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# Zinc ions block the intracellular calcium release induced by caffeine in guinea-pig taenia caeci

T. Nasu

*Department of Veterinary Pharmacology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753 (Japan)*
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**Abstract.** $Zn^{2+}$ in low concentrations (0.005 – 0.1 mM) inhibited the transient contractions in response to caffeine (25 mM) in a dose-dependent manner in smooth muscle of intact guinea-pig taenia caeci. At $Zn^{2+}$ concentrations higher than 0.1 mM, caffeine did not elicit any response. After saponin-treatment of the fibres, which leaves the $Ca^{2+}$ storage sites intact, caffeine contraction was completely inhibited by $Zn^{2+}$ at a relatively low concentration (0.03 mM). However, in Triton-X-100-treated fibres, in which the $Ca^{2+}$ release sites are destroyed, the contraction could be induced in the presence of $Zn^{2+}$ by an increase in $Ca^{2+}$. In conclusion, $Zn^{2+}$ can block the intracellular $Ca^{2+}$ release from caffeine-sensitive release sites in taenia caeci.
**Key words.** Zinc ions; caffeine; calcium store; smooth muscle.

An increase in cytoplasmic $Ca^{2+}$, the primary trigger for contraction in smooth muscle, occurs as the result of an influx of extracellular $Ca^{2+}$ or the release from intracellular $Ca^{2+}$ storage sites[1–4]. There are two mechanisms for the release of $Ca^{2+}$ from the intracellular stores, namely $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) and inositol 1,4,5-triphosphate ($IP_3$)-induced $Ca^{2+}$ release[5–7].

Zinc ions ($Zn^{2+}$) block the tonic response to $K^+$ (60 mM) by inhibition of $Ca^{2+}$ influx through voltage-operated $Ca^{2+}$ channels in the cell membranes in the taenia caeci muscle[8]. I also suggested earlier[9] that $Zn^{2+}$ did not affect the first phasic response to carbachol or histamine, since it did not inhibit the $Ca^{2+}$ release from intracellular storage sties. However, $Ca^{2+}$ storage sites were not re-supplied in the presence of $Zn^{2+}$.

Caffeine has been shown to evoke a transient contraction mediated by $Ca^{2+}$ release from stores in the ileum[3,6,10]. The present study was undertaken to examine the effects of $Zn^{2+}$ on CICR induced by caffeine in intact and skinned taenia caeci, in an attempt to further define the role of CICR in smooth muscle contraction.

## Materials and methods

Strips of taenia caeci were isolated from the caecum of male Hartley strain 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. Effects of $Zn^{2+}$ on contraction induced by caffeine in intact muscle were studied in thick fibres (1 mm in width × 2 cm in length). 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. 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 $K^+$. Caffeine was directly dissolved in the bathing solution. An appropriate amount of 0.5 or 3 mM $ZnCl_2$ stock solution dissolved in distilled water was added to the bathing solution to obtain the experimental $Zn^{2+}$ concentrations.

For the test of the effects of $Zn^{2+}$ on $Ca^{2+}$ release from $Ca^{2+}$ storage sites, thin muscle fibres (0.3 mm in width × 8 mm in length) were mounted horizontally in a bath containing a relaxing solution at 24 °C and were skinned with saponin (50 μg/ml) for 30 min. The composition of the relaxing solution was: 130 mM K-propionate, 4 mM $MgCl_2$, 4 mM $Na_2ATP$, 10 mM creatine phosphate, 10 U/ml creatine phosphokinase, 1 mM $NaN_3$, 4 mM EGTA and 20 mM trismaleate (pH 6.7). Solutions with the required $Ca^{2+}$ concentrations were prepared by adding appropriate amounts of $CaCl_2$ to the relaxing solution containing 2 mM EGTA. The $Ca^{2+}$ concentrations were calculated using the apparent binding constant of the Ca-EGTA complex of $1.6 \times 10^6\ M^{-1}$ at pH 6.7 (ref. 11). Skinned strips were contracted by changing to a contraction solution of pCa 5 for 10 min, and were subsequently relaxed by placing them in a relaxing solution containing 0.5 mM EGTA. Thereafter, ATP and creatine phosphate were excluded in order to eliminate re-uptake of $Ca^{2+}$ into the $Ca^{2+}$ storage sites. After pretreatment with 0.03 mM $Zn^{2+}$ for 10 min in the relaxing solution without ATP, creatine phosphate and EGTA, caffeine was added first and $IP_3$ was subsequently added.

In order to obtain fibres in which the $Ca^{2+}$ release sites had been destroyed, muscles were skinned in a relaxing solution containing 1% Triton-X-100 for 1 h. The $Zn^{2+}$ concentrations were calculated by the use of the apparent binding constant of Zn-EGTA of $10^{8.42}\ M^{-1}$ (ref. 12). The fibres were pretreated by $Zn^{2+}$

(0.03 mM) for 10 min before the application of $Ca^{2+}$ (pCa 5).

The following drugs were used: caffeine, saponin, Triton-X-100 (Wako Pure Chemicals, Japan) and $IP_3$ (Sigma, USA).

## Results

Caffeine (25 mM) induced rapid transient contraction in taenia caeci. The maximal tension in response to 25 mM caffeine was $97.2 \pm 3.1\%$ ($n = 30$) of the phasic response to $K^+$ (60 mM). In the presence of 0.01 mM $Zn^{2+}$ for 30 min, the first response to caffeine was reduced to $52.5 \pm 5.0\%$ ($n = 12$) of the control response. After the washing out of caffeine in the presence of 0.01 mM $Zn^{2+}$, the second response was further reduced to $25.0 \pm 5.1\%$ ($n = 12$) of the control. With higher concentrations of $Zn^{2+}$, the responses to caffeine were progressively reduced and, with 0.1 mM, virtually extinguished (fig. 1A and B).

In saponin-treated fibres, after loading of the storage sites with a $Ca^{2+}$-containing solution (pCa 5), 25 mM caffeine was first added, and after a 3 min interval, after washing out of the caffeine, 10 μM $IP_3$ was subsequently added without intervening $Ca^{2+}$ loading. Both 25 mM caffeine and 10 μM $IP_3$ elicited transient contraction, which showed maximal tension development of $9.3 \pm 0.17$ mg ($n = 12$) or $7.2 \pm 0.16$ mg ($n = 12$), respectively (fig. 2A-a). In another preparation of saponin-treated fibres, after pretreatment with 0.03 mM $Zn^{2+}$, 25 mM caffeine did not elicit any response. However, 10 μM $IP_3$ subsequently induced a transient contraction of $68 \pm 5.4\%$ ($n = 10$) of the control tension (fig. 2A-b).

In Triton-X-100-treated fibres, in which $Ca^{2+}$ release sites had been destroyed, contractions induced by $10^{-5}$ M $Ca^{2+}$ reached a maximal value of $11.1 \pm 0.21$ mg ($n = 14$) within 2 min. In another preparation of Triton-X-100-treated fibres, contraction induced by $10^{-5}$ M $Ca^{2+}$ in the presence of 0.03 mM $Zn^{2+}$ was $10.6 \pm 0.23$ mg ($n = 10$). Thus, 0.03 mM $Zn^{2+}$ did not significantly affect the contractions induced by $10^{-5}$ M $Ca^{2+}$ (fig. 2B).

## Discussion

Caffeine has been shown to elicit a transient contraction in vascular smooth muscle[15,16] accompanied by an increase in cytosolic $Ca^{2+}$ levels[2] measured by the use of fura-2 in $Ca^{2+}$-deficient solution. Bolton and Lim[3]

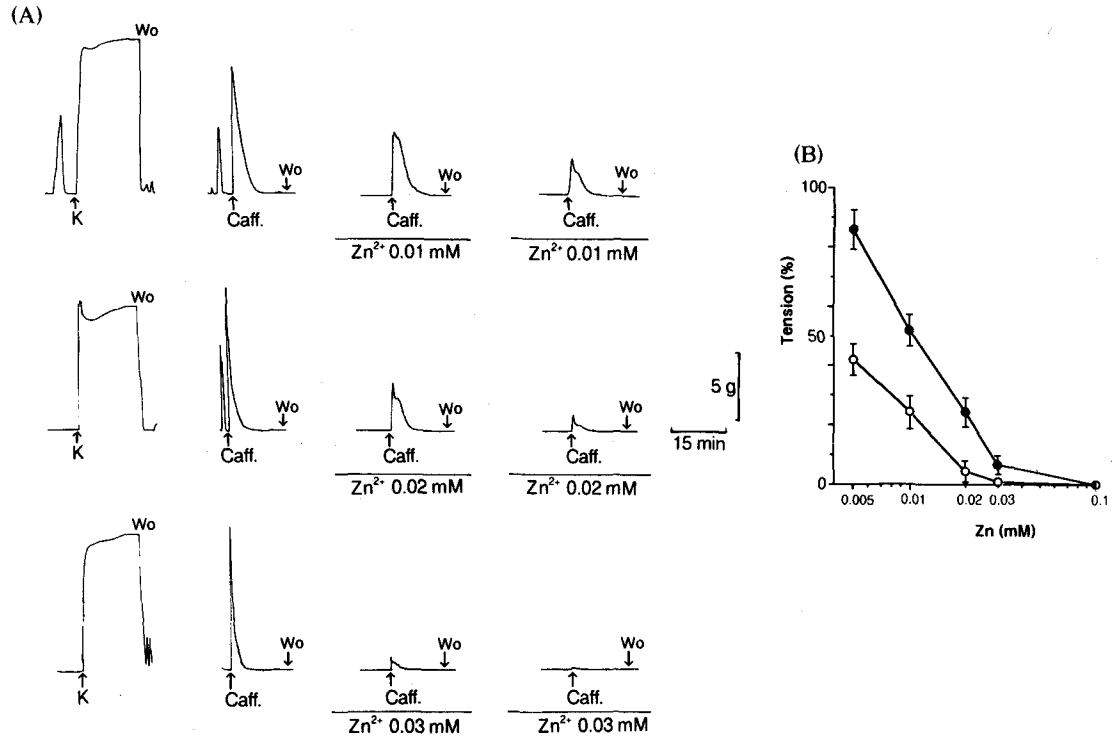


Figure 1. The effects of $Zn^{2+}$ on responses to caffeine in the intact taenia caeci.

*A* In each row, the first response to $K^+$ (60 mM, hypertonic) and the second to caffeine (25 mM) are controls. Muscles were preincubated for 30 min in $Zn^{2+}$-containing medium. Following application of caffeine in the presence of $Zn^{2+}$ for 15 min, the muscles were washed with medium containing $Zn^{2+}$ for 30 min. Caffeine was then reapplied in the presence of $Zn^{2+}$ for 15 min. K: $K^+$ (60 mM); Caff: Caffeine (25 mM); Wo: Wash-out.

*B* The first (●) and second (○) responses stimulated by caffeine in the presence of $Zn^{2+}$ were calculated as percentages of the respective responses to caffeine in the absence of $Zn^{2+}$. Each point represents the mean ± S.E. of 12 experiments.

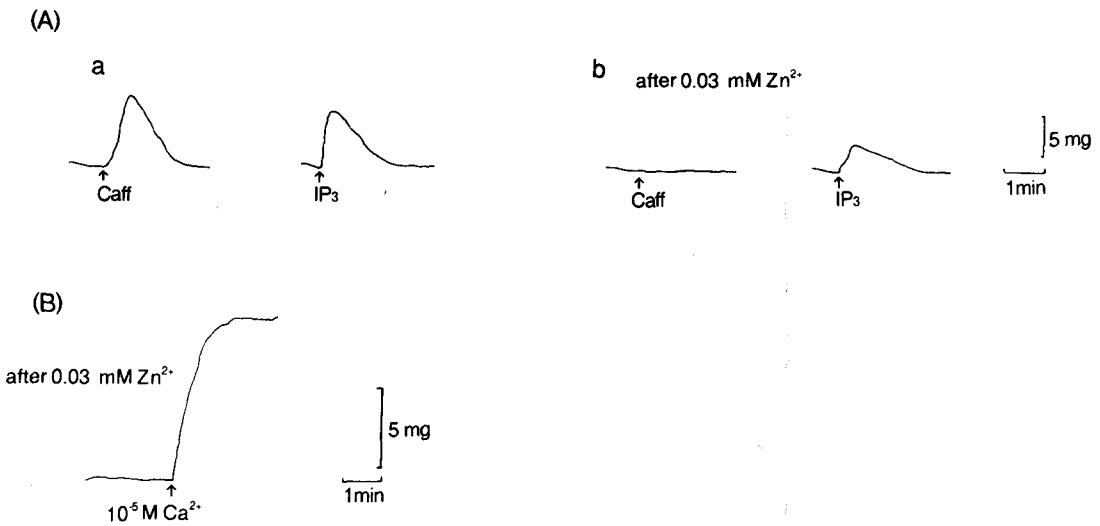


Figure 2. Effects of $Zn^{2+}$ on contraction in skinned taenia caeci. Each curve is a tracing from an actual experiment.
*A* (a) Saponin-treated fibres were contracted by changing to a contracting solution of pCa 5 for 10 min, and subsequently relaxed by a relaxing solution containing 0.5 mM EGTA (data not shown). Experiment in (a) was done in the same way as in (b) in the absence of $Zn^{2+}$. (b) After the loading with $Ca^{2+}$ in the intracellular $Ca^{2+}$ storage sites, 0.03 mM $Zn^{2+}$ was present for 10 min. Caffeine at 25 mM was then added and subsequently, with a 3 min interval for the washing out of caffeine in the presence of $Zn^{2+}$, 10 μM $IP_3$ was added to the skinned fibres. The caffeine- and the following $IP_3$-addition after $Ca^{2+}$ loading was done only once in each batch of saponin-treated fibres. Caff: Caffeine (25 mM); $IP_3$: $IP_3$ (10 μM).
*B* Muscle was skinned with Triton-X-100. The fibres were pre-treated with 0.03 mM $Zn^{2+}$ for 10 min before application of $Ca^{2+}$ (pCa 5).

showed in single dispersed ileal cells that caffeine accelerated discharge of spontaneous transient outward currents which could represent $Ca^{2+}$ release from intracellular storage sites. Iino et al.[6] and the present results showed that caffeine elicited contraction in the skinned taenia caeci after loading of $Ca^{2+}$ into the storage sites. Furthermore, caffeine greatly enhanced the amount of $Ca^{2+}$ released from the $Ca^{2+}$ storage sites in taenia caeci after $Ca^{2+}$-induction[17]. These findings support the suggestion that caffeine elicits a transient contraction by enhancing-CICR from the intracellular $Ca^{2+}$ storage sites in smooth muscles.

The present results demonstrated that $Zn^{2+}$ (0.005–0.1 mM) dose-dependently inhibited the first response to caffeine in intact taenia caeci. $Zn^{2+}$ further inhibited the response to a second application of caffeine. In addition, in fibres treated with saponin, which makes the surface membrane permeable to ions but leaves the $Ca^{2+}$ storage sites intact, $Zn^{2+}$ inhibited contraction in response to caffeine after loading of $Ca^{2+}$ into the storage sites. Furthermore, in intact taenia caeci cells, $Zn^{2+}$ appears to cross the surface membrane, as deduced from the measurement of zinc uptake and efflux[8]. These results suggest that $Zn^{2+}$ can block the intracellular $Ca^{2+}$ release from caffeine-sensitive sites. In Triton-X-100-treated fibres, in which $Ca^{2+}$ release sites are destroyed, $Zn^{2+}$ had no effect on the $10^{-5}$ M $Ca^{2+}$-induced contraction, suggesting that $Zn^{2+}$ did not inhibit the contractile elements.

In contrast, the phasic response to carbachol or histamine was produced in the presence of a higher concentration of $Zn^{2+}$ (0.3 mM) in intact taenia caeci[9]. It is known that $IP_3$ in response to activation of receptors by carbachol or histamine induced $Ca^{2+}$ release from the storage sites[5,7,13,14], which results in an initial ileal phasic contraction. In the present report, $Zn^{2+}$ had little effect on the contraction in response to $IP_3$ compared to that of caffeine in the ileal skinned fibres after loading of $Ca^{2+}$ into the storage sites. It is suggested that the intracellular $Ca^{2+}$ release from caffeine-sensitive release sites was more sensitive to $Zn^{2+}$ than those sensitive to $IP_3$ when stimulated by carbachol or histamine.

In conclusion, $Zn^{2+}$ at relatively low concentrations can block the intracellular $Ca^{2+}$ release from caffeine-sensitive release sites in taenia caeci. These experiments suggest that $Zn^{2+}$ might be a useful tool in analyzing force activation in smooth muscle because it distinguishes between caffeine- and $IP_3$-sensitive $Ca^{2+}$ release sites.

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