Br. J. Pharmac. (1985), **84**, 861–869

Some effects of nifedipine in guinea-pig isolated trachealis

F. Ahmed, R.W. Foster & R.C. Small

Department of Pharmacology, Materia Medica and Therapeutics, Manchester University Medical School, Stopford Building, Oxford Road, Manchester M13 9PT

1 In trachealis depolarized by a K^+-rich medium, nifedipine (0.001–1 μmol l^{-1}) caused concentration-dependent antagonism of $CaCl_2$-induced increase in tension, moving the $CaCl_2$ log concentration-effect curve to the right and depressing the maximal response.

2 In trachealis in normal Krebs solution, similar concentrations of nifedipine had marked antispasmogenic activity against the responses to potassium chloride (KCl) and tetraethylammonium (TEA). However, nifedipine had little, if any, antispasmogenic activity against the responses to acetylcholine or histamine.

3 Nifedipine 1 μmol l^{-1} was tested for spasmolytic activity in tissues generating tension in response to the EC_{50} of acetylcholine, KCl or $CaCl_2$. In producing spasmolysis nifedipine was most effective against $CaCl_2$ and least effective against acetylcholine.

4 Nifedipine (0.01–1 μmol^{-1}) had little or no effect on the tone of trachealis in normal Krebs solution.

5 Intracellular electrophysiological recording showed that nifedipine 1 μmol l^{-1} could abolish spontaneous slow wave activity. This was associated with very minor depolarization and little or no loss of mechanical tone. In tissues treated with TEA (8 mmol l^{-1}) nifedipine abolished spike and slow wave discharge and reduced mechanical activity to the pre-TEA level.

6 It is concluded that nifedipine prevents KCl- or TEA-induced spasm by inhibition of Ca^{2+} influx. Spasm evoked by acetylcholine or histamine and the maintenance of spontaneous tone depend largely on mechanisms for increasing the cytoplasmic concentration of free Ca^{2+} which are resistant to nifedipine.

Introduction

Potassium chloride (KCl) evokes spasm of trachealis muscle which is associated with cellular depolarization (Foster *et al.*, 1983b). The spasm is dependent on the depolarization (Coburn & Yamaguchi, 1977) and is associated with the influx of Ca^{2+} (Foster *et al.*, 1983b; Raeburn & Rodger, 1984). Both the spasm (Foster *et al.*, 1984) and the associated Ca^{2+} influx (Raeburn & Rodger, 1984) may be prevented by the organic calcium antagonist verapamil. These findings support the proposal that KCl evokes spasm of trachealis by promoting the influx of Ca^{2+} through the voltage-operated channels described by Bolton (1979). Although the evidence (Dixon & Small, 1983; Foster *et al.*, 1983a; 1984) is less complete, tetraethylammonium (TEA) may induce spasm by a similar mechanism.

In contrast, spasmogens which activate specific receptors on the surface of trachealis cells may induce tension by a mechanism distinct from that utilized by KCl or TEA. Acetylcholine, for example, evokes depolarization of trachealis (Stephens & Kroeger, 1970; Kirkpatrick, 1975; Farley & Miles, 1977; Ahmed *et al.*, 1984) but the accompanying spasm is not dependent on the depolarization (Coburn, 1979). Histamine also evokes depolarization (Kirkpatrick, 1975; Suzuki *et al.*, 1976; McCaig & Souhrada, 1980; Ahmed *et al.*, 1984) but neither acetylcholine nor histamine evoke measurable Ca^{2+} influx (Ahmed *et al.*, 1984). Furthermore, the spasm evoked by acetylcholine or histamine is resistant to concentrations of verapamil which suppress KCl- or TEA-induced spasm (Foster *et al.*, 1984). These findings are consistent with acetylcholine and histamine causing spasm mainly by releasing Ca^{2+} from intracellular sites of sequestration.

In the present study this dichotomy of spasmogen action has been investigated further by using

nifedipine as an inhibitor of Ca^{2+} entry in both mechanical and electrophysiological experiments with guinea-pig trachealis. In the tissue bath experiments we have administered nifedipine before spasmogen challenge and have measured the inhibitory effect of nifedipine on tension development (i.e. its antispasmogenic activity). We have also administered nifedipine when the response to a spasmogen was already fully developed and have measured the reduction of developed tension caused by nifedipine (i.e. its spasmolytic activity). These two types of experiment were prompted by reports (e.g. Cheng & Townley, 1983) that nifedipine can exhibit spasmolytic effects at concentrations which have no antispasmogenic activity.

Methods

Guinea-pigs (350–700g) of either sex were killed by stunning and bleeding. Tracheae were excised, cleaned of adhering fat and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis. Subsequent tissue bath or electrophysiological experiments were mainly (see below) carried out under sodium lamp illumination in order to minimize the photolysis of nifedipine.

Assessment of antispasmogenic activity of nifedipine

Spasmogen (acetylcholine, histamine, KCl or TEA) action in small segments of trachealis was studied by the construction of cumulative concentration-effect curves as previously described (Foster *et al.*, 1984). In test tissues concentration-effect curves for the spasmogens were constructed both before and after tissue equilibration (1 h) with nifedipine 0.01, 0.1, 1 or 10 μmol l^{-1}.

Where antagonism of $CaCl_2$ was measured, the trachealis was initially mounted in MOPS-buffered physiological salt solution (MOPS-PSS, Jetley & Weston, 1980) but the bath fluid was then changed to K^+-rich Ca^{2+}-free MOPS-PSS, as previously described (Foster *et al.*, 1984) to induce depolarization. Spasm evoked by exposure to the depolarizing medium was dissipated by regular changes of the K^+-rich, Ca^{2+}-free bath fluid. A cumulative concentration-effect curve for $CaCl_2$ was then constructed as described by Foster *et al.* (1984). Following $CaCl_2$ washout, test tissues were incubated with nifedipine (0.001–1 μmol l^{-1}) for 1 h. Following nifedipine equilibration, a second $CaCl_2$ concentration-effect curve was constructed.

In all the above experiments the effects of nifedipine photolysis during equilibration with test tissues were minimized by regular (every 15 min) replacement of the nifedipine-containing bath fluid. Control tissues from the same trachea were not exposed to nifedipine but otherwise were treated identically.

Assessment of spasmolytic activity of nifedipine

In order to minimize the effects of tissue tone loss, these experiments were carried out in media containing indomethacin 2.8 μmol l^{-1}. Where acetylcholine or KCl was used as the spasmogen, the bathing medium was Krebs solution. Where $CaCl_2$ was the spasmogen, depolarizing Ca^{2+}-free MOPS-PSS was used.

Initial concentration-effect curves were constructed for acetylcholine, $CaCl_2$ or KCl as described above. These concentration-effect curves were used to calculate the EC_{50} for each spasmogen. Tissues were subsequently challenged with the spasmogen EC_{50} and nifedipine (1 μmol l^{-1}) was added to test tissues 3 (acetylcholine), 10 ($CaCl_2$) or 12 min (KCl) later. The spasmolytic effects of nifedipine were monitored for 1 h. The spasmogen EC_{50} (but not the nifedipine) was then washed from the tissue. Finally, a second concentration-effect curve for the spasmogen was constructed in the presence of nifedipine (1 μmol l^{-1}). In this way spasmolytic and antispasmogenic actions of nifedipine were studied in a single test tissue. Control tissues were treated with vehicle instead of nifedipine.

Measurement of the effects of nifedipine on tone

To minimize photolysis of nifedipine, these experiments were carried out in the dark. Tissues were preincubated in Krebs solution for 1 h and then treated with nifedipine or vehicle. One hour later aminophylline (1 mmol l^{-1}) was added to determine the recorder pen position at zero tone. Tone loss occurring during tissue treatment with nifedipine or vehicle was expressed as a percentage of the tone observed at the start of treatment

Intracellular electrophysiological recording

Simultaneous recording of intracellular electrical activity and mechanical changes of a contiguous segment of trachea was performed using the technique of Dixon & Small (1983). In this technique a segment of trachea is clamped to a tissue holder which permits adjustment of the degree of tissue stretch. Impalement of trachealis cells is made from the mucosal surface and mechanical activity is recorded isometrically from an adjacent tissue segment.

The effects of nifedipine on spontaneous electrical activity were studied in single cells in each of 7 tissues. Following impalement 2–3 min were allowed to elapse to check that the electrical activity had stabilized. Nifedipine (1 μmol l^{-1}) was then added to the Krebs solution. Five minutes later the microelec-

trode was deliberately withdrawn from the cell to obtain an estimate of the resting membrane potential. The change in mechanical tone occurring during this period was also measured. Similar recordings of electrical and mechanical activity in tissues not exposed to nifedipine served as controls.

Studies of the effects on nifedipine of TEA-induced electrical activity were made in a similar manner. However, tissues were bathed in Krebs solution containing TEA 8 mmol l^{-1} and the time required for nifedipine to reduce mechanical activity to the pre-TEA level noted.

Drugs and solutions/statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. Where KCl was used as a spasmogen the stated concentration excludes the KCl provided by the formulation of the physiological salt solution. The following substances were used: acetylcholine chloride (BDH), aminophylline (BDH), calcium chloride (BDH), histamine acid phosphate (BDH), indomethacin (Sigma), nifedipine (Bayer), potassium chloride (Hopkin and Williams), tetraethylammonium bromide (Sigma). Stock solutions of acetylcholine, indomethacin and nifedipine were prepared in absolute ethanol, those of other drugs in twice-distilled water. Except in experiments were $CaCl_2$ was used as an agonist, the physiological salt solution was Krebs solution of the following composition (mmol l^{-1}): Na^+ 143.5, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 125, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11.1

The significance of differences between means was assessed using a two-tailed, unpaired *t* test or by analysis of variance

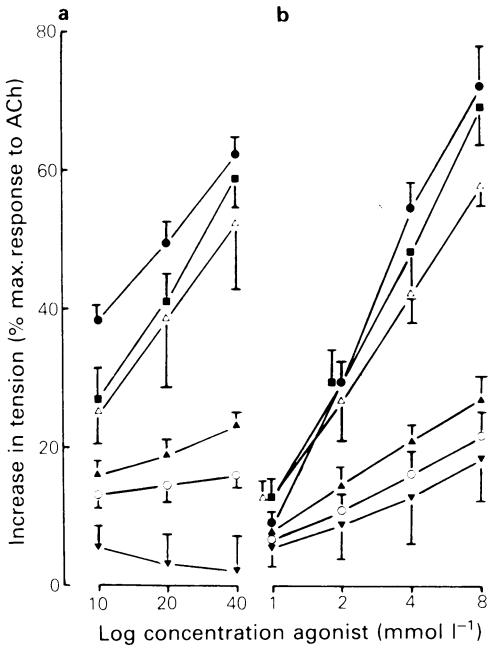

Figure 1 The antispasmogenic effects of nifedipine on the responses of the guinea-pig trachealis (in normal Krebs solution) to KCl (a) and tetraethylammonium (TEA) (b). The abscissae indicate concentration of agonist (mmol l^{-1}) on a log scale. The ordinates represent response as a % of the maximal response to acetylcholine: (●), initial log concentration-effect curve; (■), curve obtained following further incubation for 1 h in Krebs solution (control tissues); (△, ▲, ○, ▼), curves obtained after incubation with nifedipine 0.01, 0.1, 1, 10 μmol l^{-1}, respectively, for 1 h. Points represent the means of observations from at least 7 tissues at 1 h. The five initial log concentration-effect curves have been pooled. Vertical lines represent s.e. mean.

Results

Antispasmogenic activity of nifedipine

Control experiments showed that the log concentration-effect curve for KCl-induced spasmogenic activity of the trachealis underwent a slight depression on incubation of the tissue in Krebs solution for 1 h. Nifedipine (0.01–10 μmol l^{-1}) caused concentration-dependent and more marked depression of the log concentration-effect curve for KCl (Figure 1). In the presence of nifedipine 10 μmol l^{-1}, the higher concentrations of KCl (20 and 40 mmol l^{-1}) often caused active relaxation rather than an increase of tension. Under these circumstances the log concentration-effect curve for KCl had a negative slope.

Figure 1 shows that nifedipine also caused marked and concentration-dependent depression of the log concentration-effect curve for TEA. The antispasmogenic effects of nifedipine against KCl and TEA were fully developed at 1 h; longer incubation with nifedipine caused no further depression of the log concentration-effect curves for KCl and TEA.

In contrast to the log concentration-effect curves for KCl- and TEA-induced spasmogenic activity, those for histamine and acetylcholine were little modified by nifedipine (Figure 2). Control experiments showed that the log concentration-effect curve for histamine did not alter following tissue incubation (1 h) in Krebs solution. Nifedipine (0.01–10 μmol l^{-1}) appeared to shift the curve approximately 5 fold to the right – an effect which was independent of nifedipine concentration. The log concentration-effect curve for acetylcholine was unaltered by nifedipine and nifedipine had no greater action against acetylcholine or histamine following further (1 h) equilibration with the tissue.

In K^+-depolarized tissues $CaCl_2$ caused concentra-

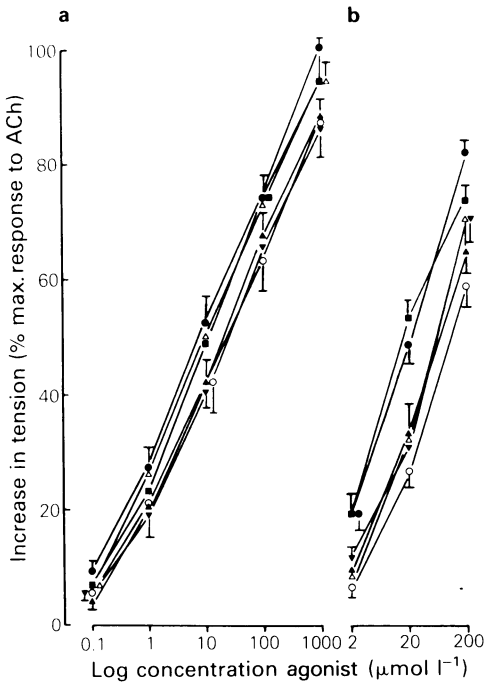

Figure 2 The antispasmogenic effects of nifedipine on the responses of the guinea-pig trachealis (in normal Krebs solution) to acetylcholine (a) and histamine (b). The abscissae indicate concentration of agonist (μmol l^{-1}) on a log scale. The ordinates represent response as a % of the initial maximal response to acetylcholine: (●), initial log concentration-effect curve; (■), curve obtained following further incubation for 1 h in Krebs solution (control tissues); (△, ▲, ○, ▼), curves obtained after nifedipine 0.01, 0.1, 1, 10 μmol l^{-1}, respectively, for 1 h. Points represent the means of observations from at least 7 tissues at 1 h. The five initial log concentration-effect curves have been pooled. Vertical lines represent s.e. mean.

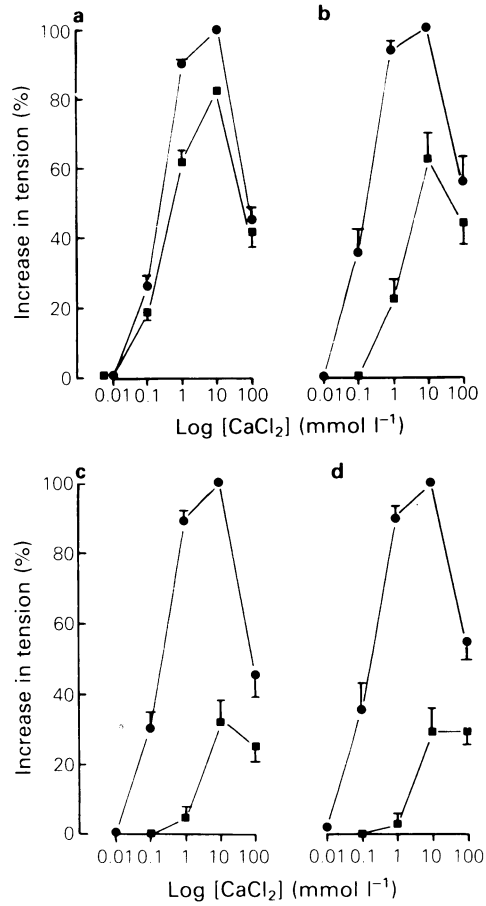

Figure 3 The antispasmogenic effects of nifedipine on the responses of guinea-pig trachealis to $CaCl_2$ in depolarizing MOPS-PSS. The abscissae indicate the concentrations of $CaCl_2$ (mmol l^{-1}) on a log scale. The ordinates indicate response as a % of the initial maximal response to $CaCl_2$. Points represent the means and vertical lines the s.e. mean (n = 7):(●), initial log concentration-effect curve for $CaCl_2$; (■), log concentration-effect curve for $CaCl_2$ obtained after tissue equilibration for 1 h in depolarizing Ca^{2+}-free MOPS-PSS (a; control), nifedipine 0.01 μmol l^{-1} (b), 0.1 μmol l^{-1} (c), 1 μmol l^{-1} (d).

tion-dependent spasm which became maximal at 10 mmol l^{-1}. Greater concentrations of $CaCl_2$ had relaxant activity. In control experiments (Figure 3a) it was observed that incubation of the tissue in depolarizing Ca^{2+}-free MOPS-PSS for 1 h caused a small rightward shift of the log concentration-effect curve for $CaCl_2$-induced increase in tension and the maximal response was slightly depressed. Nifedipine (0.001–1 μmol l^{-1}) antagonized the effect of $CaCl_2$ in a concentration-dependent manner. Figure 3 shows that this antagonism comprised both a shift to the right of the log concentration-effect curve for $CaCl_2$ and depression of the maximal response. Changes in the $CaCl_2$ log concentration-effect curve evoked by 0.001 μmol l^{-1} nifedipine were intermediate between those indicated in (a) and (b) of Figure 3. Although nifedipine (0.001–1 μmol l^{-1}) caused a progressive rightward shift of the log concentration-effect curve for $CaCl_2$, its ability to reduce the maximal response to $CaCl_2$ peaked at a concentration of 0.1 μmol l^{-1}.

Spasmolytic activity of nifedipine

Control experiments showed that the spasm evoked by the EC_{50} of acetylcholine declined slightly over a

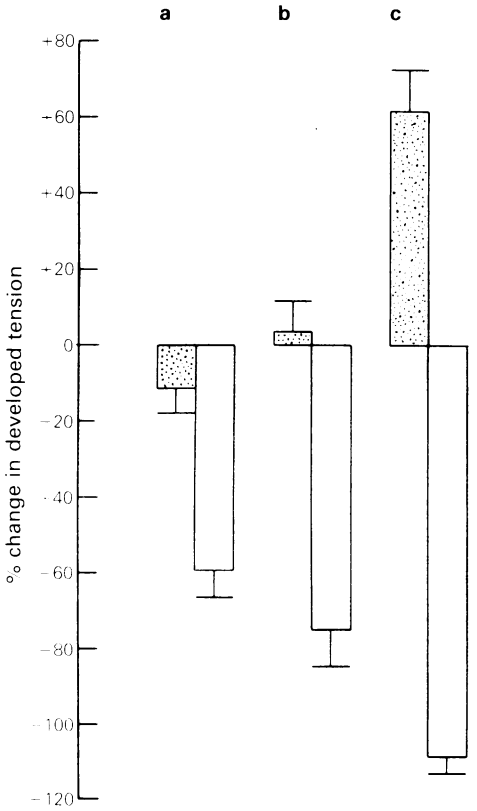

Figure 4 The spasmolytic effects of nifedipine 1 μmol l^{-1} on the responses to the EC_{50} of acetylcholine (a), KCl (b) and $CaCl_2$ (c). The ordinates show % change in developed tension occurring in 1 h. Control tissues (stippled columns) and tissues treated with nifedipine (open columns). Note that the spasmolytic effect of nifedipine against the response to $CaCl_2$ was greater than 100%. This indicates that nifedipine reduced tension to a level below that preceding the administration of the $CaCl_2$ EC_{50}. The vertical lines show s.e. mean.

period of 1 h. Spasm evoked by the EC_{50} of KCl changed little but that evoked by the EC_{50} of $CaCl_2$ increased in all tissues examined (Figure 4).

Nifedipine 1 μmol l^{-1} had spasmolytic effects in tissues generating tension in response to the EC_{50} of acetylcholine, KCl or $CaCl_2$. The initial rate at which tension was lost was similar for all three spasmogens (mean $t_{\frac{1}{2}}$ 2.5–5 min) but there were marked differences in the amplitude of the spasmolysis. When the tension changes occurring in control tissues are taken into account, it is evident that nifedipine reduced the spasm evoked by the EC_{50} of acetylcholine by approximately 50%. In contrast nifedipine totally abolished spasm evoked by $CaCl_2$ (Figure 4), reducing the developed tension to a value less than that observed before the administration of the EC_{50} of $CaCl_2$.

Following washout of the spasmogen EC_{50}, concentration-effect curves for acetylcholine, KCl or $CaCl_2$ were reconstructed in the presence of nifedipine 1 μmol l^{-1}. These curves were similar in shape and position to those obtained in experiments (Figures 1, 2 and 3) where the antispasmogenic effects of the same concentration of nifedipine were examined.

Effects of nifedipine on tone

At the start of each experiment tissues were subjected to 1g imposed tension. This generally waned rapidly. Subsequently most tissues developed tone which became maximal within the hour of preincubation in Krebs solution.

Over the next hour some tone loss was evident (Table 1) both in tissues treated with vehicle and in those treated with nifedipine (0.01–1 μmol l^{-1}). However, the mean tone loss in nifedipine-treated tissues did not differ significantly from that seen in the vehicle-treated controls.

Higher concentrations (10 μmol l^{-1}) of nifedipine also caused little tone loss but the ethanol content of the vehicle in this instance caused mild spasm and could have masked a relaxant effect of nifedipine.

Intracellular electrophysiological recording

Control experiments were performed to assess the changes in electrical activity which might result simply from holding the microelectrode within the impaled cell for 5 min. The initial electrical properties (resting membrane potential, maximal amplitude of spontaneous slow waves, slow wave frequency) of the control cells did not differ significantly from those of the test cells (Table 2). In all control cells slow wave discharge

Table 1 Loss of spontaneous tone evoked by nifedipine or vehicle in the guinea-pig isolated trachealis

	Vehicle	*Nifedipine* (μmol l^{-1})		
		0.01	*0.1*	*1*
Mean tone loss (%)	3.3 ± 7.0	11.3 ± 9.5	13.2 ± 7.2	13.0 ± 9.0

Data represent mean ± s.e. mean ($n \geq 9$).

Table 2 Effects of nifedipine 1 μmol l^{-1} on spontaneous electrical and mechanical activity of guinea-pig isolated trachealis

	Properties of cells before start of experimental period			*Measurements made over the 5 min experimental period*		
	Resting membrane potential (mV)	*Maximal amplitude of slow waves* (mV)	*Slow wave frequency* (Hz)	*Time for slow wave abolition* (min)	*Change in resting membrane potential* (mV)	*Change in mechanical tone* (mg)
Control cells	50.1 ± 2.0	13.1 ± 1.0	0.92 ± 0.07	—	−2.3 ± 0.7	+8.9 ± 12.5
Test cells	49.4 ± 2.6	12.2 ± 2.4	1.05 ± 0.5	2.40 ± 0.38	−6.4 ± 1.5	−142.9 ± 70.2

Data indicate mean ± s.e. mean of observations from 7 (test) or 9 (control) cells. The test cells only were treated with nifedipine at the start of the experimental period.

was well maintained throughout the 5 min experimental period. However, most control cells exhibited slight depolarization over this time. The mechanical tone of the contiguous segment of trachealis changed very little.

In test cells nifedipine 1 μmol l^{-1} abolished slow wave discharge within the 5 min experimental period. This was accompanied by slight cellular depolarization and a minor change in the mechanical tone of the contiguous segment of trachea (Table 2 and Figure 5).

In the presence of TEA 8 mmol l^{-1}, trachealis cells discharged spike potentials, each with a distinct plateau phase (Figure 6). Mechanical activity recorded from the contiguous segment of trachea sometimes comprised tonic tension development and sometimes phasic tension waves. In TEA-treated tissues nifedipine (1 μmol l^{-1}) at first suppressed spikes and subsequently suppressed slow waves. Spikes were abolished 2.0 ± 0.4 min and slow waves 2.9 ± 0.8 min after the addition of nifedipine. TEA-induced mechanical activity was totally suppressed 4.8 ± 1.1 min after the addition of nifedipine.

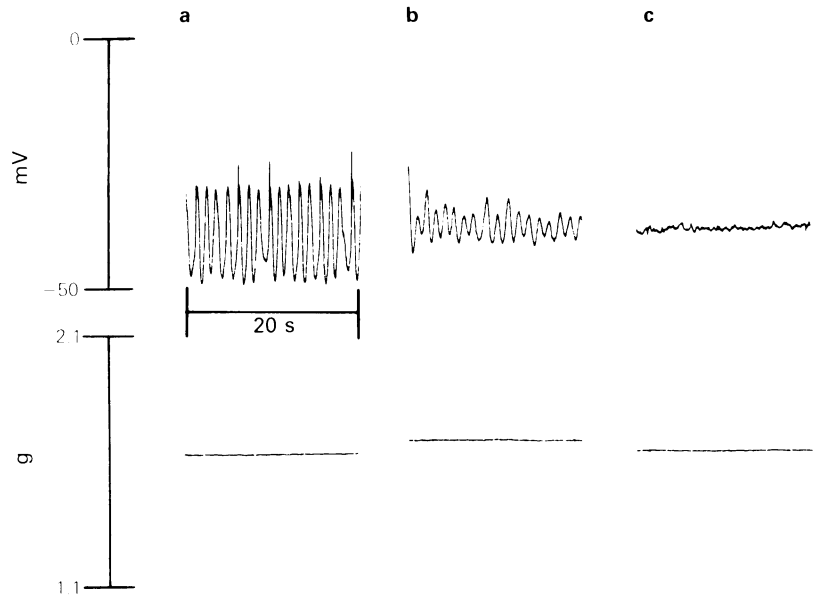

Figure 5 Simultaneous recordings of intracellular electrical activity (upper traces) and mechanical changes (lower traces) of the guinea-pig trachealis. All electrical records were taken from the same cell. (a c) Activity seen before (control), 2.5 min and 5 min after the administration of nifedipine 1 μmol l^{-1}, respectively. Note the suppression by nifedipine of spontaneous slow waves.

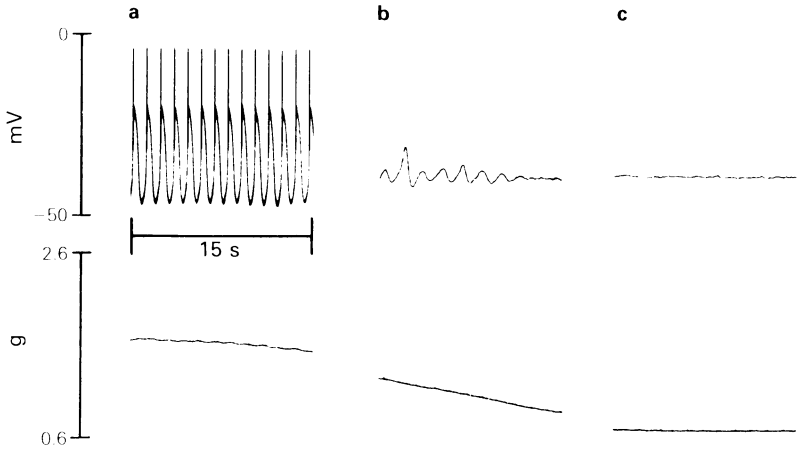

Figure 6 Simultaneous recordings of intracellular electrical activity (upper traces) and mechanical changes (lower traces) of guinea-pig trachealis in Krebs solution containing tetraethylammonium (TEA) 8 mmol l^{-1}. All electrical recordings were taken from the same cell. (a–c) Activity seen before (control), 3 min and 5 min after the administration of nifedipine 1 μmol l^{-1}, respectively. Note the suppression by nifedipine of TEA-induced spike potentials, slow waves and mechanical activity.

Discussion

In K^+ depolarized rat trachealis, nifedipine (0.01 μmol l^{-1}) antagonized the responses to $CaCl_2$ (Cheng & Townley, 1983). Nifedipine depressed markedly the log concentration-effect curve for $CaCl_2$, suggesting that the antagonism was insurmountable.

Nifedipine (0.001–1 μmol l^{-1}) caused concentration-dependent antagonism of the responses to $CaCl_2$ in the present study of guinea-pig trachealis (Figure 3). The antagonism comprised both a rightward shift of the $CaCl_2$ log concentration-effect curve and a depression of the maximal response. Depression of the maximal response to $CaCl_2$ may be due not only to the action of nifedipine but also to the relaxant action of $CaCl_2$ at concentrations in excess of 10 mmol l^{-1} (Foster *et al.*, 1984). This relaxant action confounds attempts to surmount nifedipine-induced antagonism by raising the concentration of $CaCl_2$.

The antispasmogenic activity of nifedipine against KCl in guinea-pig trachealis (Weichman *et al.*, 1983) was confirmed in the present study. Nifedipine has similar activity against TEA (Figure 1). There is evidence (Dixon & Small, 1983; Foster *et al.*, 1983a,b) that KCl- or TEA-induced spasm of guinea-pig trachealis involves both depolarization and Ca^{2+} influx. Therefore, the similar effectiveness of nifedipine against these spasmogens (Figure 1) and against $CaCl_2$ in the K^+-depolarized tissue (Figure 3) strongly suggests that nifedipine prevents KCl- or TEA-induced spasm by inhibition of Ca^{2+} influx. In the case of TEA part of this influx may occur during the upstroke of spikes or slow waves, for nifedipine rapidly suppressed (Figure 6) these calcium-dependent (Small, 1982; Foster *et al.*, 1983a; 1984) electrical phenomena.

The failure of nifedipine to exert marked antispasmogenic activity against acetylcholine or histamine (Figure 2; Cheng & Townley, 1983; Henderson *et al.*, 1983) contrasts with its effectiveness against $CaCl_2$, KCl or TEA (Figures 1 and 3). This implies either that acetylcholine and histamine promote Ca^{2+} influx through nifedipine-resistant channels or that these agents act to mobilize Ca^{2+} from intracellular sites. The former hypothesis seems improbable because spasm of guinea-pig trachealis evoked by acetylcholine or histamine is not associated with measurable Ca^{2+} influx (Ahmed *et al.*, 1984). Mobilization of Ca^{2+} from intracellular sites seems the more likely mechanism underlying the actions of acetylcholine and histamine.

In experiments where the spasmolytic effects of nifedipine were examined, the spasmogenic response to a calculated EC_{50} of $CaCl_2$ increased slowly throughout the hour following a 10 min equilibration period. The large eventual size of this tension increase (Figure 4) was not anticipated but clearly indicates that, when $CaCl_2$ is used as a spasmogen, a period well in excess of 10 min should be allowed for the equilibration of each concentration of $CaCl_2$ with the tissue, otherwise the potency of $CaCl_2$ will be underestimated.

Nifedipine 1 μmol l^{-1} had spasmolytic activity against acetylcholine, KCl, $CaCl_2$ (each applied as the EC_{50} in tissue bath experiments) and TEA (intracellular electrophysiological experiments). Assuming that nefidipine acts simply to prevent Ca^{2+} influx,

its spasmolytic effect must result both from inhibition of Ca^{2+} entry and from the operation of mechanisms which actively reduce cytosol Ca^{2+} concentration – the intracellular sequestration of Ca^{2+} or its extrusion from the cell.

Nifedipine's marked spasmolytic activity against KCl, $CaCl_2$ (Figure 4) and TEA (Figure 6) is entirely consistent with its antispasmogenic actions against these agents (Figures 1 and 3). However, the spasmolytic activity of nifedipine against acetylcholine (albeit smaller than that seen with other spasmogens) may seem incompatible with its lack of antispasmogenic activity (Figure 2a) against this agent. Nevertheless, when tested against histamine, the same concentration of nifedipine can be spasmolytic but devoid of antispasmogenic activity (Cheng & Townley, 1983).

An explanation of this phenomenon was offered by Ahmed *et al.* (1984). Acetylcholine and histamine are envisaged to stimulate tension development principally by releasing Ca^{2+} from intracellular storage sites. Since this process is not susceptible to nifedipine little antispasmogenic activity is possible. With continued activation of the muscarinic or H_1-receptor, the intracellular stores of Ca^{2+} may adapt or become partially depleted. Hence the maintenance of the developed tension may then become more dependent on Ca^{2+} influx promoted as a minor effect of acetylcholine or histamine. At this stage nifedipine can exert spasmolytic activity.

Intracellular recording from control tissues showed that spontaneous slow wave activity was well maintained over a 5 min period. However, most control cells exhibited slight depolarization during this time (Table 2). When these findings are taken into account there seems little doubt that nifedipine 1 μmol l^{-1} abolishes spontaneous slow wave discharge (Figure 5) but the small depolarization seen in the presence of this drug may partly be attributed simply to the presence of the microelectrode tip within the cell.

Although the electrical slow waves of guinea-pig trachealis may be the functional equivalent of the spikes seen in other smooth muscles (Ahmed *et al.*, 1984) the link between their discharge and the maintenance of mechanical tone is very tenuous (Small, 1982). The present finding that nifedipine can abolish slow waves without markedly lowering tissue tone further endorses the tenuous nature of the link and suggests that tone maintenance may largely depend on the release of Ca^{2+} from intracellular sites of sequestration.

Nifedipine's present failure to abolish tone is consistent with the observations of other workers (Small, 1982; Henderson *et al.*, 1983; Weichman *et al.*, 1982) who have tested organic calcium antagonists in guinea-pig trachealis subjected to imposed tension of 2 g or less. However, there is some evidence that nifedipine may have relaxant activity when a higher imposed tension is used (Fanta *et al.*, 1982; Cheng & Townley, 1983).

This work was supported in part by grants from the Asthma Research Council, the North Western Regional Health Authority and the Smith, Kline & French Foundation.

References

AHMED, F., FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1984). Some features of the spasmogenic actions of acetylcholine and histamine in guinea-pig isolated trachealis. *Br. J. Parmac.*, **83,** 227–233.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscles. *Physiol. Rev.*, **59,** 606–718.

CHENG, J.B. & TOWNLEY, R.G. (1983). Pharmacological characterization of effects of nifedipine on isolated guinea-pig and rat tracheal smooth muscle. *Archs. Int. Pharmacodyn.*, **263,** 228–244.

COBURN, R.F. (1979). Electromechanical coupling in canine trachealis muscle: acetylcholine contractions. *Am. J. Physiol.*, **236** (3), C177–C184.

COBURN, R.F. & YAMAGUCHI, T. (1977). Membrane potential-dependent and -independent tension in the canine tracheal muscle. *J. Pharmac. Exp. Ther.*, **201,** 276–284.

DIXON, J.S. & SMALL, R.C. (1983). Evidence of poor conduction of muscle excitation in the longitudinal axis of guinea-pig isolated trachea. *Br. J. Pharmac.*, **79,** 75–83.

FANTA, C.H., VENUGOPALAN, C.S., LACOUTURE, P.G. & DRAZEN, J.M. (1982). Inhibition of bronchoconstriction in the guinea-pig by a calcium channel blocker, nifedipine. *Am. Rev. Resp. Dis.*, **125,** 61–66.

FARLEY, J.M. & MILES, P.R. (1977). Role of depolarization in acetylcholine-induced contractions of dog trachealis muscle. *J. Pharmac. Exp. Ther.*, **201,** 199–205.

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. Pharmac.*, **81,** 499–507.

FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1983a). Evidence that the spasmogenic action of tetraethylammonium in guinea-pig trachealis is both direct and dependent on the cellular influx of Ca^{2+} ion. *Br. J. Pharmac.*, **79,** 255–263.

FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1983b). The spasmogenic action of potassium chloride in guinea-pig trachealis. *Br. J. Pharmac.*, **80,** 553–559.

HENDERSON, A.F., HEATON, R.W., DUNLOP, L.S. & COSTELLO, J.F. (1983). Effects of nifedipine on antigen-induced bronchoconstriction. *Am. Rev. Resp. Dis.*, **127,** 549–553.

JETLEY, M. & WESTON, A.H. (1980). Some effects of sodium

nitroprusside, methoxyverapamil (D600) and nifedipine on rat portal vein. *Br. J. Pharmac.*, **68,** 311–319.

KIRKPATRICK, C.T. (1975). Excitation and contraction in bovine tracheal smooth muscle. *J. Physiol.*, **244,** 263–281.

McCAIG, D.J. & SOUHRADA, J.F. (1980). Alteration of electrophysiological properties of airway smooth muscle from sensitised guinea-pigs. *Resp. Physiol.*, **41,** 49–60.

RAEBURN, D. & RODGER, I.W. (1984). Lack of effect of leukotriene D_4 on Ca uptake in airway smooth muscle. *Br. J. Pharmac.*, **83,** 499–504.

SMALL, R.C. (1982). Electrical slow waves and tone of guinea-pig isolated trachealis muscle: the effects of drugs and temperature changes. *Br. J. Pharmac.*, **77,** 45–54.

STEPHENS, N.L. & KROEGER, E. (1970). Effect of hypoxia on airway smooth muscle mechanics and electrophysiology. *J. Appl. Physiol.*, **28,** 630–635.

SUZUKI, H., MORITA, K. & KURIYAMA, H. (1976). Innervation and properties of the smooth muscle of the dog trachea. *Jap. J. Physiol.*, **26,** 303–320.

WEICHMAN, B.M., MUCCITELLI, R.M., TUCKER, S.S. & WASSERMAN, M.A. (1983). Effects of calcium antagonists on leukotriene D_4-induced contractions of the guinea-pig trachea and lung parenchyma. *J. Pharmac. exp. Ther.*, **225,** 310–315.

(Received July 30, 1984,
Revised September 25, 1984.
Accepted October 21, 1984.)