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Zinc ions block the second but not the first phasic response to repetitive application of carbachol and histamine in guinea-pig taenia caeci

T. Nasu

Department of Veterinary Pharmacology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753 (Japan)
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Abstract. In the presence of Zn^{2+} (0.3 mM), carbachol (10^{-6} M) or histamine (10^{-5} M) induced the phasic response in guinea-pig taenia caeci while the tonic response was markedly inhibited. However, when the muscles were kept in Zn^{2+}-containing medium following the first stimulation with either carbachol or histamine, neither application of carbachol nor of histamine elicited another phasic contraction. Caffeine (25 mM) did not induce contraction in the presence of Zn^{2+}. After the washing out of caffeine in the presence of Zn^{2+}, however, the muscle did then develop the phasic response on the application of carbachol or histamine. In conclusion, Zn^{2+} did not affect the carbachol or histamine-induced Ca^{2+} release from the storage sites. However, when Zn^{2+} was continuously present, Ca^{2+} was not supplied to the storage sites. Furthermore, carbachol and histamine mobilized a common cellular Ca^{2+} store, but they activated Ca^{2+} release channels different from the ones activated by caffeine in the Ca^{2+} storage sites.
Key words. Zn^{2+}; calcium store; smooth muscle.

Zinc ions (Zn^{2+}) have been reported to inhibit contractions due to acetylcholine, histamine and K^+ in ileal longitudinal smooth muscle[1-3]. I have shown earlier[3] that Zn^{2+} did not affect the phasic response to K^+(60 mM), but it inhibited the K^+ tonic tension mainly by inhibition of Ca^{2+} influx via the voltage-operated Ca^{2+} channels. However, when Zn^{2+} was present in the external surroundings, Ca^{2+} was not supplied to the storage sites and was not available for the second phasic response to K^+.

It is well known that vascular smooth muscle contractions in response to norepinephrine, histamine and caffeine depend on Ca^{2+} release from cellular Ca^{2+} storage sites[4-7]. Also in intestinal muscle, carbachol and caffeine induce Ca^{2+} release from Ca^{2+} storage sites[8,9]. It has been supposed that in the taenia caeci there exist two Ca^{2+} release mechanisms, namely Ca^{2+}-induced Ca^{2+} release (CICR), and inositol 1,4,5-triphosphate (IP_3)-induced Ca^{2+} release from the Ca^{2+} storage sites[10,11].

The present study was undertaken to examine the effects of Zn^{2+} on phasic responses induced by Ca^{2+} release from Ca^{2+} stores by carbachol, histamine and caffeine in the taenia caeci.

Materials and methods

Strips of the taenia caeci were isolated from the caecum of male Hartley strain guinea-pigs, b. wt 400 g, and were immersed in modified normal Tyrode 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 directly 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 the Tyrode solution. Isometric contraction of the muscle was measured by a strain gauge transducer (Nihon Kohden, RM-6000). After equilibration, the muscles were conditioned by adding 40 mM K^+.

Results

When a strip of taenia caeci muscle was treated with carbachol (10^{-6} M) or histamine (10^{-5} M), each maximal phasic tension was followed by a sustained tonic contraction. The effects of preincubation with Zn^{2+} for 30 min on tension induced by high K^+ (60 mM, hypertonic), carbachol and histamine were examined. Zn^{2+} (0.1 mM) had little effect on the phasic response to K^+ and carbachol; however, the tonic responses to K^+ and carbachol were reduced markedly, to $38.1 \pm 4.3\%$ ($n = 10$) and $9.1 \pm 0.8\%$ ($n = 10$) of the original tonic responses, respectively (fig. 1). In the presence of 0.1 mM Zn^{2+}, about 60% of the phasic response to histamine remained, but the tonic response slowly decayed completely to the baseline level. With a higher concentration of Zn^{2+} (0.3 mM), K^+, carbachol or histamine produced only a phasic response although the period was slightly shortened (fig. 2). However, Zn^{2+} blocked the second phasic response to repetitive application of carbachol or histamine (figs 1 and 2).

In the next series of experiments, after the first application of carbachol or histamine in the presence of Zn^{2+}, muscles were stimulated again with the other agonist. Carbachol (histamine) could not evoke a second con-

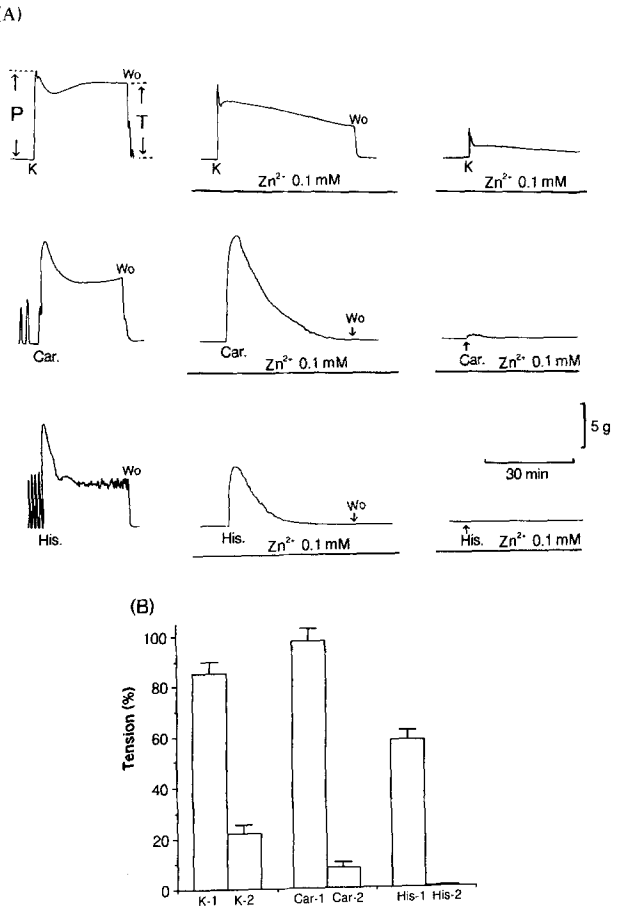


Figure 1. The effects of preincubation with Zn^{2+} on responses to K^+, carbachol and histamine in taenia coli.
A In each row, the first response to K^+ (hypertonic, 60 mM), carbachol (1×10^{-6} M) and histamine (1×10^{-5} M) was the control. Muscles were preincubated for 30 min in 0.1 mM Zn^{2+} medium before application of K^+, carbachol and histamine. Following application of K^+, carbachol and histamine in the presence of 0.1 mM Zn^{2+} for 45 min, the muscles were washed with the medium containing 0.1 mM Zn^{2+}. K^+, carbachol and histamine were then reapplied in the presence of 0.1 mM Zn^{2+}.
K: K^+ (60 mM); Car.: Carbachol (1×10^{-6} M); His.: Histamine (1×10^{-5} M); P: Phasic contraction; T: Tonic contraction; Wo: Wash-out.
B The first and second phasic responses stimulated by K^+, carbachol and histamine in the presence of 0.1 mM Zn^{2+} were calculated as percentages of phasic responses to K^+, carbachol and histamine in the absence of Zn^{2+}, respectively.
K-1, Car-1 or His-1: first phasic response to K^+, carbachol or histamine, respectively, in the presence of 0.1 mM Zn^{2+}. K-2, Car-2 or His-2: second phasic response to K^+, carbachol or histamine, respectively, in the presence of 0.1 mM Zn^{2+}. Error bars on the columns show SE of 10–12 experiments.

traction after the washing out of histamine (carbachol) in the presence of 0.3 mM Zn^{2+}, and vice versa (figs 3 and 4).

Caffeine (25 mM) induced a rapid transient contraction in taenia caeci strips (fig. 5). Caffeine has been shown to evoke a transient contraction by Ca^{2+} release from the store in the ileum[8,9]. In the presence of 0.1 mM Zn^{2+}, caffeine did not evoke any contraction. However, when K^+, carbachol or histamine was applied after the washing out of caffeine in the presence of 0.1 mM Zn^{2+}, the agonists produced phasic responses (fig. 5).

Discussion

Zn^{2+} markedly inhibited both the tonic response, and the Ca^{2+} binding at low affinity sites, induced by K^+ (60 mM). There were smaller effects on the phasic response and the high affinity Ca^{2+} site in the taenia caeci[3]. It is well established that in taenia caeci the phasic response to K^+ (40–60 mM) can be attributed to release of Ca^{2+} from a cellular store of Ca^{2+} binding

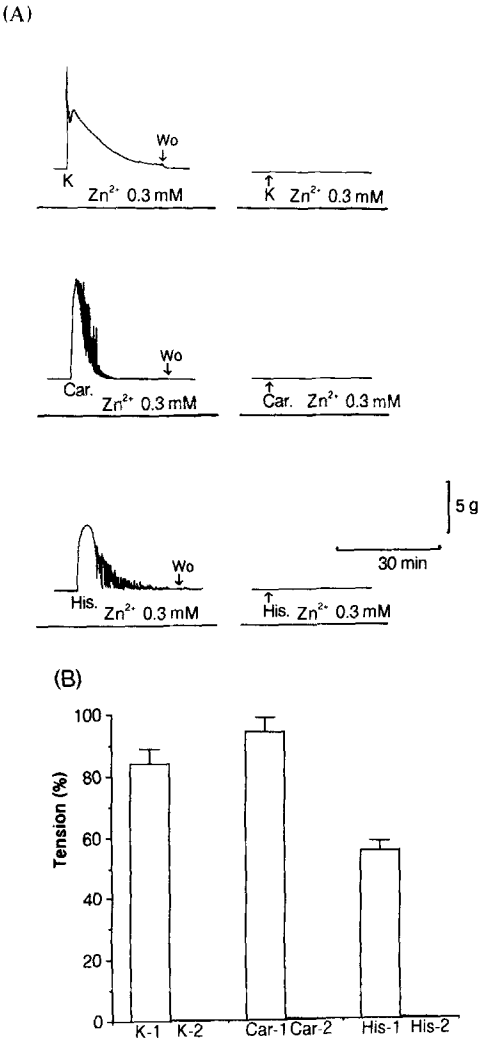
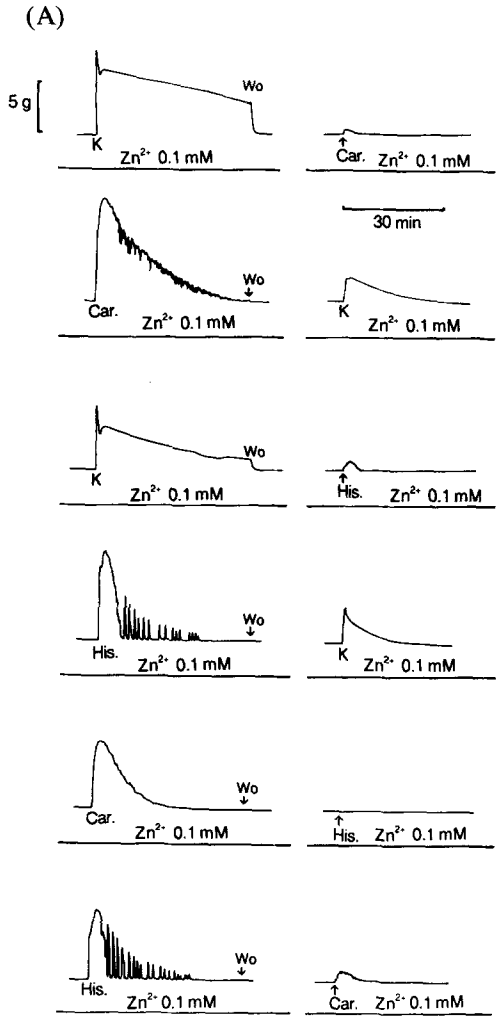


Figure 2. The effects of 0.3 mM Zn^{2+} on responses to K^+ (60 mM), carbachol (10^{-6} M) and histamine (10^{-5} M).
A In each experiment muscle was treated in the same way as in figure 1*A* except that the concentration of Zn^{2+} was 0.3 mM. The control response to K^+, or carbachol and histamine in the absence of Zn^{2+} was omitted in figure.
B Responses were expressed as a percentage of the corresponding phasic response before addition of Zn^{2+}.
K-1, Car-1 or His-1: first phasic response to K^+, carbachol or histamine in the presence of 0.3 mM Zn^{2+}, respectively. Zn^{2+} (0.3 mM) completely blocked the second phasic responses (K-2, Car-2 or His-2) to K^+, carbachol or histamine, respectively, of the same agonist as first stimulation. Error bars on the columns show SE of 10–12 experiments.



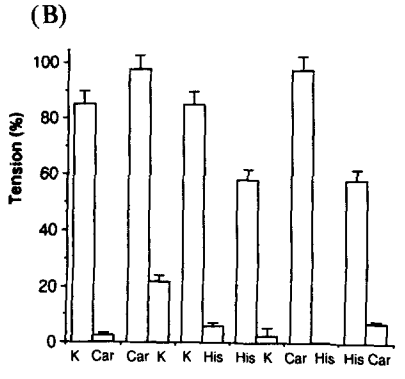


Figure 3. Effects of 0.1 mM Zn^{2+} on responses to second stimulation with a different agonist. The protocol and the concentration of each agonist of the experiments in *A* was the same as in figure 1*A*, respectively. The left bar in each row of graph *B* represents first phasic response to one agonist, the right bar is the second phasic response to another. Error bars on the columns show SE of 10 experiments.

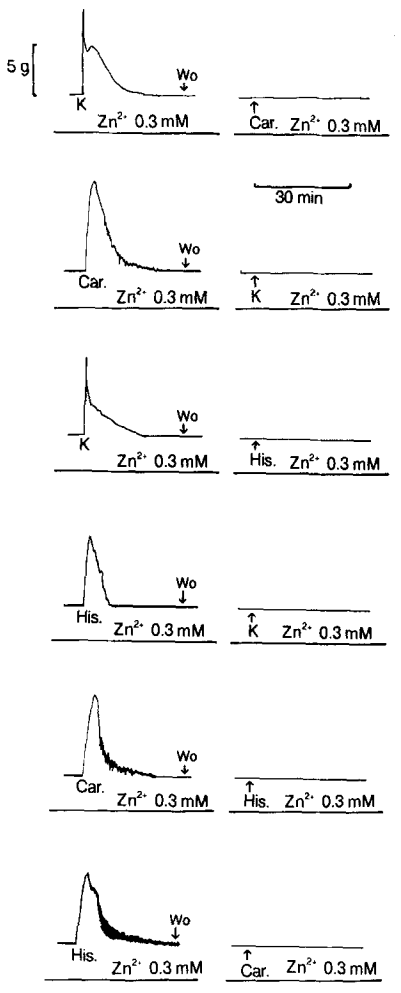


Figure 4. Effects of 0.3 mM Zn^{2+} on responses to second stimulation with a different agonist. The protocol and the concentration of each agonist of the experiments was the same as in figure 1*A*.

sites at a high affinity, as was determined by the lanthanum method, and the tonic response can be attributed to increased inward movement of Ca^{2+} from extracellular fluid[12-14]. It is also known that carbachol and histamine activate muscarinic receptors and histamine receptors, respectively, and these receptor activations evoke a production of IP_3 due to activity of triphosphoinositide phosphodiesterase[15]. IP_3 induces Ca^{2+} release from the storage sites[10,11,15,16], which results in an initial phasic contraction. This process is accompanied by the opening of receptor-linked Ca^{2+} channel in response to the acceleration of electrical membrane activity[8,17], which results in a sustained tonic contraction. The selectivity for the inhibition of the tonic response by Zn^{2+} probably indicates that Zn^{2+} inhibited the receptor-linked Ca^{2+} channel but it did not affect the Ca^{2+} release from the storage sites activated by histamine and carbachol.

However, Zn^{2+} completely inhibited the second phasic response to repetitive and sequential application of carbachol and histamine. Both the phasic response and the high affinity Ca^{2+} sites induced by K^+ in the presence of Zn^{2+} were non-responsive to a second stimulation with K^+ in taenia caeci[3]. This probably indicates that these agonists (carbachol and histamine) depleted Ca^{2+} in the storage sites sensitive to them by the first stimulation,

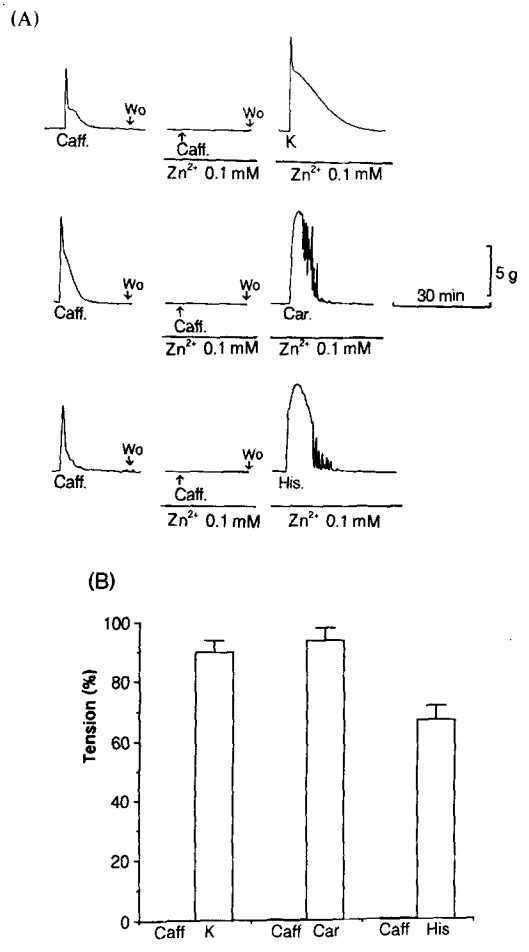


Figure 5. Effects of Zn^{2+} on response to caffeine.
A The incubation time of caffeine was 20 min. Caffeine (25 mM) did not induce the response in the presence of 0.1 mM Zn^{2+}, but after the caffeine had been washed away, the muscle developed first phasic responses to K^+ (60 mM), carbachol (10^{-6} M) or histamine (10^{-5} M).
B The phasic response stimulated by K^+, carbachol and histamine after the washing out of caffeine in the presence of 0.1 mM Zn^{2+} were calculated as percentages of the phasic response to K^+, carbachol and histamine in the absence of Zn^{2+}. Error bars on the columns show SE of 10 experiments.

and Ca^{2+} was not refilled into the Ca^{2+} storage sites in the presence of Zn^{2+}.

Caffeine did not induce a contraction in the presence of a low concentration of Zn^{2+} (0.1 mM). It is probable that the inhibition of the caffeine response by Zn^{2+} is caused by inhibition of the 'Ca^{2+}-induced Ca^{2+} release (CICR)'. However, when K^+, carbachol or histamine was applied after a wash with caffeine in the presence of 0.1 mM Zn^{2+}, the phasic response to the agonists could be produced (fig. 5). This may be because subsequent application of an agonist after caffeine application in the presence of Zn^{2+} produces IP_3 and this IP_3 releases Ca^{2+} via the IP_3 channel instead of the CICR channel. In conclusion, it was suggested that Zn^{2+} did not affect carbachol or histamine-induced Ca^{2+} release from the store sites. However, Ca^{2+} was not supplied to the store sites in the presence of Zn^{2+} in ileal muscle. I think that these results suggest the utility of Zn^{2+} as a biochemical and pharamacological tool for testing the function of the intracellular Ca^{2+} store mobilized by agonists in intact smooth muscle cells.

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