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Effect of Mn^{2+} on the Development of Tension Induced in Guinea-pig Taenia Coli by Bay K 8644

TETSUYUKI NASU AND MIYORI SASAKI

Department of Veterinary Pharmacology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753, Japan

Abstract

We have studied the effects of Mn^{2+} on the contractile response induced by Bay K 8644, a dihydropyridine Ca^{2+} agonist, on guinea-pig taenia coli. Mn^{2+} (5 mM) completely inhibited Bay K 8644 (10^{-6} M)-induced rhythmic contraction and contracture to baseline values in normal Ca^{2+} medium; thereafter, the contraction progressively increased to about 90% of the K^+ (60 mM)-induced tonic response. In Ca^{2+}-free medium in the presence of Bay K 8644 Mn^{2+} also evoked contraction and a concomitant increase in Mn^{2+} influx into the cytoplasm.

These results suggest that during the opening of voltage-dependent Ca^{2+} channels activated by Bay K 8644, Mn^{2+} can enter cytoplasm through the channels and induce contraction in taenia coli.

The decrease in Ca^{2+} influx through the slow Ca^{2+} channels evoked by manganese ions (Mn^{2+}) has long been considered to reduce K^+-induced contractions in the ileum (Osa 1974) and taenia coli (Nonomura et al 1966), and responses to K^+ or noradrenaline in the portal vein (Collins et al 1972).

In contrast, Mn^{2+} evokes contraction in Ca^{2+}-free, high-K^+ medium in ileal longitudinal muscle (Nasu et al 1994; Nasu & Shibata 1997), aorta (Shibata 1969) and uterus (Sakai & Uchida 1981). It has been shown that Mn^{2+} penetrates the cell membranes of ileal (Nasu et al 1994), vascular (Chen & Van Breemen 1993) and vas deferens (Tsunobuchi & Gomi 1990) smooth muscles in high-K^+ medium. In guinea-pig myocardium in Na^+- and Ca^{2+}-free medium, Mn^{2+} also evokes a slow inward current by electrical stimulation (Ochi 1976). These results suggest that Mn^{2+} passes through voltage-dependent Ca^{2+} channels activated by K^+ or by electrical stimulation.

In essence, voltage-dependent Ca^{2+} channels in smooth muscle can be activated directly by cellular depolarization either with high concentrations of K^+ or with Ca^{2+}-channel activators (Janis & Triggle 1984). It has been demonstrated using the patch-clamp technique that the dihydropyridine derivative Bay K 8644 prolongs the open state of voltage-dependent Ca^{2+} channels in intestinal (Xiong et al 1995), vascular (Strübing et al 1993) and tracheal (Green et al 1993) smooth muscle cells and cardiac cells (Hess et al 1984); Bay K 8644 has been shown to enhance intracellular Ca^{2+} concentrations in smooth muscle cells (Ozaki et al 1991).

This study was undertaken to examine whether Mn^{2+} can penetrate Ca^{2+} channels and induce contraction in taenia coli during the opening of the Ca^{2+} channels evoked by Bay K 8644.

Correspondence: T. Nasu, Department of Veterinary Pharmacology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753, Japan.

Materials and Methods

Preparation, physiological solution and tension recording

Strips of taenia coli were isolated from the caecum of male Hartley-strain guinea-pigs, 400 g, and immersed in modified normal Tyrode solution saturated 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. The solution with added K^+ (60 mM) was prepared by adding an appropriate amount of 2 M KCl solution to the normal medium. The stock solution of 0·5 M $MnCl_2$ was prepared and diluted appropriately with Tyrode solution.

The muscle strips were suspended at a resting tension of 0·6 g and left to equilibrate for 40 min

with several changes of the Tyrode solution. After equilibration, the tissue was conditioned by adding 40 mM K^+ to the bath. Isometric contraction of the muscle was measured by means of a strain-gauge transducer (Nihon Kohden, RM-6000).

Quick release was performed to assess the intensity of the active state of a Mn^{2+}-induced contraction by the method of Bose & Bose (1975). One end of the strip was connected to the strain gauge of the transducer. The transducer was moved down by micrometer calliper by 1 mm at the velocity of 1 mm per 0·1 s to change strip length (1·5 cm).

Manganese uptake

To determine tissue Mn^{2+} concentrations in taenia coli, each muscle strip was tied at each end with thread and mounted on a glass rod under a resting tension of 0·6 g. The strips were then incubated for an appropriate time in different media containing 5 mM Mn^{2+}. The strips were washed successively, for 30 min, with Ca^{2+}- and Mg^{2+}-free Tyrode solution containing 5 mM EDTA, a chelating agent which does not penetrate the cell membrane of guinea-pig taenia coli (Brading & Jones 1969). After removal from the bath they were blotted on filter paper, weighed, transferred to a quartz cuvette containing 0·5 mL of a 1:1 mixture of $HClO_4$ (60%) and HNO_3 (60%), and heated in a muffle furnace at 200°C for 3 h. The samples were dissolved in 0·1 M HCl and Mn^{2+} concentrations were measured by atomic absorption spectrophotometry (Hitachi, Z-8200).

Chemicals

R-(+)-Bay K 8644 from Funakoshi (Tokyo, Japan) was dissolved in 99·5% ethyl alcohol and further diluted with distilled water such that the final concentration of ethyl alcohol did not exceed 0·01%. All experiments were conducted in the dark to avoid light-induced degradation of the drug. Other chemicals used were of analytical grade.

Statistics

All data are expressed as means ± s.e.m. of results from the number of tissues indicated. Student's *t*-test was used to compare data; $P < 0.05$ was considered to indicate significance.

Results

Effects of Mn^{2+} on the response of taenia coli in Ca^{2+}-free, K^+ medium

When 5 mM Mn^{2+} was added to Ca^{2+}-free, K^+ (60 mM) medium, initial rapid contraction was followed by a gradual increase in tension

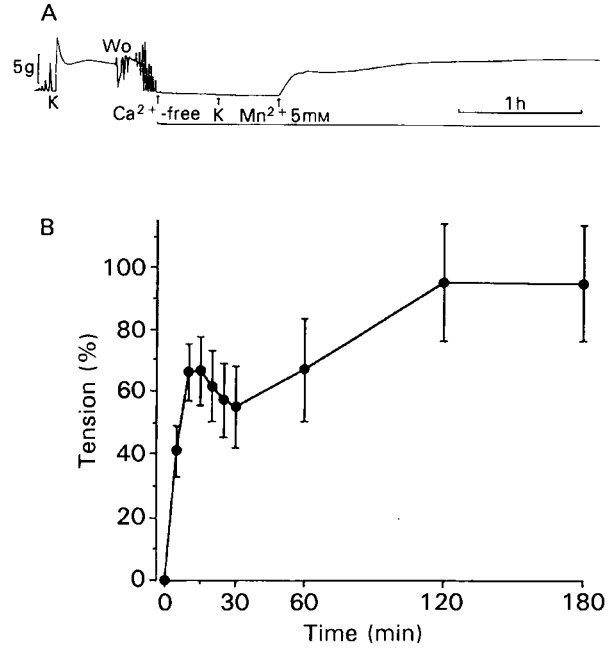


Figure 1. Effects of Mn^{2+} on the mechanical response of guinea-pig taenia coli in Ca^{2+}-free, K^+ (60 mM) medium. A. The muscles were suspended in Ca^{2+}-free medium for 30 min, with several changes of medium, then Mn^{2+} (5 mM) was added: Wo, wash-out; K, 60 mM K^+. B. The responses after addition of Mn^{2+} (5 mM) to the Ca^{2+}-free, K^+ (60 mM) medium, expressed as percentages of the tonic tension developed 30 min after addition of hypertonic 60 mM K^+ to normal Ca^{2+} (2·5 mM) medium. Each point represents the mean ± s.e.m. of results from eight experiments.

(Figure 1). The development of tension induced by 5 mM Mn^{2+} in the Ca^{2+}-free, K^+ medium 3 h after appearance of the response reached 94·8 ± 18·7% (n = 8) of the original K^+ (60 mM)-induced tonic levels in normal Ca^{2+} (2·5 mM) medium.

Effects of Mn^{2+} on the response of taenia coli in the presence of Bay K 8644

Bay K 8644 alone (10^{-6} M) induced a strong rhythmic contraction in normal Ca^{2+} medium; tonic contracture with cessation of the rhythmic reactivity developed 5·6 ± 0·9 h (n = 8) after addition of the Ca^{2+} agonist (Figure 2).

When 5 mM Mn^{2+} was added after 30 min of the rhythmic contraction evoked by 10^{-6} M Bay K 8644, the rhythmic response was inhibited to baseline. The suppressed response persisted for more than 2 h. Thereafter, the muscle progressively contracted until 5 h after the reappearance of tone,

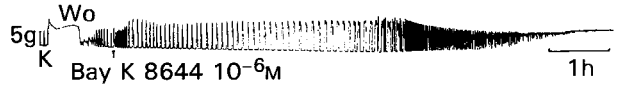


Figure 2. The effect of Bay K 8644 on the response of taenia coli in normal Ca^{2+} (2·5 mM) medium. The contractile responses evoked by 10^{-6} M Bay K 8644 were apparent for much longer than 9 h.

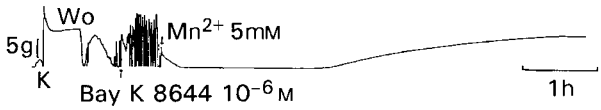


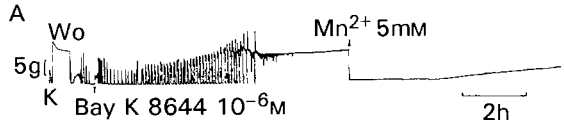
Figure 3. Effects of Mn^{2+} on rhythmic contraction evoked by Bay K 8644 in normal Ca^{2+} medium. Mn^{2+} (5 mM) was added 30 min after the start of the rhythmic contraction induced by 10^{-6} M Bay K 8644.

and the response induced by 5 mM Mn^{2+} in the presence of 10^{-6} M Bay K 8644 reached 87·8 ± 13·1% (n = 8) of the original K^+-induced tonic levels (Figure 3).

When 5 mM Mn^{2+} was added to the state of contracture evoked by 10^{-6} or 10^{-5} M Bay K 8644, the contractures were completely inhibited to baseline and thereafter tension also developed progressively (Figure 4).

In Ca^{2+}-free medium Bay K 8644 alone did not induce contraction in taenia coli. However, Mn^{2+} (5 mM) induced the contraction in Ca^{2+}-free medium, depending on the concentration of Bay K 8644 present (Figure 5).

The quick release method was used as a measure of a series of values of elastic stiffness during Mn^{2+}-induced contraction in the presence of Bay K 8644 in Ca^{2+}-free medium. The length (1·5 cm) of



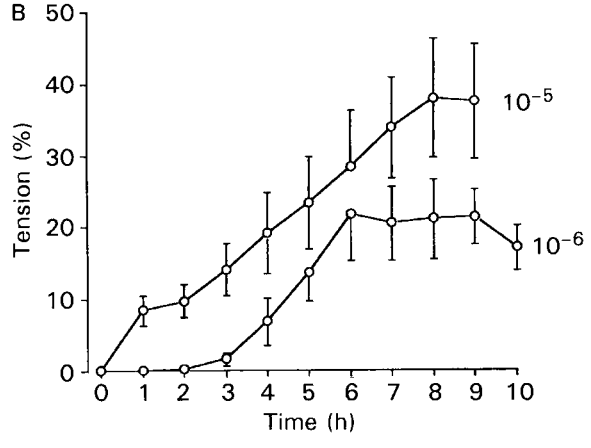
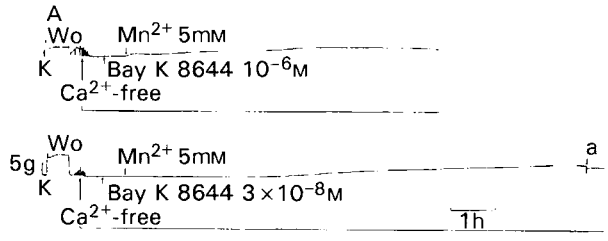


Figure 4. Effects of Mn^{2+} on the state of contracture evoked by Bay K 8644 in normal Ca^{2+} medium. A. Mn^{2+} (5 mM) was added 8 h after the start of contracture induced by addition of 10^{-6} M Bay K 8644. B. The responses obtained after addition of 5 mM Mn^{2+}, expressed as percentages of the tonic tension developed 30 min after addition of hypertonic 60 mM K^+ to normal Ca^{2+} medium. The number accompanying each plot indicates the concentration (M) of Bay K 8644. Each point represents the mean ± s.e.m. of results from eight experiments.



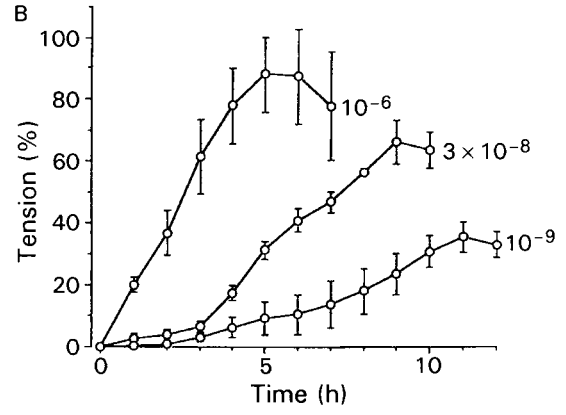


Figure 5. Effects of Mn^{2+} on response in the presence of Bay K 8644 in Ca^{2+}-free medium. A. Mn^{2+} (5 mM) was added after suspension for 30 min in Ca^{2+}-free medium containing 10^{-6} or 3×10^{-8} M Bay K 8644. At point 'a', 11 h after application of Mn^{2+}, the length (1·5 cm) of the taenia coli strip was rapidly reduced by 1 mm. B. The responses after addition of Mn^{2+} (5 mM) to Ca^{2+}-free medium containing Bay K 8644, expressed as percentages of the tonic tension developed 30 min after addition of hypertonic 60 mM K^+ to normal Ca^{2+} medium. The number accompanying each curve indicates the concentration (M) of Bay K 8644. Each point represents the mean ± s.e.m. of results from eight experiments.

the taenia coli strip was rapidly reduced by 1 mm 11 h after application of 5 mM Mn^{2+} in the presence of 3×10^{-8} M Bay K 8644 in a Ca^{2+}-free medium. After shortening of the strip, the tension rapidly decreased; this was followed by an initial fast and then subsequently slow re-development of tension during a 30-min period (Figure 5).

Manganese uptake in the presence of Bay K 8644

The amount of manganese in tissue immersed in normal Ca^{2+} medium in the absence of Bay K 8644 was 0·15 ± 0·01 mmol kg^{-1} wet weight (n = 12) 5 h after application of 5 mM Mn^{2+}. After pretreatment with 10^{-6} M Bay 8644 for 30 min in normal Ca^{2+} medium, the tissue manganese level 5 h after application of 5 mM Mn^{2+} increased to 0·92 ± 0·11 (n = 8) mmol kg^{-1} wet weight.

In Ca^{2+}-free medium in the presence of 5 mM Mn^{2+} the increase in manganese uptake which accompanied the development of tension (compared with the control values in the absence of Bay K 8644) depended on the concentration of Bay K

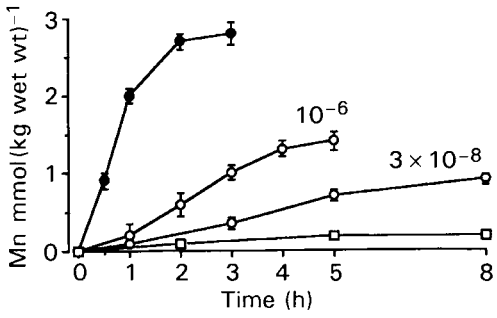


Figure 6. Manganese uptake by taenia coli. Mn^{2+} (5 mM) was added to Ca^{2+}-free medium (□) or to Ca^{2+}-free medium 30 min after addition of Bay K 8644 (○) or 60 mM K^+ (●). The number accompanying each curve indicates the concentration (M) of Bay K 8644. Each point represents the mean ± s.e.m. of results from eight experiments.

8644 used for pretreatment. However, the rate and extent of manganese uptake after pretreatment with Bay K 8644 was lower than in the K^+ (60 mM) medium (Figure 6).

Discussion

In this study, Bay K 8644 (10^{-6} M) induced rhythmic contraction and, after approximately 5·5 h, the development of contracture in taenia coli in normal Ca^{2+} medium. The contracture evoked in taenia coli by 10^{-6} M Bay K 8644 could be reduced by low concentrations (1×10^{-7} M) of nifedipine, a dihydropyridine Ca^{2+} antagonist, whereas reduction of the first rhythmic contraction required much higher concentrations (Nasu et al 1997). Thus, in taenia coli the extent of the contracture, relative to the rhythmic contraction induced by Bay K 8644 strongly depended on the extracellular Ca^{2+} level. It has been shown that Bay K 8644 had no effect on Ca^{2+}-induced contraction in skinned artery (Kanmura et al 1984), suggesting that Bay K 8644 has no effect on contractile proteins.

The rhythmic contraction and the contrature induced in taenia coli by Bay K 8644 in normal Ca^{2+} medium were both completely inhibited by 5 mM Mn^{2+}, presumably because Mn^{2+} first reduces the Bay K 8644-induced Ca^{2+} influx at the cell membranes. Thereafter, Mn^{2+} uptake from the medium was higher in the presence of Bay K 8644 than in its absence.

In Ca^{2+}-free medium Mn^{2+} also increased the development of tension in taenia coli to an extent depending on the concentration of Bay K 8644. Furthermore, nifedipine dose-dependently inhibited both the development of tension and the manganese uptake induced in taenia coli, by Mn^{2+}, in the presence of Bay K 8644 in Ca^{2+}-free medium (Nasu & Sasaki 1998). It is probable that Mn^{2+} is progressively accumulated in the cytoplasm which EDTA cannot reach, through voltage-dependent Ca^{2+} channels activated by Bay K 8644; in the cytoplasm it induces contraction.

When 5 mM Mn^{2+} was added to the Ca^{2+}-free medium in the absence of Bay K 8644 or K^+, development of tension was not observed, although a very small amount of manganese uptake was measured. This might indicate that Mn^{2+} enters the restricted cytoplasmic space near the inner surface of the cell membrane through a leaky pathway and that Mn^{2+} entering in this manner does not induce contraction. In addition, 5 mM Mn^{2+} did not induce ileal contraction in the presence of carbachol (5×10^{-7} M) or histamine (5×10^{-6} M) (Nasu et al 1994) which had smaller effects on membrane depolarization (Bülbring & Burnstock 1960).

The Mn^{2+}-induced maximum contraction in the presence of 1×10^{-6} M Bay K 8644 in Ca^{2+}-free medium was comparable with that in Ca^{2+}-free, 60 mM K^+ medium. However, during Mn^{2+}-induced maximum contractions, the uptake of manganese in the presence of Bay K 8644 was lower than occurred with the 60 mM K^+ medium. These results suggest that Bay K 8644 increases the Mn^{2+}-sensitivity of contractile elements.

Incidentally, it has been shown that Mn^{2+} directly induces contractions in skinned fibres of smooth muscles (Savineau et al 1988). Quick-release experiments indicate that Mn^{2+}-induced contraction in the presence of Bay K 8644 in Ca^{2+}-free medium is a result of active interaction between actin and myosin.

In conclusion, we have shown that in the taenia coli of guinea-pig during the prolongation of opening time of voltage-dependent Ca^{2+} channels activated by Bay K 8644, Mn^{2+} can enter the cytoplasm through the channels and induce contraction. It is also possible that Bay K 8644 increases the Mn^{2+}-sensitivity of contractile elements.

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