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Calcium Antagonist Properties of Cinnarizine, Trifluoperazine and Verapamil in Guinea-pig Normal and Skinned Trachealis Muscle

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Abstract—In guinea-pig trachealis, depolarized by a K^+-rich medium, Ca^{2+} (0·01–10 mM) caused concentration-related spasm. Verapamil (0·5–5 μM), cinnarizine (10–100 μM) and trifluoperazine (16–160 μM) each produced concentration-dependent antagonism of Ca^{2+} characterized by a rightward and downward displacement of the log concentration-effect curve for Ca^{2+}. The rank order of potencies of these antagonists, measured as the IC75 against Ca^{2+} (10 mM)-induced contraction of depolarized trachea, was verapamil (5·6 μM) > cinnarizine (59 μM) > trifluoperazine (91 μM). In skinned trachea, verapamil in concentrations up to 100 μM did not modify the concentration-effect curve for Ca^{2+}. In contrast, cinnarizine (59–177 μM) diminished the sensitivity and trifluoperazine (273 μM) decreased the responsiveness of the tissue to Ca^{2+}. In skinned trachea, trifluoperazine (91 μM) produced greater inhibition of Ca^{2+} (10 μM)-induced contraction after 120 min than after 30 min of incubation. Verapamil (100 μM) and cinnarizine (177 μM) were devoid of inhibitory effect against the 10 μM Ca^{2+} standard. In skinned trachea, changes in the Ca^{2+} concentration-effect curve produced by cinnarizine (177 μM) were reversed after washout whilst those induced by trifluoperazine (273 μM) persisted. It is concluded that distinct differences exist between the three calcium antagonists examined. The action of verapamil is restricted to the plasmalemma. That of cinnarizine and trifluoperazine is exerted both on the plasma membrane and upon the intracellular contractile machinery.

The term "calcium antagonist" was originally introduced by Fleckenstein et al (1969) as a group name for specific inhibitors of Ca^{2+} influx into cardiac and smooth muscle. Drugs embraced by this term now comprise a very large group of agents which do not constitute a homogeneous class since substantial differences in structural/functional properties and clinical profiles exist among them (Spedding 1981, 1982, 1985; Snyder & Reynolds 1985). Hence, "calcium antagonist" has become a broader term for drugs which (i) lower cytosolic Ca^{2+} concentration by inhibiting Ca^{2+} entry into the cell and/or (ii) block the effect of Ca^{2+} by acting on Ca^{2+} binding cytosolic proteins. It is clear that a calcium antagonist may have various mechanisms and sites of action (Janis & Scriabine 1983; Triggle & Swamy 1983; Rampe et al 1985).

A subgroup of calcium antagonists is thought to inhibit smooth muscle contraction by selective blockade of plasmalemmal Ca^{2+} channels and the term "calcium-entry channel blockers" probably is a more precise description for these drugs. Thus, substances such as verapamil, diltiazem and the dihydropyridines have a major site of action according to ligand binding studies (Ferry & Glossmann 1982), analysis of ^{45}Ca fluxes (Weston 1978; Thorens & Haeusler 1979) and electrophysiological studies (Foster et al 1984; Ahmed et al 1985), at the plasmalemmal Ca^{2+} channels. Cytosolic sites of action may contribute to their effects but probably only at higher than therapeutic concentrations (Janis & Scriabine 1983).

In contrast, another subgroup of calcium antagonists consists of highly lipophilic weakly basic drugs such as cinnarizine and flunarizine. The inhibitory effects of these drugs are time and Ca^{2+} concentration-dependent and not readily reversible (Spedding 1982). This clearly distinguishes them from the established calcium-entry channel blockers and suggests a predominantly intracellular site for their action.

A third subgroup of calcium antagonists comprises phenothiazine antipsychotic agents like trifluoperazine (Spedding 1982). Trifluoperazine binds calmodulin in a calcium-dependent fashion (Levin & Weiss 1979; Sparrow et al 1981) thus preventing activation of the calmodulin-dependent myosin light chain kinase (Cassidy et al 1980) which represents again an intracellular site of action.

Since the early work of Filo et al (1965), skinning smooth muscle of its plasmalemma has been used to examine the complex relationship between cytosolic Ca^{2+} and contraction (Gordon 1978; Cassidy et al 1981; Iino 1981; Walsh et al 1982). The study of the effects of calcium antagonists in skinned smooth muscle preparations may help to ascertain the relative relevance of their plasmalemmal versus intracellular sites of action (Cassidy et al 1980; Spedding 1983).

In the present study, the effects of verapamil, cinnarizine and trifluoperazine on Ca^{2+}-induced contractions of normal and skinned guinea-pig trachealis have been compared to determine the relative contribution of extra- (plasmalemma) versus intra-cellular targets to the action of each calcium antagonist.

Materials and Methods

Guinea-pigs of either sex (350–450 g) were killed by stunning and bleeding. Tracheae were excised, cleaned of adhering fat and connective tissue and opened by cutting longitudinally

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through the cartilage rings diametrically opposite the trachealis.

Membrane intact tracheal preparations

Small segments of trachea were set up for the isometric recording of tension changes, as previously described (Foster et al 1983). The tissues were subjected initially to an imposed tension of 1 g and were bathed by a MOPS-buffered physiological salt solution (MOPS-PSS) (Jetley & Weston 1980). After equilibration the bath fluid was changed to a K^+-rich, Ca^{2+}-free MOPS-PSS as previously described (Foster et al 1984). This produced a spasm which was dissipated by regular changes of the K^+-rich, Ca^{2+}-free bath fluid. The spasmogenic response to $CaCl_2$ was studied by constructing cumulative concentration-effect curves. The contact time for each concentration of $CaCl_2$ was sufficient to allow the development of the full tension rise attainable by that concentration (15 min).

An initial concentration-effect curve for $CaCl_2$ was 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 curves for $CaCl_2$ 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 antagonist. The inhibitory effect of each concentration of each calcium antagonist was measured as the reduction (%) in the initial spasmogenic effect of $CaCl_2$ 10 mM. The concentration of calcium antagonist causing 75% reduction in the spasm evoked by $CaCl_2$ 10 mM (IC75) was derived by interpolation.

Skinned tracheal preparations

Segments of trachea were prepared as described above and then skinned of their plasmalemmal membranes as previously reported (Cortijo et al 1987b). Tissue segments were incubated (4 h at 4°C) in a 1% (v/v) Triton X-100 solution which contained (mM): KCl 50, sucrose 150, EGTA 5, imidazole 20 and dithioerythritol 0·5 (pH 7·4). 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 and dithioerythritol 0·5 (pH 6·7), with 50% glycerol at −20°C for up to 10 days.

Under an imposed tension of 1 g, segments of skinned trachea 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. All tissues were allowed to equilibrate in this medium for 20 min before commencing the construction of cumulative concentration-response curves for Ca^{2+}. For the relaxing solution used in these experiments, a temperature of 20°C and pH 6·7, the apparent stability constant for Ca^{2+}-EGTA solutions was 1·2 μM, a value calculated from the stability constant of EGTA-metal ion complexes (Martell 1971), using the equations described by Portzhel et al (1964). This value of the binding constant was used to calculate the amount of $CaCl_2$ to be added to the relaxing solution to achieve the required bath concentration of free Ca^{2+}. 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 until an increase of contractile force of 10 mg was observed. Further tension development was induced by addition of increasing Ca^{2+} concentrations and tension was allowed to plateau (20 min) before incrementing Ca^{2+} concentration. When the tension became maximal it was dispelled by repeatedly washing with Ca^{2+}-free relaxing solution and the tension was allowed to dissipate for 90 min before constructing a second concentration-response curve. At the end of each experiment the preparation was challenged with acetylcholine 100 μM.

Assessment of antagonism of Ca^{2+}

In test tissues a single concentration of the antagonist was examined on each tissue after an initial control concentration-response curve was constructed for Ca^{2+}. The antagonist was present for 30 min before and throughout the second concentration-effect curve to Ca^{2+}. Control tissues were treated similarly except that they were not exposed to the antagonist. For each antagonist, two concentrations were examined, its IC75 as determined in unskinned tissues (see above) and a concentration which was either 100 μM for verapamil or three times its IC75 for cinnarizine and trifluoperazine. The inhibitory effect of each treatment was measured as the reduction (%) in the initial spasmogenic effect of Ca^{2+} (10 μM).

Assessment of the influence of the incubation time of the antagonist

In test tissues three successive Ca^{2+} (10 μM) challenges were performed. Verapamil (100 μM), cinnarizine (177 μM) or trifluoperazine (91 μM) was present for 30 min or 120 min before and throughout the second and third challenge respectively. Acetylcholine (100 μM) was added to the tissue once full relaxation was achieved after the third Ca^{2+} challenge. Control tissues were treated in a similar way except that they were not exposed to calcium antagonists. The response to the second and third Ca^{2+} challenge was expressed as a proportion (%) of that in the first challenge.

Assessment of reversibility of the antispasmogenic action of cinnarizine and trifluoperazine

In test tissues three consecutive concentration-effect curves to Ca^{2+} (0·2–0·8 μM) were constructed. Cinnarizine (177 μM) or trifluoperazine (273 μM) was present for 30 min before and throughout the second concentration-response curve to Ca^{2+}. These agents were then removed from the bathing medium so that the third concentration-effect curve for Ca^{2+} was obtained in the absence of antagonist. Control tissues were treated identically but were not exposed to the antagonists. The maximal effect in the second and the third Ca^{2+} concentration-effect curves was expressed as a proportion (%) of that in the first curve.

Drugs and solutions/statistical analysis of results

The substances used were: acetylcholine chloride (Sigma), adenosine-5-triphosphate (ATP disodium salt, Sigma), cinnarizine (Janssen), dithioerythritol (Sigma), ethyleneglycol-bis(β-amino-ethylether)-*N*,*N*-tetraacetic acid (EGTA, Sigma), glycerol (Merck), imidazole (Sigma), trifluoperazine

(Sigma), Triton X-100 (Merck), sucrose (Merck), verapamil hydrochloride (Biosedra-Knoll, Paris, France). Concentration of drugs is expressed in terms of the molar concentration of the active species.

The significance of differences between means was assessed by two-tailed paired or unpaired *t*-test as appropriate. A difference between means was assumed to be significant when $P < 0.05$.

Results

Studies with membrane intact trachea preparations

In K^+-depolarized tissues, $CaCl_2$ caused concentration-dependent contraction which became maximal at 10 mM. Further concentration increments evoked some relaxation. In control experiments (Fig. 1a) it was observed that repetition of the concentration-effect curve to $CaCl_2$ caused a small rightward and downward shift of the log concentration-response curve for $CaCl_2$-induced increase in tension. Verapamil (0·5 and 5 μM), cinnarizine (10 and 100 μM) and trifluoperazine (16 and 160 μM) each antagonized $CaCl_2$ in a concentration-dependent manner. Fig. 1 (b–d) 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. The log concentration-effect curves of the three calcium antagonists as inhibitors of the contraction produced by $CaCl_2$ (10 mM) are shown in Fig. 2. A significant difference existed between the position and slope of the verapamil log concentration-effect curve and those of cinnarizine and trifluoperazine. The mean (mean−s.e. mean, mean+s.e. mean) IC75 values against $CaCl_2$ 10 mM were 5·6 (3·2, 9·3) μM for verapamil, 59 (45, 78) μM for cinnarizine and 91 (66, 133) μM for trifluoperazine.

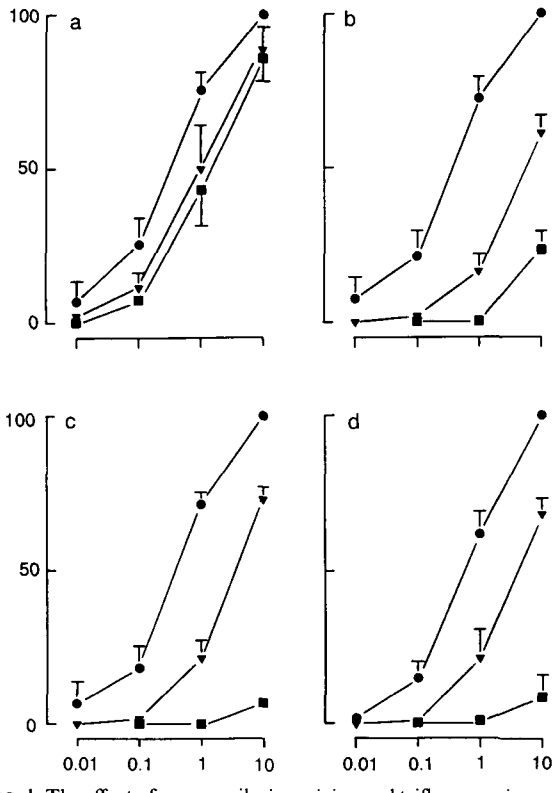

FIG. 1. The effect of verapamil, cinnarizine and trifluoperazine on the response of guinea-pig trachea in K^+-rich, Ca^{2+}-free, MOPS-PSS to $CaCl_2$. The abscissae indicate the concentration of $CaCl_2$ (mM) on a log scale. The ordinates indicate spasm as a % of the response to $CaCl_2$ 10 mM in the absence of antagonists. Points represent the means and vertical lines the s.e. mean, (n=7) (●) initial log concentration-effect curve for $CaCl_2$; (▼) second log concentration-effect for $CaCl_2$ obtained after tissue equilibration for 80 min in (a) depolarizing Ca^{2+}-free, MOPS-PSS, control, (b) verapamil 0·5 μM, (c) cinnarizine 10 μM, (d) trifluoperazine 16 μM; (■) third log concentration-effect curve for $CaCl_2$ obtained after an additional 80 min period of equilibration in the (a) same solution, control, (b) verapamil 5 μM, (c) cinnarizine 100 μM, (d) trifluoperazine 160 μM.

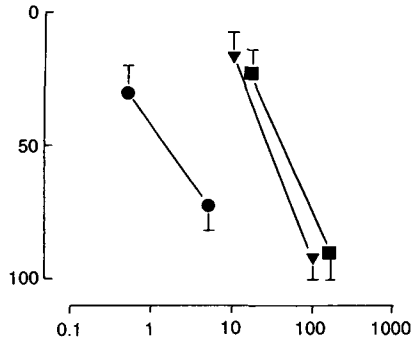

FIG. 2. The inhibitory effect of verapamil (●), cinnarizine (▼) and trifluoperazine (■) on the responses of the guinea-pig trachea in K^+-rich, Ca^{2+}-free, MOPS-PSS to Ca^{2+} 10 mM. The abscissa indicates concentration of the antagonists (μM) on a log scale. The ordinate represents inhibition expressed as % reduction in the initial response to Ca^{2+} (10 mM). Points represent the means and bars the s.e. mean (n=7).

Studies with skinned tracheal preparations

Segments of skinned trachea developed tension in response to low concentrations of Ca^{2+}. The log concentration-effect curve for Ca^{2+} obtained in these experiments always had a steep slope, the threshold concentration of Ca^{2+} was within

Table 1. Effect of verapamil, cinnarizine and trifluoperazine on −log threshold concentration, pD_2 values and maximal effect of Ca^{2+} in skinned trachea.

		−log threshold free Ca^{2+} concentration	pD_2	% decrease of maximal effect of B relative to A
Control	A	6·36±0·04	6·35±0·04	
	B	6·41±0·05	6·41±0·05*	14·89±9·80
Verapamil	A	6·36±0·09	6·33±0·15	
5·6 μM	B	6·46±0·04	6·48±0·07	12·70±14·30
Cinnarizine	A	6·41±0·05	6·42±0·02	
59 μM	B	6·20±0·07*	6·21±0·06*	19·90±9·20
Trifluoperazine	A	6·42±0·02	6·43±0·02	
91 μM	B	6·42±0·02	6·42±0·04	38·40±13·81
Control	A	6·44±0·04	6·36±0·04	
	B	6·49±0·05	6·46±0·06*	19·40±8·20
Verapamil	A	6·42±0·05	6·36±0·07	
100 μM	B	6·44±0·05	6·46±0·07	32·70±7·72
Cinnarizine	A	6·49±0·05	6·38±0·07	
177 μM	B	6·38±0·04*	6·21±0·04*	35·17±10·00
Trifluoperazine	A	6·32±0·05	6·23±0·07	
273 μM	B	NR	NR	97·30±1·20*

A, B = first and second concentration-response curve for Ca^{2+}, respectively. The second curve was generated in the absence (control) or presence of the stated concentration of the antagonist. NR = values not obtained because of lack of response to Ca^{2+}.

Values = mean ± s.e. mean (n=6). * $P < 0.05$ with respect to A.

the range 0·2–0·4 μM and maximal response was attained at 10 μM. Hence the threshold concentration and EC50 were very close (Table 1). The second concentration-effect curve for Ca^{2+} obtained in the same preparation was slightly displaced to the left with a small depression of its maximum compared to the initial concentration-effect curve. The value of the pD_2 of the second curve was increased ($P<0·05$) compared with the corresponding values in the first curve. A similar trend of change was observed for the threshold concentration but differences between the first and second curves did not achieve statistical significance. The acetylcholine 100 μM challenge terminating each experiment did not produce any tension increment.

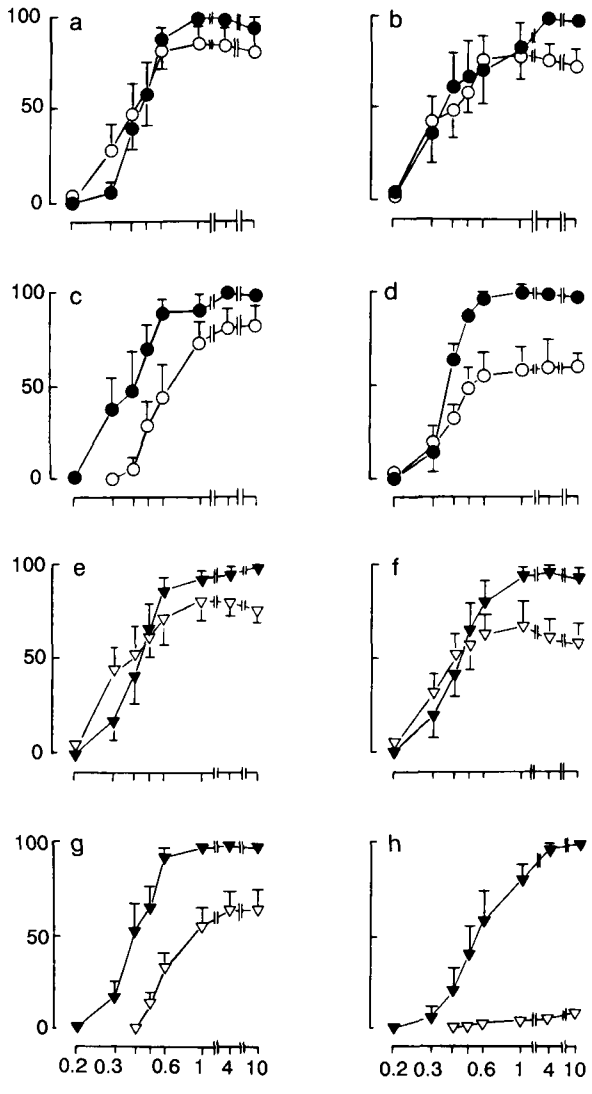

FIG. 3. Effects of verapamil, cinnarizine and trifluoperazine on cumulative Ca^{2+} concentration effect curves in skinned trachea. The abscissae indicate concentration of Ca^{2+} (μM) on a log scale. The ordinates represent spasmogenic effect (% of the initial maximal effect of Ca^{2+}). Points represent the means and bars the s.e. mean (n = 6). Upper panels (●) initial log concentration-effect curves for Ca^{2+}; (○) second log concentration-effect curves for Ca^{2+} in the absence (a) or presence of verapamil 5·6 μM (b), cinnarizine 59 μM (c) or trifluoperazine 91 μM (d). Lower panels: (▼) initial log concentration-effect curves for Ca^{2+}; (▽) second log concentration-effect curves for Ca^{2+} in the absence (e) or presence of verapamil 100 μM (f), cinnarizine 177 μM (g) or trifluoperazine 273 μM (h).

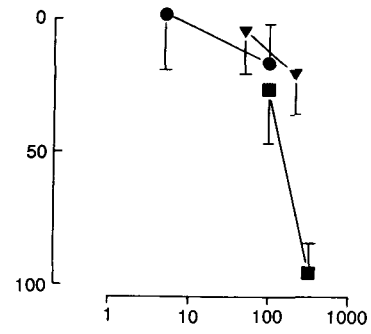

FIG. 4. The inhibitory effect of verapamil (●), cinnarizine. (▼) and trifluoperazine (■) on the responses of the guinea-pig skinned trachea to Ca^{2+} 10 μM. The abscissa indicates concentration of antagonist (μM) on a log scale. The ordinate represents inhibition as a % of the initial response to Ca^{2+}. Points represent the means and bars the s.e. mean (n = 6).

Antagonism of Ca^{2+}

The effects of the calcium antagonists on Ca^{2+}-induced activation of the contractile machinery are shown in Fig. 3 and Table 1. When changes occurring in the time-matched control tissues were taken into account, it was observed that 30 min incubation with verapamil (5·6 and 100 μM) produced no inhibition (Fig. 3b, f; Table 1). Cinnarizine (59 and 177 μM) decreased the sensitivity but not the responsiveness of the preparation to Ca^{2+} (Fig. 3c, g; Table 1). Trifluoperazine (273 μM) diminished the responsiveness of skinned trachea to Ca^{2+} (Fig. 3d, h; Table 1).

The log concentration-effect curves of the three calcium antagonists as inhibitors of the spasm produced by Ca^{2+} (10 μM) in skinned trachealis are shown in Fig. 4. A significant difference existed between the position and slope of the verapamil and cinnarizine log concentration-effect curves and that of trifluoperazine.

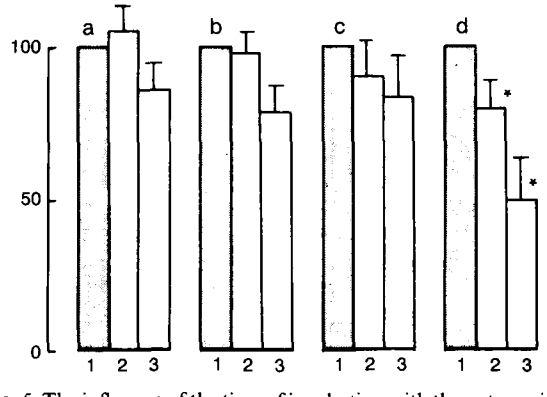

FIG. 5. The influence of the time of incubation with the antagonist on its antagonism of Ca^{2+} in skinned trachea. The abscissae indicate the first, second and third (1, 2, 3) challenge with Ca^{2+} 10 μM. The ordinate indicates the magnitude of the response as a % of the first Ca^{2+} challenge (shaded columns). Open columns indicate the response to the second and third challenge with Ca^{2+} in the absence (a) or presence of verapamil 100 μM (b), cinnarizine 177 μM (c) or trifluoperazine 91 μM (d). The time of incubation with the antagonist was 30 min before the second Ca^{2+} and 120 min before the third Ca^{2+} challenge. Column heights represent the mean values of 6 experiments and vertical lines indicate the s.e. mean. * = significant difference ($P<0·05$) from time-matched controls.

Influence of incubation time of the antagonist

To study the influence of the incubation time on the inhibitory effect of these calcium antagonists, three successive challenges with Ca^{2+} 10 μM were carried out. Control experiments showed that only for the third challenge, was the contractile response slightly depressed (Fig. 5). Acetylcholine (100 μM) never produced contraction of the skinned preparations. When test tissues were compared with their appropriate time-matched controls, it was observed that only trifluoperazine (91 μM) depressed the maximal tension evoked by Ca^{2+}, whereas verapamil (100 μM) and cinnarizine (177 μM) were ineffective. The longer incubation time (120 min) also resulted in a greater inhibition of the Ca^{2+}-induced response (Fig. 5d).

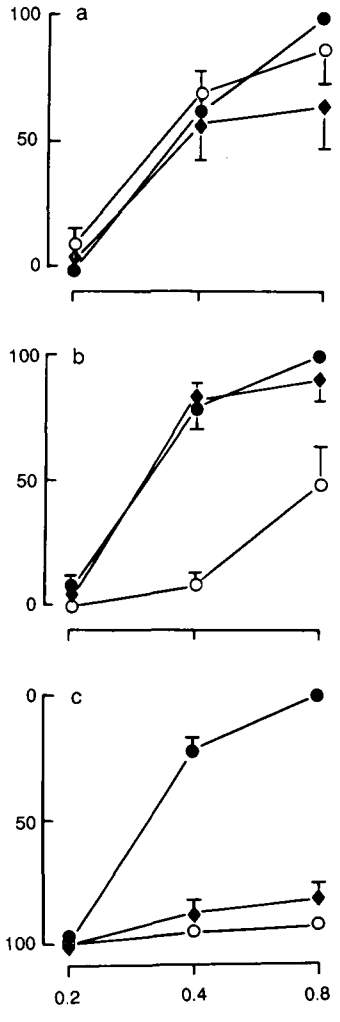

FIG. 6. The reversibility of the antagonism produced by cinnarizine and trifluoperazine of the responses to Ca^{2+} in skinned trachea. The abscissae indicate concentration of Ca^{2+} (μM) on a log scale. The ordinates represent spasmogenic effect (% of the initial maximal effect of Ca^{2+}). Points represent the means and bars the s.e. mean of at least 6 experiments. (●) Initial log concentration-effect curve for Ca^{2+}; (○) second log concentration-effect curve for Ca^{2+} in control tissues (a) or tissues treated with 177 μM cinnarizine (b) or 273 μM trifluoperazine (c); (◆) third log concentration-effect curve for Ca^{2+} obtained in the absence of antagonists (a, b, c).

Reversibility of the antispasmogenic action of cinnarizine and trifluoperazine

To study the reversibility of the calcium antagonism produced by cinnarizine and trifluoperazine, three successive concentration-response curves to Ca^{2+} were obtained in skinned trachealis with the second curve generated in the presence and the third curve in the absence of the antagonist. Fig. 6a shows the three successive concentration-effect curves for Ca^{2+} in control preparations. The antagonism elicited by cinnarizine (177 μM) was reversed by washing as shown by a third curve to Ca^{2+} not significantly different from its time-matched control (Fig. 6b). In contrast, the inhibition produced by trifluoperazine was still present when the third curve to Ca^{2+} was generated indicating that its antagonism of Ca^{2+} had not been reversed by washout.

Discussion

The first part of this study shows that three calcium antagonists, verapamil, cinnarizine and trifluoperazine, are able to inhibit Ca^{2+}-induced contractions of guinea-pig trachea bathed in a K^+-rich, Ca^{2+}-free, MOPS-PSS. The antagonism produced by these calcium antagonists appeared to be qualitatively similar in this test and was characterized by a rightward and downward displacement of the log concentration-effect curve for $CaCl_2$ when compared to appropriate time-matched controls. These results confirm and extend those previously published for these and other calcium antagonists in trachealis (Foster et al 1984; Ahmed et al 1985).

Although verapamil, cinnarizine and trifluoperazine had similar effect as calcium antagonists under these conditions there were large differences in their potencies. The rank order of potencies measured by their IC75 against $CaCl_2$ (10 mM)-induced contraction in depolarized trachea was verapamil (5·6 μM) > cinnarizine (59 μM) > trifluoperazine (91 μM). These differences could be accounted for by differences in their chemical structure and physicochemical properties which govern accessibility to and affinity for a common site of action or could arise from their having different mechanisms or sites of action. The procedure of skinning the plasmalemmal membrane from guinea-pig trachealis muscle by using the detergent Triton X-100 method (Sparrow et al 1984; Allen et al 1986a, b; Cortijo et al 1987b) was used in the second part of this study, to further analyse the effects of the three calcium antagonists.

The failure of acetylcholine to evoke spasm has been used (Ito & Itoh 1984; Sparrow et al 1984; Allen et al 1986a, b; Cortijo et al 1987b) as an index of the functional completeness of the skinning process in trachealis muscle. Since acetylcholine (100 μM) never evoked spasm from our preparations of skinned trachea, we assume that our skinning of this tissue was functionally complete. A leftward shift of the second log concentration-effect curve for Ca^{2+} relative to the initial curve was a constant feature in the present and previously reported (Cortijo et al 1987b) experiments. Whatever its underlying mechanism, which is beyond the scope of the present work, this leftward shift must be controlled for in experiments designed to investigate the effects of antagonists on the Ca^{2+}-induced responses.

In the skinned trachealis, verapamil 5·6 and 100 μM was

without effect. This is in contrast with the effects of cinnarizine and trifluoperazine which displaced to the right the log concentration-effect curve for Ca^{2+} (cinnarizine 59 and 177 μM, trifluoperazine 273 μM) and depressed the maximal effect attainable with Ca^{2+} (trifluoperazine 273 μM). This finding allows us to extend to the trachea the conclusions of Spedding (1983) and Cortijo et al (1987a) that cinnarizine and trifluoperazine interfere with Ca^{2+} activation of the intracellular contractile machinery whilst verapamil does not share this property. Thus, verapamil is able completely to suppress Ca^{2+}-induced contraction of unskinned depolarized trachea at about 10 μM but it is without effect, at 100 μM, in skinned trachea. Hence, the action of verapamil is mediated within the cell membrane and does not involve direct inhibition of Ca^{2+} activation of the contractile proteins. In contrast, cinnarizine and trifluoperazine need a one order of magnitude higher concentration (about 100 μM) than verapamil to abolish $CaCl_2$-induced contraction of unskinned trachea. At this concentration, cinnarizine and trifluoperazine produce some inhibition of the Ca^{2+}-induced contraction of skinned trachea.

It is interesting to note that, although skinned trachea preparations are sensitive to lower concentrations of Ca^{2+} than intact tissues, greater concentrations of calcium antagonists (cinnarizine and trifluoperazine) are needed to obtain the same degree of inhibition of the Ca^{2+}-induced responses in skinned smooth muscle preparations than in intact tissues (Cassidy et al 1980; Mrwa et al 1980; Sparrow et al 1981; Spedding 1983; Cortijo et al 1987a). This does not necessarily mean that the inhibition of the Ca^{2+}-induced activation of the contractile proteins produced by these antagonists could not operate at lower concentrations in intact preparations (Spedding 1983).

Additional experiments show that the same concentration of trifluoperazine (91 μM) produced greater inhibition of Ca^{2+}-induced contraction in skinned trachea after 120 min of incubation than after 30 min. On the other hand, the generation of a concentration-response curve to Ca^{2+} in skinned trachea in the presence of trifluoperazine (273 μm) resulted in inhibition which persisted, after washout, during a successive curve to Ca^{2+}. This was not observed after cinnarizine 177 μM. These experiments indicate that the calcium antagonistic action of trifluoperazine, but not that of cinnarizine, was time-dependent and not readily reversible by washing.

In conclusion, the findings of the present study demonstrate that cinnarizine and trifluoperazine, although exhibiting certain differences, may inhibit Ca^{2+}-induced tracheal spasm by two mechanisms: one seen at low concentrations in unskinned tissues involving the plasmalemma and the other detectable at higher concentrations in skinned tissues representing a direct interference with Ca^{2+} activation of contractile proteins. Therefore, distinct differences exist between the three calcium antagonists used. Verapamil appears to have an action restricted to the plasma membrane, while cinnarizine and trifluoperazine may have actions both at the level of plasmalemma and at the intracellular contractile machinery.

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References

Ahmed, F., Foster, R. W., Small, R. C. (1985) Some effects of nifedipine in guinea-pig isolated trachealis. Br. J. Pharmacol. 84: 861–869

Allen, S. L., Cortijo, J., Foster, R. W., Morgan, G. P., Small, R. C., Weston, A. H. (1986a) Mechanical and electrical aspects of the relaxant action of aminophylline in guinea-pig isolated trachealis. Ibid. 88: 473–483

Allen, S. L., Boyle, J. P., Cortijo, J., Foster, R. W., Morgan, G. P., Small, R. C. (1986b) Electrical and mechanical effects of BRL34915 in guinea-pig isolated trachealis. Ibid. 89: 395–405

Cassidy, P., Hoar, P. E., Kerrick, W. G. L. (1980) Inhibition of Ca^{2+}-activated tension and myosin light chain phosphorylation in skinned smooth muscle strips by the phenothiazines. Pflügers Arch. 387: 115–120

Cassidy, P. S., Kerrick, W. G. L., Hoar, P. E., Malenick, D. A. (1981) Exogenous calmodulin increases Ca^{2+} sensitivity of isometric tension activation and myosin phosphorylation in skinned smooth muscle. Ibid. 392: 115–120

Cortijo, J., Foster, R. W., Small, R. C. (1987a) Differentiation of calcium antagonists with respect to their effects in normal and skinned taenia caeci preparations. J. Pharm. Pharmacol. 39: 283–289

Cortijo, J., Dixon, J. S., Foster, R. W., Small, R. C. (1987b) Influence of some variables in the Triton X-100 method of skinning the plasmalemmal membrane from guinea-pig trachealis muscle. J. Pharmacol. Methods 18: 253–266

Ferry, D. R., Glossmann, H. (1982) Evidence for multiple receptor sites within the putative calcium channel. Naunyn-Schmiedebergs Arch. Pharmacol. 321: 80–83

Filo, R. S., Bohr, D. F., Rüegg, J. C. (1965) Glycerinated skeletal and smooth muscle: calcium and magnesium dependence. Science 147: 1581–1583

Fleckenstein, A., Tritthart, H., Fleckenstein, B., Herbest, A., Grün, G. (1969) Ein neue Gruppe Kompetitiver Ca^{++} Antagonisten (Iproveratril, D 600, Prenylamin) mit starken Hemmeffekten auf die elecktromechanische Koppelung im Warmblüter-Myokard. Pflügers Arch. 307: R25

Foster, R. W., Small, R. C., Weston, A. H. (1983) Evidence that the spasmogenic action of tetraethylammonium in guinea-pig trachealis is both direct and dependent on the cellular influx of calcium ion. Br. J. Pharmacol. 79: 255–263

Foster, R. W., Okpalugo, B. I., Small, R. C. (1984) Antagonism of Ca^{2+} and other actions of verapamil in guinea-pig isolated trachealis. Ibid. 81: 499–507

Gordon, A. R. (1978) Contraction of detergent-treated smooth muscle. Proc. Natl. Acad. Sci. U.S.A 75: 3527–3530

Iino, M. (1981) Tension responses of chemically skinned fibre bundles of the guinea-pig taenia caeci under various ionic environments. J. Physiol. (London) 320: 449–467

Ito, Y., Itoh, T. (1984) Effects of isoprenaline on the contraction-relaxation cycle in the cat trachea. Br. J. Pharmacol. 83: 677–686

Janis, R. A., Scriabine, A. (1983) Sites of action of Ca^{2+} channel inhibitors. Biochem. Pharmacol. 32: 3499–3507

Jetley, M., Weston, A. H. (1980) Some effects of sodium nitroprusside, methoxyverapamil (D600) and nifedipine on rat portal vein. Br. J. Pharmacol. 68: 311–319

Levin, R. M., Weiss, B. (1979) Selective binding of antipsychotics and other psychoactive agents to the calcium-dependent activator of cyclic nucleotide phosphodiesterase. J. Pharmacol. Exp. Ther. 208: 454–459

Martell, A. E. (1971) Organic Ligands: Stability Constants of Metal Ion Complexes. The Chemical Society London, Oxford, Alden Press, pp 733–734

Mrwa, V., Peterson, J. W., Rüegg, J. C. (1980) Effects of trifluoperazine on smooth muscle actomyosin. Arzneim. Forsch. 30: 114–116

Portzhel, H., Caldwell, P. C., Rüegg, J. C. (1964) The dependence of contraction and relaxation of the muscle fibers from the crab Maia

squinado on the internal concentration of free calcium ions. Biochim. Biophys. Acta 79: 581–591

Rampe, D., Su, C. M., Yousif, F., Triggle, D. J. (1985) Calcium channel antagonists: pharmacological considerations. Br. J. Clin. Pharmacol. 20: 247S–254S

Snyder, S. H., Reynolds, I. J. (1985) Calcium-antagonist drugs. Receptor interactions that clarify therapeutic effects. New Engl. J. Med. 313: 995–1002

Sparrow, M. P., Mrwa, U., Hofmann, F., Rüegg, J. C. (1981) Calmodulin is essential for smooth muscle contraction. FEBS Letts. 125: 141–145

Sparrow, M. P., Pfitzer, G., Gagelmann, M., Rüegg, J. C. (1984) Effects of calmodulin, Ca^{2+} and cAMP protein kinase on skinned tracheal smooth muscle. Am. J. Physiol. 246: C308–C314

Spedding, M. (1981) Marked differences in the effects of "Ca^{2+}-antagonists" on $CaCl_2$-induced contractions in K^+-depolarized smooth muscle. Br. J. Pharmacol. 72: 144P

Spedding, M. (1982) Assessment of the "Ca^{2+}-antagonist" effects of drugs in K^+-depolarized smooth muscle. Differentiation of antagonist sub-groups. Naunyn-Schmiedebergs Arch. Pharmacol. 318: 234–240

Spedding, M. (1983) Direct inhibitory effects of some calcium-antagonists and trifluoperazine on the contractile proteins in smooth muscle. Br. J. Pharmacol. 79: 225–231

Spedding, M. (1985) Calcium antagonist subgroups. Trends in Pharmacol. Sci. 6: 109–114

Thorens, S., Haeusler, G. (1979) Effects of some vasodilators on calcium translocation in intact and fractioned vascular smooth muscle. Eur. J. Pharmacol. 54: 79–91

Triggle, D. J., Swamy, V. C. (1983) Calcium antagonists. Some chemical-pharmacologic aspects. Circ. Res. 52: 117–128

Walsh, M. P., Bridenbough, R., Hartshorne, D. J., Kerric, W. H. L. (1982) Phosphorylation-dependent activated tension in skinned gizzard muscle fibres in the absence of Ca^{2+}. J. Biol. Chem. 257: 5987–5990

Weston, A. H. (1978) The effects of noradrenaline on electrical activity and calcium fluxes in rat portal vein. In: Szabadi, E., Bradshaw, C. M., Bevan, P. (eds) Recent Advances in the Pharmacology of Adrenoceptors, Amsterdam, Elsevier, pp 15–22