J. Pharm. Pharmacol. 1987, 39: 283–289
Received September 23, 1986

Differentiation of calcium antagonists with respect to their effects in normal and skinned taenia caeci preparations

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In taenia preparations, depolarized by a K^+-rich medium, Ca^{2+} caused contraction and cinnarizine (0·4–100 μM), trifluoperazine (2–100 μM) and verapamil (0·02–10 μM) caused concentration-dependent antagonism of Ca^{2+}, displacing the Ca^{2+} log concentration-effect curve to the right and depressing the maximal response. Equieffective (IC75) antispasmogenic concentrations were selected. The antispasmogenic effects of verapamil were readily offset by removing the drug from the bathing fluid but those of the other drugs were not. The calcium antagonists (antispasmogenic IC75) were then tested for spasmolytic activity in tissues generating tension in response to the EC80 of Ca^{2+}. Verapamil was more effective in producing spasmolysis than cinnarazine or trifluoperazine. In skinned taenia preparations, verapamil (100 μM) did not inhibit Ca^{2+}-induced contractions. High concentrations of cinnarizine (100 μM) and trifluoperazine (100 μM) inhibited Ca^{2+}-induced activation of the contractile proteins. However, antispasmogenic IC75s from intact taenia were not able to produce this effect on skinned preparations. It is concluded that there are differences between calcium antagonists. The action of verapamil on intact taenia is mainly exerted on the plasma membrane. Cinnarizine and trifluoperazine act both on the plasma membrane and upon the intracellular contractile machinery.

The calcium antagonists are a heterogeneous group of agents with dissimilar structural, electrophysiological and pharmacological properties. Fleckenstein et al (1969) observed that these agents share effects on cardiac muscle which are similar to those of calcium removal. By blocking the voltage-dependent calcium channels in cell membranes, these agents interfere with the calcium influx needed to translate membrane depolarization into cellular contractile events. However, attempts to characterize calcium antagonists by analysis of ^{45}Ca-fluxes in smooth muscle have led to conflicting results regarding the site of action of these drugs (Weston 1978; Thorens & Haeusler 1979; Church & Zsoter 1980) and it is likely that they possess differing mechanisms of action.

Fleckenstein (1981), Spedding (1981, 1982a, b, 1983) and Ferry & Glossman (1982) have proposed a classification of calcium antagonists into three distinct subgroups on the basis of structural and pharmacological differences. One of these subgroups consists of lipophilic weakly basic drugs, such as cinnarizine and flunarizine, which are readily distinguished from other calcium antagonists, such as verapamil and diltiazem, in that their inhibitory effects are time- and Ca^{2+} concentration-dependent and are not readily reversible (Spedding 1982a). These features of cinnarizine and flunarizine could indicate an intracellular site of action.

Another subgroup of calcium antagonists comprises the phenothiazine antipsychotic agents including trifluoperazine and chlorpromazine (Spedding 1982a). Trifluoperazine has been demonstrated to bind calmodulin in calcium-dependent fashion (Levin & Weiss 1979; Mrwa et al 1980; Sparrow et al 1981) and consequently to prevent activation of the calmodulin-dependent myosin light chain kinase (Cassidy et al 1980).

Experimental evidence therefore suggests that certain calcium antagonists (for example verapamil) act on the external surface of, or within, the plasma membrane whilst others, like cinnarizine and trifluoperazine, produce the calcium antagonistic action by acting intracellularly.

In the present study using Ca^{2+}-induced contractions in guinea-pig taenia caeci as an experimental model, we have compared verapamil, cinnarizine and trifluoperazine. We have tested equieffective concentrations of these three calcium antagonists as measured on intact smooth muscle, for direct inhibitory effect on the contractile proteins, using taenia preparations in which the sarcolemma had been chemically disrupted. By comparing the actions of

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these agents on intact and skinned tissue we hoped to determine the contribution of the cytoplasmic membrane to the action of each calcium antagonist.

METHODS

Guinea-pigs of either sex (250 to 600 g) were killed by stunning and bleeding. Taenia caeci were dissected cleanly from the caecal circular muscle.

General protocol for experiments with taenia caeci

Strips of taenia, of about 10 mm length, were set up under a resting tension of 1 g in isolated organ baths, containing Krebs solution (composition mM: NaCl 118; KCl 4·75; $CaCl_2 \cdot 6H_20$ 2·55; $MgSO_4 \cdot 7H_2O$ 1·2; KH_2PO_4 1·19; $NaHCO_3$ 25; glucose 11), or K^+-rich, Ca^{2+}-free physiological salt solution (PSS) (composition mM: NaCl 118; KCl 40; $MgSO_4 \cdot 7H_2O$ 1·2; KH_2PO_4 1·19; $NaHCO_3$ 25; glucose 11). The temperature was maintained at 37 °C and the solutions were gassed with 95% O_2 + 5% CO_2. Spasmogenic responses to agonists were measured under isometric conditions, recorded using Ether type UF1 transducers and displayed on a Grass 79C polygraph.

Antagonism of Ca^{2+}

In experiments where antagonism of Ca^{2+} was measured, the taenia was initially mounted in Krebs solution. The bath fluid was then changed for the K^+-rich, Ca^{2+}-free PSS. This evoked marked spasm which was dissipated by regular changes of the K^+-rich, Ca^{2+}-free bath fluid. An initial concentration-effect curve for Ca^{2+} (6 min contact time) was then constructed, succeeded by two further cumulative curves. In test tissues calcium antagonists were present for 30 min before and throughout the second and the third concentration-effect curve to Ca^{2+}, but a calcium antagonist concentration increment was made 30 min before the third curve. Control tissues were treated similarly, except that they were not exposed to calcium antagonists. The inhibitory effect of each concentration of each calcium antagonist was quantified as a proportion (%) of the initial spasmogenic effect of Ca^{2+}, 3·2 and 10 mM. The concentrations of calcium antagonists causing 75% reduction in the spasm evoked by Ca^{2+} 10 mM (IC75) were derived by interpolation.

Measurement of the spasmolytic activity of calcium antagonists

In tissues equilibrated with K^+-rich, Ca^{2+}-free PSS, an initial cumulative concentration effect curve to Ca^{2+} was constructed to select a suitable near-maximally effective concentration (10 mM). Repeated applications of this Ca^{2+} standard were made at 15 min intervals, over 1 h. Six minutes after the final application and without washing, the equieffective antispasmogenic concentrations (IC75) of cinnarizine, trifluoperazine or verapamil were added to the tissue bath fluid. Suppression of the spasm was recorded, observation being made every 5 min for 1 h and the half-time derived. Four preparations were worked simultaneously—i.e. a time-matched control and a test preparation for each of the three calcium antagonists.

Reversibility of the antagonist effects

In tissues equilibrated with K^+-rich, Ca^{2+}-free PSS, the antispasmogenic IC75 of antagonists were added to the tissue baths and incubated for three half-times (calculated from data obtained from the spasmolytic activity experiments). After this incubation period, 10 mM Ca^{2+} was added and the response recorded. This constituted the response at time zero. The antagonist and Ca^{2+} were then removed by washing. Further applications of Ca^{2+} 10 mM were repeated every 15 min for 2 h to assess reversibility of the actions of the calcium antagonists.

Skinned taenia preparations

Segments of taenia were prepared as described above and then skinned of their plasma membranes essentially as described by Sparrow et al (1981). Tissue segments were incubated (4 h at 4 °C) in a 1% (v/v) Triton X-100 solution which contained (mM): EGTA 5; KCl 50; sucrose 150; imidazole 20 (pH 7·4) and dithioerythritol 0·5. After rinsing for 15 min in a solution of the same composition but without Triton X-100, tissues were stored in a solution of (mM): EGTA 4; $MgCl_2$ 10; ATP 7·5; NaN_3 1; imidazole 20 (pH 6·7) and dithioerythritol 0·5 with 50% glycerol at −20 °C for up to 10 days.

With an imposed tension of 0·5 g, segments of skinned taenia were set up at 20 °C for isometric recording of tension changes in 5 mL of relaxing solution containing (mM): EGTA 4; $MgCl_2$ 10; ATP 7·5; KH_2PO_4 6; NaN_3 1 and imidazole 20 adjusted to pH 6·7 with KOH. The relaxing solution did not contain added calmodulin. Contraction was induced by addition of $CaCl_2$ in small volume to increase the free Ca^{2+} concentration by varying the ratio of EGTA/Ca-EGTA, which was calculated using the equations described by Portzehl et al (1964) and the apparent binding constant for Ca^{2+}/EGTA of 1·2 μM^{-1}. The Ca^{2+} threshold for initiation of tension was detected by increasing the free Ca^{2+} concentration at 5 min intervals beginning at 0·2 μM

and ascending cumulatively in steps of 1·47-fold until an increase in tension of 10 mg was observed. $CaCl_2$ additions were then adjusted to give a free Ca^{2+} concentration of 5–40 μM (Sparrow et al 1981) ascending in two-fold steps and allowing up to 20 min for the response to plateau. The maximal developed tension was dispelled by repeatedly washing with relaxing solution to restore the free Ca^{2+} concentration to <10 nM. Such cumulative free Ca^{2+} concentration-effect curves were obtained at 60–90 min intervals. At the end of each experimental day each preparation was challenged with acetylcholine 100 μM.

The experimental design used to assess antagonism of Ca^{2+} was similar to that used in the experiments with normal taenia in a K^+-rich, Ca^{2+}-free medium—that is, two concentrations of the same calcium antagonist were examined on each tissue after an initial control concentration-effect curve was constructed for Ca^{2+}. Control tissues were treated similarly, except that they were not exposed to modifying agents. The maximal effect in the second and the third curve was expressed as a proportion (%) of that in the first curve.

Effect of exogenous calmodulin on sensitivity and responsiveness to Ca^{2+}

Segments of skinned taenia were prepared and arranged for tension recording as described above. Tension development was induced by addition of $CaCl_2$ cumulatively in amounts calculated to give free Ca^{2+} concentrations of 0·2–20 μM. Following construction of an initial log concentration-effect curve for Ca^{2+}, test tissues were bathed in a relaxing solution containing calmodulin (0·1 μM) for 2 h. The log concentration-effect curve for Ca^{2+} was then reconstructed. Control tissues were treated similarly but were not exposed to exogenous calmodulin.

Drugs and statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species—the following substances were used: acetylcholine chloride (Sigma), adenosine-5′-triphosphate (ATP disodium salt, Sigma), calmodulin (Sigma), cinnarizine (Janssen), dithioerythritol (BDH), ethylene glycol-bis (β-amino-ethylether) *N-N′*-tetraacetic acid (EGTA, Sigma), imidazole (Sigma), methacholine (Sigma), sodium azide (BDH), trifluoperazine (Merck, Sharpe and Dohme), Triton X-100 (BDH), verapamil (Knoll).

The significance of differences between two means was assessed using a one- or two-tailed unpaired *t*-test. Larger numbers of groups were assessed using analysis of variance and the Studentized range test. A difference between means was assumed to be significant when $P < 0.05$.

RESULTS

Tissue bath studies with normal taenia preparations

When the bath fluid was changed from Krebs solution to a K^+-rich, Ca^{2+}-free PSS, tissues generated tension which became maximal after approximately 3 min. Despite regular changes of the bath fluid the tension developed by the tissue required approximately 20 min for dissipation. This established the incubation time in K^+-rich, Ca^{2+}-free PSS for subsequent experiments—addition of Ca^{2+} (0·1 to 10 mM) at this time evoked smoothly developing spasm which was concentration-dependent (E_{max} = 6·62 ± 0·32 g; pD_2 = 2·66 ± 0·035; n = 24). Verapamil (0·02–10 μM), cinnarizine (0·4–100 μM) and trifluoperazine (2–100 μM) each caused a concentration-dependent rightward shift and depression of the log concentration-effect curve to Ca^{2+}. The log concentration-effect curves of the three calcium antagonists are shown in Fig. 1. Ca^{2+} 3·2 mM was more inhibited than Ca^{2+} 10 mM. The slopes of the three log concentration-effect curves against Ca^{2+} 10 mM at their midpoints did not differ significantly. Analysis of variance showed a significant difference ($P < 0.05$) between the positions of the cinnarizine and trifluoperazine log concentration-effect curves at each Ca^{2+} concentration and highly significant

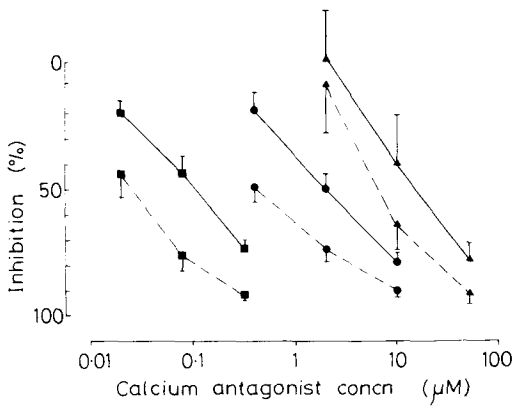

FIG. 1. The inhibitory effects of cinnarizine (●), trifluoperazine (▲) and verapamil (■) on the responses of the guinea-pig unskinned taenia (in K^+-rich, Ca^{2+}-free PSS) to Ca^{2+}. The abscissa indicates concentration of antagonists (μM) on a log scale. The ordinate represents inhibition as a proportion (%) of the initial response to Ca^{2+}. Solid lines represent inhibition of Ca^{2+} 10 mM and broken lines that of Ca^{2+} 3·2 mM. Points represent the means of observations from at least 6 tissues; bars represent s.e.m.

differences between each of those curves and that of verapamil. The IC75 against Ca^{2+} 10 mM and corresponding relative potencies are shown in Table 1.

Spasmolytic activity of the antagonists

Spasm induced by 10 mM Ca^{2+} ranged between 63–88% of the maximum Ca^{2+}-induced contraction and was reproducible over 1 h. Suppression of Ca^{2+}-induced spasm (spasmolysis) occurred rapidly following the addition of verapamil (0·4 μM) to the bathing medium and followed an approximately exponential time course. The rate constant, k, was calculated to be 0·124. The relaxant effects of cinnarizine and trifluoroperazine (10 and 45 μM, respectively) developed much more slowly (k = 0·036 and 0·022, respectively) (Table 1). After 30 min the Ca^{2+}-induced spasm in the control preparation also started to decline, this process reaching 34% after a further 30 min.

Table 1. Features of the effects of calcium antagonists on responses to Ca^{2+} of guinea-pig taenia caeci maintained in K^+-rich, Ca^{2+}-free PSS.

Antagonist	IC75 (μM)	Potency relative to verapamil	Half-time of relaxation during spasmolysis (min)	Half-time of recovery from anti-spasmogenic effect (min)
Verapamil	0·4	1	5·6	20·5
Cinnarizine	10	0·04	19·2	—
Trifluoperazine	45	0·0089	31·8	170·8

Reversibility of antagonism

The effects of verapamil (0·4 μM) were readily reversed by washing. Restitution of the pretreatment response occurred in 90 min. Antagonism by cinnarizine (10 μM) or trifluoperazine (45 μM) was not so readily reversed by washing; after a 2 h wash, responses to 10 mM Ca^{2+} in the trifluoperazine-treated tissue had recovered to only 35% of the pretreatment response. During this period the antagonism due to cinnarizine had increased by approximately 14%. As recovery did not occur with the cinnarizine-treated preparation, it was not possible to calculate its half-time for recovery (Table 1).

Skinned taenia preparations

Segments of taenia which had been pretreated with Triton X-100 contracted in response to low concentrations of Ca^{2+} (0·5–40 μM). The concentration-response curve was steep and the threshold occurred at 4·1–7 μM. However, in the second and the third curves obtained from the same preparation, the threshold Ca^{2+} concentration was decreased ($P <$ 0·01) 5- and 9-fold, respectively. Fig. 2a shows the relationship between Ca^{2+} concentration and tension development and time. The initial maximal response was obtained with 40 μM Ca^{2+} (2·11 ± 0·16 g, n = 34). In subsequent Ca^{2+} concentration-effect curves the maximal response slowly declined (about 10%) over 4 h. Half-maximal activation was achieved with Ca^{2+} concentrations of 11–13 μM (n = 34); this was lower ($P < 0·01$) at the second trial.

The acetylcholine 100 μM challenge terminating each experiment did not produce any tension increment.

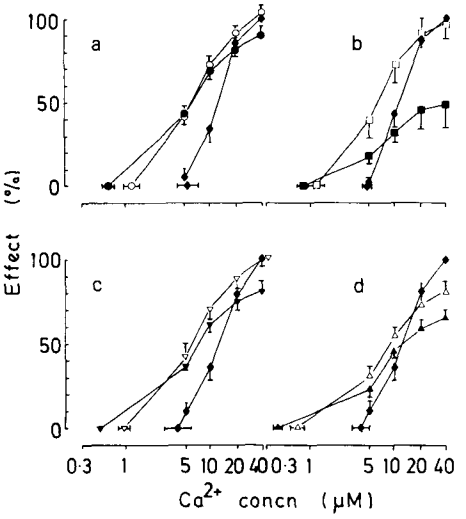

FIG. 2. Effects of calcium antagonists on cumulative Ca^{2+} concentration effect curves in skinned taenia. The abscissae indicate concentration of Ca^{2+} (μM) on a log scale. The ordinates represent effect (% of the initial maximal effect of Ca^{2+}). Points represent the means and bars the s.e.m. (n = 5–9). Initial log concentration-effect curve for Ca^{2+} (◆); second log concentration-effect curve for Ca^{2+} in control tissues (○) or tissues treated with 45 μM trifluoperazine (□), 0·4 μM verapamil (▽) or 10 μM cinnarizine (△); third log concentration-effect curve for Ca^{2+} in control tissues (●) or tissues treated with 100 μM trifluoperazine (■), 100 μM verapamil (▼) or 100 μM cinnarizine (▲).

The effects of the calcium antagonists on Ca^{2+}-induced activation of the contractile proteins are shown in Fig. 2b, c, d. When changes observed in the time-matched control tissues were taken into account, it was evident that 30 min incubation with cinnarizine (10 and 100 μM) or trifluoperazine (100 μM) depressed the maximal tension evoked by Ca^{2+}, whereas verapamil (100 μM) was ineffective. The effects of cinnarizine (100 μM) in depressing the maximal response to Ca^{2+} were not significantly ($P < 0·05$) different from those of trifluoperazine

(100 μM). In contrast to effects on the maximal response to Ca^{2+}, the threshold Ca^{2+} concentration for initiation of tension and the EC50 were not modified ($P > 0.05$) by incubation with cinnarizine, trifluoperazine or verapamil (Table 2).

Experiments with added calmodulin

The use of time-matched control tissues showed that the log Ca^{2+} concentration-effect curves moved to the left when reconstructed following further tissue incubation in relaxing solution (Fig. 3a). Treatment of test tissues with calmodulin (0·1 μM) caused the same leftward shifts in the log Ca^{2+} concentration-effect curves, indicating that calmodulin had no significant effect on the Ca^{2+} sensitivity (control taenia strips 6·19 ± 1·98-fold decrease in threshold concentration; test strips 4·19 ± 1·09-fold, n = 6, $P > 0.05$). Nor did it affect the Ca^{2+} concentration at which 50% of maximal tension was developed, which was decreased 2·91 ± 0·55-fold in control tissues and 2·04 ± 0·36-fold when calmodulin was present. The peak tension developed was similarly increased in control (+22·0 ± 3·2%) and test preparations (+25·7 ± 5·3%).

DISCUSSION

The present experiments confirm that several calcium antagonists are able to inhibit Ca^{2+}-induced contractions in K^+-depolarized smooth muscle (Ferrari 1970; Sanner & Prusa 1980; Spedding 1982a; Foster et al 1984). Their remarkable differences in potency (verapamil > cinnarizine > trifluoperazine) could be accounted for by their differences in chemical structure, governing their affinity for a common site of action, or could arise from their having different mechanisms of action.

Table 2. Failure of 30 min incubation with cinnarizine (10 μM ○, 100 μM ★), trifluoperazine (45 μM ○, 100 μM ★) and verapamil (0·4 μM ○, 100 μM ★) to modify the threshold and pD_2 for responses of skinned taenia preparations to Ca^{2+}. Data represent mean ± s.e.m. (n = 5–9).

	Control	Cinnarizine	Tri-fluoperazine	Verapamil
Threshold ($-\log_{10}$ Ca^{2+} concentration)				
First curve	5·27 ± 0·12	5·4 ± 0·09	5·32 ± 0·05	5·39 ± 0·14
○ Second curve	5·94 ± 0·08	6·17 ± 0·09	5·94 ± 0·1	6·03 ± 0·06
★ Third curve	6·21 ± 0·06	6·4 ± 0·08	6·08 ± 0·1	6·31 ± 0·03
pD_2				
First curve	4·92 ± 0·04	4·93 ± 0·06	4·94 ± 0·04	4·90 ± 0·05
○ Second curve	5·20 ± 0·06	5·19 ± 0·07	5·27 ± 0·08	5·22 ± 0·07
★ Third curve	5·28 ± 0·05	5·17 ± 0·05	5·27 ± 0·1	5·26 ± 0·05

The effects of these calcium antagonists were also assessed by measuring their relaxant effects against established Ca^{2+} contractions and the reversibility of their antispasmogenic action. These tests revealed marked kinetic differences between the antagonist—whereas verapamil caused relaxation rapidly, the other drugs were only slowly spasmolytic when they were used in concentrations which had equally marked antispasmogenic effects. Such a difference may underlie or compound the reported lesser spasmolytic than antispasmogenic activity of cinnarizine which has been ascribed to a low affinity for its site of action in the presence of calcium. This explanation was first offered by Spedding (1982a)

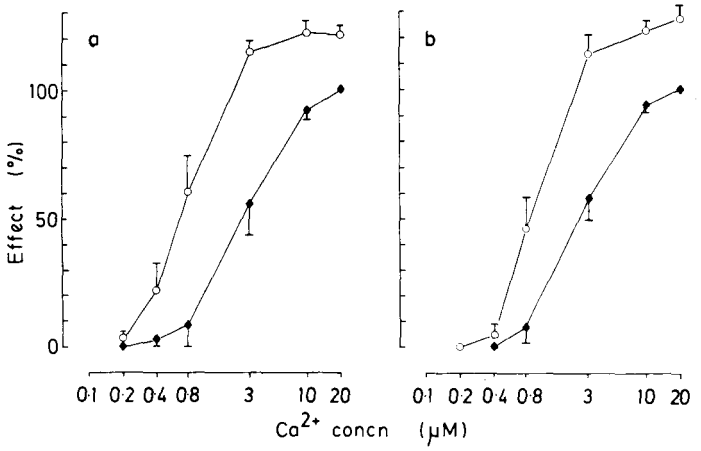

FIG. 3. Effect of calmodulin on cumulative Ca^{2+} concentration-effect curves in skinned taenia. The abscissae indicate concentration of Ca^{2+} (μM) on a log scale. The ordinates represent effect (% of the initial maximal effect of Ca^{2+}). Points represent the means and bars the s.e.m. (n = 6). (◆) Initial log concentration-effect curve for Ca^{2+} in (a) control and (b) test tissues. (○) Subsequent log concentration-effect curve for Ca^{2+} in (a) control tissues and in (b) calmodulin (0·1 μM)-treated test tissues.

who described reduction in the antagonist potency of cinnarizine but not of verapamil when Ca^{2+} was included in the depolarizing solution during the incubation period with the antagonist. The effects of verapamil were readily reversed by washing but the antagonist effects of cinnarizine and trifluoperazine were not reversed over 2 h. These results support the findings of Fleckenstein (1981), Spedding (1981, 1982a) and Ferry & Glossmann (1982).

It is notable that, in our hands, cinnarizine appeared less potent than Spedding (1982a) found it to be. However, in our experiments the agonist Ca^{2+} was also of lower potency. Since the action of cinnarizine is dependent on the concentration of Ca^{2+} in the medium (Spedding 1982a), the cinnarizine potency differences may have arisen from the differences in Ca^{2+} concentrations used. On the other hand, our potency of cinnarizine does agree with that reported by Spedding (1984).

Spedding (1983), using Ca^{2+} 20 μM challenges in skinned smooth muscle, showed that verapamil did not directly modify the sensitivity of the intracellular contractile machinery to Ca^{2+}. In contrast, cinnarizine and trifluoperazine inhibited Ca^{2+}-induced activation of the contractile proteins. The present study has extended these observations.

The failure of acetylcholine to evoke spasm has been used (Ito & Itoh 1984; Allen et al 1986) as an index of the functional completeness of skinning of trachealis muscle. Since acetylcholine (100 μM) never evoked spasm from our preparations of skinned taenia caeci, we may assume that our skinning of this tissue was also functionally complete.

The second and third cumulative concentration-effect curves for Ca^{2+} constructed on a single skinned taenia preparation were displaced to the left compared with the initial curve, indicating sensitization of the tissue to Ca^{2+}. A similar phenomenon was observed by Sparrow et al (1981) and was attributed by them (in the absence of the appropriate time-matched controls) to the effects of their having added calmodulin. However, in the present study, tissue sensitization to Ca^{2+} was observed in the absence of exogenous calmodulin and was not modified by its addition. Clearly the tissue sensitization to Ca^{2+} is not a consequence of the action of exogenous calmodulin. How, then, may such sensitization be brought about?

One possible explanation of the tissue sensitization to Ca^{2+} is that EGTA from the skinning and storage solutions may penetrate into the skinned smooth muscle. On first exposure of the skinned tissue to Ca^{2+} this tissue-sequestered EGTA may represent a site of loss for Ca^{2+}. Consequently, during the construction of the initial concentration-effect curve, the apparent Ca^{2+} threshold would be raised relative to that in the second or third curve.

Alternatively, the phenomenon of Ca^{2+} sensitization may result from the Ca^{2+}-buffering capacity of the smooth muscle cells. Muscle from a wide variety of sources has been shown to contain Ca^{2+} binding proteins (parvalbumins) which are a group of closely related substances with molecular weights of about 12 000 Daltons and possessing two Ca^{2+} binding sites (Collins 1976). These may constitute a Ca^{2+} buffering system which is saturated during the construction of the first Ca^{2+} concentration-effect curve. The true sensitivity of the contractile machinery to Ca^{2+} may subsequently be revealed.

In the skinned taenia caeci, cinnarizine (10 and 100 μM) and trifluoperazine (100 μM) depressed maximal responses to Ca^{2+} whereas verapamil (100 μM) did not. This prompts us to endorse the conclusion of Spedding (1983) that cinnarizine and trifluoperazine depress Ca^{2+} activation of the intracellular contractile machinery and that verapamil does not share this property.

Verapamil, cinnarizine and trifluoperazine each suppressed Ca^{2+}-induced spasm in the unskinned, K^+-depolarized taenia, the order of drug potency being verapamil > cinnarizine > trifluoperazine. Again these findings confirm the earlier work of Spedding (1982a).

Since verapamil suppressed Ca^{2+}-induced spasm of unskinned taenia in the concentration range 0·02–1·0 μM and was without effect at 100 μM in the skinned tissue, it seems highly likely that, in the unskinned tissue, the action of verapamil is mediated within the cell membrane and does not involve direct interference with Ca^{2+} activation of the intracellular contractile machinery.

Trifluoperazine (2–45 μM) suppressed Ca^{2+}-induced spasm of the unskinned taenia (Spedding 1982a, present study). However in skinned tissue trifluoperazine (45 μM) was without effect on Ca^{2+}-induced spasm. Interference with Ca^{2+} activation of the intracellular contractile machinery was observed only at 100 μM trifluoperazine. This implies that trifluoperazine has two actions in the unskinned tissue. One action is seen at low concentration and involves the plasma membrane. The other action is seen at higher concentration (100 μM) and represents direct interference with Ca^{2+} activation of the contractile machinery.

Cinnarizine, too, may interfere with Ca^{2+}-induced spasm by two mechanisms. It suppressed Ca^{2+}-

induced spasm of intact tissue in the concentration range 0·4–10 μM but experiments with skinned tissue revealed effects on the intracellular contractile machinery in the range 10–100 μM. Clearly the action of cinnarizine in unskinned tissue cannot be regarded as restricted to the plasma membrane.

In conclusion, these experiments have demonstrated distinct differences between the three calcium antagonists used. Verapamil may have a single action mediated within the plasma membrane, while cinnarizine and trifluoperazine may have actions both at the level of the plasma membrane and within the cell.

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