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AN ANALYSIS OF THE ACTION OF CATIONS OF THE LANTHANIDE SERIES ON THE MECHANICAL RESPONSES OF GUINEA-PIG ILEAL LONGITUDINAL MUSCLE

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SUMMARY

1. The inhibitory effects of lanthanide cations (Ln^{3+}) on mechanical responses and ^{45}Ca uptake in guinea-pig ileal longitudinal smooth muscle were studied.

2. Ln^{3+} strongly inhibited the phasic and tonic component of the response to the muscarinic agonist cis-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (CD) the two components being affected to the same extent. Inhibition was also obtained for the responses evoked by high K^+ but here the effect was mainly on the phasic response, the tonic component was merely delayed.

3. Other members of the Ln^{3+} series, with the exception of cerium, were found to be more effective than lanthanum in their ability to inhibit the CD response. Thulium, Tm^{3+}, the thirteenth member of the series was the most effective cation.

4. Analysis of ^{170}Tm uptake revealed at least two components. The concentration–dependence of one component, saturating at $2{\cdot}5 \times 10^{-6}$ Tm, corresponded closely to that of the inhibitory effect of Tm^{3+} on contraction.

5. ^{170}Tm uptake as a function of time showed a secondary rise after 30 min of exposure to the lanthanide.

6. Although $2{\cdot}5 \times 10^{-6}$ M-Tm^{3+} produced 90% inhibition of the CD and the high K^+ induced responses significant reduction of ^{45}Ca uptake by the muscle was only detected when much higher Tm^{3+} concentrations ($\geqslant 10^{-3}$ M-Tm^{3+}) were used.

7. It is concluded that Ln^{3+} combine with membrane sites specifically involved in Ca^{2+} translocation during excitation–contraction coupling.

INTRODUCTION

The mechanical responses of smooth muscle are thought to be controlled by both extra- and intracellular Ca^{2+} stores, the relative use of which depends upon the tissue, the stimulant and the ionic environment (Bohr, 1964; Daniel, 1964; Hurwitz & Suria, 1971; Triggle, 1971, 1972). The problems associated with attempts to correlate smooth muscle activity with transmembrane Ca^{2+} fluxes arise, in part, from the large amount of exchangeable Ca^{2+} compared with the small quantity actually needed to sustain mechanical response (Lüllmann, 1970; van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973).

Lanthanum, La^{3+}, is known to inhibit Ca^{2+} fluxes across artificial and biological membranes (van Breemen, 1968, 1969; Weiss & Goodman, 1969; van Breemen & de Weer, 1970; Langer & Frank, 1972; van Breemen *et al.* 1973) and to displace Ca^{2+} bound to membranes (Mayer, van Breemen & Casteels, 1972; van Breemen *et al.* 1973). Because of these properties La^{3+} can inhibit many Ca^{2+}-utilizing processes (Weiss, 1974). Van Breemen (van Breemen, Farinas, Gerba & McNaughton, 1972; van Breemen *et al.* 1973) has proposed that the ability of La^{3+} to displace extracellular Ca^{2+} and inhibit Ca^{2+} fluxes will permit the determination of changes in cellular Ca^{2+} associated with mechanical response. Use of this 'lanthanum method' has led to the conclusion that in rabbit aorta K^+ contractions are associated with a much larger ^{45}Ca uptake than are angiotensin contractions (van Breemen *et al.* 1973) and that in guinea-pig taenia coli a large influx of ^{45}Ca accompanies K^+ contractions (Mayer *et al.* 1972).

The responses of the guinea-pig ileal longitudinal smooth muscle to muscarinic agonists and to high K^+ concentration are rapidly lost in Ca^{2+}-free media and are very dependent upon the extracellular Ca^{2+} concentration (Chang & Triggle, 1973) suggesting an important role for extracellular Ca^{2+} in the excitation–contraction coupling process. La^{3+} is known to be a powerful inhibitor of guinea-pig intestinal smooth muscle contraction (Goodman & Weiss, 1971; Chang & Triggle, 1973) and in the present study it and other cations of the lanthanide series (Ln^{3+}) have been used to analyse the role of extracellular Ca^{2+} in the contractile process.

METHODS

Tissue preparation and incubation. Male white guinea-pigs weighing 300–500 g were decapitated and about 20 cm of the terminal portion of the ileum was removed and placed in aerated Tris-buffered Tyrode solution at 37° C. The ileal longitudinal muscle was separated from the underlying circular muscle by a method essentially the same as that described by Rang (1964). The incubation media employed was a Tris-buffered Tyrode solution of the following composition (mM): NaCl, 125; KCl,

2·55; $CaCl_2$, 1·78; dextrose, 5·56; tris-(hydroxymethyl)aminomethane (Tris), 23·85. This solution was adjusted to pH 7·45 with 4 N-HCl and aerated with air (CO_2-free).

Recording of isotonic contractions. Muscle segments of approximately 2 cm length were suspended in jacketed glass baths of 10 ml. capacity containing Tris-Tyrode maintained at 37° C and one end of the muscle was fixed while the other end was connected to a light lever (magnification 9 : 1, tension 300 mg). Mechanical responses were recorded on smoked paper. Tissues were equilibrated for 30 min and the Tris-Tyrode changed every 10 min. Following equilibration a cumulative concentration–response curve was produced to either cis-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (CD) or K^+ and the occasional insensitive tissue was discarded. Following incubation for a further 60 min two control responses were determined at 60 min intervals to supramaximal concentrations of agonist, $5{\cdot}0 \times 10^{-7}$ M CD or 80 mM-K^+: the mean of these two responses was taken to represent the 100 % values of the phasic and tonic components of the response. Tissues in which the effects of Ln^{3+} were studied were used only once and then discarded.

^{170}Tm uptake determinations. Tissues, prepared as described, were incubated in 10 ml. Tris-Tyrode for 60 min and then transferred to vials containing varying concentrations of ^{170}Tm, thulium, for 5 min or to vials containing a single concentration of ^{170}Tm for varying periods of time. ^{170}Tm solutions were made from stock solutions of 5×10^{-4} and 5×10^{-2} M containing a theoretical level of 0·0284 mc/ml. which were diluted with Tris-Tyrode as required. Tissues were then washed for a total period of 5 min by transferring to vials containing 10 ml. Tris-Tyrode for 4·5 and 0·5 min, blotted gently with tissue paper, dried to constant wt. at 30–35°, weighed and solubilized overnight at 37° C in a medium of the following composition: 1·5 ml. 50 : 50 mixture of propanol/Packard Soluene plus 0·5 ml. 30 vol. H_2O_2. The solubilized material was then suspended in a mixture of 3 ml. H_2O and 10 ml. Packard Instagel and counted in a scintillation counter. Samples were corrected for background and tissue ^{170}Tm levels were determined by the use of external thulium standards prepared in a manner identical to that described for the tissues.

^{45}Ca uptake determinations. Tissues were pre-incubated for 1 hr in Tris-Tyrode and then transferred to new medium containing ^{45}Ca (0·5 μc/ml.), with or without CD or K^+, for times corresponding to the peak phasic and tonic components of the agonist response. In experiments designed to study the effect of Tm^{3+} on ^{45}Ca uptake, before the transfer to the ^{45}Ca Tris-Tyrode containing varying Tm^{3+} concentrations, the tissues were incubated for 5 min in Tris-Tyrode containing the same concentration of Tm^{3+}. The uptake of ^{45}Ca into intracellular compartments was determined by a modification of the method developed by van Breemen *et al.* (1973): after response in the ^{45}Ca or ^{45}Ca/Tm^{3+} Tris-Tyrode media, tissues were incubated for 3 min in ^{45}Ca Tris-Tyrode of the same specific activity containing 10 mM-La^{3+} and for a further 50 min in a Ca^{2+}-free Tris-Tyrode containing 10 mM-La^{3+}. Tissues were then blotted, dried, solubilized and counted as previously described.

RESULTS

Time courses of CD and K^+ responses

Time courses of CD ($5{\cdot}0 \times 10^{-7}$ M) and K^+ (80 mM) responses were compared, these concentrations being chosen because they produce maximum responses. The biphasic response to CD (Fig. 1*A*) consists of an initial rapid phasic response followed by partial relaxation and a more slowly developing tonic response. The contractile response to 80 mM-K^+

(Fig. 1 *B*) was similar to the response produced by CD but could readily be distinguished by its different time course. The phasic component of the CD response required $26 \pm 1{\cdot}6$ (S.E. of mean) sec ($n = 20$) to reach its peak and this was significantly longer ($P < 0{\cdot}05$) than that for K^+ ($8{\cdot}8 \pm 0{\cdot}3$ sec). Similarly, the tonic component of the CD response did not begin until 430 ± 21 sec had elapsed from the time of CD addition, whereas the corresponding component of the K^+ response was already started after $37{\cdot}5 \pm 5{\cdot}3$ sec. However, the time for both tonic components to reach equilibrium was not significantly different ($P < 0{\cdot}05$) for the CD (971 ± 75 sec) or for the K^+ (1070 ± 42 sec) responses.

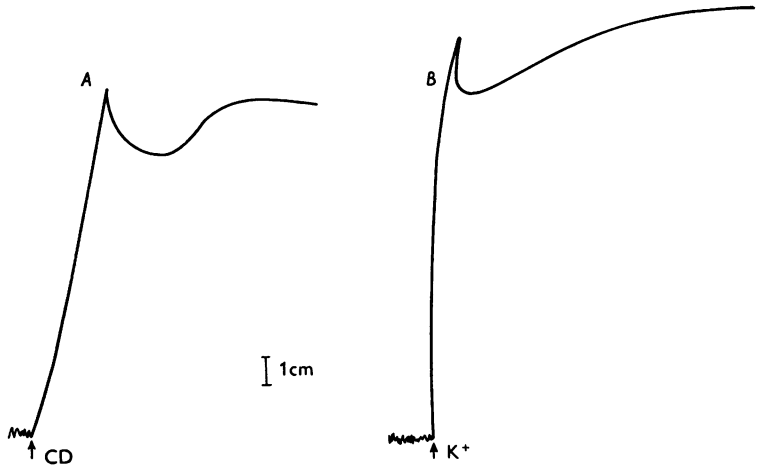

Fig. 1. Isotonic responses (tracings of kymograph records) of guinea-pig ileal longitudinal muscle to: *A*, 5×10^{-7} M, CD and *B*, 80 mM-K^+.

Effects of lanthanides and other cations on contractile responses

The resting tone and spontaneous activity of the ileal longitudinal muscle were both reduced by Ln^{3+} concentrations (Fig. 4) which inhibited the responses produced by CD and K^+. The concentration–response curves for La^{3+}, Tm^{3+} and cerium, Ce^{3+}, inhibition of the responses produced by 5×10^{-7} M CD are shown in Fig. 2. Table 1 shows the ED_{50}s, determined, from similar concentration–response curves, for La^{3+} and Tm^{3+} and Fig. 3 those for all the lanthanides studied (except Ce^{3+}). A standard 5 min incubation period was used since preliminary experiments with La^{3+} and Tm^{3+} indicated that inhibitory activity had reached its maximum at this time. The data in Fig. 3 show that although there is little difference in the inhibitory effect between neighbouring lanthanides there is a general trend of increasing inhibition with decreasing ionic radius, the maximum effect being reached with thulium which is approximately six to ten times more

active than lanthanum. Beyond thulium in this series there is a reversal of this trend as ytterbium and lutetium are less effective inhibitors than thulium. At the lower lanthanide concentrations the phasic component of response is slightly more sensitive than the tonic component but this small differential sensitivity is lost with increasing lanthanide concentration and complete inhibition of both components is achieved with the same

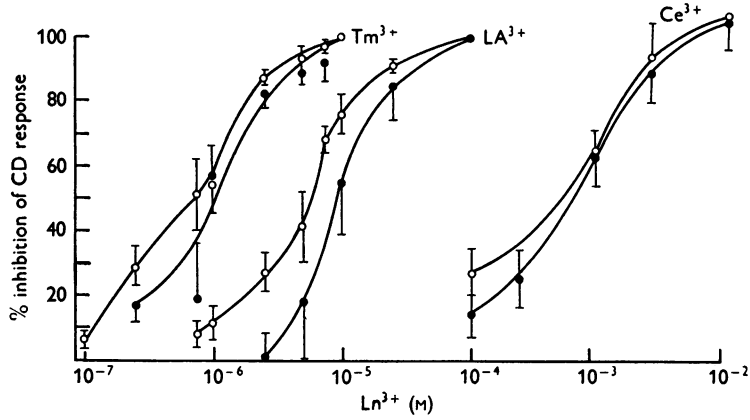

FIG. 2. Dose–response curves for La^{3+}, Tm^{3+} and Ce^{3+} inhibition of phasic (○) and tonic (●) components of response to CD (5×10^{-7} M) of guinea-pig ileal longitudinal muscle.

TABLE 1. Inhibitory ED_{50} concentrations of La^{3+} and Tm^{3+} on the CD and K^+ induced phasic components of response

	ED_{50}, M CD ($5{\cdot}0 \times 10^{-7}$ M)	ED_{50}, M K^+ (80 mM)
La^{3+}	$4{\cdot}7 \times 10^{-6}$ (2·2–9·9)	$4{\cdot}5 \times 10^{-6}$ (2·4–9·6)
Tm^{3+}	$8{\cdot}0 \times 10^{-7}$ (0·37–1·8)	$9{\cdot}5 \times 10^{-7}$ (0·33–1·6)

95 % confidence limits in parentheses.

lanthanide concentration (Fig. 2). The inhibitory effects of these low concentrations of Ln^{3+} were reversible on washing, the $t_{\frac{1}{2}}$ for recovery from Tm^{3+} being $45 \pm 4{\cdot}4$ min ($n = 5$). However, with increasing concentration and time of incubation the reversibility was progressively lost.

Thorium (Th^{4+}) was also examined and shown to be very ineffective, a concentration of 2×10^{-4} producing only $8{\cdot}3 \pm 2\%$ ($n = 4$) inhibition of the tonic component and leaving unaltered the phasic component of the CD response. Similarly, with the uranyl cation, UO_2^{2+}, complete inhibition of the phasic and tonic components of response was attained only at the high concentration of 10^{-2} M. On the other hand, ruthenium red ($(NH_3)_5$

$Ru\text{-}O\text{-}Ru(NH_3)_4\text{-}O\text{-}Ru\text{-}(NH_3)_5)^{6+}$ was significantly less active than La^{3+} in inhibiting CD responses, and at 10^{-4} M produced $26 \pm 2{\cdot}5$ and $62 \pm 22\%$ ($n = 6$) inhibition of the phasic and tonic components respectively.

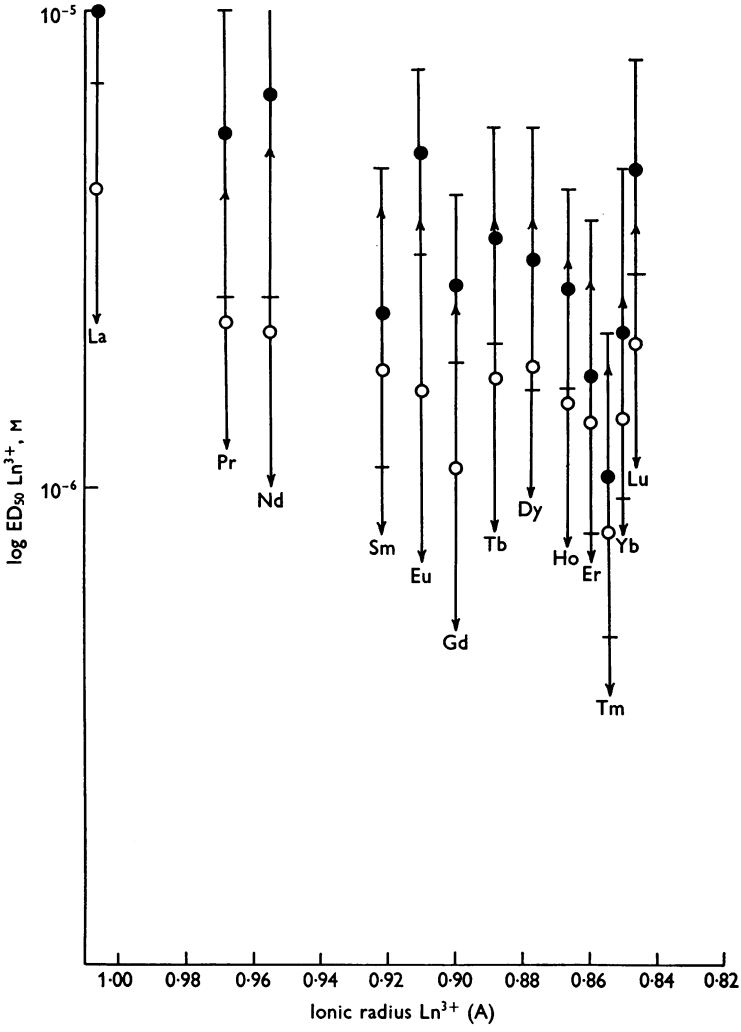

Fig. 3. Dependence of inhibition of CD response upon Ln^{3+} ionic radius. ED_{50}s of Ln^{3+} for inhibition of phasic (○) or tonic (●) components of response are shown including the 95% confidence limits (↔, phasic; ⊢, tonic).

Thulium uptake

The results with a 5 min incubation period, chosen to correspond to the exposure times to Ln^{3+} used in one of the series of pharmacological experiments, are shown in Fig. 5. ^{170}Tm binding is very sensitive to tissue damage and deliberate stretching of the tissue before exposure to ^{170}Tm increased tissue uptake. Thus, after 5 min incubation with 10^{-6} M ^{170}Tm the control tissue contained $61{\cdot}5 \pm 5{\cdot}3$ p-mole Tm/mg tissue (dry wt.) but stretching of the tissue, which irreversibly abolished tissue sensitivity to

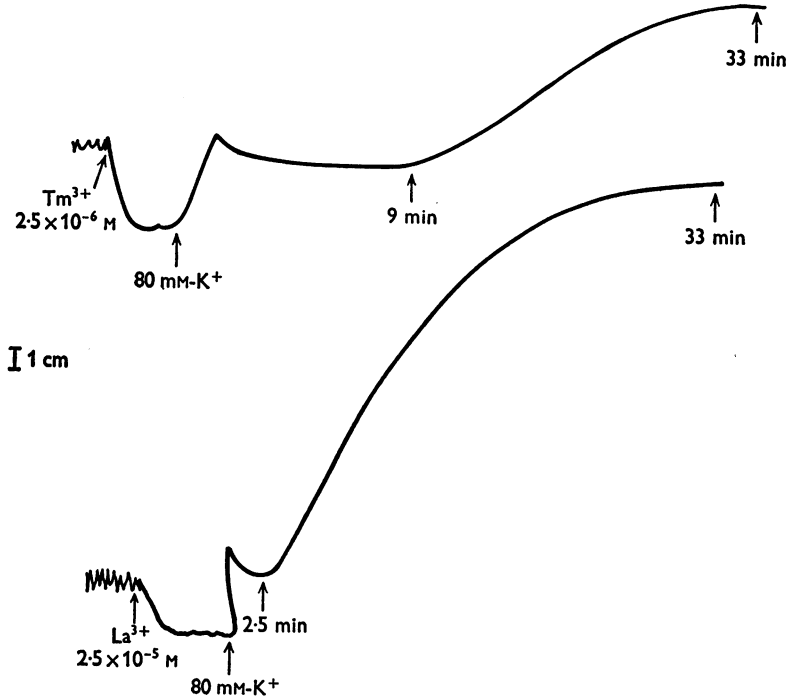

Fig. 4. Effects of Tm^{3+} ($2{\cdot}5 \times 10^{-6}$ M) and La^{3+} ($2{\cdot}5 \times 10^{-5}$ M) upon responses of guinea-pig ileal longitudinal muscle to 80 mM-K^+.

CD or K^+, increased ($P < 0{\cdot}05$) this to $305{\cdot}5 \pm 65{\cdot}8$ ($n = 6$) p-mole Tm/mg tissue (dry wt.). A more detailed examination of ^{170}Tm uptake (in undamaged preparations) reveals a two component system (Fig. 5): a high affinity low capacity site ($K \sim 0{\cdot}7 \times 10^{-6}$ M, capacity $= 77{\cdot}4 \pm 21{\cdot}0$ p-mole Tm^{3+}/mg tissue dry wt.) saturating at $2{\cdot}5 \times 10^{-6}$ M Tm^{3+} and a second high capacity site ($\sim$ 5000 p-mole/mg tissue dry wt.) showing apparent saturation at $\sim 10^{-4}$ M-Tm^{3+}. A significant increase in Tm^{3+} uptake from $77{\cdot}4 \pm 13{\cdot}0$ to $134{\cdot}0 \pm 10{\cdot}0$ p-mole/mg tissue ($n = 10$, $P < 0{\cdot}05$) was observed when tissue was exposed to Ca^{2+}-free Tris-Tyrode

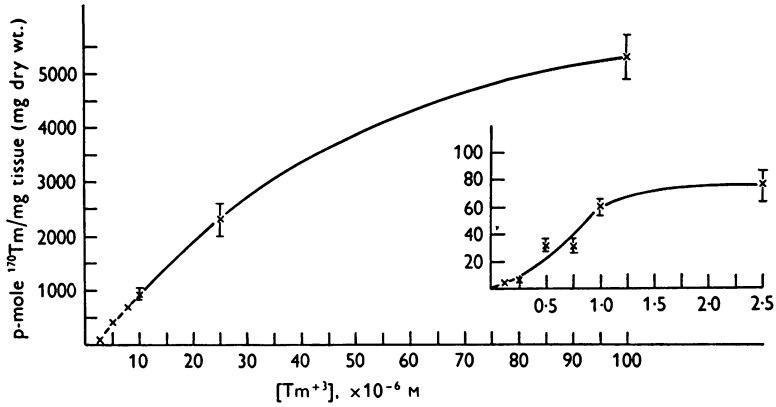

Fig. 5. Uptake of ^{170}Tm by guinea-pig ileal longitudinal muscle. A 5 min incubation period with ^{170}Tm was used. The inset shows the lower range of the uptake curve.

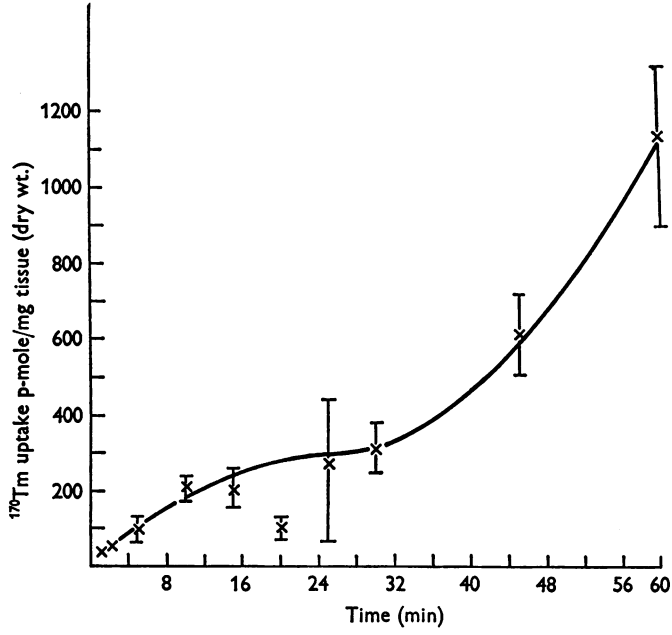

Fig. 6. Uptake of ^{170}Tm ($2{\cdot}5 \times 10^{-6}$ M) in guinea-pig ileal longitudinal muscle at various times of exposure to the tracer.

for 60 min before and during a 5 min period of exposure to $2{\cdot}5 \times 10^{-6}$ M-Tm^{3+}. Further experiments using the Tm^{3+} concentration of $2{\cdot}5 \times 10^{-6}$ M for varying incubation times revealed an initial phase of Tm^{3+} uptake which appeared to attain a plateau after about 30 min (Fig. 6) but was followed by a later phase of progressive uptake.

Lanthanum in ^{45}Ca uptake studies

The effects of Tm^{3+} on ^{45}Ca uptake in the presence of 5×10^{-7} M CD or 80 mM-K^+ were determined by the method of van Breemen (van Breemen *et al.* 1972, 1973) in which a high La^{3+} concentration is used to displace extracellular Ca^{2+} in estimating ^{45}Ca uptake by the cells. Tissues were incubated in ^{45}Ca media containing appropriate Tm^{3+} concentrations and were then post-incubated in a media containing 10 mM-La^{3+}. Figs. 7 and 8 show the ^{45}Ca uptake measured at times corresponding to the peak phasic and tonic components of the CD and high K^+ responses. The values for ^{45}Ca uptake during the tonic component have been corrected for ^{45}Ca uptake occurring during the phasic component (^{45}Ca $uptake_{tonic}$ = ^{45}Ca $uptake_{total} - {}^{45}Ca$ $uptake_{phasic}$). Figs. 7 and 8 show that there is a significant increase of ^{45}Ca uptake during both components of the K^+ response and with the phasic component of the CD response but that there is no observed increase of ^{45}Ca uptake during the tonic component of the CD response. During the phasic and tonic components of the K^+ response the increases in ^{45}Ca uptake were 0·15 and 0·39 m-mole Ca^{2+}/kg tissue wet wt. respectively and during the phasic component of the CD response an increased uptake of 0·34 m-mole Ca^{2+}/kg wet wt. was observed. At concentrations of 10^{-6}–10^{-4} M, Tm^{3+} did not decrease Ca^{2+} uptake during the CD or K^+ responses, but reduction of uptake to control levels or below was observed with 10^{-3} M-Tm^{3+}.

DISCUSSION

The present study of fourteen of the fifteen lanthanide cations demonstrates their high activity in antagonizing CD- and K^+-induced contractions of the guinea-pig ileal longitudinal smooth muscle. The dependence of activity upon ionic radius, although not very marked, reaches a maximum at Tm^{3+} and this type of sequence resembles that for lanthanide complexation with carboxylate ligands (Moeller, Martin, Thompson, Ferrus, Feistel & Randall, 1965) and is consistent with cation binding to a high field strength anionic site (Eisenman, 1965; Diamond & Wright, 1969). The response to Ce^{3+} and to the non-lanthanide Th^{4+}, both of which are approximately 1000-fold less active than Tm^{3+}, is puzzling since these ions have ionic radii very similar to that of Ca^{2+} (Ce^{3+}, 0·99 Å; Th^{4+},

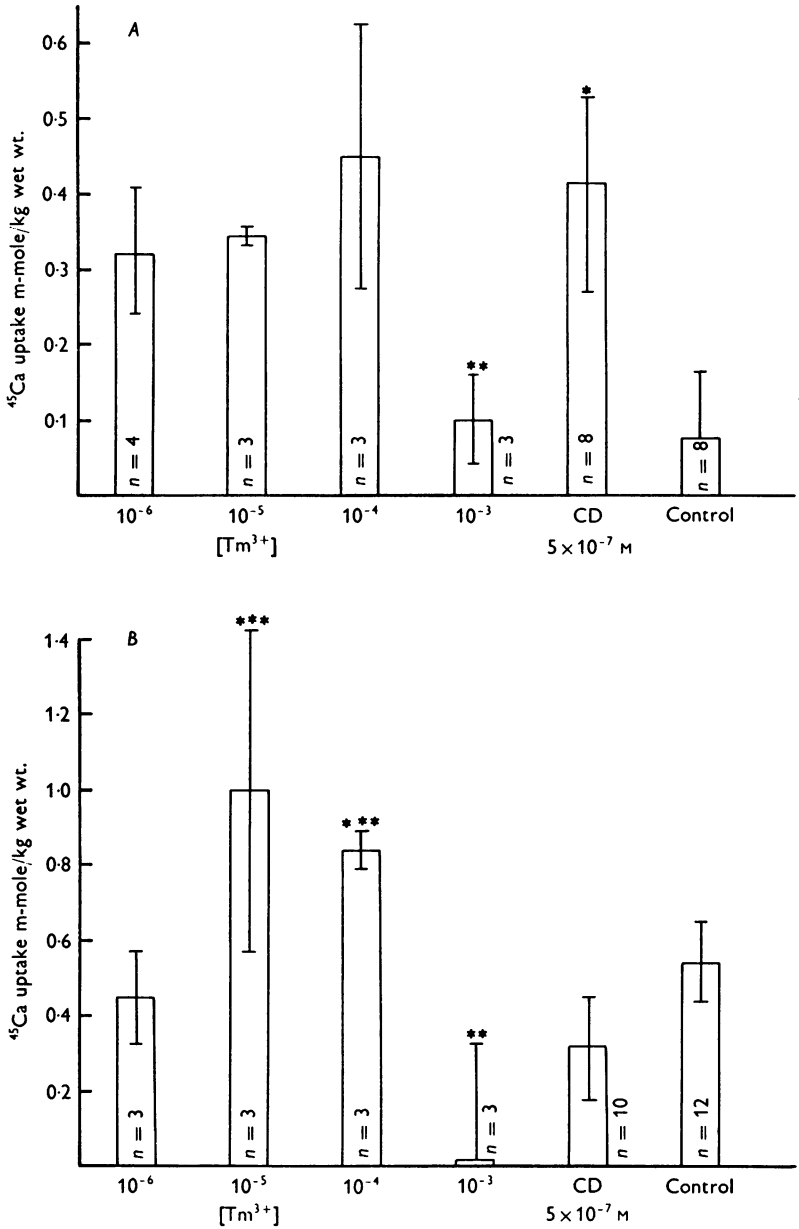

Fig. 7. The uptake of ^{45}Ca and its modification by CD and Tm^{3+} in ileal longitudinal muscle as determined by the lanthanum method. Ordinate: tissue ^{45}Ca content in m-mole/kg wet weight. The individual columns represent (from right to left), control $^{45}Ca^{2+}$ uptake, uptake in the response to 5×10^{-7} M CD either in the absence, or in the presence of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M-Tm^{3+}. *A*, uptake after 30 sec; *B*, uptake after 30 min. *, significant increase in ^{45}Ca uptake from control; **, significant decrease from CD-treated tissues ***, significant increase above CD-treated tissue.

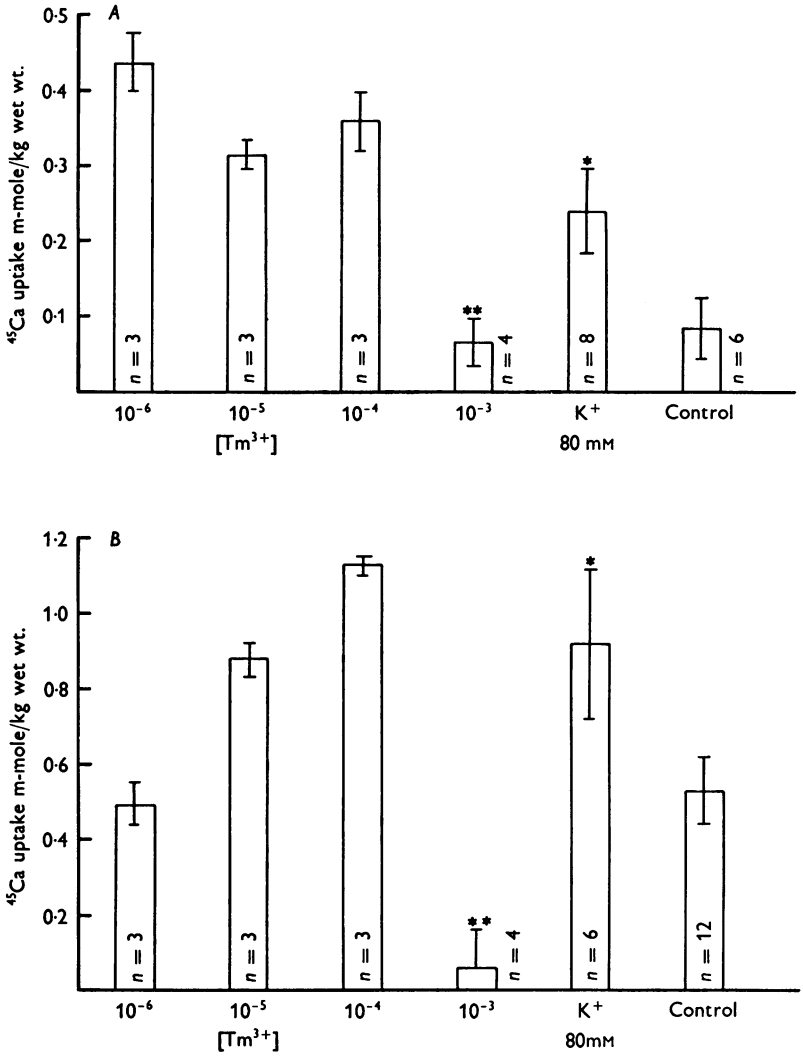

Fig. 8. The uptake of ^{45}Ca and its modification by high (80 mM) K^+ and various concentrations of Tm^{3+} determined by the lanthanum method. Presentation of results analogous to that in Fig. 7. ^{45}Ca uptake was measured at 15 sec (*A*) and 30 min (*B*).

0·98 Å; Ca^{2+}, 0·99 Å; Bagnall, 1972). Possibly, the formation of the tetravalent CeO^{2+} and ThO^{3+} species in aqueous solution (Cotton & Wilkinson, 1972) may underlie this lack of activity. The inactivity of UO_2^{2+}, which has high affinity for phosphate groups in membranes and phospholipids (Bungenberg de Jong, 1952; Rothstein & Meier, 1951;

Barton, 1968), would also be consistent with the view that Ln^{3+} binding occurs at carboxylate ligands. These sites are probably not glycoproteins for ruthenium red, which has high affinity for glycoproteins (Luft, 1965; Gustafson & Pihl, 1967) and is equipotent with La^{3+} in inhibiting both Ca^{2+} uptake in mitochondria (Lehninger & Carafoli, 1971; Moore, 1971) and Ca^{2+}-binding to a mitochondrial glycoprotein (Sottocasa, Sandri, Panfili, de Bernard, Gazzotti, Vasington & Carafoli, 1972), is 100-fold less active than La^{3+} in inhibiting CD responses in the ileal muscle.

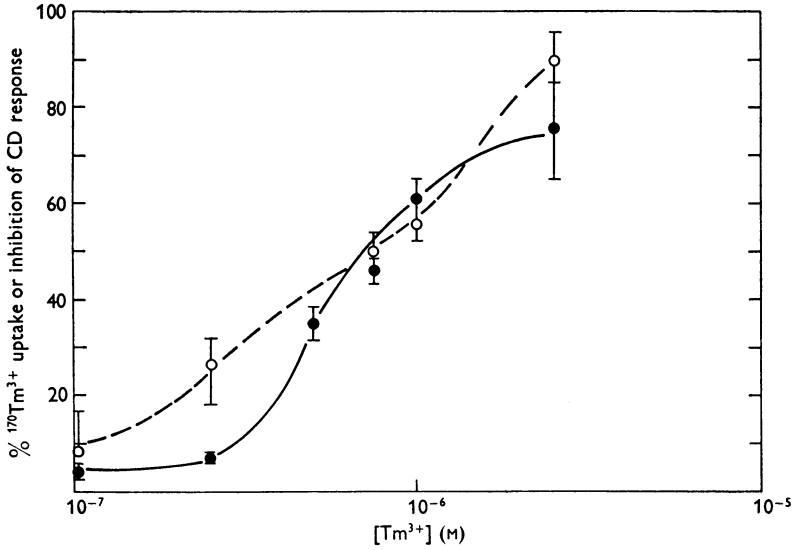

Fig. 9. Comparison of ^{170}Tm uptake, —●—, with Tm^{3+} inhibition, – – ○ – –, of phasic component of CD (5×10^{-7} M) response in guinea-pig ileal longitudinal muscle. Binding and inhibition are both expressed in relative (0–100 %) units.

The uptake of ^{170}Tm shows both concentration and time-dependent components. The concentration-dependence of the high affinity low capacity component is similar to that with which Tm^{3+} inhibits the CD response (Fig. 9) suggesting a correlation between this component of thulium uptake and the inhibitory effect. On the other hand, the rapid onset and ready reversibility of the inhibition point to superficial membrane sites being involved in this process. Since there are in ileal longitudinal muscle 6×10^8 cells/g tissue (Paton & Rang, 1965) and the surface area is approximately $0{\cdot}8 \times 10^{-5}$ cm^2/cell (Goodford, 1967) the density of these high affinity sites is $2{\cdot}45 \times 10^4/\mu m^2$ or one site per 4000 Å^2. Apparently, the activity of the Ln^{3+} in this system is mediated through the occupancy of only a small fraction of the total membrane surface by these

cations. This is consistent with the idea that Ln^{3+} displace superficially bound Ca^{2+} of critical importance in excitation–contraction coupling or, alternatively, that Ca^{2+} channels in the membrane are blocked. The latter suggestion is favoured since the CD responses are also very sensitive to the agents verapamil and D-600 (R. Ticku & D. J. Triggle, unpublished) which serve as organic Ca^{2+}-channel antagonists (Fleckenstein, 1972; Kohlhardt, Bauer, Krause & Fleckenstein, 1973). This high sensitivity to La^{3+} and the organic Ca^{2+} antagonists is shared by cardiac muscle where excitation–contraction coupling is also critically dependent upon the availability of superficially bound Ca^{2+} (Langer & Frank, 1972; Langer, Serena & Nudd, 1974) and Ca^{2+} translocation through specific Ca^{2+} channels (Kohlhardt *et al.* 1973).

The other components of ^{170}Tm uptake may represent binding to membrane sites of lower affinity or reduced accessibility, but the increasing irreversibility of inhibition found with increasing Ln^{3+} concentration or time of incubation is also consistent with intracellular uptake. Although it is generally assumed that La^{3+} does not enter cells the evidence is not conclusive (Weiss, 1974) and La^{3+} can certainly enter damaged cells (Bannister, 1972; Bloom, Brady & Langer, 1974) and mitochondria (Reed & Bygrave, 1974), and the amount of La^{3+} uptake in uterine tissue is too great to be accommodated at the cell surface (Hodgson, Kidwai & Daniel, 1972). The delayed K^+ contraction seen in the presence of La^{3+} or Tm^{3+} may arise from the Ca^{2+} displacing activity of a slow intracellular accumulation of Ln^{3+} and Mayer *et al.* (1972) have shown that La^{3+} induces in guinea-pig taenia coli a similar delayed contraction and increase in membrane permeability.

A significant increase in ^{45}Ca uptake occurs during the phasic components of the K^+ and CD responses and during the tonic component of the K^+ response. Mayer *et al.* (1972) using the same technique with the guinea-pig taenia coli have also reported an increase in ^{45}Ca uptake during the K^+ response. The lack of additional ^{45}Ca uptake during the CD tonic component appears inconsistent with the high dependence of this component upon extracellular Ca^{2+} and its sensitivity to inhibition by Ln^{3+}. However, the situation may be complicated by the processes underlying the prolonged period of 'fade' of phasic component of the CD response, during which Ca^{2+} may be extruded from the cell.

The ability of low Ln^{3+} concentrations to inhibit mechanical responses without apparent inhibition of Ca^{2+} movements suggests that the Ca^{2+} uptake measured by the lanthanum method may over-estimate the amount of extracellular Ca^{2+} used in the contraction and that the Ca^{2+} influx specifically associated with mechanical response is not readily measurable. Much of the Ca^{2+} which enters the cell may not be available to the

contractile machinery or is rapidly converted from a free to a bound form. Freeman & Daniel (1973) have also provided evidence indicating the difficulty of measuring the Ca^{2+} uptake associated with contraction by the lanthanum method. It is also possible that the apparent inability of low Ln^{3+} concentrations to inhibit Ca^{2+} entry results from a compensation of an actual inhibitory effect on agonist induced Ca^{2+} influx by a non-specific promotion of Ca^{2+} entry, the latter component being reduced with increasing Ln^{3+} concentrations. Mayer *et al.* (1972) have provided evidence for such a promotion of Ca^{2+} influx by low La^{3+} concentrations in the guinea-pig taenia coli. Finally, it cannot be ignored that the lanthanide cations may have actions quite distinct from membrane Ca^{2+} displacement and that their possible intracellular effects on Ca^{2+} dependent processes must be more seriously considered.

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