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Antagonism of Ca^{2+} and other actions of verapamil in guinea-pig isolated trachealis

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1 In trachealis bathed by a K^+-rich, Ca^{2+}-free physiological salt solution, calcium chloride ($CaCl_2$) at 0.01 to 10 mmol l^{-1} evoked concentration-dependent spasm. Verapamil (0.1 to 10 μmol l^{-1}) was an effective antagonist of $CaCl_2$.

2 Spasm evoked by acetylcholine, histamine, potassium chloride (KCl) and tetraethylammonium (TEA) was studied in trachealis bathed by normal Krebs solution. Verapamil (0.1 to 10 μmol l^{-1}) markedly suppressed spasm evoked by KCl and TEA. In contrast the actions of acetylcholine and histamine were much less affected by verapamil.

3 Spasm evoked by prostaglandin E_2 was studied in trachealis bathed by Krebs solution containing indomethacin (2.8 μmol l^{-1}). Verapamil (0.1 to 10 μmol l^{-1}) had little or no effect against prostaglandin E_2-induced spasm.

4 Verapamil (0.1 to 10 μmol l^{-1}) had relatively little effect on the tone of trachealis bathed by normal Krebs solution. In contrast bathing in Krebs solution lacking $CaCl_2$ caused almost complete tone loss.

5 Extracellular electrophysiological recording showed that verapamil (10 μmol l^{-1}) suppressed not only TEA-evoked spasm but also TEA-evoked slow waves and spike potentials. Verapamil also abolished the transient period of slow wave activity associated with the spasm evoked by KCl.

6 Intracellular electrophysiological recording showed that TEA-induced spike activity was resistant to tetrodotoxin (3 μmol l^{-1}). However, verapamil (10 μmol l^{-1}) abolished the tetrodotoxin-resistant spikes without increasing the resting membrane potential.

7 It is concluded that verapamil suppresses TEA- or KCl-induced spasm, slow waves or spikes by inhibition of Ca^{2+} influx. Spasm evoked by acetylcholine, histamine and prostaglandin E_2 depends on mechanisms for increasing the cytoplasmic concentration of free Ca^{2+} which are resistant to verapamil. The failure of verapamil markedly to depress tissue tone is consistent with the proposal that tone results from the activity of endogenous prostaglandins.

Introduction

Agonists may evoke contraction of smooth muscle by releasing Ca^{2+} from intracellular sites of sequestration or by promoting the entry of extracellular Ca^{2+}. The cellular entry of Ca^{2+} may be facilitated by the opening of receptor-operated or voltage-dependent Ca^{2+} channels (Bolton, 1979).

Spasm of airway smooth muscle evoked by KCl or tetraethylammonium (TEA) can be suppressed by verapamil (Coburn, 1977; Farley & Miles, 1978; Cerrina *et al.*, 1982) but verapamil is less effective in suppressing spasm evoked by acetylcholine (Farley & Miles, 1978; Cerrina *et al.*, 1982), histamine or leukotriene D_4 (Cerrina *et al.*, 1982). Verapamil is believed to block voltage-dependent Ca^{2+} channels (Coburn, 1977). The differential sensitivity of bronchoconstrictor agents to the suppressant effects of verapamil may therefore be a reflection of the different mechanisms by which bronchoconstrictors increase the concentration of free Ca^{2+} in the cytoplasm of the airway smooth muscle cell.

As yet there have been no direct studies in respiratory tract smooth muscle of the ability of verapamil to suppress Ca^{2+}-induced spasm. In the present work with guinea-pig trachealis we have therefore sought to measure Ca^{2+} antagonism by verapamil. Concentrations of verapamil causing marked antagonism of Ca^{2+} were then tested against a variety of bronchoconstrictor agents with the dual intent of assessing

the selectivity of action claimed by earlier workers and of attempting to relate suppressant effects of verapamil to antagonism of Ca^{2+}.

Prostaglandin E_2 was included in the group of bronchoconstrictor agents studied. Its inclusion was prompted by evidence (Farmer *et al.*, 1974) that prostaglandins have a role in the generation of tone in guinea-pig isolated trachea, that prostaglandin E_2 is synthesized by the tissue (Gryglewski *et al.*, 1976) and is one of the most potent spasmogenic prostaglandins (Coleman & Kennedy, 1980), and also by the observation that organic calcium antagonists have relatively little effect against the tension spontaneously developed by guinea-pig trachea (Fanta *et al.*, 1982; Small, 1982). It was therefore reasoned that study of the interaction between verapamil and prostaglandin E_2 might yield evidence pertinent to the proposed role of prostaglandins in maintaining tissue tone.

Methods

Guinea-pigs (350–750 g) of either sex were killed by stunning and bleeding. Tracheae were excised from the animals, cleaned of adhering fat and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis.

General protocol of tissue bath experiments

Small segments of trachea were set up for the isometric recording of tension changes as previously described (Foster *et al.*, 1983a). The effects of spasmogens were studied by constructing cumulative concentration-effect curves. The contact time for each concentration of a particular spasmogen was such as to allow the development of almost all the tension rise attainable by that concentration of agonist. For acetylcholine this was 3 min, for $CaCl_2$ 10 min, for histamine 6 min, for prostaglandin E_2 6 min, for TEA 5 min and for KCl 12 min. In test tissues, spasmogen action was examined both before and after tissue equilibration (1 h) with verapamil. Control tissues from the same trachea were not exposed to verapamil but otherwise were treated identically.

Antagonism of $CaCl_2$

In experiments where antagonism of $CaCl_2$ was measured, the trachealis was initially mounted in a medium (see below) containing normal amounts of Ca^{2+} and K^+. The bath fluid was then changed for a K^+-rich, Ca^{2+}-free medium (see below). This evoked marked spasm which was dissipated by regular (15 min intervals) changes of the K^+-rich, Ca^{2+}-free bath fluid. A concentration-effect curve for $CaCl_2$ was then constructed. Tissues were treated with vehicle or verapamil only when the Ca^{2+}-induced spasm had been fully dissipated by washing with K^+-rich, Ca^{2+}-free medium.

Measurement of the effects of verapamil or Ca^{2+}-free Krebs solution on tone

Initially tissues were set up in normal Krebs solution. Aminophylline (1 mmol l^{-1}) was then added to determine the recorder pen position at zero tone. Following aminophylline washout, tissue tone was allowed to reach its peak value. At this time the tissue was treated with Ca^{2+}-free Krebs solution, verapamil or vehicle. The tone loss occurring over the next hour was measured as a % of the peak tone achieved following the aminophylline washout.

Extracellular electrophysiological recording

Segments of trachea were set up for the extracellular recording of electrical and mechanical activity as previously described (Small, 1982). The effects of agonists ((TEA and KCl) were studied by their addition to the Krebs solution superfusing the tissue. Cumulative concentration-effect curves for the agonists were constructed as described for the tissue bath studies.

Following the construction of an initial agonist concentration-effect curve, verapamil (0.1–10 μmol l^{-1}) was added to the superfusion fluid and allowed to equilibrate with the tissue for 1 h. The effects of the agonist were then re-examined in the presence of verapamil.

Intracellular electrophysiological recording

Simultaneous recording of intracellular electrical activity and mechanical changes of a contiguous segment of trachea was performed as described by Dixon & Small (1983).

Microelectrode impalements of trachealis cells were made in tissues bathed by Krebs solution containing TEA 8 mmol l^{-1}. During the course of an impalement, tetrodotoxin (3 μmol l^{-1}) was added to the superfusion fluid and allowed to equilibrate with the tissue for 10 min. Verapamil 10 μmol l^{-1} was then added to the superfusion fluid and the effects of the drug combination on spike activity were then studied for as long as the microelectrode tip remained within the cell.

Drugs and solutions/statistical analysis of results

Drug concentrations are throughout expressed in

terms of molar concentration of the active species. Where KCl was used as an agonist the stated concentration excludes the KCl provided by the formulation of the physiological salt solution. The following substances were used: acetylcholine chloride (BDH), calcium chloride (BDH), histamine acid phosphate (BDH), indomethacin (Sigma), potassium chloride (Hopkin and Williams), prostaglandin E_2 (Sigma), tetrodotoxin (Sigma) and verapamil hydrochloride (Knoll). Stock solutions of acetylcholine and indomethacin were prepared in absolute ethanol, those of other drugs in twice distilled water. The stock solution of prostaglandin E_2 was renewed daily.

The physiological salt solution (PSS) used in most experiments of the present study was Krebs solution as described by Small (1982). Indomethacin ($2.8\,\mu mol\,l^{-1}$) was present in the Krebs solution in all experiments where the effects of prostaglandin E_2 were examined.

The experiments where $CaCl_2$ was used as an agonist required that a depolarizing Ca^{2+}-free PSS be employed. The depolarizing, Ca^{2+}-free PSS of Spedding (1982) contained K^+ at $40\,mmol\,l^{-1}$ and this concentration of K^+ evokes profound (approximately 40 mV) depolarization of guinea-pig trachealis (Foster *et al.*, 1983b). Accordingly, the depolarizing, Ca^{2+}-free media used in the present study also contained K^+ at $40\,mmol\,l^{-1}$. These media were prepared by altering the formulation of Krebs solution (Small, 1982) or MOPS-PSS (Jetley & Weston, 1980) respectively. $CaCl_2$ was omitted from each formulation and the concentration of KCl was raised to achieve a final K^+ concentration of $40\,mmol\,l^{-1}$. The concentration of NaCl was reduced to preserve isosmolality.

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

Results

Responses to depolarizing, Ca^{2+}-free media and subsequent addition of $CaCl_2$

When the bath fluid (Krebs or MOPS-PSS) was changed to a depolarizing, Ca^{2+} free medium, tissues generated tension which became maximal after approximately 3 min. Despite regular changes of the depolarizing, Ca^{2+}-free bath fluid, the tension developed by the tissue required approximately 70 min to fall back to the level seen prior to exposure to the depolarizing medium.

In both types of depolarizing medium, addition of $CaCl_2$ (0.01 to $10\,mmol\,l^{-1}$) at this time evoked smoothly-developing spasm which was concentration-dependent. The spasmogenic effect of $CaCl_2$ was clearly maximal at $10\,mmol\,l^{-1}$ since higher concentrations evoked some relaxation. The shape and position of the log concentration-effect curve of $CaCl_2$ obtained in depolarizing, Ca^{2+}-free MOPS-PSS did not significantly differ from that obtained in depolarizing, Ca^{2+}-free Krebs solution (Table 1).

Table 1 Features of the log concentration-effect curve for $CaCl_2$ as obtained in depolarizing, Ca^{2+}-free media

	Depolarizing Ca^{2+}-free Krebs solution	*Depolarizing Ca^{2+}-free MOPS-PSS*
Maximal response (mg)	829 ± 80	770 ± 101
$-\log_{10} EC_{50}$ ($mol\,l^{-1}$)	-3.98 ± 0.05	-3.97 ± 0.15
Slope of linear portion of curve (% per log unit)	47.9 ± 1.3	45.5 ± 1.6

Data represent mean ± s.e.mean ($n = 7$).

In the depolarizing, Ca^{2+}-free Krebs solution a precipitate appeared when $CaCl_2$ was used in concentrations of $10\,mmol\,l^{-1}$ or greater. For this reason all subsequent experiments involving the use of $CaCl_2$ as an agonist were carried out in depolarizing, Ca^{2+}-free MOPS-PSS.

Antagonism of $CaCl_2$ by verapamil

Control experiments (Figure 1a) revealed that the log concentration-effect curve for $CaCl_2$ was slightly depressed following an hour of incubation in depolarizing, Ca^{2+}-free MOPS-PSS.

Verapamil ($0.1-10\,\mu mol\,l^{-1}$) caused a concentration-dependent rightward shift of the log concentration-effect curve of $CaCl_2$. In the presence of verapamil the maximal response to $CaCl_2$ was clearly depressed and this may in part have been due to the relaxant action of $CaCl_2$ observed at concentrations greater than $10\,mmol\,l^{-1}$ (Figure 1b, c, d).

The mean relationship between log [verapamil] and log (Ca^{2+} concentration-ratio -1) showed a slope (0.61) significantly different from unity ($P < 0.01$). However, the line could be interpreted as inflected, the lower half showed a slope (0.86) not significantly different from unity and the upper half a much lesser slope (0.36).

Effect of verapamil on spasm evoked by acetylcholine, histamine, KCl and TEA

The actions of acetylcholine, histamine, KCl and TEA were examined in tissues bathed by normal Krebs solution. Control experiments showed that the

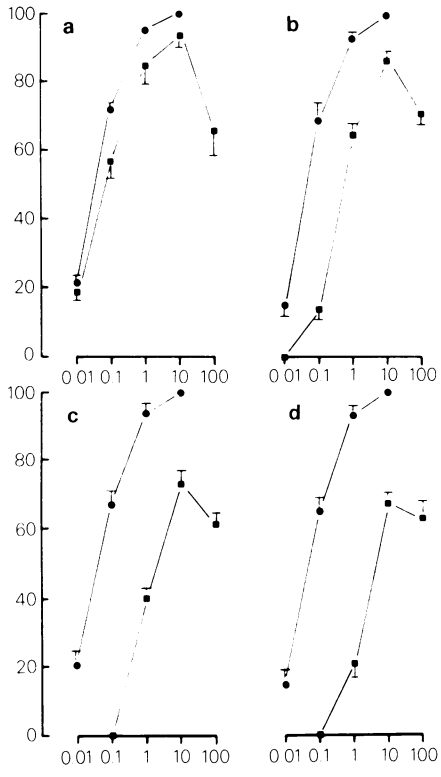

Figure 1 Guinea-pig trachealis bathed by depolarizing MOPS-PSS: antagonism of $CaCl_2$ by verapamil. The abscissae indicate the concentration of $CaCl_2$ (mmol l^{-1}) on a log scale. The ordinates indicate responses as a % of the initial maximal response to $CaCl_2$. Points represent the means and vertical bars 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 with MOPS-PSS (a) or verapamil 0.1 μmol l^{-1}(b), 1 μmol l^{-1}(c), 10 μmol l^{-1}(d).

log concentration-effect curves for acetylcholine and histamine underwent some depression following incubation in Krebs solution for 1 h. This effect was quite marked in the case of histamine. When control changes in agonist action were taken into account, it was clear that tissue incubation for 1 h with verapamil (1–10 μmol l^{-1}) had little or no effect against the spasm evoked either by acetylcholine or histamine (Figure 2). Incubation of tissues with verapamil for a further hour did not produce greater effects against acetylcholine- or histamine-induced spasm.

Control experiments revealed that log concentration-effect curves for KCl and TEA also underwent some depression following tissue incubation for 1 h. In contrast to the spasm evoked by acetylcholine or histamine, that evoked by KCl or TEA was markedly suppressed following incubation of tissues with verapamil (0.1–10 μmol l) for 1 h (Figure 3). Incubation of tissues with verapamil for a further hour did not produce greater effects against KCl or TEA-induced spasm.

Effect of verapamil on spasm evoked by prostaglandin E_2

Indomethacin 2.8 μmol l^{-1} evoked loss of tissue tone which became maximal after approximately 30 min. In the presence of indomethacin, prostaglandin E_2 (0.11–14.2 μmol l^{-1}) evoked tonic (smoothly developing) spasm which was concentration-dependent. The maximal response to prostaglandin E_2 was observed at a concentration of 14.2 μmol l^{-1} and was equivalent to approximately 45% of the maximal response to acetylcholine measured in the same medium.

Concurrent control experiments showed that the log concentration-effect curve for prostaglandin E_2 underwent slight depression following incubation with indomethacin-containing Krebs solution for 1 h. Incubation of test tissues with verapamil (0.1–10 μmol l^{-1}) for 1 h failed to modify the log concentration-effect curve for prostaglandin E_2 (Figure 4).

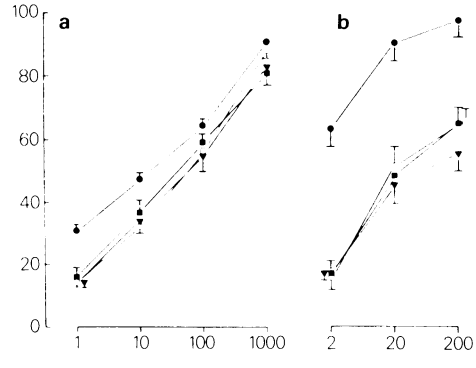

Figure 2 Guinea-pig trachealis bathed by normal Krebs solution: effect of verapamil on spasm evoked by (a) acetylcholine and (b) histamine. The abscissae indicate the concentration of agonist (μmol l^{-1}) on a log scale. The ordinates represent response as a % of the initial maximal response to acetylcholine: (●) = initial log concentration-effect curve; (■) = curve obtained following further incubation for 1 h with Krebs solution (control tissues); (○) = curve obtained after verapamil 1 μmol l^{-1} for 1 h; (▼) = curve obtained after verapamil 10 μmol/l for 1 h. Data points represent the means from a minimum of six experiments. Vertical bars represent s.e.mean.

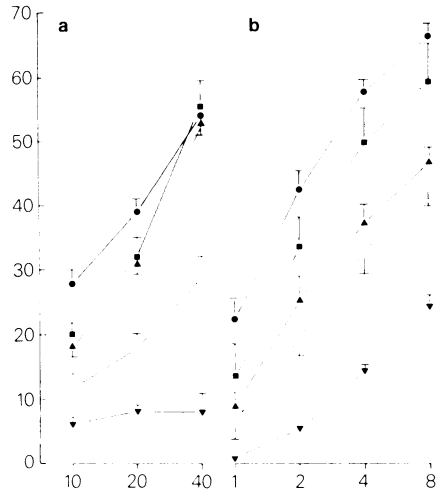

Figure 3 Guinea-pig trachealis bathed by normal Krebs solution: suppression by verapamil of spasm evoked by KCl (a) and tetraethylammonium (b). The abscissae indicate concentration of agonist (mmol l^{-1}) on a log scale. The ordinates represent response as a % of the maximal response to acetylcholine: (●) = initial log concentration-effect curve; (■) = curve obtained following further incubation for 1 h with Krebs solution (control tissues); (▲) = curve obtained after verapamil 0.1 μmol l^{-1} for 1 h; (○) = curve obtained after verapamil 1 μmol l^{-1} for 1 h; (▼) = curve obtained after verapamil 10 μmol l^{-1} for 1 h. Points represent the means from a minimum of six experiments. Vertical bars represent s.e.mean.

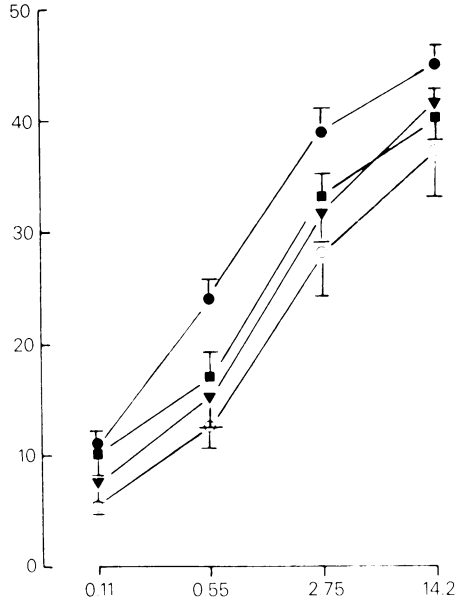

Figure 4 Guinea-pig trachealis bathed by Krebs solution containing indomethacin 2.8 μmol l^{-1}: effect of verapamil on spasm evoked by prostaglandin E_2. The abscissae indicate the concentration of prostaglandin E_2 (μmol l^{-1}) on a log scale. The ordinates represent response as a % of the maximal response to acetylcholine; (●) = initial log concentration-effect curve for prostaglandin E_2; (■) = curve obtained after further incubation for 1 h with Krebs solution (control tissues); (○) = curve obtained after verapamil 1 μmol l^{-1} for 1 h; (▼) = curve obtained after verapamil 10 μmol l^{-1} for 1 h. Points represent the means from a minimum of seven experiments. Vertical bars represent s.e.mean.

Effects of verapamil on tone

Aminophylline (1 mmol l^{-1}) evoked tissue relaxation which became maximal after approximately 5 min. Following aminophylline washout, tissue tone rose again and after approximately 15 min reached a peak which was greater than the pre-aminophylline value.

Over the next hour some tone loss was evident both in tissues treated with vehicle and in those treated with verapamil (0.1 – 10 μmol l^{-1}). When the effects seen in vehicle-treated tissues are compared with the effects seen in verapamil-treated tissues it is evident that verapamil itself evoked only minor loss of tone (Table 2). In this respect verapamil contrasted markedly with Ca^{2+} withdrawal which caused almost total loss of tone.

Table 2 Guinea-pig isolated trachealis: loss of spontaneous tone evoked by Ca^{2+}-withdrawal, verapamil and vehicle

	Ca^{2+}-free Krebs solution	*Krebs solution + vehicle*	*Krebs solution + verapamil* 0.1 μmol l^{-1}	1 μmol l^{-1}	10 μmol l^{-1}
Mean tone loss (%)	96.7	26.7	33.9	31.7	37.9
	±	±	±	±	±
s.e.mean	2.2	2.0	7.1	5.9	3.9

Data represent mean ± s.e.mean ($n = 7$). Analysis of variance revealed no significant differences between verapamil- and vehicle-treated tissues.

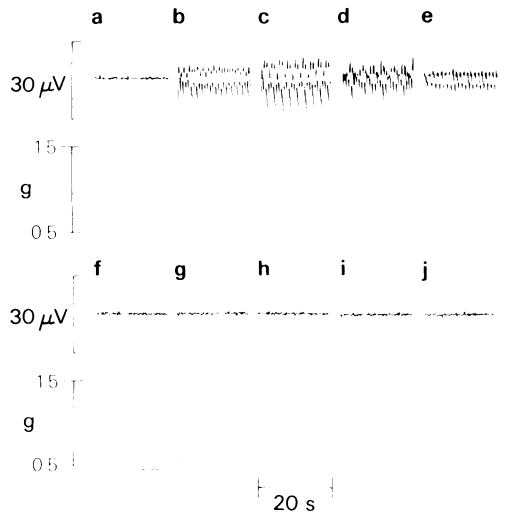

Figure 5 Guinea-pig trachealis: simultaneous recordings of extracellular electrical activity (upper trace) and mechanical changes (lower trace). All records taken from the same preparation. (a-e) Activity seen prior to and 5 min after tetraethylammonium (TEA) 1, 2, 4, 8 mmol l^{-1} respectively; (f-j) corresponding activity observed after tissue equilibration with verapamil (10 μmol l^{-1}) for 1 h. Note the suppression by verapamil of TEA-induced spasm, electrical slow waves and spikes.

Extracellular electrophysiological recording

In all preparations of trachealis examined, TEA (1–8 mmol l^{-1}) evoked spasm which was associated with the promotion of electrical slow waves and sometimes spike activity. Control experiments showed that these effects of TEA could be reproduced following incubation in normal Krebs solution for 1 h.

The interaction between verapamil (10 μmol l^{-1}) and TEA was studied in 7 preparations. In all 7 tissues equilibration with verapamil for 1 h caused suppression not only of spasm but also of the electrical slow waves and spikes induced by TEA (Figure 5).

The electrical events accompanying KCl-induced spasm comprised solely the transient promotion of slow wave activity. This slow wave promotion was seen with KCl 10 mmol l^{-1} but not at higher concentrations. Following equilibration with verapamil (10 μmol l^{-1}) for 1 h, the slow wave activity and spasm induced by KCl were both suppressed (Figure 6).

Intracellular electrophysiological recording

In the presence of TEA 8 mmol l^{-1}, trachealis cells often exhibited a continuous discharge of action potentials. Each action potential usually comprised an initial spike followed by a plateau phase (Figure 7a, b). The mechanical activity recorded from a contiguous segment of tissue sometimes comprised tonic tension development and sometimes phasic tension waves as previously reported (Dixon & Small, 1983).

Tetrodotoxin (3 μmol l^{-1}) did not abolish the TEA-induced spike discharge or mechanical activity (Figure 7c). In contrast, verapamil (10 μmol l^{-1}) abolished action potentials within 10 min. Initially verapamil depressed the fast spike component of the action potential so that the action potential took on the appearance of a slow wave. This residual slow wave activity then rapidly decreased in amplitude until regular oscillations of membrane potential finally ceased (Figure 7d). At this time the resting membrane potential was slightly smaller (less negative) than the pre-tetrodotoxin value. Mechanical activity could still be recorded when action potentials had been abolished in individual cells. However, mechanical activity became fully depressed following 20–30 min superfusion with verapamil.

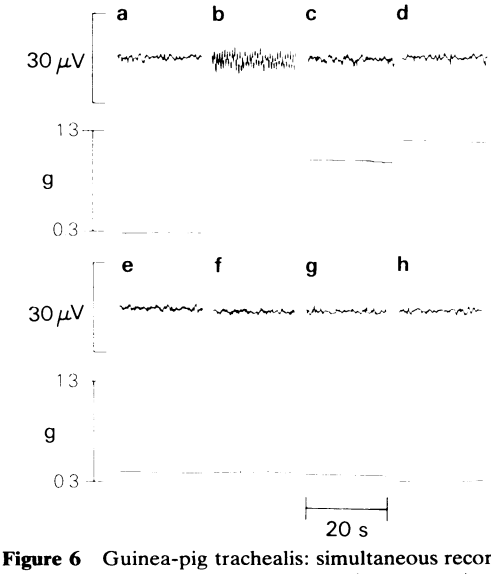

Figure 6 Guinea-pig trachealis: simultaneous recordings of extracellular electrical activity (upper trace) and mechanical changes (lower trace). All records taken from the same preparation. (a-d) Activity seen prior to and 12 min after KCl 10, 20, 40 mmol l^{-1} respectively; (e-h) corresponding activity observed after tissue equilibration with verapamil (10 μmol l^{-1}) for 1 h. Note the suppression by verapamil of KCl-induced spasm and electrical slow waves.

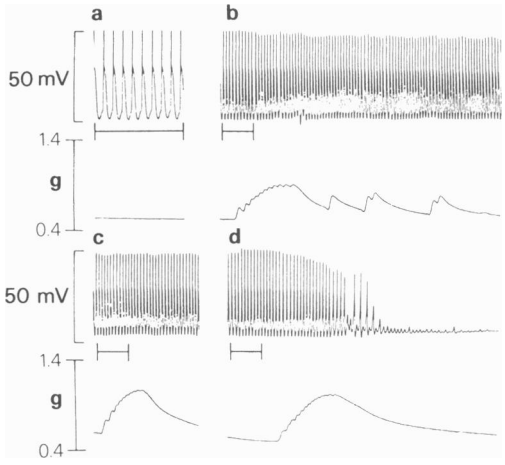

Figure 7 Guinea-pig trachealis bathed by Krebs solution containing tetraethylammonium (TEA) 8 mmol l^{-1}: simultaneous recordings of intracellular electrical activity (upper trace) and mechanical changes of a contiguous segment of trachea (lower trace). All electrical records from the same cell. In each panel the time axis calibration = 10 s. (a and b) Consecutive recordings illustrating control activity (c) activity seen after 10 min superfusion with tetrodotoxin 3 μmol l^{-1}, (d) activity seen after a further 1.5 min superfusion with tetrodotoxin 3 μmol l^{-1} + verapamil 10 μmol l^{-1}. Note ability of verapamil to suppress spike activity while causing little or no change in resting membrane potential.

Discussion

Several groups of workers have assessed the ability of verapamil to antagonize Ca^{2+} acting on K^+-depolarized smooth muscle. In rabbit ear artery (Ferrari, 1970), rabbit pulmonary artery (Haeusler, 1972) and guinea-pig taenia caeci (Spedding, 1982) verapamil was found to cause parallel rightward shifts of the log concentration-effect curve of Ca^{2+} without depression of the maximal response. However, in cat nictitating membrane (Haeusler, 1972) and rabbit aorta (Sanner & Prusa, 1980) the antagonism was clearly not of the simple, competitive kind since changes occurred either in the slope or in the maximal response of the Ca^{2+} log concentration-effect curve. In guinea-pig trachealis (present study) competition between Ca^{2+} and verapamil is suggested by the progressive and largely parallel (when $[Ca^{2+}] < 10$ mmol l^{-1}) rightward shift of the Ca^{2+} log concentration-effect curve. This shift shows the relationship predicted on the simple competition hypothesis between increment of antagonist concentration (when [verapamil] < 1 μmol l^{-1}) and antagonism.

The presumed site for such competition is one controlling access of exogenously applied Ca^{2+} to its site of spasmogenic action. On this interpretation of the evidence two features perturb the simple competitive relationship. Firstly, concentrations of $Ca^{2+} > 10$ mmol $^{-1}$ seem to have another action, antispasmogenic and not antagonized by verapamil. Secondly (and suggested by the small size of the rightward shift of the Ca^{2+} log concentration effect curve seen on increasing the concentration of verapamil from 1 to 10 μmol l^{-1}), higher concentrations of Ca^{2+} may gain entry by another route, not susceptible to verapamil. Both impede our ability to collect (convincing) evidence for competition over a sufficiently wide range of concentration and make the alternative hypothesis, that the antagonism is not of a competitive nature, equally likely.

There is evidence (Dixon & Small, 1983; Foster et al., 1983a,b) that spasm of guinea-pig trachealis evoked by TEA or KCl represents a direct action on the smooth muscle cells and involves both depolarization and the influx of Ca^{2+}. Our present observation that verapamil can suppress tracheal spasm evoked by these agents confirms the findings of earlier workers (Coburn, 1977; Farley & Miles, 1978; Cerrina *et al.*, 1982). That verapamil may suppress TEA or KCl induced spasm by interfering with Ca^{2+} influx is strongly suggested by the similar effectiveness of verapamil against TEA or KCl (Figure 3) and Ca^{2+} in the K^+-depolarised trachealis (Figure 1). It has been suggested (Spedding, 1982) that Ca^{2+}-induced contraction of K^+ depolarized smooth muscle is mediated by Ca^{2+} influx through voltage-dependent channels. In view of this it seems not unreasonable to propose that the spasmogenic actions of TEA and KCl in guinea-pig trachealis depend on the opening of voltage-dependent Ca^{2+} channels and that this may be prevented by verapamil.

We have previously shown (Small, 1982; Foster *et al.*, 1983a) that trachealis slow waves may be suppressed by methoxyverapamil (D600) and by Ca^{2+}-deprivation. The present observations that verapamil suppresses TEA or KCl-induced slow waves confirms that slow waves are Ca^{2+}-dependent events and suggests that voltage-dependent Ca^{2+}-channels may be activated during the upstroke of a slow wave.

It is likely that high concentrations of TEA, by reducing K^+ conductance, allow fuller Ca^{2+} channel activation and therefore the production of spike potentials. Tetrodotoxin (3 μmol l^{-1}) virtually abolished nerve-mediated responses of guinea-pig trachealis to field stimulation (Foster *et al.*, 1983a) yet did not abolish TEA-induced spike potentials of trachealis cells (present study). This suggests that trachealis spikes differ from the Na^+-carried spikes of mammalian neurones. That trachealis spikes may be Ca^{2+}-carried is suggested by both their resistance to tetrodotoxin and their suppression by verapamil.

Acetylcholine and histamine have spasmogenic actions in guinea-pig trachealis, which are resistant to tetrodotoxin but are selectively antagonized by atropine and mepyramine respectively (Foster *et al.*, 1983a,b). These findings are consistent with acetylcholine and histamine acting directly on the trachealis cells at muscarinic and H_1-receptors respectively. In that event, activation of these receptors mediates the rise in cytoplasmic free Ca^{2+} necessary for tension development. As yet there have been no reports of whether acetylcholine or histamine can promote the cellular influx of Ca^{2+} in guinea-pig trachealis. However, the resistance of spasm induced by these agents to removal of extracellular Ca^{2+} has been equated with their releasing Ca^{2+} from intracellular sites of sequestion (Cerrina *et al.*, 1982).

We have observed that concentrations of verapamil known to antagonize powerfully the effect of Ca^{2+} in K^+-depolarised guinea-pig trachea, have little effect against acetylcholine- or histamine-induced spasm. We have therefore confirmed the selectivity of verapamil claimed by earlier workers (Cerrina *et al.*, 1982) and can rule out the possibility that the principal effect of acetylcholine and histamine might be the opening of voltage-dependent Ca^{2+} channels. The ability of histamine to depolarize guinea-pig trachealis (McCaig & Souhrada, 1980) may be a phenomenon mediated by the activation of H_1-receptors and presumably does not lead to significant secondary opening of voltage dependent Ca^{2+} channels.

The calcium antagonists, nifedipine and D600, have relatively minor effects against the spontaneous mechanical tone of guinea-pig trachealis (Fanta *et al.*, 1982; Small, 1982). When the tone loss observed in vehicle-treated control tissues was compared with that seen in verapamil-treated trachea (Table 2) it became evident that verapamil, too, has little effect on trachealis tone. Clearly, the administration of organic calcium antagonists is not equivalent to removal of extracellular Ca^{2+} since the latter procedure caused almost total tone loss (Table 2) and was less able to discriminate among spasmogens (Foster *et al.*, 1983b).

That spasm induced by prostaglandin E_2 cannot be mediated by the opening of voltage-dependent Ca^{2+} channels is suggested by the failure of verapamil (Figure 4) to suppress prostaglandin-induced spasm. Farmer *et al.* (1974) proposed that prostaglandins are responsible for the maintenance of tone in guinea-pig isolated trachea. If this hypothesis is valid, it is likely that prostaglandin E_2 will be one of the prostanoids involved, for prostaglandin E_2 can be synthesized by the tissue (Gryglewski *et al.*, 1976) and is one of the most potent of the spasmogenic prostaglandins (Coleman & Kennedy, 1980). The failure of verapamil to suppress either the spontaneous tone of the trachea or the spasm evoked by prostaglandin E_2 is consistent with the proposed role of prostaglandins in tone production.

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