Effects of calcium modulators on vagally-mediated constriction in the guinea-pig isolated trachea

¹Dorothy J. McCaig & Susan Aitken

School of Pharmacy, The Robert Gordon Institute of Technology, Schoolhill, Aberdeen AB9 1FR

1 The effects of calcium modulators on tracheal constriction evoked by vagal stimulation were examined in the isolated, innervated trachea of the guinea-pig. Responses were assessed in the Krebs-filled trachea as changes in intraluminal pressure (ILP), increases and decreases reflecting constriction and dilatation, respectively.

2 Preparations had a positive resting ILP, indicating significant spontaneous tone. Verapamil and nifedipine reduced baseline ILP, whilst Bay K 8644 had mixed effects.

3 Verapamil and nifedipine attenuated vagal responses in a concentration-dependent manner. At lower concentrations attenuation was due entirely to postjunctional effects but at higher concentrations prejunctional effects may have contributed to attenuation.

4 Verapamil and nifedipine attenuated vagal responses in the absence or presence of flurbiprofen, indicating that their effects are largely independent of the generation of cyclo-oxygenase products. Nifedipine, however, was less effective in reducing responses to low frequency vagal stimulation (up to 5 Hz) when flurbiprofen was present.

5 Bay K 8644 augmented vagal responses, the degree varying widely between preparations.

6 It was concluded that influx of Ca^{2+} through voltage-operated Ca^{2+} channels contributes significantly to vagally-mediated tracheal constriction in normal trachea and in trachea where endogenous release of cyclo-oxygenase products is inhibited.

Keywords: Trachea; vagus nerve; acetylcholine; Bay K 8644; nifedipine; flurbiprofen; verapamil

Introduction

Airway hyperreactivity is a hallmark of bronchial asthma manifest as a greatly increased sensitivity to a wide range of bronchoconstricting stimuli (Empey, 1982). It has been suggested that excessive permeability of airway smooth muscle cells to Ca^{2+} ions might underly airway hyperreactivity (Middleton, 1980). Increased Ca^{2+} influx from the extracellular fluid could occur if, for example, the number or frequency of opening of voltage-operated Ca^{2+} -channels (VOCs) in the smooth muscle cell membrane were increased. Evidence in support of this concept has been obtained recently in passively sensitized human airway smooth muscle (Black *et al.*, 1989). In the airway smooth muscle of the guinea-pig, and to some extent the dog and ox, there is spontaneous influx of Ca^{2+} ions through VOCs which can be recorded electrophysiologically as regular or irregular depolarizations, often termed slow waves (Small & Foster, 1988). The trachea isolated from albumin-sensitized guinea-pigs, which serve as an animal model of bronchial asthma, exhibits changes consistent with increased Ca^{2+} influx, including increased incidence of slow waves and augmented depolarization and constriction in response to vagal stimulation (McCaig, 1987). The long-term aim of this work is to examine the possibility that altered Ca^{2+} -channel behaviour contributes to vagal hyperresponsiveness and to airway hyperreactivity in general in albumin-sensitized guinea-pigs. There is limited information available, however, on the effects of drugs that modulate VOC behaviour on vagal responses in normal airways. As a first step, therefore, we have examined the effects of two VOC blockers, verapamil and the dihydropyridine, nifedipine, on vagally-mediated constriction in the guinea-pig isolated trachea. The effects of another dihydropyridine, Bay K 8644, which promotes VOC opening (Freedman & Miller, 1984), were studied for comparison. Finally, since the coupling of Ca^{2+} influx to the development of tone is influenced by products of the cyclo-oxygenase pathway of arachidonic acid

metabolism (McCaig & Rodger, 1988), the effects of verapamil and nifedipine were compared in the absence and presence of the cyclo-oxygenase inhibitor, flurbiprofen.

Methods

Preparation

Guinea-pigs (male, 300–550 g) were killed by a blow to the head. The trachea, with, or without the right vagus nerve and recurrent laryngeal branch as appropriate, was excised and mounted horizontally in a chamber through which Krebs solution at 37°C flowed at a rate of 5 ml min⁻¹ (Blackman & McCaig, 1983). The composition of the Krebs solution was (mM): Na^+ 127, K^+ 5.9, Ca^{2+} 1.2, Mg^{2+} 1.2, Cl^- 121, H_2PO_4^- 1.2, SO_4^{2-} 1.2, HCO_3^- 25, glucose 11; the solution was equilibrated both in the chamber and the reservoir with a gas mixture of 95% O_2 and 5% CO_2 .

The trachea was filled with Krebs solution then the caudal end was closed and the rostral end attached to a pressure transducer (Statham) for continuous monitoring of intraluminal pressure (ILP) on a pen recorder (electromed). Increases and decreases in ILP directly reflect tracheal constriction and dilatation, respectively. The vagus nerve was stimulated pre-ganglionically through a suction electrode with rectangular pulses of amplitude 40 V and duration 1 ms for 5 s at frequencies of 1–50 Hz.

Procedure

After a 20 min equilibration period a frequency-response curve was obtained to vagal stimulation or the response to applied acetylcholine (ACh, 10^{-4} M) was assessed. Tissues were then exposed to one of the test drugs. In some experiments the vagus nerve was stimulated at 20 Hz for 5 s every 90 or 120 s during exposure to the test drug in order to follow the time course of the response. After 20–30 min, a second frequency-

¹ Author for correspondence.

response curve or response to ACh was obtained. Some tissues were then exposed to up to 2 higher concentrations of the test drug and responses monitored as before. In some experiments flurbiprofen, 10^{-6} M, was added to the reservoir of Krebs solution to prevent the generation of cyclooxygenase products. The stability of the tissues over time was assessed by obtaining responses repeatedly in the absence of drug. The dihydropyridines were dissolved in ethanol which produced a final bath concentration of 0.1% ethanol. The effects of this concentration of ethanol alone were examined.

Drugs

The following drugs were used: acetylcholine chloride (ACh, Sigma), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(trifluoromethylphenyl)-pyridine-5-carboxylate, Bayer), flurbiprofen (Boots), nifedipine (Bayer) and verapamil hydrochloride (Sigma).

Acetylcholine, verapamil and flurbiprofen were dissolved in distilled water. Nifedipine and Bay K 8644 were dissolved in ethanol and the experiments were performed in a darkened room with the chamber covered in aluminium foil to minimize photolysis of the drugs. Flurbiprofen and verapamil were added to the reservoir of Krebs solution. All other drugs were added directly to the chamber with the perfusion pump off.

Analysis of data

Results are expressed as the mean $\pm$ s.e.mean. Statistical significance was assessed by two-way analysis of variance and *t* tests for independent or paired data, as appropriate. The level of significance was set at $P < 0.05$.

Results

Effects of drugs on resting intraluminal pressure

In the absence of flurbiprofen tracheal preparations had significant spontaneous resting tone, as reflected by a positive intraluminal pressure (ILP of $+3.4 \pm 0.4$ cmH₂O, $n = 8$). Verapamil evoked a small decrease in ILP which developed over 10–15 min and ranged from -0.6 ± 0.3 cmH₂O at 10^{-7} M to -1.1 ± 0.6 cmH₂O at 10^{-5} M. Nifedipine also elicited a gradual but more substantial fall in ILP, ranging from -1.0 ± 0.8 cmH₂O at 10^{-7} M to -4 ± 1 cmH₂O at 10^{-5} M. Bay K 8644, 10^{-6} M, reduced ILP by less than 0.5 cmH₂O in two tissues and increased it slightly in four others ($+1.8 \pm 0.6$ cmH₂O) over a 5 min period, the increase subsiding thereafter in the continued presence of the drug. In the presence of flurbiprofen, 10^{-6} M, spontaneous tone was abolished as reported previously (McCaig, 1989).

Effect of flurbiprofen on responses to vagal stimulation

Preganglionic stimulation of the vagus nerve evoked frequency-dependent increases in ILP, reflecting tracheal constriction (Figure 1). When frequency-response curves were generated repeatedly at 30–40 min intervals in the absence of any drug (time-match controls) response amplitude tended to decrease slightly, but responses at 20 and 50 Hz only were significantly reduced in the fourth such frequency-response curve.

In the presence of flurbiprofen, 10^{-6} M, the frequency-response curve was shifted to the left (Figure 1). Responses at lower frequencies of stimulation (up to 5 Hz) were increased in amplitude to a greater degree than those at higher frequencies, the maximum response was reached at 20 Hz (cf. 50 Hz in the absence of flurbiprofen) and responses declined thereafter such that at 50 Hz the response was significantly smaller in the presence of flurbiprofen. In addition there was no diminution of response amplitude over time in flurbiprofen-treated tissues.

Figure 1 Relationship between vagal responses (expressed as percentage control maximum increase in intraluminal pressure, ordinate scale) and frequency of stimulation (abscissa scale) in guinea-pig isolated trachea in the absence (●) ($n = 19$) and presence (○) ($n = 11$) of flurbiprofen, 10^{-6} M. Values are the mean and bars represent s.e.mean. Asterisks denote values significantly different from corresponding value in the absence of flurbiprofen at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Effect of verapamil on constrictor responses

Verapamil, 10^{-7} M, in the absence of flurbiprofen, had no significant effect on the amplitude of responses to vagal stimulation (Figure 2a). At a concentration of 10^{-6} M verapamil, vagal responses were attenuated (Figures 2a and 3a) at stimulation frequencies of 5 Hz and above. At lower frequencies of stimulation, however, the effect was variable and, despite increasing the number of experiments to 9, the changes were not significant. Verapamil, 10^{-5} M, reduced response amplitude significantly at all frequencies of stimulation by 60–80% (Figure 2a). Attenuation was evident after 5 min, reached a peak after 30 min contact with the drug and was significantly greater at higher concentrations of verapamil. In 4 tissues, for example, exposed to 10^{-7} , 10^{-6} and 10^{-5} M verapamil successively, responses at 10 Hz were attenuated by $14 \pm 5\%$, $39 \pm 8\%$ and $60 \pm 3\%$, respectively (values all significantly different at $P < 0.05$).

In the presence of flurbiprofen, verapamil also attenuated vagal responses, significant reductions in amplitude occurring at all concentrations tested and at all frequencies of stimulation (except 50 Hz at 10^{-7} M, verapamil, Figure 2b). Attenuation was concentration-dependent (e.g. at 20 Hz, 10^{-7} M: $-17 \pm 6\%$; 10^{-6} M: $-36 \pm 5\%$; 10^{-5} M: $-52 \pm 5\%$, values all significantly different at $P < 0.05$). It was noted that responses at lower frequencies of stimulation (up to 5 Hz) were more profoundly inhibited than those at higher frequencies (> 10 Hz), at any concentration of verapamil in flurbiprofen-treated tissues (e.g. 10^{-6} M verapamil at 2 Hz, $-66 \pm 8\%$; at 20 Hz $-36 \pm 5\%$; values significantly different at $P < 0.05$). Comparison of Figures 2(a) and (b) suggested that verapamil might be more effective in reducing vagal responses in the presence of flurbiprofen, particularly at lower concentrations of verapamil and lower frequencies of vagal stimulation. Statistical analysis, however, indicated that the effects of verapamil at any concentration were not significantly different in flurbiprofen-treated tissues.

Application of ACh, 10^{-4} M, elicited increases in ILP amounting to approximately 80% of the ACh maximum, which were highly reproducible and did not deteriorate over time. All experiments with ACh were performed in the absence of flurbiprofen. Verapamil significantly attenuated this response at each concentration tested (10^{-7} to 10^{-5} M, Figure 4), reductions ranging from 36 to 44%. Attenuation was not significantly greater at higher concentrations of verapamil. At

Figure 2 Relationship between vagal responses (expressed as percentage control maximum increase in intraluminal pressure, ordinate scale) and frequency of stimulation (abscissa scale) in guinea-pig isolated trachea before (●) and 20–30 min after addition of verapamil, 10^{-7} M (○), 10^{-6} M (□) and 10^{-5} M (■) in the absence (a) and presence (b) of flurbiprofen, 10^{-6} M. Values are the mean of 4 to 9 observations and bars represent s.e.mean. Asterisks denote values significantly different from corresponding control values at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

10^{-5} M verapamil, responses to ACh were significantly less attenuated than vagal responses (e.g. ACh, $-44 \pm 7\%$; vagal stimulation at 50 Hz, $-68 \pm 9\%$; values significantly different at $P < 0.05$).

Effect of nifedipine on constrictor responses

In control experiments it was established that ethanol, 0.1%, had no effect on the frequency-response curve to vagal stimulation (results not shown). Nifedipine, 10^{-7} M, in the absence of flurbiprofen did not significantly attenuate vagal responses either (except at 5 Hz) but at higher concentrations, 10^{-6} M and 10^{-5} M, responses were significantly reduced in a concentration-dependent manner at all frequencies of stimulation (range 10^{-6} M: 35–68%; 10^{-5} M: 66–97%; see Figures 3b and 5a). Attenuation increased with concentration of nifedipine (e.g. at 20 Hz, $-9 \pm 20\%$; $-49 \pm 13\%$ and $-71 \pm 10\%$ at 10^{-7} M, 10^{-6} M and 10^{-5} M, respectively, all values significantly different at $P < 0.05$). Responses at lower frequencies of stimulation were reduced more markedly by nifedipine (e.g. at 10^{-5} M responses at 2 Hz were reduced by $93 \pm 3\%$ and at 20 Hz by $71 \pm 10\%$, difference significant at $P < 0.025$). Attenuation of responses began after approximately 3 min and peaked after 16 min contact with nifedipine.

In the presence of flurbiprofen, 10^{-6} M, attenuation of vagal responses occurred also but was less marked, significant mainly at higher frequencies of stimulation and not clearly concentration-dependent (Figure 5b). When the effects of

Figure 3 Effect of drugs on vagally-induced increases in intraluminal pressure in guinea-pig isolated trachea. (a) Responses before and 20–30 min after exposure to verapamil (Verap) 10^{-6} M; (b) responses before and 20–30 min after exposure to nifedipine (Nif), 10^{-6} M; (c) responses before, 20–25 min after addition of Bay K 8644, 10^{-6} M, and 3–8 min after start of washout. Traces for each drug are taken from a single trachea. Response amplitude was reduced in the presence of the test drug in (a) and (b) and increased in (c), at each frequency of stimulation.

nifedipine, 10^{-6} M or 10^{-5} M, were compared in the absence and presence of flurbiprofen (Figure 5a and b) it was evident that at low frequencies of stimulation attenuation was considerably less marked in the presence of flurbiprofen (10^{-6} M). At 5 Hz, for example, in the presence of nifedipine, 10^{-6} M, responses were reduced by $63 \pm 12\%$ without flurbiprofen but only by $16 \pm 13\%$ with flurbiprofen present (values significantly different at $P < 0.05$).

Figure 4 Histogram showing the effect of drugs on the response to applied acetylcholine (ACh), 10^{-4} M, in guinea-pig isolated trachea. Responses are expressed as percentage of the control response to ACh, in the absence of drug. Values are the mean of 8 observations and bars represent s.e.mean. Asterisks denote values significantly different from the control response to ACh at * $P < 0.001$.

Figure 5 Relationship between vagal responses (expressed as percentage control maximum increase in intraluminal pressure, ordinate scale) and frequency of stimulation (abscissa scale) in guinea-pig isolated trachea before (●) and 20–30 min after the addition of nifedipine 10^{-7} M (■); 10^{-6} M (□) and 10^{-5} M (▲) in the absence (a) and presence (b) of flurbiprofen, 10^{-6} M. Values are the mean of 4 to 9 observations and bars represent s.e.mean. Asterisks denote values significantly different from corresponding controls: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 6 Relationship between vagal responses (expressed as percentage control maximum increase in intraluminal pressure, ordinate scale) and frequency of stimulation (abscissa scale) in guinea-pig isolated trachea before (●) and 20–30 min after (○) the addition of Bay K 8644, 10^{-6} M. Values are the mean of 5 observations and bars represent s.e.mean. Asterisks denote values significantly different from corresponding controls: * $P < 0.05$, ** $P < 0.025$; *** $P < 0.01$.

Nifedipine, 10^{-7} to 10^{-5} M, also significantly reduced the amplitude of the response to applied ACh (10^{-4} M, Figure 4). The degree of attenuation increased with concentration of nifedipine from $-34 \pm 5\%$ at 10^{-7} M to $-53 \pm 4\%$, at 10^{-5} M (values significantly different at $P < 0.05$). At 10^{-5} M nifedipine, responses to ACh were reduced significantly less than those to vagal stimulation at frequencies up to and including 20 Hz.

Effect of Bay K 8644 on vagal responses

The amplitude of vagally-mediated increases in ILP was increased in the presence of Bay K 8644, 10^{-6} M (no flurbiprofen present) (Figures 3c and 6). The degree of augmentation varied considerably between tissues. At a stimulation frequency of 50 Hz, for example, increases ranged from 5–116%. Within a few minutes of commencing washout of Bay K 8644 an unexpected pattern of response was observed in 4 of 5 preparations with responses at low frequencies of stimulation (up to 5 Hz) transiently greatly increased in amplitude (Figure 3c). At higher frequencies the response amplitude was unchanged in the early stages of washout. Responses at all frequencies of stimulation declined in amplitude towards control levels over 30 min in drug-free Krebs solution. No augmentation of vagal responses was observed in 3 tissues exposed to lower concentrations of Bay K 8644 (10^{-7} M and 5×10^{-7} M).

Discussion

These results show that two Ca^{2+} -channel blockers, verapamil and nifedipine attenuate vagally-mediated constriction in the guinea-pig isolated trachea, indicating that a significant component of this response is due to Ca^{2+} influx through VOCs. Similar results have been obtained in bovine trachea with nifedipine (Cuss *et al.*, 1985) and in human airways with methoxyverapamil (Kannan & Davies, 1988). Farley & Miles (1978) observed in canine trachealis that responses to low concentrations of acetylcholine were more susceptible to inhibition by VOC blockers than responses to high concentrations. They concluded that there is significant involvement of Ca^{2+} influx through VOCs in airway constriction elicited by low concentrations of agonists but that at high concentrations constriction is due mainly to potential-independent mechanisms, involving influx of Ca^{2+} through receptor-operated Ca^{2+} -channels (ROCs) and release of intracellularly-stored Ca^{2+} . In the present work a significant VOC-dependent component was evident in responses at all frequencies of vagal stimulation.

In keeping with the result of Farley & Miles (1978) in canine trachea other groups have shown that low concentrations of cholinomimetics are more susceptible to Ca^{2+} antagonists than higher concentrations in species including guinea-pig, rabbit and dog (Weiss *et al.*, 1985; Baba *et al.*, 1986; Bengtsson *et al.*, 1987). In contrast neither verapamil nor nifedipine had significant effects on the constriction induced by ACh at any concentration in guinea-pig trachea in the work of Foster *et al.* (1984) and Ahmed *et al.* (1985). In the present work, both verapamil and nifedipine consistently reduced the response to a single dose of ACh, suggesting a significant contribution from VOC opening even at this relatively high concentration of ACh.

In the absence of flurbiprofen, attenuation of vagal responses increased with increasing concentrations of verapamil or nifedipine. At concentrations of 10^{-7} or 10^{-6} M of these drugs the responses to vagal stimulation and applied ACh were attenuated to a similar degree. This strongly suggests that their effects can be accounted for entirely by postjunctional blockade of the L type VOCs in the smooth muscle cell membrane (see Spedding, 1987). At the highest concentration of verapamil or nifedipine tested, 10^{-5} M, vagal responses were attenuated more than those to applied ACh, suggesting a pre-junctional component to the inhibition. It has been demon-

strated, however, that Ca^{2+} antagonists selectively block L-type channels and do not affect the N-type channels which are thought to predominate in nerve-terminals and are involved in transmitter release (Miller, 1987; Spedding, 1987). Stimulation of the vagus nerve in this preparation is preganglionic and an effect on ganglionic transmission cannot be ruled out since L-type channels are thought to be present on neuronal cell bodies (Spedding, 1987). Alternatively, ACh released from nerve-terminals may have effects which differ from those of applied ACh. If, for example, the distribution of VOCs in the smooth muscle cell membrane were asymmetrical, with clustering at sites close to nerve terminals, a greater part of the action of neuronally-derived ACh might depend on VOC activation.

The development of spontaneous tone in guinea-pig trachea depends on cyclical influx of Ca^{2+} ions through VOCs, recorded as slow waves in single trachealis cells, since it is abolished in Ca^{2+} -free medium or reduced by VOC blockade (Small, 1982; McCaig, 1986). In the presence of cyclo-oxygenase inhibitors, such as indomethacin or flurbiprofen, slow wave activity continues unabated but is no longer coupled to tension development, indicating a requirement for cyclo-oxygenase products in the coupling process (Boyle *et al.*, 1988; McCaig & Rodger, 1988; McCaig, 1989). On the other hand endogenous cyclo-oxygenase products have been shown to suppress cholinergic neurotransmission in canine airways (Ito & Tajima, 1981) and cyclo-oxygenase inhibition augments vagal constriction in guinea-pig trachea (McCaig, 1989). In the present work it was found that flurbiprofen augmented vagal responses and prevented the deterioration in vagal response amplitude that occurred over several hours. Verapamil and nifedipine attenuated vagal responses both in the absence and presence of flurbiprofen. Nifedipine, however, was less effective in the presence of flurbiprofen, especially at lower frequencies of stimulation (<10 Hz). This suggests that a smaller component of the constriction is dependent on VOC opening in flurbiprofen-treated tissues where cholinergic neurotransmission is not being naturally dampened by endogenous cyclo-oxygenase products. This is not borne out by the results for verapamil, however, which were not significantly different in the presence of flurbiprofen. This differential effect of flurbiprofen on nifedipine could be related to the fact that

nifedipine and verapamil each bind to specific but distinct sites within the Ca^{2+} channel (Towart & Schramm, 1984).

A reduction in resting tone of guinea-pig trachea in response to methoxyverapamil has been reported (Small, 1982) but neither nifedipine nor verapamil was found to affect resting tone significantly (Foster *et al.*, 1984; Ahmed *et al.*, 1985). In the present work both verapamil and nifedipine reduced baseline tone, which is consistent with the suppression of slow wave activity by these drugs. Bay K 8644 had variable effects on spontaneous tone. Similar results have been obtained in guinea-pig (Allen *et al.*, 1985) and human airways (Black *et al.*, 1989). In addition, Bay K 8644 had no effect on membrane potential or slow wave discharge in guinea-pig trachealis (Allen *et al.*, 1985). Bay K 8644 promotes Ca^{2+} influx through VOCs seemingly by binding to and activating the dihydropyridine binding site thereby prolonging the open state of the channel (Freedman & Miller, 1984; Reuter *et al.*, 1985). It seems, therefore, that Bay K 8644 has a limited capacity to activate VOCs in trachea at rest. In contrast Bay K 8644 augmented responses to vagal stimulation, sometimes by very large amounts, indicating that Bay K 8644 can activate VOCs involved in the cholinergic neurotransmission process. Whilst it is likely that these VOCs are associated with the smooth muscle cell membrane, a prejunctional effect on transmitter release cannot be excluded (Perney *et al.*, 1986).

In conclusion the Ca^{2+} antagonists verapamil and nifedipine reduce vagal constriction in guinea-pig isolated trachea, whilst the Ca^{2+} -channel activator Bay K 8644 augments this response. These results indicate significant involvement of VOCs in vagally-mediated constriction. The effects of verapamil and nifedipine occur whether or not the enzyme cyclo-oxygenase is inhibited but nifedipine is much less effective in reducing responses to low frequency vagal stimulation in the presence of flurbiprofen. At higher concentrations of verapamil or nifedipine attenuation may involve prejunctional effects on cholinergic neurotransmission.

The support of the Nuffield and SmithKline Foundations, the Scottish Home and Health Department, the National Asthma Campaign and Lilly Research is gratefully acknowledged. We thank Boots for their kind gift of flurbiprofen and Mr B. De Jonckheere for technical assistance.

References

- AHMED, F., FOSTER, R.W. & SMALL, R.C. (1985). Some effects of nifedipine in guinea-pig isolated trachealis. *Br. J. Pharmacol.*, **84**, 861–869.
- ALLEN, S.L., FOSTER, R.W., SMALL, R.C. & TOWART, R. (1985). The effects of the dihydropyridine Bay K 8644 in guinea-pig isolated trachealis. *Br. J. Pharmacol.*, **86**, 171–180.
- BABA, K., SATAKE, T., TAKAGI, K. & TOMITA, T. (1986). Effects of verapamil on the response of the guinea-pig tracheal muscle to carbachol. *Br. J. Pharmacol.*, **88**, 441–449.
- BENGTTSSON, B., KHAN, A.R. & WEIBER, R. (1987). Low potency of Ca^{2+} antagonists in smooth muscle from different levels of the respiratory tract. *Acta Physiol. Scand.*, **131**, 249–256.
- BLACK, J.L., MARTHAN, R., ARMOUR, C.L. & JOHNSON, P.R.A. (1989). Sensitization alters contractile responses and calcium influx in human airway smooth muscle. *J. Allergy Clin. Immunol.*, **84**, 440–447.
- BLACKMAN, J.G. & McCAIG, D.J. (1983). Studies on an isolated innervated preparation of guinea-pig trachea. *Br. J. Pharmacol.*, **80**, 703–710.
- BOYLE, J.P., DAVIS, J.M., FOSTER, R.W., GOOD, D.M., KENNEDY, I. & SMALL, R.C. (1988). Spasmogen action in guinea-pig isolated trachealis: involvement of membrane K^{+} -channels and the consequences of K^{+} -channel blockade. *Br. J. Pharmacol.*, **93**, 319–330.
- CUSS, F.M., PALMER, J.D. & BARNES, P.J. (1985). Effect of nifedipine on autonomic control of airway smooth muscle *in vitro*. *Amer. Rev. Resp. Dis.*, **131**, A283.
- EMPEY, D.W. (1982). Bronchial hyper-reactivity. *Eur. J. Resp. Dis.*, **63**, Suppl 117, 33–42.
- FARLEY, J.M. & MILES, P.R. (1978). The sources of calcium for acetylcholine-induced contractions of dog tracheal smooth muscle. *J. Pharmacol. Exp. Ther.*, **207**, 340–346.
- FOSTER, R.W., OKPALUGO, B.I. & SMALL, R.C. (1984). Antagonism of Ca^{2+} and other actions of verapamil in guinea-pig isolated trachealis. *Br. J. Pharmacol.*, **81**, 499–507.
- FREEDMAN, S.B. & MILLER, R.J. (1984). Calcium channel activation: a different type of drug action. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 5580–5583.
- ITO, Y. & TAJIMA, K. (1981). Actions of indomethacin and prostaglandins on neuro-effector transmission in the dog trachea. *J. Physiol.*, **319**, 379–392.
- KANNAN, M.S. & DAVIES, C. (1988). Mode of action of calcium antagonists on responses to spasmogens and antigen challenge in human airway smooth muscle. *Resp. Physiol.*, **74**, 15–24.
- McCAIG, D.J. (1986). Electrophysiology of neuroeffector transmission in the isolated, innervated trachea of the guinea-pig. *Br. J. Pharmacol.*, **89**, 793–801.
- McCAIG, D.J. (1987). Comparison of autonomic responses in the trachea isolated from normal and albumin-sensitive guinea-pigs. *Br. J. Pharmacol.*, **92**, 809–816.
- McCAIG, D.J. (1989). Effects of flurbiprofen on the isolated, innervated trachea of the guinea-pig. *J. Physiol.*, **409**, 88P.
- McCAIG, D.J. & RODGER, I.W. (1988). Effects of leukotriene D_4 on the mechanical and electrical properties of guinea-pig isolated trachealis. *Br. J. Pharmacol.*, **94**, 729–736.
- MIDDLETON, E. (1980). Antiasthmatic drug therapy and calcium ions: review of the pathogenesis and role of calcium. *J. Pharm. Sci.*, **69**, 243–251.
- MILLER, R.J. (1987). Multiple calcium channels and neuronal function. *Science*, **235**, 46–52.
- PERNEY, T.M., HIRNING, L.D., LEEMAN, S.E. & MILLER, R.J. (1986). Multiple calcium channels mediate neurotransmitter release from peripheral neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 6656–6659.

- REUTER, H., PORZIG, H., KOKUBUN, S. & PROD'HOM, B. (1985). 1,4-Dihydropyridines as tools in the study of Ca^{2+} channels. *Trends Neurosci.*, **8**, 396–400.
- SMALL, R.C. (1982). Electrical slow waves and tone of guinea-pig isolated trachealis muscle: effects of drugs and temperature changes. *Br. J. Pharmacol.*, **77**, 45–54.
- SMALL, R.C. & FOSTER, R.W. (1988). Electrophysiology of the airway smooth muscle cell. In *Asthma: Basic Mechanisms and Clinical Management* ed. Barnes, P.J., Rodger, I.W. & Thomson, N.C., pp. 35–56. London: Academic Press.
- SPEEDING, M. (1987). Three types of Ca^{2+} channels explain discrepancies. *Trends Pharmacol. Sci.*, **8**, 115–117.
- TOWART, R. & SCHRAMM, M. (1984). Recent advances in the pharmacology of the calcium channel. *Trends Pharmacol. Sci.*, **5**, 111–113.
- WEISS, G.B., PANG, I-H. & GOODMAN, F.R. (1985). Relationship between $^{45}\text{Ca}^{2+}$ movements, different calcium components and responses to acetylcholine and potassium in tracheal smooth muscle. *J. Pharmacol. Exp. Ther.*, **233**, 389–394.

(Received April 2, 1991

Revised May 31, 1991

Accepted June 18, 1991)