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THE EFFECTS OF LANTHANUM AND THULIUM ON THE MECHANICAL RESPONSES OF RAT VAS DEFERENS

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SUMMARY

1. The contractile responses of rat vas deferens to noradrenaline and K^+ are composed of phasic and tonic components both of which are dependent upon the concentration of extracellular Ca^{2+}.

2. Lanthanum, La^{3+}, and thulium ions, Tm^{3+}, inhibited the noradrenaline and K^+ induced responses, complete inhibition being obtained at approximately 10^{-3} M-Ln^{3+}.

3. La^{3+} and Tm^{3+} were equally effective in inhibiting noradrenaline and K^+ responses. The phasic and tonic components of the noradrenaline response were equally sensitive to lanthanide cations, Ln^{3+}, but the phasic component of the K^+ response was more sensitive than the tonic component.

4. ^{170}Tm binding did not show any saturable component over the concentration range in which inhibition of the pharmacological response was obtained.

5. It is suggested that the actions of Ln^{3+} in the rat vas deferens are mediated through some kind of membrane stabilization rather than via a specific Ca^{2+} binding site concerned with excitation–contraction coupling, the mechanism previously postulated for the Ln^{3+} action in guinea-pig ileal longitudinal muscle.

INTRODUCTION

Our understanding of the sources of calcium involved, and the role played by this ion, in excitation–contraction coupling in smooth muscle is far from complete (Daniel, 1964; Hurwitz & Suria, 1971; van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973). The lanthanide cations, Ln^{3+}, appear to serve as substitutes or antagonists for Ca^{2+} in a variety of cellular and subcellular processes (Weiss, 1974) and a comparative analysis

of their actions in smooth muscles may be valuable in analysing differences in Ca^{2+} dependence and utilization.

As has previously been shown, the contractile responses of guinea-pig ileal longitudinal smooth muscle to increased K^+ concentration and to muscarinic agonists are highly dependent upon extracellular Ca^{2+} and are very sensitive to the lanthanides (Chang & Triggle, 1973; Triggle & Triggle, 1975). In the present paper the effects of Ca^{2+}, lanthanum, La^{3+}, and thulium, Tm^{3+}, have been studied in the rat vas deferens on the responses induced either by high K^+ concentration or by application of noradrenaline, *NA*.

METHODS

Tissue preparation and incubation. Male Wistar rats weighing 120–150 g were killed by a blow to the head, the vasa deferentia removed, cleaned of mesenteric investment and mounted under a resting tension of 350 mg in 10 ml. jacketed organ-baths and incubated at 37° C in aerated Tyrode solution of the following composition (mM): NaCl, 137; KCl, 2·86; $MgCl_2$, 1·05; $CaCl_2$, 1·8; NaH_2PO_4, 0·31; $NaHCO_3$, 11·9; dextrose 5·5. Isotonic contractions to noradrenaline or K^+ were recorded with a muscle transducer (Harvard Model 386). The effects of La^{3+} and Tm^{3+} on noradrenaline and K^+ responses were measured in a Tris-Tyrode solution of the following composition (mM): NaCl, 137; KCl, 2·86; $MgCl_2$, 1·05; $CaCl_2$, 1·8; dextrose 5·5; tris(hydroxymethyl)aminomethane (Tris) 10·0; adjusted to pH 7·45 with 4 N-HCl.

^{170}Tm uptake. The uptake of ^{170}Tm in rat vas deferens was determined as described for the guinea-pig ileal longitudinal smooth muscle (Triggle & Triggle, 1975).

RESULTS

Effects of lanthanum and thulium on contractile responses

Noradrenaline produces a biphasic response consisting of an initial rapid phasic and a slower sustained tonic component (Fig. 1). Quite similar responses were obtained in fluids of high K^+ concentration (Fig. 2) save that the tonic component was more pronounced in this case. Noradrenaline was used at a concentration of 10^{-4} M since this concentration (determined from dose–response curves) gave a well defined maximum response with clearly separate phasic and tonic components. K^+ was used at a sub-maximum concentration of 40 mM to give optimum separation of the phasic and tonic components. There was little difference in responses produced in Tyrode or Tris-Tyrode but relaxation after agonist wash-out was much slower in the latter solution. Responses to noradrenaline and K^+ were determined after 60 min initial incubation in Tyrode or Tris-Tyrode and were measured for 10 min. When Tris-Tyrode was employed tissues were relaxed by washing with Tyrode and, before determination of a second response, were reincubated in Tris-Tyrode for 30 min. The second response to noradrenaline or K^+ was used as the control.

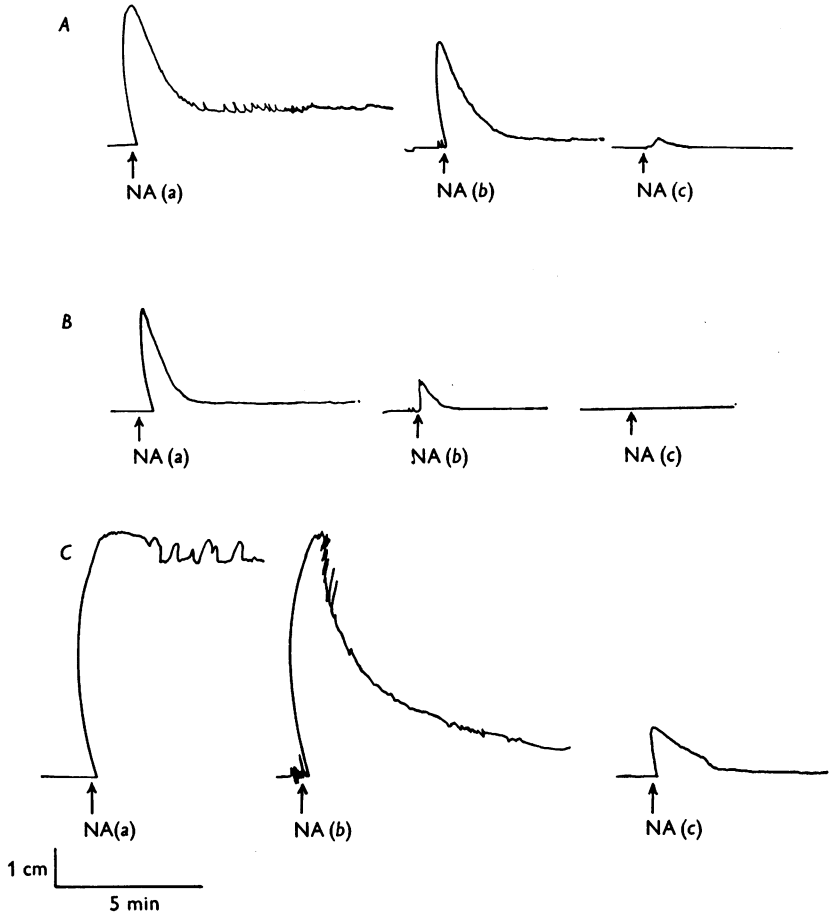

Fig. 1. Effects of varying extracellular Ca^{2+} concentrations, $[Ca^{2+}]_o$, and $[Ca^{2+}]_o$ removal on noradrenaline (10^{-4} M) responses in rat vas deferens. *A*, the control response (*a*) is in 1·8 mM-Ca^{2+} and subsequent responses are measured 0 (*b*) and 3 min (*c*) after switching to nominally Ca^{2+}-free medium. *B* and *C*, the control responses are in 1·0 and 8·0 mM-Ca^{2+} respectively.

The inhibitory effects of La^{3+} and Tm^{3+} on the phasic and tonic components of the noradrenaline and K^+ responses in Tris-Tyrode solution are summarized in Fig. 3 and are expressed as percentage inhibition relative to the second control response. La^{3+} and Tm^{3+} do not differ significantly in their inhibitory activity towards either noradrenaline or K^+ responses. The phasic and tonic components of the noradrenaline response are approximately equally sensitive to Ln^{3+} but the phasic component of the K^+ response is clearly more sensitive than the tonic component at Ln^{3+}

concentrations $< 10^{-3}$ M. An increase in exposure time of the tissue to La^{3+} from 5 to 30 min did not increase the extent of inhibition.

When La^{3+} or Tm^{3+} concentrations greater than 10^{-3} M were employed a slow and small contractile response ($\sim$ 10% of that seen with noradrenaline) was often observed in the absence of any stimulant.

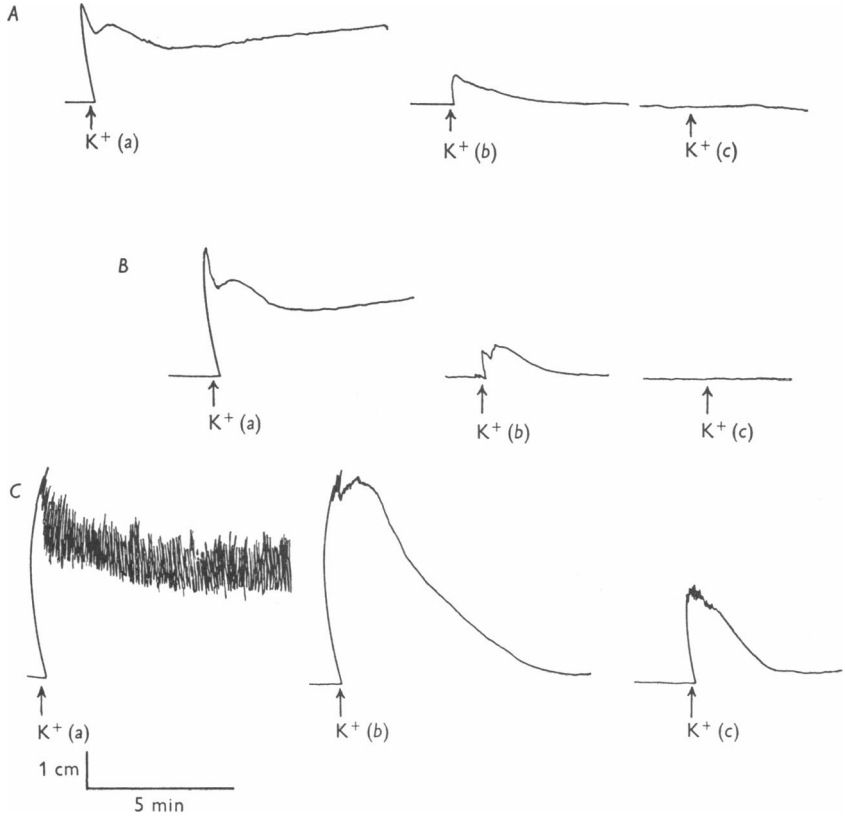

Fig. 2. Effects of varying $[Ca^{2+}]_o$ concentrations and $[Ca^{2+}]_o$ removal on K^+ (40 mM) responses in rat vas deferens. The protocol was analogous to that of Fig. 1.

Calcium dependence of contractile response

Both the phasic and tonic components of the noradrenaline and K^+ responses were markedly affected by varying Ca^{2+} concentrations. These results are summarized in Table 1 and are expressed as percentage increases or decreases in the phasic and tonic components relative to the magnitude of these components in 1·8 mM Ca^{2+} Tyrode. Increasing the extracellular Ca^{2+} concentration causes an increase in the phasic and tonic

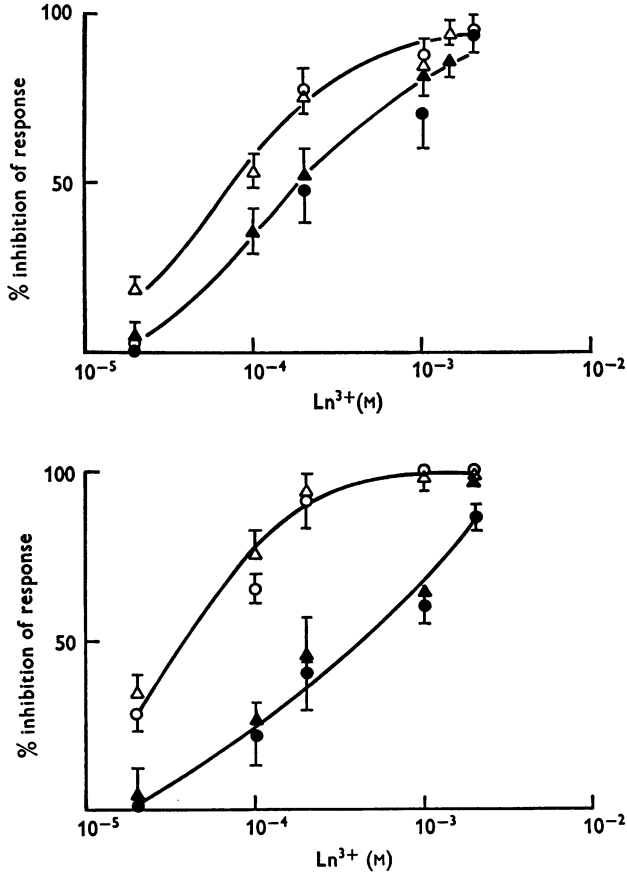

Fig. 3. Dose–response curves for La^{3+} and Tm^{3+} inhibition of noradrenaline (*A*) and K^+ (*B*) responses of rat vas deferens showing: La^{3+} inhibition of phasic (○) and tonic (●); Tm^{3+} inhibition of phasic (△) and tonic (▲) components.

components of both noradrenaline and K^+ responses with a relatively greater increase in the tonic components. At the elevated Ca^{2+} level (8·0 mM) the ratio of phasic to tonic component becomes equal for both K^+ and noradrenaline responses whilst at the normal (1·8 mM) and reduced Ca^{2+} level (1·0 mM) the phasic to tonic ratio is significantly higher for the noradrenaline responses. A decrease in extracellular Ca^{2+} causes an approximately equal decrease in the phasic and tonic components of both the K^+ and noradrenaline responses.

Replacement of normal Tyrode by a Ca^{2+}-free Tyrode results in a rapid loss of response to both noradrenaline and K^+ (Figs. 1*A* and 2*A*). Responses evoked immediately after Ca^{2+} withdrawal consist solely of a phasic

TABLE 1. Effects of variation of extracellular Ca^{2+} concentration, $[Ca^{2+}]_o$, on phasic and tonic components of noradrenaline and K^+ responses in vas deferens

	% change in noradrenaline response ± S.E. of mean (n = 10)			% change in K^+ response ± S.E. of mean (n = 10)		
$[Ca^{2+}]_o$	Phasic	Tonic	Phasic/tonic	Phasic	Tonic	Phasic/tonic
1·8 mM	Control = 100 %		6·1 ± 1·03	Control = 100 %		2·5 ± 0·5
1·0 mM	− 60·9 ± 4·2	− 69·9 ± 6·8	8·3 ± 1·3	− 43·3 ± 4·6	− 40·5 ± 4·5	2·2 ± 0·5
8·0 mM	+ 53·6 ± 5·9	414·8 ± 8·8	1·8 ± 0·3	+ 29·5 ± 6·9	+ 92·2 ± 14·7	1·8 ± 0·3

component which is greatly reduced or abolished by incubation of the tissue for 3 min in Ca^{2+}-free Tyrode. Variation of the Ca^{2+} concentration in the incubating media before replacement by Ca^{2+}-free Tyrode did not affect the immediate loss of the tonic component but did influence the apparent rate at which the phasic response was lost. Incubation in 1·0 mM-Ca^{2+} Tyrode before switching to Ca^{2+}-free Tyrode results in a significant loss of response at time zero and a complete loss of response after 3 min (Figs. 1*B* and 2*B*). The phasic component to noradrenaline and K^+ is unchanged at time zero after switching from an 8 mM Ca^{2+}-Tyrode and is still measurable after 3 min (Figs. 1*C* and 2*C*).

^{170}Tm uptake

A 5 min incubation period was chosen to reproduce the exposure time used in the pharmacological experiments. The concentration dependent ^{170}Tm uptake is illustrated in Fig. 4 which also shows the absence of saturation even with concentrations as high as 10^{-3} M.

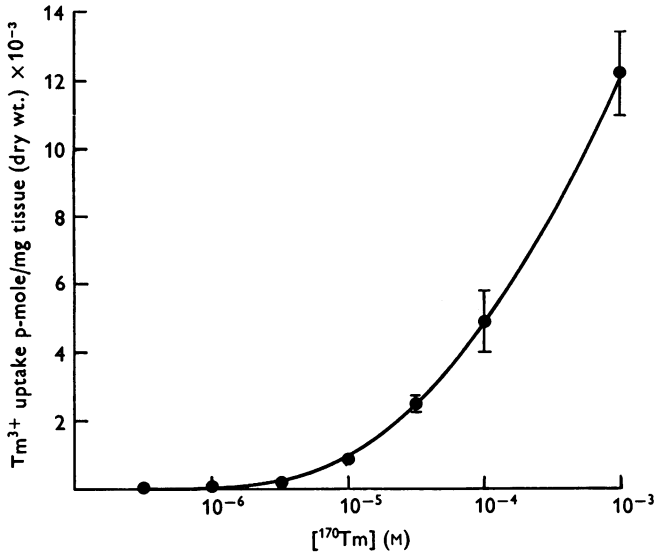

Fig. 4. Uptake of ^{170}Tm by rat vas deferens using 5 min incubation period.

DISCUSSION

The insensitivity of the contractile responses of the rat vas deferens to La^{3+} and Tm^{3+} is in marked contrast to the high sensitivity of the guinea-pig ileal longitudinal smooth muscle preparation reported in the preceding paper (Triggle & Triggle, 1975). This difference in behaviour is probably

not simply related to a different dependence of the contractile responses on extracellular Ca^{2+} in these two preparations since, for example, the rate of loss of response of the vas deferens in Ca^{2+}-free media is at least as rapid as that observed in the ileal smooth muscle. More likely, it reflects a difference in the properties of the calcium binding sites which in the cell membrane of these two types of muscle control the process of excitation–contraction coupling. That La^{3+} or Tm^{3+} only fully inhibit vas deferens contractions at such high concentrations as 0·5–1·0 mM may suggest, for example, that the lanthanides act at sites where Ca^{2+} exerts a membrane stabilizing action similar to that previously described for the same concentration range in lobster axon (Takata, Pickard, Lettvin & Moore, 1966), crayfish axon (Hartz & Ulbricht, 1973), skeletal muscle (Andersson & Edman, 1974) and *Xenopus* nerve (Vogel, 1974).

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