Antagonism of calcium by zinc in guinea-pig isolated taenia caeci and trachealis muscle

B. Sarriá,¹ J. Cortijo, M. Martí-Cabrera, *E. Morcillo & J. Esplugues

Departament de Farmacologia y Farmacotecnia, Facultat de Medicina, Universitat de València and

*Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Alcalá de Henares, Madrid, Spain

1 In guinea-pig isolated taenia caeci and trachealis bathed in a K^+ -rich, Ca^{2+} -free medium, $CaCl_2$ (0.01–10 mM) produced a concentration-dependent contraction. Zn^{2+} (0.01–1 mM), Cd^{2+} (0.01–1 mM), verapamil (0.01–100 μ M) and trifluoperazine (1–100 μ M) were effective antagonists of $CaCl_2$ -induced responses.

2 Zn^{2+} and Cd^{2+} in concentrations from 0.01 to 1 mM were without effect on the tone of taenia and trachea in normal Tris solution. Conversely, Zn^{2+} and Cd^{2+} , in concentrations of 1 mM, caused contraction of these tissues in a K^+ -rich, Ca^{2+} -free medium. Zn^{2+} (1 mM)-induced contractions of taenia and trachea were completely inhibited by verapamil (10 μ M).

3 In taenia and trachea skinned of their plasma membranes, tension development induced by Ca^{2+} (10 μ M or 1 μ M, respectively) was unaffected by verapamil (100 μ M), whereas trifluoperazine (100 μ M) depressed the maximal tension produced by Ca^{2+} . Segments of skinned preparations contracted in response to low concentrations of Zn^{2+} (10 μ M) or Cd^{2+} (10 μ M).

4 It is concluded that Zn^{2+} may suppress Ca^{2+} -induced spasm by a direct action on the binding sites of the Ca^{2+} channel.

Introduction

The term calcium-blocking agents refers to drugs that block the entry of Ca^{2+} through the Ca^{2+} channels (Spedding, 1985; Godfraind *et al.*, 1986). The first method of achieving Ca^{2+} channel blockade was the use of inorganic cations which interact with Ca^{2+} channels in a direct and reversible manner (Hurwitz, 1986).

Zinc, a divalent cation, is involved in many biological functions (Prasad, 1985). Thus, some of the observed physiological effects of Zn^{2+} may be related to competition between Zn^{2+} and several other cations such as Cd^{2+} , Ca^{2+} and Cu^{2+} but its exact mechanism of action has not yet been defined. Experimental evidence *in vitro* suggests the existence of a Zn^{2+} – Ca^{2+} antagonism because Zn^{2+} depresses the contractile responses to different Ca^{2+} -dependent agonists in rat aorta (Cortijo *et al.*, 1985). However, the characteristics of this antagonism are different from those reported for other calcium-blocking agents such as verapamil (Triggle, 1982).

This suggests the possibility that the Zn^{2+} – Ca^{2+} antagonism could be the result of an intracellular modification in the regulation of the excitation–contraction coupling in the smooth muscle. This is in keeping with previous hypotheses proposed to explain some intracellular events promoted by Zn^{2+} in blood cells (Brewer *et al.*, 1979). Nevertheless, further studies are necessary to discover whether this divalent cation possesses calcium-blocking properties in other tissues and, if this is proved to be so, to study the nature of its calcium antagonistic action.

Therefore, the main objective of this study was to provide more direct evidence that this cation can produce calcium antagonism. To achieve this, we have assessed its ability to inhibit calcium-induced contractions in guinea-pig isolated taenia caeci and trachealis muscle, comparing its effects with those obtained with other calcium-blocking agents such as Cd^{2+} , verapamil and trifluoperazine. Furthermore, we have tested these four calcium-blocking agents on preparations in which the sarcolemma had been chemically disrupted. By comparing the effects of these agents on intact and skinned tissues we tried to

¹ Author for correspondence.

determine whether they act on the external surface of, or within, the plasma membrane or conversely if they produce their calcium antagonistic action by acting intracellularly.

Methods

Guinea-pigs of either sex (350–450 g) were killed by stunning and bleeding. Strips of taenia were obtained from the caecum. Tracheae were excised, cleaned of adhering fat and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis.

Antagonism of calcium

Strips of taenia or small segments of trachea were mounted, for the isometric recording of tension changes, as previously described by Cortijo *et al.* (1987a) and Foster *et al.* (1983). The tissues were subjected to a tension of 1 g in a Tris buffer solution containing normal amounts of Ca^{2+} and K^{+} (composition mM: NaCl 134.8, KCl 4.75, CaCl_2 2.55, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, Tris 5.0, HCl 5.0 and glucose 10.1). After equilibration, the bath fluid was changed to a Ca^{2+} -free (neither Ca^{2+} nor a Ca^{2+} -chelating agent was added) depolarizing solution containing 55 mM KCl in equimolar replacement for NaCl. This produced a spasm which was dissipated by regular (20 min) changes of the K^{+} -rich, Ca^{2+} -free bath fluid. These solutions were aerated with O_2 at 37°C and the pH adjusted to 7.4 with HCl 0.5 N. The Ca^{2+} concentration of the Ca^{2+} -free medium did not exceed $6\text{ }\mu\text{M}$ calculated from the impurity limits of the constituents. Changes in tone were recorded with a Hewlett-Packard FTA 100-1 (1 g) isometric force transducer connected to a Phillips PM-8222 pen recorder via a HP-8808B carrier amplifier. 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 complete development of the tension attainable at that concentration (15 min). In these experiments the strips were divided into pairs. In test tissues, the effect of CaCl_2 was examined before and after tissue equilibration (20 min) with the calcium-blocking agents. Tissues were treated with the antagonist once the CaCl_2 induced spasm had been fully dissipated by washing with K^{+} -rich, Ca^{2+} -free medium. Only one concentration of the antagonist was tested in each strip. Paired tissues from the same taenia or trachea were not exposed to calcium-blocking agents but otherwise were treated identically, giving reproducible concentration-response curves to CaCl_2 . The comparison of the effects produced by the calcium antagonist was made

with their appropriate time-matched controls i.e. the concentration-response curve to CaCl_2 obtained in the presence of a certain concentration of the calcium antagonist was compared to the second concentration-response curve generated in the paired tissue in the absence of antagonist. No differences were found among these time-matched control concentration-response curves to CaCl_2 hence they were pooled for statistical purposes and presented as the control group. The inhibitory effect of each concentration of each calcium-blocking agent was quantified as a proportion (%) of the CaCl_2 (10 mM)-induced contraction in the absence of antagonist. The concentration of calcium-blocking agent causing a 50% reduction in the spasm produced by CaCl_2 10 mM (IC_{50}) was derived by interpolation and expressed as $-\log$ molar concentration.

Measurement of the effect of Zn^{2+} and Cd^{2+} on tone

In taenia and trachea batched in either normal Tris solution or K^{+} -rich, Ca^{2+} -free Tris solution, cumulative concentration-effect curves to Zn^{2+} (0.01–1.0 mM) or Cd^{2+} (0.01–1.0 mM) were constructed. In tissues equilibrated with K^{+} -rich, Ca^{2+} -free Tris solution the response to a single concentration (1 mM) of Ca^{2+} , Zn^{2+} and Cd^{2+} was generated to obtain the values of the latency and the initial velocity of contraction. The time elapsed between the time of drug addition to the bath and a just discernible increase in isometric tension was the latency which is expressed in s. The initial velocity of contraction was derived from the tangent to the initial part of the tension record and is expressed in mg s^{-1} . In another set of experiments, the contractile response to single concentration (1 mM) of Zn^{2+} (taenia and trachea bathed in K^{+} -rich, Ca^{2+} -free Tris solution) was generated in the absence (time-matched control) or presence (20 min of incubation time) of verapamil (10 μM). The inhibitory effect was quantified as a proportion (%) of the contraction obtained in the absence of antagonist.

Experiments with taenia and trachea skinned of their plasma membranes

Segments of taenia or trachea were prepared as described above and then skinned of their plasma membranes as previously reported (Cortijo *et al.*, 1987a, b). 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,

Na_2N 1, imidazole 20) and dithioerythritol 0.5 (pH 6.7), with 50% glycerol at $-20^{\circ}C$ for up to 7 days.

With an imposed tension of 0.5 g, segments of skinned taenia or tracheae were set up at $20^{\circ}C$ 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, Na_2N 1 and imidazole 20, adjusted to pH 6.7 with KOH. The relaxing solution did not contain added calmodulin. Tension development was induced by addition of $CaCl_2$ in an amount calculated to yield a free Ca^{2+} concentration of $10\ \mu M$ (taenia) or $1\ \mu M$ (trachea). When the tension became maximal (25 min) it was subsequently dispelled by repeatedly washing with relaxing solution (90 min). The apparent stability constants used for Ca^{2+} -EGTA, Zn^{2+} -EGTA and Cd^{2+} -EGTA solutions were $1.2\ \mu M$, $0.0162\ \mu M$ and $10.8\ \mu M$ at $20^{\circ}C$ and pH 6.7, which were calculated from the stability constants of EGTA-metal ion complexes (Martell, 1971), using the equations described by Portzehl *et al.* (1964).

The experimental design used was: in test tissues three successive Ca^{2+} challenges were performed. Verapamil ($100\ \mu M$) or trifluoperazine ($100\ \mu M$) was present for 20 min before and throughout the second Ca^{2+} challenge. Zn^{2+} ($10\ \mu M$) or Cd^{2+} ($10\ \mu M$) were also added 20 min before the second Ca^{2+} challenge but because they produce a contraction, the second and third Ca^{2+} challenge were not carried out. Acetylcholine ($100\ \mu M$) was added to the tissue once full relaxation was achieved either after the third Ca^{2+} challenge (experiments with verapamil or trifluoperazine) or after the contraction elicited by Zn^{2+} or Cd^{2+} . Control tissues were treated similarly except that they were not exposed to calcium blocking agents. The effect in the second and third challenge was expressed as a proportion (%) of that in the first challenge.

Drugs and solutions/statistical analysis of results

The following substances were used: acetylcholine (Sigma), adenosine-5'-triphosphate (ATP disodium salt, Sigma), cadmium chloride (Merck), dithioerythritol (Sigma), ethyleneglycol-bis(β -amino-ethylether)-*N,N*-tetraacetic acid (EGTA, Sigma), glycerol (Merck), imidazole (Sigma), trifluoperazine (Sigma), Tris (Merck), Triton X-100 (Merck), sucrose (Merck), verapamil hydrochloride (Biosedra-Knoll, Paris, France), zinc sulphate (Merck). Concentration of drugs is expressed in terms of the molar concentration of the active species.

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

Results

Experiments in normal taenia preparations

When the bath fluid was changed from Tris solution to a K^+ -rich, Ca^{2+} -free, Tris solution, the 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 was the period of incubation in K^+ -rich, Ca^{2+} -free, Tris solution used in subsequent experiments. Addition of $CaCl_2$ (0.1 to 100 mM) at this time produced a concentration-dependent smoothly developing spasm, $E_{max} = 5.31 \pm 0.25$ g; $pD_2 = 3.30 \pm 0.05$; these values are the mean of 72 experiments corresponding to the time-matched controls. Verapamil, trifluoperazine, Zn^{2+} and Cd^{2+} caused a concentration-dependent right downward shift of the concentration-response curve to $CaCl_2$ (Figure 1, Table 1). The IC_{50} against $CaCl_2$ 10 mM and the corresponding relative potencies are shown in Table 2.

Experiments in normal trachea preparations

Spasm evoked by exposure to the depolarizing medium was dissipated by regular changes of the K^+ -rich, Ca^{2+} -free Tris solution in approximately 70 min. This established the incubation time in this medium for subsequent experiments. A cumulative concentration-effect curve for $CaCl_2$ was then constructed as described above, $E_{max} = 905.7 \pm 127.2$ mg; $pD_2 = 3.93 \pm 0.10$; these values are the mean of 72 experiments corresponding to the time-matched controls. Verapamil, trifluoperazine, Zn^{2+} and Cd^{2+} antagonized the effect of $CaCl_2$ in a concentration-dependent manner producing a right downward shift of the concentration-effect curve for $CaCl_2$ (Figure 2, Table 1). The IC_{50} against $CaCl_2$ 10 mM and the corresponding relative potencies are shown in Table 2.

Effects of Zn^{2+} and Cd^{2+} on tone

Zn^{2+} (0.01 to 1 mM) and Cd^{2+} (0.01 to 1 mM) were without effect on the tone of taenia caeci and trachealis in normal Tris solution but the higher concentrations were able to contract tissues bathed in a K^+ -rich, Ca^{2+} -free medium. This contraction was reversed by washing. Figure 3 shows the contraction elicited by a single concentration (1 mM) of Ca^{2+} , Zn^{2+} or Cd^{2+} in taenia caeci and trachea bathed in a K^+ -rich, Ca^{2+} -free medium. The characteristics of the Zn^{2+} and Cd^{2+} -induced contractions are different (greater latency and lesser initial velocity) from those of the contraction elicited by Ca^{2+} (Table 4).

Figure 1 The effect of verapamil, trifluoperazine, Zn^{2+} and Cd^{2+} on the response of guinea-pig taenia caeci (in K^+ -rich, Ca^{2+} -free, Tris solution) to CaCl_2 . The abscissae indicate concentration of CaCl_2 (mM) on a log scale. The ordinates indicate response as a % of the maximal response to CaCl_2 in the absence of antagonists. Points represent the means and vertical lines the s.e.mean; (●), time-matched control concentration-effect curve for CaCl_2 in the absence of antagonists ($n = 18$); (○, □, ▽), curves obtained after verapamil (a) (0.01, 0.1, 1 μM), trifluoperazine (b) (1, 10, 100 μM), Zn^{2+} (c) (0.01, 0.1, 1 mM) and Cd^{2+} (d) (0.01, 0.1, 1 mM) respectively ($n = 6$ for each concentration of the antagonist).

Figure 2 The effect of verapamil, trifluoperazine, Zn^{2+} and Cd^{2+} on the response of guinea-pig trachea (in K^+ -rich, Ca^{2+} -free, Tris solution) to CaCl_2 . The abscissae indicate concentration of CaCl_2 (mM) on a log scale. The ordinates indicate response as a % of the maximal response to CaCl_2 in the absence of antagonists. Points represent the means and vertical lines the s.e.mean; (●), time-matched control concentration-effect curve for CaCl_2 in the absence of antagonists ($n = 18$); (○, □, ▽), curves obtained after verapamil (a) (0.01, 0.1, 1 μM), trifluoperazine (b) (1, 10, 100 μM), Zn^{2+} (c) (0.01, 0.1, 1 mM) and Cd^{2+} (d) (0.01, 0.1, 1 mM) respectively ($n = 6$ for each concentration of the antagonist).

Table 1 Maximal effects (E_{max}) and EC_{50} values for $CaCl_2$ in the absence and presence of various concentrations of antagonists, in the guinea-pig taenia caeci and trachealis suspended in a Ca^{2+} -free, depolarizing solution

	<i>Taenia caeci</i>		<i>Trachealis</i>	
	E_{max} (g)	pD_2	E_{max} (g)	pD_2
<i>Verapamil</i>				
Control	5.01 ± 0.31	3.30 ± 0.05	0.937 ± 0.131	3.97 ± 0.08
$0.01 \mu M$	5.11 ± 0.29	$2.70 \pm 0.07^*$	0.834 ± 0.116	3.33 ± 0.10
$0.1 \mu M$	$2.55 \pm 0.51^*$	$2.30 \pm 0.06^*$	$0.618 \pm 0.098^*$	$2.39 \pm 0.12^*$
$1 \mu M$	$1.18 \pm 0.41^*$	$1.60 \pm 0.10^*$	$0.328 \pm 0.091^*$	$2.25 \pm 0.04^*$
<i>Trifluoperazine</i>				
Control	5.39 ± 0.36	3.25 ± 0.04	0.893 ± 0.099	4.09 ± 0.11
$1 \mu M$	5.55 ± 0.41	$2.74 \pm 0.11^*$	0.839 ± 0.092	$3.18 \pm 0.09^*$
$10 \mu M$	$3.21 \pm 0.47^*$	$2.90 \pm 0.13^*$	$0.645 \pm 0.086^*$	$2.56 \pm 0.14^*$
$100 \mu M$	$1.95 \pm 0.63^*$	$1.77 \pm 0.09^*$	$0.218 \pm 0.043^*$	$2.57 \pm 0.06^*$
Zn^{2+}				
Control	5.11 ± 0.39	3.24 ± 0.08	0.826 ± 0.123	3.80 ± 0.15
$0.01 mM$	5.23 ± 0.81	$2.35 \pm 0.09^*$	0.606 ± 0.197	3.31 ± 0.19
$0.1 mM$	$3.40 \pm 0.38^*$	$2.01 \pm 0.04^*$	$0.422 \pm 0.157^*$	$3.19 \pm 0.34^*$
$1 mM$	$1.46 \pm 0.34^*$	$1.91 \pm 0.11^*$	$0.220 \pm 0.080^*$	$2.87 \pm 0.32^*$
Cd^{2+}				
Control	5.52 ± 0.42	3.37 ± 0.07	1.006 ± 0.114	3.90 ± 0.90
$0.01 mM$	$3.49 \pm 0.59^*$	$2.52 \pm 0.06^*$	0.611 ± 0.202	3.35 ± 0.06
$0.1 mM$	$2.16 \pm 0.77^*$	$2.38 \pm 0.20^*$	$0.308 \pm 0.071^*$	$3.08 \pm 0.02^*$
$1 mM$	$0.95 \pm 0.28^*$	$1.43 \pm 0.11^*$	$0.195 \pm 0.041^*$	$2.31 \pm 0.21^*$

Data are mean $\pm$ s.e.mean of 18 experiments for time-matched controls and 6 experiments for each concentration of the antagonist in treated tissues. * $P < 0.05$ compared to control.

In addition, Zn^{2+} (1 mM)-induced contractions of taenia and trachea were completely inhibited (100% inhibition) by verapamil (10 μM).

Skinned taenia and trachea preparations

Control experiments showed that skinned taenia and trachea responded to an initial Ca^{2+} challenge (10 μM and 1 μM , respectively) by generating tension which reached a peak value (1.62–1.97 g, for taenia and 0.298–0.465 g for trachea) within 25 min. Responses to subsequent Ca^{2+} challenges became progressively smaller (about 15% for taenia and about 40% for trachea). Acetylcholine (100 μM) never produced contraction of the skinned preparations.

When test tissues were compared with their appropriate time-matched controls, it was evident that trifluoperazine (100 μM) depressed the maximal tension evoked by Ca^{2+} , whereas verapamil (100 μM) was ineffective (Figure 4). Segments of skinned taenia and trachea contracted in response to low concentrations of Zn^{2+} (10 μM) and Cd^{2+} (10 μM) (Figure 5). The magnitude of these contractions was similar to those elicited by Ca^{2+} in time-matched skinned tissues.

Discussion

The present experiments show that Zn^{2+} , which is without effect on the tone of taenia caeci and trachealis in normal Tris solution, causes a contraction of

Table 2 IC_{50} values of calcium blocking agents for responses to $CaCl_2$ (10 mM) of guinea-pig taenia and trachea maintained in K^+ -rich, Ca^{2+} -free, Tris solution

	<i>Verapamil</i>	<i>Trifluoperazine</i>	Zn^{2+}	Cd^{2+}
<i>IC₅₀ (–log molar concentration)</i>				
Taenia caeci	7.15 ± 0.12	4.93 ± 0.07	4.49 ± 0.48	4.60 ± 0.58
Trachealis	5.70 ± 0.32	4.37 ± 0.48	4.19 ± 0.82	4.10 ± 0.56
<i>Potency relative to verapamil</i>				
Taenia caeci	1	0.0060	0.0021	0.0028
Trachealis	1	0.0470	0.0300	0.0240

Figure 3 Original traces showing the contraction produced by a single concentration (1 mM) of Ca^{2+} , Zn^{2+} or Cd^{2+} when added to guinea-pig isolated taenia caeci (left panel) or trachealis (right panel) bathed in a K^{+} -rich, Ca^{2+} -free, Tris solution.

Table 3 Increase of resting tone produced by Zn^{2+} and Cd^{2+} in the guinea-pig taenia caeci and trachea bathed in K^{+} -rich, Ca^{2+} -free medium

	<i>Taenia caeci</i>	<i>Trachealis</i>
Zn^{2+}		
0.01 mM	NR	NR
0.1 mM	NR	0.045 ± 0.015
1 mM	1.8 ± 0.25	0.123 ± 0.024
Cd^{2+}		
0.01 mM	NR	NR
0.1 mM	NR	0.031 ± 0.019
1 mM	0.965 ± 0.17	0.101 ± 0.013

Numbers indicate the response in g. NR indicates the absence of response after drug addition. Data represents mean $\pm$ s.e.mean ($n = 9$)

Figure 4 The effect of verapamil (ii) and trifluoperazine (iii), on responses of skinned taenia caeci (a) and trachea (b) to Ca^{2+} (i). The abscissae indicate the first, second and third (1, 2, 3) challenge with $10 \mu\text{M}$ Ca^{2+} (taenia) or with $1 \mu\text{M}$ Ca^{2+} (trachea) and with $100 \mu\text{M}$ acetylcholine (ACh). The ordinates indicate response as a % of the first Ca^{2+} challenge. Solid columns indicate the presence of verapamil $100 \mu\text{M}$ and hatched columns the presence of trifluoperazine $100 \mu\text{M}$. Column heights represent the mean value of at least 6 experiments and vertical lines indicate the s.e.mean. There was a significant difference ($P < 0.05$) between time-matched control tissues and trifluoperazine treated tissues.

Table 4 Latency (L) and initial velocity (V) of the contraction produced by a single concentration (1 mM) of Ca^{2+} , Zn^{2+} or Cd^{2+} when added to guinea-pig isolated taenia caeci or trachealis bathed in a K^{+} -rich, Ca^{2+} -free, Tris solution

Agonist	<i>Taenia caeci</i>		<i>Trachealis</i>	
	L (s)	V (mg s^{-1})	L (s)	V (mg s^{-1})
Ca^{2+}	24.0 ± 1.8	30.00 ± 5.80	30.0 ± 2.3	2.89 ± 0.57
Zn^{2+}	72.0 ± 27.2	$9.40 \pm 1.69^*$	$132.0 \pm 49.1^*$	$0.59 \pm 0.12^*$
Cd^{2+}	$180.0 \pm 63.0^*$	$1.44 \pm 0.26^{*\dagger}$	$252.0 \pm 86.3^*$	$0.16 \pm 0.03^{*\dagger}$

* $P < 0.01$ compared to Ca^{2+} , $\dagger P < 0.05$ compared to Zn^{2+} . $n = 6$ for each group.

Figure 5 Responses to Zn^{2+} (i) and Cd^{2+} (ii) in skinned taenia caeci (a) and trachea (b). Open columns indicate responses to Ca^{2+} (1) ($10\text{ }\mu\text{M}$ for taenia or $1\text{ }\mu\text{M}$ for trachea). Lined columns indicate the response to Zn^{2+} $10\text{ }\mu\text{M}$ and stippled columns the response to Cd^{2+} $10\text{ }\mu\text{M}$. Acetylcholine (ACh) $100\text{ }\mu\text{M}$ produced no response. The ordinates indicate the magnitude of the response as a % of the Ca^{2+} -induced response. Column heights represent the mean values of at least 6 experiments and vertical lines indicate the s.e. mean.

these tissues when bathed in K^{+} -rich, Ca^{2+} -free medium or in skinned preparations. Zn^{2+} also produces an antagonism of the responses to CaCl_2 in normal (unskinned) but not in the skinned tissues. A similar profile of activity is found for Cd^{2+} in this study.

The effects of Zn^{2+} and Cd^{2+} on resting tone under the above experimental conditions may be explained by taking into account the structure and functional properties of Ca^{2+} channels (Hurwitz,

1986). Since Ca^{2+} has a higher affinity than Zn^{2+} or Cd^{2+} for the binding sites of the Ca^{2+} channel (Hagiwara & Byerly, 1981) it is understandable that this channel is readily permeable for these cations only in the absence of extracellular Ca^{2+} , thus permitting Zn^{2+} and Cd^{2+} to enter the cell and express their ability to activate the contractile machinery which is also unveiled when applied to skinned preparations. The conclusion that Zn^{2+} can enter the cell to cause contraction is reinforced by the finding that verapamil blocked such a contraction elicited in a K^{+} -rich, Ca^{2+} -free, Tris solution.

Zn^{2+} , as well as Cd^{2+} , verapamil and trifluoperazine, produced in this study a concentration-related, right downward displacement of the concentration-response curve to CaCl_2 in normal taenia and trachea bathed in a Ca^{2+} -free depolarizing solution as previously reported in other preparations under similar experimental conditions (Tand *et al.*, 1970; Hayashi & Toda, 1977; Spedding, 1983; Foster *et al.*, 1984; Cortijo *et al.*, 1987a; Sanz *et al.*, 1988).

The Zn^{2+} - Ca^{2+} antagonism 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. As a matter of fact, Zn^{2+} inhibition of calmodulin has been proposed (Brewer *et al.*, 1979; Brewer, 1980) as the molecular mechanism of Zn^{2+} action. However we have demonstrated in skinned taenia and trachea that low concentrations ($10\text{ }\mu\text{M}$) of Zn^{2+} and Cd^{2+} are able to produce contractions *per se*. As expected, in the skinned preparations, only trifluoperazine depresses the Ca^{2+} -induced contraction whereas verapamil is ineffective, thus confirming the idea that verapamil works mainly in the cell membrane while trifluoperazine is capable of interfering with the contractile machinery (Cortijo *et al.*, 1987a).

Therefore the antagonism of Ca^{2+} produced by Zn^{2+} and other cations such as Cd^{2+} appears to be exerted at the level of the plasma membrane. These cations have reasonably high affinity for the binding sites of the Ca^{2+} channel but traverse it at low or even negligible speeds (Hagiwara & Byerly, 1981) so they behave as potent competitive inhibitors of Ca^{2+} influx through Ca^{2+} channels.

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