted that treatment with albumin alone induces significant airway hyper-reactivity in guinea-pigs *in vivo* (Stewart & Fennessy, 1983; Daly *et al*, 1984). This suggests that impaired β-adrenoceptor-mediated relaxation is not a prerequisite for the development of airway hyper-reactivity.

In conclusion, sensitized guinea-pig trachealis muscle exhibits normal β-adrenoceptor-mediated relaxant responses *in vitro* but a marked hyper-responsiveness to excitatory vagal stimulation. Electrophysiologically, sensitized trachealis is depolarized in the resting state and the amplitude of the vagally-mediated e.j.p. is increased significantly, but is not yet clear whether these changes are pre- or post-junctional in origin.

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