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Effects of Calcium Antagonists on Rat Normal and Skinned Fundus

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Abstract—Calcium chloride ($CaCl_2$) (0·1–25 mM, in K^+-depolarized tissue), KCl (10–112 mM) and acetylcholine (1×10^{-9} M^{-1} mM) produced concentration-dependent contractions of rat isolated fundus. Verapamil (0·01–100 μM), cinnarizine (1–100 μM), trifluoperazine (10–500 μM) and dantrolene (50–250 μM) each produced a concentration-related rightward and downward shift of the log concentration-effect curve for $CaCl_2$. The rank order of potencies of these antagonists, measured as the IC50 against Ca^{2+} (25 mM)-induced contraction of depolarized fundus, was verapamil (2·5 μM) > cinnarizine (8·7 μM) > trifluoperazine (85·1 μM) > dantrolene (> 250 μM). Cinnarizine (0·5 mM) and trifluoperazine (0·5 mM), but neither verapamil nor dantrolene depressed Ca^{2+} (20 μM)-evoked contraction of rat skinned fundus preparations. In intact preparations of rat fundus, verapamil had greater inhibitory effects on contractions produced by KCl than against those elicited by acetylcholine while trifluoperazine depressed to the same extent the responses to these two spasmogens. Dantrolene was without effect on contractions elicited by KCl or acetylcholine. Cinnarizine inhibited acetylcholine-induced responses but enhanced contractions to KCl. Augmentation of KCl-induced responses by cinnarizine is resistant to verapamil (1 μM). This enhancing effect of cinnarizine was not observed for KCl-induced contraction of guinea-pig fundus or rat gastro-oesophageal sphincter. In the rat fundus, cinnarizine (1–100 μM) produced an additional and concentration-related contraction when added on the plateau contraction to KCl (100 mM). The enhancing effect and the direct contraction produced by cinnarizine are at least partly dependent on extracellular Ca^{2+}. It is concluded that distinct differences exist between the calcium antagonists examined. The action of verapamil is restricted to the plasmalemma whereas cinnarizine and trifluoperazine also act on the intracellular contractile machinery. Dantrolene is scarcely effective as a calcium antagonist in rat fundus.

The calcium antagonists are a large and heterogeneous group of substances with diverse mechanisms and sites of action, and different pharmacological and clinical profiles (Triggle & Swamy 1983; Spedding 1985; Godfraind et al 1986). The term "calcium-entry channel blockers" designates a subgroup of calcium antagonists (nifedipine, verapamil, diltiazem, and related compounds) with a major site of action on voltage-dependent Ca^{2+} channels at the plasmalemma.

Another subgroup of calcium antagonists consists of highly lipophilic, weakly basic drugs, such as cinnarizine, with a predominant intracellular site of action (Spedding 1982). Phenothiazines like trifluoperazine are calmodulin antagonists (Weiss et al 1980). Dantrolene interferes with Ca^{2+} release from the sarcoplasmic reticulum of skeletal muscle but it has also an inhibitory effect on smooth muscle (Ward et al 1986).

The main clinical use of calcium antagonists is in the treatment of cardiovascular disorders. However, the study of the effects of calcium antagonists on nonvascular smooth muscle provides information on other mechanisms of action, the basis of certain side effects, or new therapeutic applications (Godfraind et al 1986). Gastrointestinal function is also related to calcium and may be modified by calcium antagonists (Castell 1985). A previous in-vivo study from this laboratory showed that calcium antagonists (verapamil, diltiazem, cinnarizine) delayed gastric emptying in the rat (Brage et al 1986).

Removal of the plasmalemma of smooth muscle has been used to detect a direct action of calcium antagonists which reduce the sensitivity of the intracellular contractile machinery to Ca^{2+} (Cassidy et al 1980; Spedding 1983). There are only a few studies on skinned intestinal smooth muscle and none on skinned gastric muscle (for review see Meisheri et al 1985).

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The aim of the present study was to characterize the effects of various calcium antagonists on the contractions of rat isolated fundus to $CaCl_2$, KCl and acetylcholine. Additional experiments were carried out for cinnarizine in guinea-pig isolated fundus and rat gastro-oesophageal sphincter. The effect of the antagonists was also studied on Ca^{2+}-induced contractions of rat skinned fundus.

Materials and Methods

Animals and tissue preparation

Adult Wistar rats, 200–250 g, of either sex were killed by stunning and bleeding. The abdomen was opened and the stomach excised and transferred to a Petri dish containing the buffer solution for tissue preparation. The fundal region of the stomach was dissected out and strips were prepared essentially as described by Vane (1957). Strips were mounted in organ baths containing buffer solution (mM): NaCl 118·00, KCl 4·69, $CaCl_2$ 2·50, KH_2PO_4 1·19, $MgSO_4$ 1·18, $NaHCO_3$ 24·99 and glucose 11·10), at 37°C, gassed with carbogen to maintain a pH close to 7·4. Isometric recording of tension changes was achieved by a Hewlett-Packard FTA 100-1 (1 g) force transducer connected to a Philips PM-8222 pen recorder via an HP8805 B carrier amplifier. In preliminary experiments we examined a range of resting tensions (0·5–4 g) finding that 2 g gave optimal contractions to KCl (50 mM) or acetylcholine (1 mM). This tension was used in

subsequent experiments. An equilibration period of 60 min was permitted with changes of buffer solution every 15 min.

Assessment of the effects of calcium antagonists in membrane-intact preparations of rat fundus

Cumulative concentration-response curves to $CaCl_2$ (0·1–25 mM), KCl (10–112 mM) and acetylcholine (1 nM–1 mM) were constructed. Responses to $CaCl_2$ were obtained in a Ca^{2+}-free, depolarizing (KCl 55 mM), Tris-buffer solution as previously outlined (Sarriá et al 1989).

After an initial dose-response curve for an agonist had been obtained, the tissues were allocated randomly to test or time-matched control groups and a second concentration-effect curve was constructed. A certain concentration of a calcium antagonist was present in test tissues for 20 min before and during the construction of the second concentration-effect curve. Only one concentration of the antagonist was tested in each strip. Tissues were exposed to the antagonist once agonist-induced contraction had been fully dissipated by washing with the appropriate buffer solution. Paired tissues from the same animal were not exposed to the calcium antagonist but otherwise were treated identically, giving reproducible concentration-effect curves to the agonists. The comparison of the effects produced by the calcium antagonist was made with their time-matched controls, i.e. the concentration-effect curve to each agonist obtained in the presence of a certain concentration of the calcium antagonist was compared with the second concentration-effect curve generated in the paired tissue in the absence of antagonist. No differences were found among these time-matched control concentration-effect curves to each agonist hence they were pooled for statistical purposes and presented as the control group. The inhibitory effect of each concentration of each calcium antagonist was quantified as a proportion (%) of the maximal effect of the agonist in the absence of antagonist. The concentration of calcium antagonist causing 50% inhibition (IC50) was derived by interpolation and expressed as negative log molar concentration.

Experiments with cinnarizine

Guinea-pigs of either sex, 350–400 g, were killed and strips from the fundus of their stomach prepared as already described in this study. Preparations of rat gastro-oesophageal sphincter were obtained as previously outlined (Morales-Olivas et al 1985).

In the rat fundus, cumulative concentration-effect curves for KCl were obtained in the absence or presence of cinnarizine with the preparations bathed in a buffer solution containing a low amount of calcium (0·60 mM). In addition, cumulative concentration-response curves to KCl were generated in normal calcium (2·50 mM) solution in the absence or presence of cinnarizine (0·1 mM), verapamil (1 μM), or cinnarizine (0·1 mM) plus verapamil (1 μM). In the guinea-pig fundus, cumulative concentration-response curves to KCl were generated before and after cinnarizine in the presence of a buffer solution with a normal amount of calcium (2·50 mM). In the rat gastro-oesophageal sphincter, cumulative concentration-response curves to KCl and acetylcholine were obtained in the absence or presence of cinnarizine with a buffer solution containing either a normal (2·50 mM) or a low (0·60 mM) concentration of calcium. In these experiments the protocol was as described above and the effects of cinnarizine were established by comparison with control, time-matched, preparations.

In other experiments, a plateau contraction of rat fundus was obtained by KCl (100 mM) and then a known concentration of cinnarizine was added. In these experiments, responses to KCl and cinnarizine were examined in the presence of either a normal calcium (2·50 mM) or a low calcium (0·60 mM) buffer solution.

Separate experiments were performed in rat fundus as follows. After equilibration in normal calcium solution, the response to a single concentration of KCl (50 mM) was obtained (first challenge). The medium was changed for a Ca^{2+}-free solution containing EGTA (0·1 mM) and after 20 min a second challenge was carried out. In test tissues, cinnarizine (0·1 or 0·5 mM) was present for 20 min before and throughout the second challenge with KCl. In control tissues, responses to KCl were determined in the absence of antagonist. The response to the second challenge was expressed as a proportion (%) of that in the first challenge.

Antagonism of calcium in rat skinned fundus preparations

Fundus strips were prepared as described above and then skinned of their plasmalemmal membranes as previously reported (Cortijo et al 1987). 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 containing (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 fundus 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 (pH 6·7). The relaxing solution did not contain added calmodulin. All tissues were allowed to equilibrate in this medium for 20 min. Tension development was induced by addition of $CaCl_2$ in an amount calculated to yield a free Ca^{2+} concentration of 20 μM. When the tension became maximal it was subsequently dispelled by repeatedly washing with relaxing solution. The experimental design used was: in test tissues two successive Ca^{2+} challenges were performed. A certain concentration of a calcium antagonist was present for 20 min before and throughout the second Ca^{2+} challenge. Acetylcholine (100 μM) was added to the tissue once full relaxation was achieved after the second challenge. Control tissues were treated similarly except that they were not exposed to calcium antagonist. The effect in the second challenge was expressed as a proportion (%) of that in the first challenge.

Drugs and solutions

The following substances were used: acetylcholine (Sigma, St Louis, MO, USA), adenosine-5′-triphosphate (ATP disodium salt, Sigma), cinnarizine (Esteve Lab., Barcelona, Spain), dantrolene (Alonga Lab., Barcelona, Spain), dithioerythritol (Sigma), ethyleneglycol-bis (β-amino-ethylether)-*N*-*N*-tetracetic acid (EGTA, Sigma), glycerol (E. Merck, Darmstadt, Germany), imidazole (Sigma), sodium

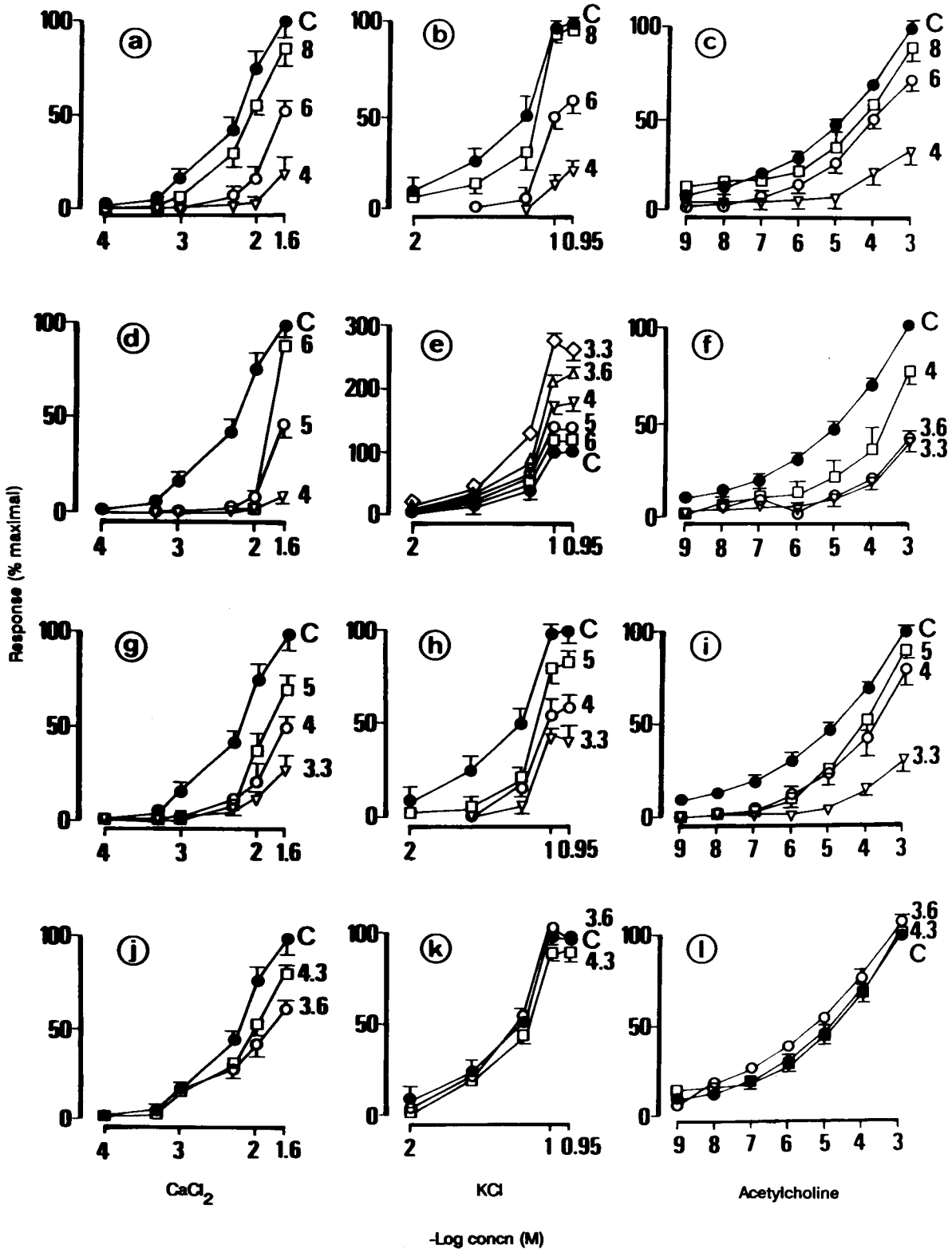

FIG. 1. Effects of calcium antagonists on the responses of rat fundus to $CaCl_2$ (in K^+-rich, Ca^{2+}-free, Tris solution) (a, d, g, j), KCl (b, e, h, k) and acetylcholine (c, f, i, l). The ordinates indicate response as a % of the maximal response to each agonist in the absence of antagonist. Points represent the means and vertical lines the s.e.m. (n=6). Time-matched control concentration-effect curves for agonists in the absence of antagonist are represented by ●, C. Open symbols represent concentration-effect curves obtained in the presence of different concentrations (indicated at −log M near each curve) of verapamil (a, b, c), cinnarizine (d, e, f), trifluoperazine (g, h, i) and dantrolene (j, k, l).

azide (NaN_3, E. Merck), sucrose (E. Merck), trifluoperazine (Sigma), Triton X-100 (E. Merck), verapamil (Biosedra-Knoll, Paris, France). Drugs were dissolved in the bathing buffer solution with the exception of cinnarizine which was prepared in absolute ethanol. Previous experiments demonstrated that addition of the vehicle to the bath did not alter drug-induced responses (data not shown).

Statistical analysis

Data are presented as means ± s.e.m. Two-tailed Student's *t*-test was used for statistical analysis of the data. A difference between means was taken to be significant for $P < 0.05$.

Results

Calcium antagonists in membrane intact preparations of rat fundus

Cumulative addition to the bath of increasing concentrations of $CaCl_2$, KCl and acetylcholine resulted in concentration-dependent contractions of rat fundus. The initial concentration-response curve to these spasmogens and the second curve constructed in untreated (no antagonist present) tissues were not significantly different. Data from the first curves are not shown while data from the second curves are shown in Fig. 1, and served as controls (time-matched) in assessing the effects of calcium antagonists. Maximal effect and −log EC50 for spasmogens in the absence of antagonists (time-matched controls) were, respectively, as follows: 3·56±0·28 and 2·15±0·03 g (n=6) for $CaCl_2$; 1·53±0·08 and 1·26±0·02 g (n=6) for KCl; and 1·37±0·16 and 4·97±0·09 g (n=6) for acetylcholine. Incubation with calcium antagonists tested in this study did not alter the resting tension of the preparations. Verapamil and trifluoperazine produced a concentration-dependent rightward and downward shift of the log concentration-effect curve of spasmogens (Fig. 1). The efficacy of these antagonists to depress the contraction produced by the spasmogens is shown in Fig. 1, and their relative potencies are shown in Table 1.

Cinnarizine inhibited responses to $CaCl_2$ and acetylcholine but enhanced, in a concentration-dependent manner, those elicited by KCl (Fig. 1).

Dantrolene produced small inhibitions of $CaCl_2$-induced contraction and was without effect on contractions elicited by KCl and acetylcholine.

Experiments with cinnarizine

The finding that cinnarizine enhanced KCl-induced contractions of rat fundus instead of the expected inhibition prompted us to perform additional experiments with this calcium antagonist. Contractions of rat fundus in a low calcium solution were also enhanced by cinnarizine although augmentation of KCl-induced responses started at a higher concentration (0·1 mM) of cinnarizine compared with the small concentration (1 μM) already effective in the normal calcium solution (compare Fig. 1e with Fig. 2a). Verapamil, in a concentration (1 μM) sufficient to inhibit KCl-induced contraction of rat fundus in normal calcium solution, did not

Table 1.-Log IC50 values of calcium antagonists for responses to $CaCl_2$ (25 mM), KCl (112 mM) and acetylcholine (ACh, 1 mM) in rat isolated fundus.

	Verapamil	Cinnarizine	Trifluoperazine	Dantrolene
$CaCl_2$	5·60±0·23	5·06±0·31◆#	4·07±0·35◆#	<3·6
KCl	5·82±0·33	NC	3·55±0·34	NC
ACh	4·82±0·36*	3·68±0·19	3·60±0·42	NC

Data are mean ± s.e.m. of 6 experiments. NC = not calculated. *$P < 0.05$ compared with $CaCl_2$ or KCl. ◆$P < 0.05$ compared with verapamil. #$P < 0.05$ compared with cinnarizine.

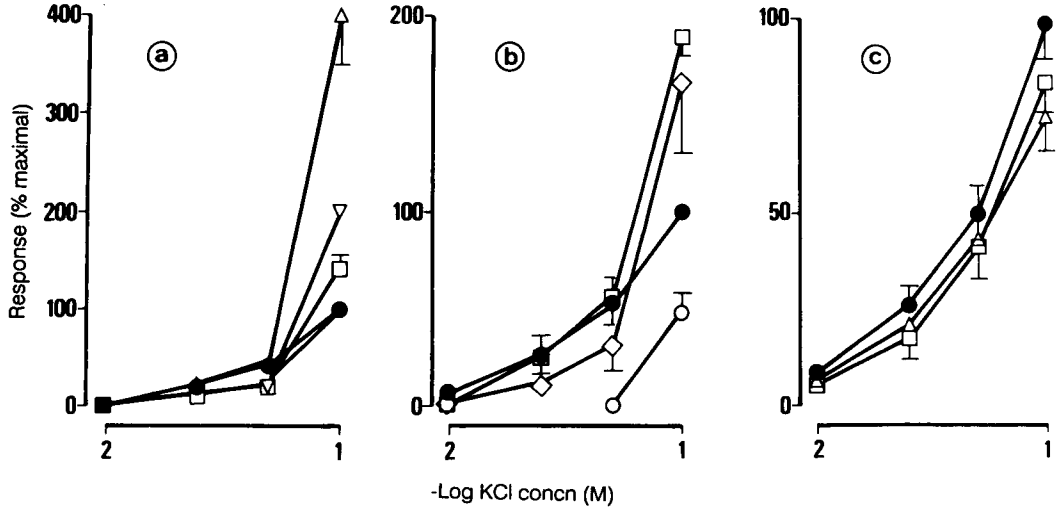

FIG. 2. Effects of cinnarizine on the response of rat (a, b) and guinea-pig (c) fundus to KCl. The ordinates indicate response as a % of the maximal response in the absence of antagonist. Points represent the means and vertical lines the s.e.m. (n = 6). Time-matched control concentration-effect curves for KCl in the absence of antagonist are represented by ●. Panel 'a' shows the effects of cinnarizine 0·01 (○), 0·1 (□), 0·25 (▽) and 0·5 (△) mM on KCl-induced contraction of rat fundus bathed in a low calcium (0·60 mM) medium. Panel 'b' shows the effects of cinnarizine 0·1 mM (□), verapamil 1 μM (○) and cinnarizine 0·1 mM plus verapamil 1 μM (◇) on KCl-induced contraction of rat fundus bathed in normal calcium solution. Panel 'c' shows the effects of cinnarizine 0·1 (□) and 0·5 (△) mM on KCl-induced contraction of guinea-pig fundus.

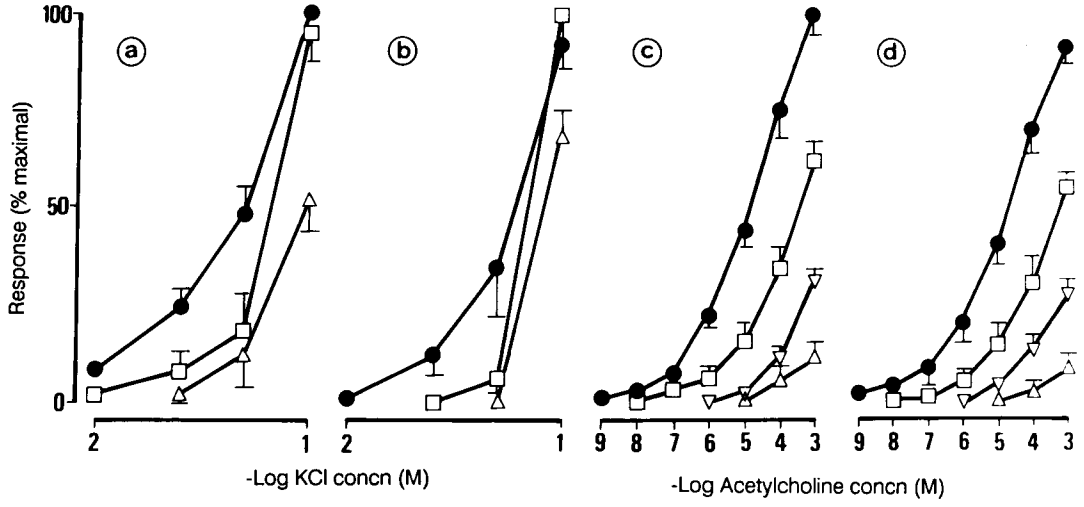

FIG. 3. Effects of cinnarizine on the responses to KCl (a, b) and acetylcholine (c, d) in the rat isolated gastro-oesophageal sphincter. Responses were obtained in normal (2·5 mM) (a, c) or low (0·60 mM) (b, d) calcium media. The ordinates indicate response as a % of the maximal response to each spasmogen in the absence of cinnarizine. Points represent the means and vertical lines the s.e.m. (n = 6). Time-matched control concentration-effect curves for spasmogens in the absence of cinnarizine are represented by ●. Open symbols represent concentration-effect curves obtained in the presence of cinnarizine 0·10 (□), 0·25 (▽) or 0·50 (△) mM.

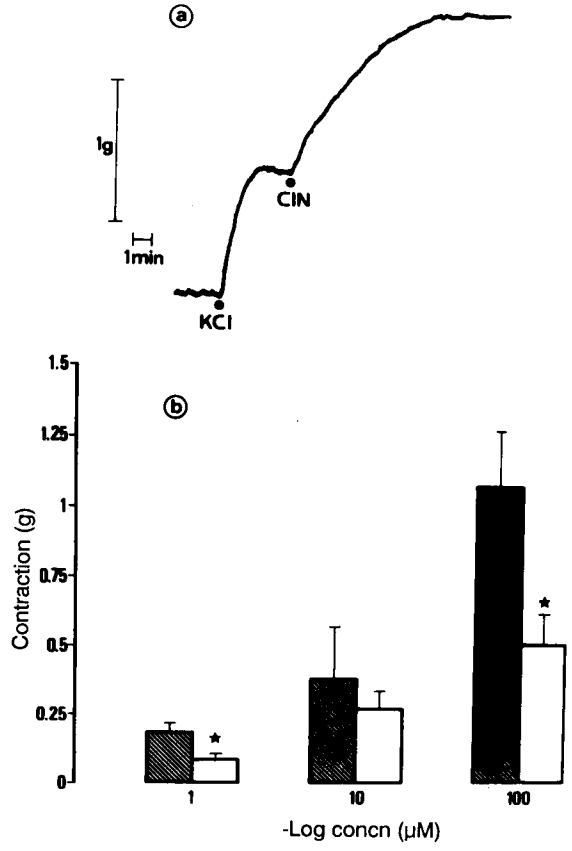

FIG. 4. Effects of cinnarizine in rat isolated fundus precontracted with KCl. Panel 'a' reproduces an original trace showing the contraction produced by cinnarizine (CIN) 0·1 mM on top of the plateau response to KCl (100 mM). Panel 'b' shows the additional contraction produced by cinnarizine (1, 10 or 100 μM) on top of the plateau response to KCl (100 mM) in rat fundus bathed in normal (2·5 mM, ▩) or low (0·60 mM, □) calcium solution. Column height represents the mean value of 5 experiments and vertical line indicates the s.e.m. ★$P < 0.05$ compared to normal calcium.

impede the enhancing effect of cinnarizine on KCl-induced spasm (Fig. 2b).

The enhancing effect of cinnarizine was not observed in guinea-pig fundus where this antagonist depressed KCl-induced contractions (Fig. 2c). Contraction to KCl and acetylcholine in rat gastro-oesophageal sphincter were also inhibited by cinnarizine irrespective of whether these contractions were obtained in normal or low calcium media (Fig. 3).

Cinnarizine (1–100 μM), when added to rat fundus strips previously contracted to a plateau by KCl (100 mM), produced a slowly developing contraction (Fig. 4a). The size of the cinnarizine-induced contractions was smaller in preparations bathed in a low calcium medium (Fig. 4b).

In other experiments in rat fundus, two consecutive challenges with KCl (50 mM) were obtained, the first in normal calcium solution and the second in zero-calcium (plus EGTA 0·1 mM) solution. In control preparations, the response to the second challenge was 43 ± 8% of that obtained for the first challange. In preparations treated with cinnarizine (0·1 or 0·5 mM) the responses to the second challenge were 45 ± 7 and 42 ± 8%, respectively (not significantly different from values in the absence of cinnarizine). This indicates that cinnarizine did not enhance responses to KCl generated in a buffer solution deprived of extracellular calcium.

Antagonism of calcium in rat skinned fundus preparations

Control experiments showed that skinned fundus responded to an initial Ca^{2+} challenge (20 μM) by generating tension which reached a peak value of 0·272 ± 0·023 g (n = 64) within 15 min. Responses to a subsequent Ca^{2+} challenge were smaller (62 ± 6% of first challenge). The acetylcholine (100 μM) challenge terminating each experiment did not produce any tension increment. When test tissues were compared with their appropriate time-matched controls, it

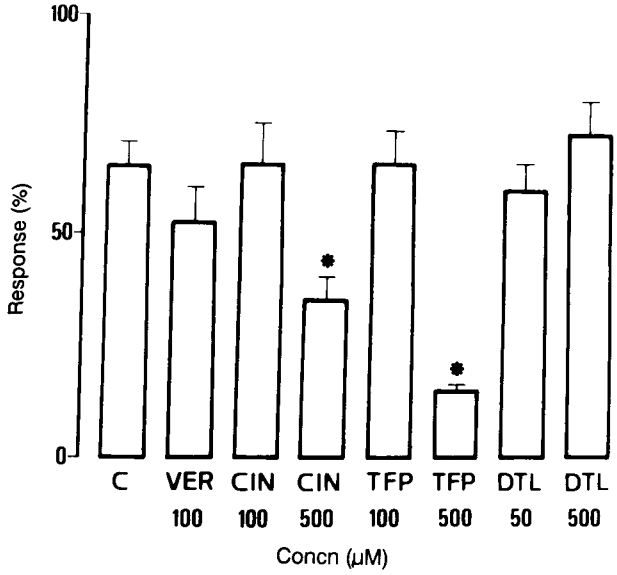

FIG. 5. Effects of calcium antagonists on the response of rat skinned fundus to Ca^{2+}. The abscissae indicate the second challenge with Ca^{2+} (20 μM) in the absence (time-matched control, C) or presence of verapamil (VER), cinnarizine (CIN), trifluoperazine (TFP) or dantrolene (DTL) in concentrations (μM) as indicated. The ordinate represents response as a % of the first Ca^{2+} (20 μM) challenge. Column heights represent the mean value of at least 8 experiments and vertical lines indicate the s.e.m. $*P < 0.05$ vs control.

was observed that only cinnarizine (0·5 mM) and trifluoperazine (0·5 mM) depressed Ca^{2+} (20 μM)-evoked contraction (Fig. 5).

Discussion

In intact (unskinned) rat fundus strips, contraction was elicited in three different ways: exogenously applied Ca^{2+} acting on K^+-depolarized tissues, which allows for the analysis of the direct interaction between Ca^{2+} and calcium antagonists in this preparation; KCl-induced contraction; and acetylcholine-induced contraction. Presumably, the mechanism underlying contraction of rat fundus by KCl is depolarization by K^+ leading to Ca^{2+} entry through voltage-dependent channels as shown in other intestinal smooth muscles (Godfraind et al 1986). Activation of muscarinic receptors by acetylcholine results in contraction of rat fundus which is almost exclusively dependent on the mobilization of intracellularly stored Ca^{2+} (Donoso et al 1986). Representatives of different subgroups of calcium antagonists (verapamil, cinnarizine, trifluoperazine and dantrolene) were selected to characterize their ability to interfere with the responses to these spasmogens.

The first part of this study shows that the calcium antagonists tested inhibit Ca^{2+}-induced contraction of rat fundus bathed in Ca^{2+}-free, depolarizing (K^+ 55 mM), buffer solution. The antagonism produced by these substances appeared to be qualitatively similar and was characterized by a concentration-related, rightward and downward displacement of the log concentration-response curve to $CaCl_2$ compared with appropriate time-matched controls.

The rank order of potencies was verapamil > cinnarizine > trifluoperazine > dantrolene. These results confirm and extend those previously published for these and other calcium antagonists in rat fundus (Donoso et al 1986) and other smooth muscle preparations (Spedding 1985; Godfraind et al 1986; Cortijo et al 1990).

Verapamil displayed a specific inhibitory effect on the contractile action of KCl when compared with acetylcholine. This selective effect was exerted at concentrations effective in displacing the calcium concentration-response curves. This finding extends to the rat fundus the selectivity of Ca^{2+}-entry channel blockers in inhibiting pharmacologically-induced contraction previously reported in other smooth muscle preparations (Godfraind et al 1986). The specificity of verapamil for KCl vs acetylcholine was not observed with the calmodulin antagonist trifluoperazine which is consistent with results from other tissues (Sanz et al 1988). A similar trend was observed for cinnarizine but this antagonist enhanced instead of depressed the contractions produced by KCl.

The antagonism exerted by the antagonists tested in this study may be due to an action on sarcolemma interfering with transmembrane fluxes of Ca^{2+} or to some type of intracellular action yielding to an interference with Ca^{2+}-induced contraction (Spedding 1985; Godfraind et al 1986). The procedure of skinning the plasmalemmal membrane from rat fundus by using the detergent Triton X-100 method (Sparrow et al 1984; Cortijo et al 1987) was used to further analyse the effects of these calcium antagonists. Since acetylcholine did not evoke spasm from our preparations of skinned fundus, we assume that the skinning process was functionally complete. The response to the second Ca^{2+} challenge was consistently found to be smaller than that to the first Ca^{2+} challenge as previously reported for skinned preparations of guinea-pig taenia caeci and trachealis (Sarriá et al 1989). Therefore, the effect of calcium antagonists can be compared with the appropriate time-matched controls. In the skinned fundus only cinnarizine and trifluoperazine depressed the effect of Ca^{2+}. This finding confirms for the rat fundus, the previous reports (Spedding 1983; Cortijo et al 1990) that cinnarizine and trifluoperazine interfere with Ca^{2+} activation of the intracellular contractile machinery whilst verapamil or dantrolene do not. This indicates that the action of verapamil or dantrolene is mainly mediated within the cell membrane. Previous findings with dantrolene in other smooth muscle preparations (Mahmoudian et al 1981; Sanz et al 1990) are in agreement with this site of action. It should be noted in this study as in previous studies that 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 tissues as in intact preparations (Spedding 1983; Cortijo et al 1990). However, it is possible that the direct inhibition of Ca^{2+} activation of the contractile proteins produced by these antagonists operates at lower concentrations in intact tissues (Spedding 1983).

Enhancement by cinnarizine of the contraction of rat fundus by KCl was an unexpected finding which is difficult to explain. This enhancing effect of cinnarizine was not observed for KCl-induced contraction of guinea-pig fundus or rat gastro-oesophageal sphincter. In the rat fundus, cinnarizine did not alter the resting tension of the preparation but produced an additional and concentration-related

contraction when added on the plateau contraction to KCl. Experiments carried out in a low calcium medium indicate that both the enhancing effect and the direct contraction produced by cinnarizine are at least partly dependent on extracellular Ca^{2+}. The mechanism involved in the enhancing effect of cinnarizine was insensitive to verapamil. Interestingly, it has been reported by Ichida et al (1988) that ω-conotoxin, which is an inhibitor of Ca^{2+} influx through N- and L-type calcium channels in certain tissues, caused concentration-dependent contraction of rat fundus. This contraction was dependent on extracellular Ca^{2+} but resistant to verapamil. In addition, ω-conotoxin enhanced contractions to high concentrations of KCl in rat uterus. These effects were not observed in rat ileum or guinea-pig taenia coli (Ichida et al 1988). We are not aware of other reports in the literature showing this paradoxical effect of cinnarizine. This effect probably lacks any physiological relevance provided that cinnarizine is competent in inhibiting gastric emptying in the rat (Brage et al 1986).

In conclusion, the present study characterizes the effect of four calcium antagonists in rat intact and skinned fundus. Verapamil appears to have an action restricted to the plasma membrane while cinnarizine and trifluoperazine may have actions at the level of the plasmalemma and at the intracellular level. Dantrolene is scarcely effective as a calcium antagonist. The study confirms both the existence of distinct differences between the calcium antagonists tested and that tissues vary widely in their response to this category of drugs.

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