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Blockade by zinc ions of K^+-induced contraction and calcium in guinea pig *Taenia coli*

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Abstract. Preincubation with 0.3 mM Zn^{2+} markedly inhibited both the tonic response and Ca^{2+} binding at low affinity sites induced by K^+ (60 mM), with smaller effects on the phasic response and the high affinity Ca^{2+} sites, in *Taenia coli*. However, when the muscle was kept in Zn^{2+}-containing medium following the first stimulation with the K^+, the phasic response and the high affinity Ca^{2+} sites were more severely inhibited during the second stimulation with K^+. This probably indicates that Zn^{2+} reduced the tonic tension response to K^+ mainly by inhibiting Ca^{2+} influx at the cell membranes of *Taenia coli*. However, when Zn^{2+} is continuously present, Ca^{2+} is not supplied at the storage sites and is not available for the phasic response to a second stimulation with K^+.

Key words. Zinc ions; calcium; ileal muscle.

It is well known that zinc ions (Zn^{2+}) are required as a cofactor to activate metalloenzymes such as alcohol dehydrogenase, carbonic anhydrase and carboxypeptidase. Cadmium (Cd^{2+}) or mercury (Hg^{2+}) ions (both members of the zinc group of heavy metals in the periodic table) inhibited the contractions of vascular[1-3] and intestinal[4-6] smooth muscle preparations in vitro. The main presumed mechanism of the action of Cd^{2+} [2,3,5] or Hg^{2+} [6] is to abolish the Ca^{2+} influx through voltage-operated Ca^{2+} channels in the cell membranes. It has also been reported that Zn^{2+} noncompetitively inhibits contractile responses to acetylcholine, histamine or high concentrations of K^+ in ileal smooth muscle[7-9]. However, there are few studies which have tested the effects of Zn^{2+} on calcium movement in smooth muscle. The present study was therefore undertaken to examine zinc uptake and efflux in *Taenia coli* and the effects of Zn^{2+} on high and low affinity binding sites for Ca^{2+}, in an attempt to define further how Zn^{2+} inhibits muscle contraction.

Materials and methods

Strips of *Taenia coli* were dissected from male Hartley guinea pigs, body weight 400 g, and were immersed in modified normal Tyrode's solution bubbled with 100% O_2 at 37 °C. The solution contained (mM): NaCl, 123.7; KCl, 2.7; $CaCl_2$, 2.5; $MgCl_2$, 1.0; tris(hydroxymethyl) aminomethane 25 and glucose 5.5. The pH of the solution was adjusted to 7.4 with HCl at 37 °C. Zinc ions, as $ZnCl_2$, were added to the bath solution.

The muscle strips were suspended at a resting tension of 0.6 g and allowed to equilibrate for 40 min with several changes of normal solution. After equilibration, the muscles were conditioned by adding 40 mM K^+. Isometric contraction of the muscle was measured by a strain gauge transducer (Nihon Kohden, RM-6000). ^{45}Ca uptake was measured by a lanthanum method. The muscles were exposed to 0.025 or 2.5 mM Ca^{2+} (+^{45}Ca (5 μ Ci/ml), New England Nuclear), 60 mM K^+ medium containing Zn^{2+} for 4 or 30 min, respectively, after which they were rinsed with a lanthanum solution ($LaCl_3$ 68.7, glucose 5.5 and Tris-HCl 25 mM, pH 7.4), which was aerated with 100% O_2 at 1 °C for 50 min. The strips were blotted and then treated with a solubilizer (Soluene TM-350, Packard) and the radioactivities were counted with a liquid scintillation spectrophotometer (Aloka, LSC-602).

To determine tissue zinc concentrations, the strips were removed from the bath after incubation in a medium containing 0.3 mM Zn^{2+}. They were blotted on filter paper, then weighted, put into quartz cuvettes containing 0.1 ml $HClO_4$ and heated in a muffle furnace at 480 °C for 2.5 h. The samples were dissolved in 0.01 N HCl, and Zn^{2+} concentrations were measured with an atomic absorption spectrophotometer (Hitachi, 308).

Results

The effects of preincubation with Zn^{2+} on tension induced by high K^+ (60 mM, hypertonic) were examined in *Taenia coli*. After 30 min of incubation with 0.1, 0.2 and 0.3 mM Zn^{2+}, the tonic response to K^+ decreased markedly to 14 ± 4, 5 ± 0.08 and 0% of the original tonic response, respectively, with smaller effects on the phasic response. With Zn^{2+} present continuously after the first exposure to high K^+, the muscles were washed with normal medium (+0.1–0.3 mM Zn^{2+}). The phasic responses to the second stimulation with K^+ were now markedly inhibited in the presence of 0.1–0.3 mM Zn^{2+} (fig. 1A and B).

To determine if washing has any effect on the recovery of tension after Zn^{2+} treatment, muscles were incubated in 0.1, 0.2 or 0.3 mM Zn^{2+} for 120 min as shown in

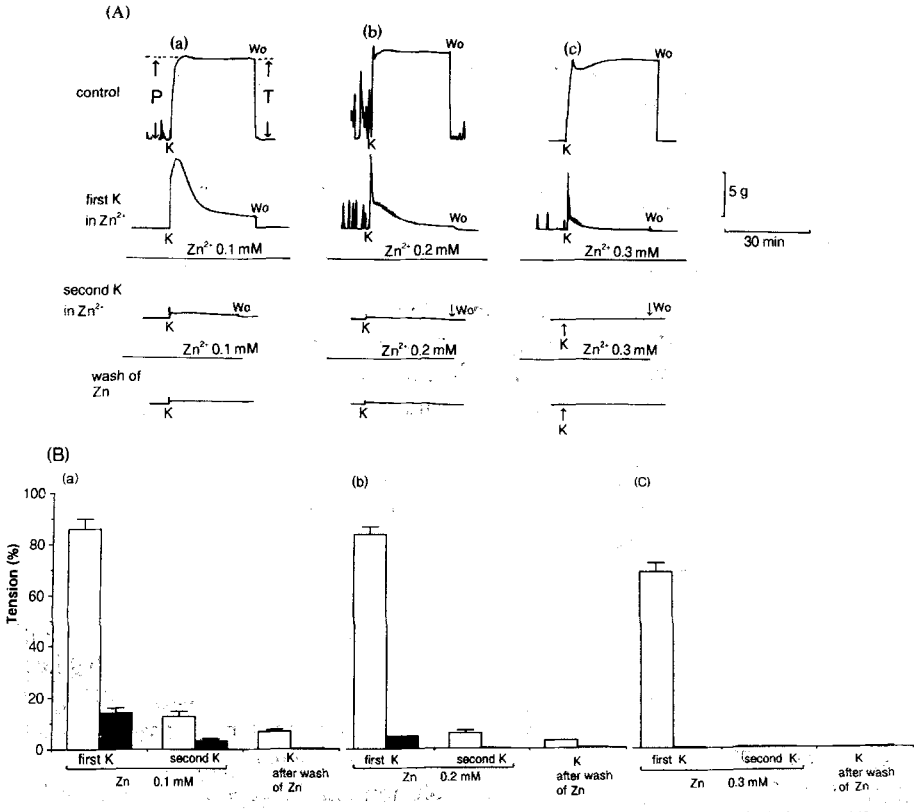

Figure 1. The effects of preincubation with Zn^{2+} on responses to high K^+ (60 mM, hypertonic) and the effects of a wash with normal medium on recovery of the high K^+-induced response after Zn^{2+} treatment in *Taenia coli*.
A Muscles were preincubated for 30 min in Zn^{2+} medium before the addition of high K^+. Following incubation in the high K^+ medium containing Zn^{2+} for 30 min, the muscles were washed with medium containing Zn^{2+} for 30 min. The high K^+ was then reapplied in the presence of Zn^{2+}. After 30 min, the muscles were washed with normal medium for 60 min. Then high K^+ was added. The tensions in response to each concentration of Zn^{2+} in (a), (b) and (c) were recorded from different strips. K, 60 mM K^+; Wo, wash-out of the K^+; P, phasic contraction; T, tonic contraction.
B The time course of the experiments is the same as in *A*. The first and second phasic or tonic responses to 60 mM K^+ in the presence of 0.1 (a), 0.2 (b) or 0.3 (c) mM Zn^{2+} were calculated as per cent of their corresponding phasic or tonic responses to 60 mM K^+ in the absence of Zn^{2+}. The responses to high K^+ after wash with normal medium after treatment with Zn^{2+} are also shown. Error bars on the columns show S.E. of 12 experiments. □, Phasic response; ■, tonic response.

figure 1A, then washed with normal medium for 60 min. After returning to fresh normal medium, high K^+ was applied. However, both phasic and tonic responses to K^+ were still inhibited in spite of the washings (fig 1A and B).

The effects of Zn^{2+} on Ca^{2+}-induced responses in depolarized *Taenia coli* were examined. Zn^{2+} reduced the size of the maximal response to Ca^{2+} without shifting the concentration-response curves for Ca^{2+} to the right (fig. 2A).

To test the effects of Zn^{2+} on Ca^{2+} binding at high and low affinity sites during 60 mM K^+-induced contraction, muscles were loaded with ^{45}Ca for 4 or 30 min in 0.025 or 2.5 mM Ca^{2+} medium (+60 mM K^+) containing 0.3 mM Zn^{2+}. High K^+ (60 mM) did increase the Ca^{2+} binding at high and low affinity sites. When first stimulation with high K^+ was in the presence of 0.3 mM Zn^{2+}, the Ca^{2+} binding at low affinity sites was significantly inhibited with smaller effects on the high affinity sites. In the next series of experiments, after muscles had been stimulated with high K^+ in the presence of 0.3 mM Zn^{2+} for 30 min, they were washed with normal medium containing 0.3 mM Zn^{2+}. At this point, muscles were loaded with ^{45}Ca for 4 or 30 min in 0.025 or 2.5 mM Ca^{2+} medium (60 mM K^+), respectively. Under these conditions, 0.3 mM Zn^{2+} significantly blocked the Ca^{2+} binding at the high affinity sites (fig. 2B).

The zinc uptake by ileal muscle stabilized after 30–60 min in 0.3 mM Zn^{2+} medium (fig. 3A). The tissue/medium Zn^{2+} concentration ratio at the equilibrium level was 2.83. This indicates that muscles accumulated a far greater amount of zinc than the extracellular space.

To study changes in zinc efflux, the muscles were incubated in 0.3 mM Zn^{2+} medium for 60 min and then washed with normal medium or Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA, a non-penetrating chelator[10]. The zinc content of the muscles reached equilibrium after 60 min and about 43% (with normal medium) or 25% (with medium containing EDTA) of

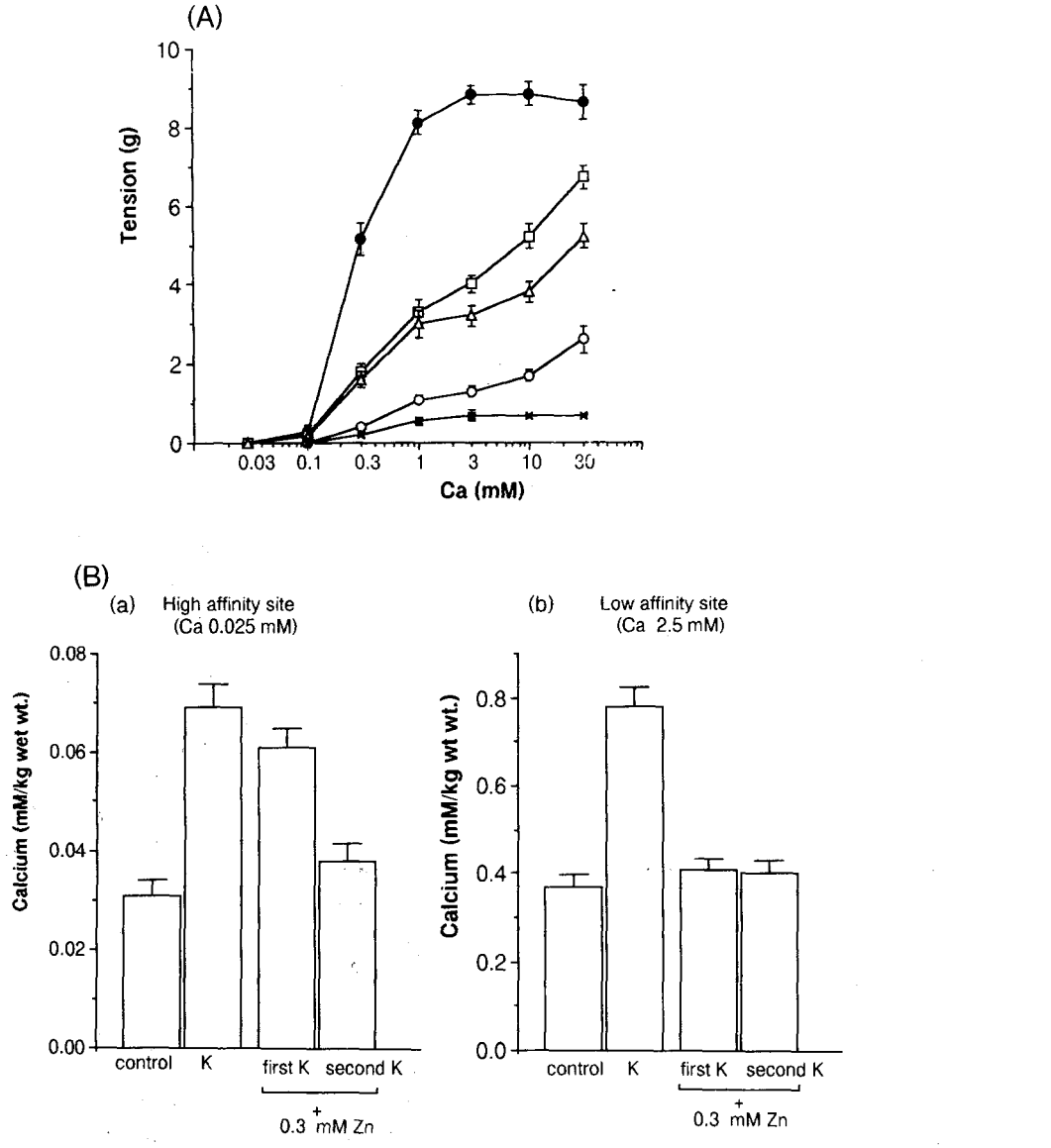

Figure 2. *A* Effects of Zn^{2+} on Ca^{2+}-induced contraction in depolarized *Taenia coli*. The concentration-tension relationships were obtained by increasing the concentration of Ca^{2+} in isotonic 120 mM K^+ medium containing Zn^{2+}. Each point represents 10 experiments (mean ± S.E.). Control (●); 0.005 (□), 0.01 (△), 0.03 (○), 0.1 (×) mM Zn^{2+}

B The effects of Zn^{2+} on La^{3+}-resistant uptake at high (a) and low (b) affinity Ca^{2+} binding sites in *Taenia coli*. For the test of Zn^{2+} on high affinity Ca^{2+} sites, muscles were preincubated in 0.025 mM Ca^{2+} medium for 60 min before loading in the 0.025 mM Ca^{2+} medium; K^+ (0.025 mM Ca^{2+}) medium or Zn^{2+} added K^+ (0.025 mM Ca^{2+}) medium, respectively, each containing ^{45}Ca. The loading time with ^{45}Ca was 4 min. For the test of Zn^{2+} on low affinity Ca^{2+} sites ^{45}Ca was added in normal Ca^{2+} (2.5 mM) medium; K^+ (2.5 mM Ca^{2+}) medium or Zn^{2+} added K^+ (2.5 mC Ca) medium, respectively, and each loading time was 30 min. Muscles were preincubated with Zn^{2+} for 30 min before the addition of high-K^+ in the test of both affinity sites. In the next series of experiments, after muscles had been stimulated with high K^+ in presence of 0.3 mM Zn^{2+} for 30 min, they were washed with normal medium containing 0.3 mM Zn^{2+}. At this point, muscles were stimulated a second time with K^+ medium (0.025 or 2.5 mM Ca^{2+} + ^{45}Ca). Error bars on the columns show S.E. of 10–12 experiments.

the original tissue zinc concentrations were retained after the washings.

Discussion

Zn^{2+} had a non-specific antagonistic action on Ca^{2+} concentration response similar to N_2 gas, 2,4-dinitrophenol[11] and heavy metals such as Cd^{2+} [5], Hg^{2+} [6] and Cr^{6+} [12]. Conversely, the Ca^{2+} antagonists, verapamil and nifedipine[11] and the heavy metals, Co^{2+} [13] and Pb^{2+} [14] caused a rightward shift of the Ca^{2+} response-curve in ileal muscle. From this, it is postulated that Zn^{2+} may interfere at some level in the sequence of the contraction process besides the inhibition of Ca^{2+} influx at the cell membrane. In fact, Zn^{2+} has been reported to inhibit the respiration of isolated mitochondria[15]. In *Taenia coli*, Zn^{2+} inhibited the tonic response to K^+

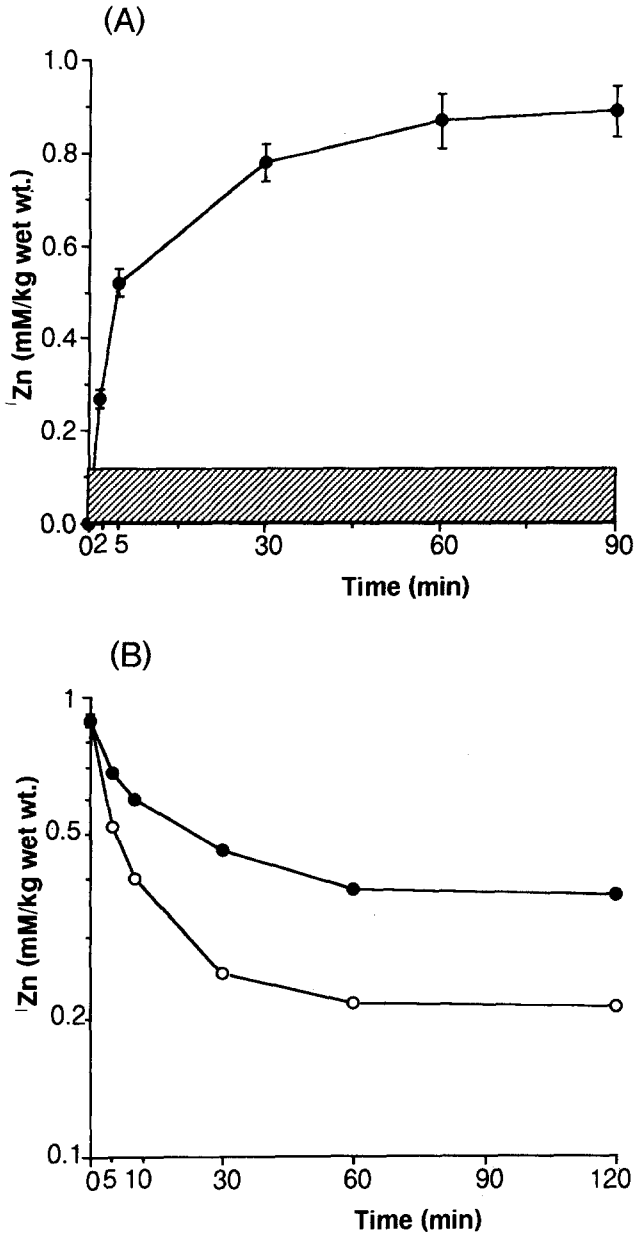

Figure 3. Time course of zinc uptake *A* and zinc efflux *B* in *Taenia coli*.
A $ZnCl_2$ (0.3 mM) was added at time 0. Assuming that the extracellular space is saturated with the same concentration of substrate as the external medium, the hatched part (0.3 mM Zn^{2+} in external medium $\times 0.41$ of tissue/medium ratio in extracellular space measured by ^{14}C-sorbitol) represents the Zn^{2+} in the extracellular space.
B Muscles were incubated with 0.3 mM Zn^{2+} medium for 60 min and were washed with normal medium (●) or Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA (○). Each point represents the mean of 10 experiments (mean ± S.E.).

with small effects on the phasic one similar to the metabolic inhibitors, N_2 gas or 2,4-dinitrophenol[16].

It is well established that in *Taenia coli* the phasic response to K^+ can be attributed to the release of Ca^{2+} from a cellular store, and the tonic response is caused by increasing Ca^{2+} influx[17,18]. Scatchard plots of La^{3+}-resistant residual Ca^{2+} uptake show that two distinguishable Ca^{2+} binding sites of high and low affinities exist in ileal[19] and vascular[20] muscles. Ca^{2+} at high affinity sites has been thought to be associated with Ca^{2+} release in norepinephrine-induced contraction, and increase in Ca^{2+} at low affinity sites is found in K^+-induced contraction in aorta[20]. In the present report, Zn^{2+} inhibited K^+-induced Ca^{2+} uptake at low affinity sites with a smaller effect on the high affinity Ca^{2+} sites in *Taenia coli*. The results suggest that Zn^{2+} reduced the tonic tension in response to K^+ primarily by inhibiting Ca^{2+} influx, with a lesser effect on Ca^{2+} release from Ca^{2+} storage sites.

The phasic response to K^+ and the Ca^{2+} uptake at high affinity sites was still inhibited even on a second stimulation with K^+. This suggests that Zn^{2+} inhibits Ca^{2+} supply into Ca^{2+} storage sites.

Zinc uptake increased along with the duration of Zn^{2+} incubation and it reached an equilibrium level after 30–60 min in *Taenia coli*. This may explain the report by Schnieden and Small[7] that the inhibitory action of Zn^{2+} on contraction increased with an increase in exposure time (15–51 min) in ileum. The zinc fraction not eliminated by EDTA may accumulate in the intracellular compartment, to which EDTA has no access.

Sarria et al.[8] have shown that zinc in millimolar concentrations did not inhibit the contraction of skinned *Taenia coli*. The inhibition by Zn^{2+} of the K^+-induced phasic and tonic responses was not restored after washing with normal medium in *Taenia coli* (fig. 1), suggesting that Zn^{2+} bound both to Ca^{2+} storage sites and voltage operated Ca^{2+} channels is not easily eliminated.

In conclusion, in *Taenia coli* Zn^{2+} inhibited the tonic response to K^+ mainly by inhibition of Ca^{2+} influx at voltage operated Ca^{2+} channels, with a smaller effect on the phasic response. However, when Zn^{2+} is continuously present, Ca^{2+} is not supplied at the storage sites and is not available for the phasic response.

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