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The specific effect of Mn^{2+} on the tonic components of receptor-mediated contractions in isolated vas deferens of the guinea pig

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Abstract

Intracellular accumulation of Mn^{2+} augmented the contractions induced by norepinephrine and acetylcholine in the guinea pig isolated vas deferens. Contractions repeatedly induced by norepinephrine, acetylcholine, or a high concentration of K^+ decreased depending on the incubation time in Ca^{2+}-free medium. The rate of decrease of all contractions was delayed by intracellularly accumulated manganese. In the Mn^{2+}-loaded preparations, the tonic components of the contractions induced by norepinephrine and acetylcholine, but not K^+, were highly resistant to extracellular Ca^{2+} elimination. Ryanodine abolished the initial phasic component but did not affect the tonic component of norepinephrine- and acetylcholine-contractions in Mn^{2+}-loaded preparations in Ca^{2+}-free medium. In Ca^{2+}-depleted preparations, the tonic contraction induced by norepinephrine was restored after the Mn^{2+}-loading procedure, and the magnitude of this tonic contraction was comparable to the tonic component of the norepinephrine contraction in the normal medium before Mn^{2+} loading. The tonic contraction was reproducible in medium without either Mn^{2+} or Ca^{2+}. These results suggested that intracellular Mn^{2+} can support norepinephrine-induced tonic contractions. In the Ca^{2+}-depleted Mn^{2+}-loaded preparations, K^+ also induced a tonic contraction in the presence of extracellular Mn^{2+}. However, this contraction was much smaller than that induced by norepinephrine. These results suggested that intracellular Mn^{2+} augmented contractions not only via an increase in intracellular Ca^{2+} availability but also via the direct action of Mn^{2+} on contractile mechanisms, and that this action is highly specific for developing and/or maintaining tonic contractions mediated by receptor activation in the guinea pig isolated vas deferens.

Keywords: Vas deferens, guinea pig; Mn^{2+}; Contraction; Norepinephrine; Acetylcholine

1. Introduction

Extracellular Mn^{2+} inhibits Ca^{2+} influx through cell membranes and therefore inhibits contractions of smooth muscle (Nonomura et al., 1966; Anderson et al., 1971; Shimodan and Sunano, 1981). However, under some conditions, Mn^{2+} initiates or accelerates mechanical responses in various smooth muscles and the myocardium (Shibata, 1969; Osa, 1974; Delahayes, 1975; Sakai and Uchida, 1981). In the guinea pig vas deferens, Mn^{2+} enters smooth muscle cells through L-type voltage-dependent Ca^{2+} channels activated by a high concentration of K^+, norepinephrine or acetylcholine, and accumulates in the cells (Tsunobuchi and Gomi, 1990b). The most potent stimulant of manganese accumulation is K^+. Intracellular manganese augmented contractions induced by these stimulants in a concentration-dependent manner (Ushijima and Gomi, 1991, 1995). Mn^{2+} enhances or supports contractions (Itoh et al., 1982; Sakai et al., 1983; Jamieson et al., 1983) and we suggested that this is due to the increased availability of intracellular Ca^{2+} caused by the antagonizing effects of intracellular Mn^{2+} on Ca^{2+} extrusion and/or Ca^{2+} sequestration to the sarcoplasmic reticulum (Ushijima and Gomi, 1991). However, the possibility that Mn^{2+} directly activates contractile proteins could not be excluded (Milanov and Stoyanov, 1982; Sunano, 1984; Hoar and Kerrick, 1988; Lategan and Brading, 1988). In this study, we examined the effects of Ca^{2+} depletion on contractions in the Mn^{2+}-loaded guinea pig vas deferens to evaluate

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the contribution of Ca^{2+} to the effect of Mn^{2+}, and to evaluate the direct action of Mn^{2+} on the contractile mechanisms.

2. Materials and methods

Male guinea pigs of the Hartley strain weighing 350–550 g were killed by a blow to the neck. The vasa deferentia were excised and the serous membrane was stripped away. A strip of 8–10 mm in length was prepared from the epididymal portion of the vas deferens. Each preparation was suspended under a 0.3 g load in a 10 ml organ bath containing aerated Hepes-Locke-Ringer solution (normal medium) of the following composition (mM): NaCl 154; KCl 5.6; $MgCl_2$ 2.1; $CaCl_2$ 2.2; glucose 2.8; Hepes 4.4, adjusted to pH 7.4 at 30°C with 1 N NaOH. For Ca^{2+}-free medium, $CaCl_2$ was omitted from the normal medium and Na_2EGTA was added at a final concentration of 0.2 mM. Isotonic high-K^+ medium (100 mM K^+) was prepared by replacing 94.4 mM NaCl in the normal or the Ca^{2+}-free medium with an equimolar concentration of KCl. Isotonic contractions were recorded at a 12.5-fold magnification using an isotonic transducer (Nihon Kohden, model TD-112S).

After equilibration in normal medium for 1 h, 10 μM norepinephrine, 10 μM acetylcholine or 100 mM K^+ was applied to elicit a control contraction. The magnitudes of the phasic and tonic components of these contractions, except that induced by high K^+, were 100 and about 90% of the corresponding components of the maximal contractions induced by 100 μM of each agonist. K^+ at 100 mM induced the maximal contraction. Unless otherwise stated, to load Mn^{2+} in the smooth muscle cells, the preparations were exposed to 2.1 mM Mn^{2+} for 135 min. During this period, 100 mM K^+ was applied for 5 min every 15 min, and the K^+ with Mn^{2+} were applied 9 times. In the experiments described in Section 3.3 (Results), the exposure time to Mn^{2+} was shortened from 135 min to 90 min to load less Mn^{2+}. For studies using Ca^{2+}-depleted preparations (Section 3.5), the exposure time to Mn^{2+} was also 90 min with 6 applications of K^+. In spite of the shorter exposure time, the manganese content of these preparations was more than that of the preparations exposed to Mn^{2+} for 135 min with extracellular Ca^{2+}, since manganese accumulation is accelerated in Ca^{2+}-free medium (Tsunobuchi and Gomi, 1990b). These repeated applications of K^+ in the presence of Mn^{2+} are designated as the Mn^{2+}-loading procedure. Preparations in which Mn^{2+} was accumulated through the Mn^{2+}-loading procedure are termed the Mn^{2+}-loaded preparations.

To determine the effects of extracellular Ca^{2+} removal, the preparations were incubated in Ca^{2+}-free medium for various periods (1–60 min). An agonist was added for 5 or 10 min, and then the preparations were incubated in normal medium for 25 min. The incubation time in the Ca^{2+}-free medium was increased stepwise in the same preparation.

In some experiments, the manganese content of the preparation was measured using an atomic absorption spectrophotometer (z-8000, Hitachi, Tokyo, Japan). After recording the mechanical responses, the preparations were washed 4 times with Ca^{2+}-free medium. Each preparation was then blotted, weighed and dissolved in 1 ml HNO_3 for 72 h at room temperature. The sample was diluted with distilled water to measure the manganese content.

To measure ^{45}Ca tissue spaces (either total or residual after incubation in Ca^{2+}-free medium), preparations were incubated in normal medium containing $^{45}Ca^{2+}$ (2 μCi/ml) for 195 min. During this period, the preparations were exposed to K^+ in the presence or absence of Mn^{2+} as described above. After the incubation with $^{45}Ca^{2+}$, the preparations were washed in Ca^{2+}-free medium for 5 s, 15 min or 60 min, then blotted, weighed and solubilized with Protozol. Radioactivity was measured by means of scintillation spectrometry.

Results are expressed as mean values ± S.E.M. and Student's paired and unpaired t-tests were used for statistical analysis. $P < 0.05$ was considered to be significant.

The drugs applied were: (−)-norepinephrine bitartrate (Sigma Chemical Co., St. Louis, MO, USA), acetylcholine chloride (Daiichi Pharmaceutical Co., Tokyo, Japan), manganese chloride (Nacalai Tesque, Kyoto, Japan), ryanodine and caffeine (Wako Pure Chemical Industries, Osaka, Japan), ^{45}Ca and Protozol (E.I. Du Pont de Nemours Co., Boston, MA, USA). Norepinephrine was dissolved in 0.1 N HCl and diluted with 0.1% $NaHSO_3$. Acetylcholine and $MnCl_2$ were dissolved in de-ionized and distilled water. Ryanodine was dissolved in ethanol. The final concentration of ethanol in the bathing medium was 0.3%. At this concentration, the vehicle did not affect the contractions.

3. Results

3.1. Effects of the Mn^{2+}-loading procedure on the contractions induced by KCl, norepinephrine and acetylcholine

The K^+-induced contraction was composed of phasic and tonic components, whereas norepinephrine (10 μM) and acetylcholine (10 μM) induced a small transient contraction (initial phasic component) followed by a large transient (2nd phasic component) and a

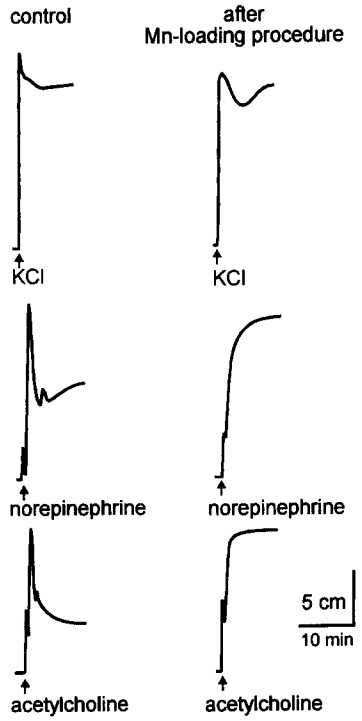

Fig. 1. The effects of intracellularly accumulated manganese on the contractions induced in the isolated vas deferens of the guinea pig. The left column shows the control contractions induced by 100 mM K^+ (upper), 10 μM norepinephrine (middle) and 10 μM acetylcholine before the Mn^{2+}-loading procedure. The right column shows the corresponding contractions induced in the Mn^{2+}-loaded preparations in the normal medium.

sustained contraction (tonic component) (Fig. 1). After the Mn^{2+}-loading procedure, the 2nd phasic components of both norepinephrine- and acetylcholine-induced contractions disappeared while the phasic component of the K^+-induced one remained, although it was decreased to $87.8 \pm 7.8\%$ ($n = 8$, Fig. 1). The tonic component of the K^+-induced contraction was not affected. The initial phasic and tonic components of norepinephrine-induced contractions in the Mn^{2+}-loaded preparations were augmented to $145.9 \pm 21.5\%$ and $186.2 \pm 16.1\%$ ($n = 7$) respectively of the corresponding components before Mn^{2+} loading. These two components of acetylcholine-induced contractions were also augmented after Mn^{2+} loading to $111.0 \pm 15.0\%$ and $291.0 \pm 3.8\%$ ($n = 3$), respectively. The manganese content of these Mn^{2+}-loaded preparations was 3.77 ± 0.23 mmol/kg wet weight of tissue ($n = 8$).

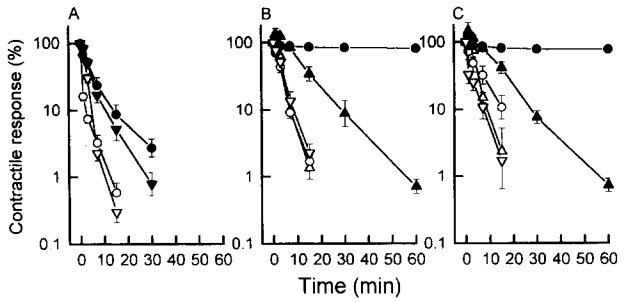

Fig. 2. The effects of eliminating extracellular Ca^{2+} on the contractions repeatedly induced by 100 mM K^+ (A), 10 μM norepinephrine (B) and 10 μM acetylcholine (C) in Ca^{2+}-free medium. Unfilled and filled symbols show the contractions induced in the Mn^{2+}-non-loaded and loaded preparations, respectively. (△ ▲) initial phasic component; (▽ ▼) phasic (A) or 2nd phasic (B, C) component; (○●) tonic component. These components of all the contractions are expressed as percentages of the corresponding components of the contractions induced in the normal medium. The abscissa represents the incubation time (min) in the Ca^{2+}-free medium. Vertical bars show S.E.M. (A: $n = 8$, B: $n = 7$, C: $n = 3$).

3.2. Effects of extracellular Ca^{2+} elimination on the contractions induced by KCl, norepinephrine and acetylcholine

In the normal preparations, evoked contractions were reduced depending on the incubation time in Ca^{2+}-free medium (Fig. 2). A 15-min incubation in Ca^{2+}-free medium abolished or markedly reduced the contractions induced by all agonists to less than 10%. The rate of decrease of the K^+-induced contraction was faster than that induced by norepinephrine and acetylcholine. There was no difference among the components in each contraction.

In the Mn^{2+}-loaded preparations, the rates of decrease of contractions in Ca^{2+}-free medium were significantly slower than those in the normal preparations (Fig. 2). The K^+-induced contractions were reduced to less than 5% after a 30-min incubation in Ca^{2+}-free medium. The manganese content of these preparations

Table 1
The effects of Mn^{2+} loading on the calcium content of the guinea pig isolated vas deferens incubated in Ca^{2+}-free medium

	Calcium content (μmol/kg wet weight of tissue)		
Incubation time (min)	0	15	60
Control	1020.0 ± 103.1	479.1 ± 44.3	188.5 ± 9.8
Mn^{2+} loading	850.5 ± 66.9 [a]	374.6 ± 54.1 [a]	202.9 ± 22.9

Values are means ± S.E.M. ($n = 5$). Significance of difference between the average calcium content in the control preparations and that in the Mn^{2+}-loaded preparations was determined using Student's unpaired t-test ([a] $P < 0.05$).

was 3.47 ± 0.14 mmol/kg wet weight of tissue ($n = 8$). This was not significantly different from the Mn^{2+} content before Ca^{2+} elimination. The initial phasic components of norepinephrine- and acetylcholine-induced contractions were also slowly reduced, but the tonic components of the contractions induced by the two agonists were remarkably resistant to Ca^{2+} elimination. After a 60-min incubation in Ca^{2+}-free medium, the magnitude of norepinephrine- and acetylcholine-induced tonic components was about 80% of those before the elimination of extracellular Ca^{2+}, whereas the initial phasic components were abolished (Fig. 2B and C). The manganese content of these preparations was 3.07 ± 0.09 mmol/kg wet weight of tissue ($n = 12$), which was slightly lower than the content before the Ca^{2+} elimination.

As shown in Table 1, the calcium content of the tissue was significantly reduced after Mn^{2+} loading. The calcium contents in Mn^{2+}-loaded and non-loaded preparations were also decreased with the elimination of extracellular Ca^{2+}. They fell to the same level after a 60-min incubation in Ca^{2+}-free medium.

3.3. Effects of extracellular Mn^{2+} on the norepinephrine-induced contraction of Mn^{2+}-loaded preparations in Ca^{2+}-free medium

When the exposure to Mn^{2+} was shortened from 135 min to 90 min with 6 applications of K^+ during Mn^{2+} loading in normal medium, the increase in manganese content and potentiation of the tonic component of the norepinephrine-induced contraction was significantly less (cf. Section 3.1). The manganese content of these preparations was 2.41 ± 0.07 mmol/kg wet weight of tissue ($n = 6$). The initial phasic and tonic components of the norepinephrine-induced contractions were augmented to 152.5 ± 13.6% and 129.0 ± 5.1% ($n = 12$) respectively of the corresponding components before Mn^{2+} loading. Furthermore, when the Mn^{2+}-loading time was shorter, the magnitude of the tonic component resistant to Ca^{2+} elimination was also less. The initial phasic component of the norepinephrine-induced contraction was abolished by a 40-min incubation in Ca^{2+}-free medium, while the tonic component was 54.1 ± 4.3% ($n = 12$) of the augmented tonic component before Ca^{2+} elimination (Fig. 3).

When these preparations loaded with less Mn^{2+} were stimulated by norepinephrine every 40 min in Ca^{2+}-free medium, the tonic component of the norepinephrine-induced contraction remained even after a 6-h incubation in Ca^{2+}-free medium, although the magnitude was significantly decreased throughout the incubation period. When 2.1 mM Mn^{2+} was applied during resting periods to prevent leakage of intracellular Mn^{2+} after the 1st contraction in Ca^{2+}-free medium to the end of the experiment, the decrease

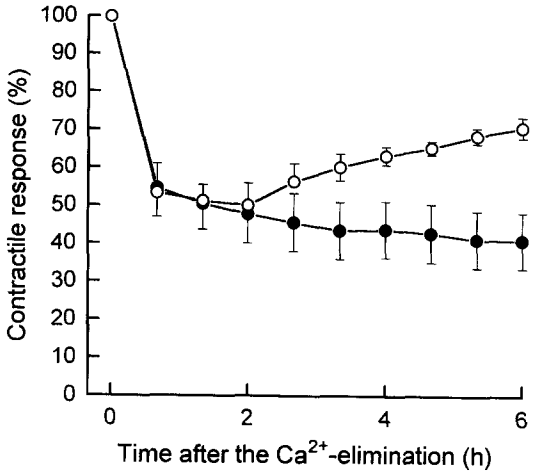

Fig. 3. The effects of extracellular Mn^{2+} on the tonic contractions of Mn^{2+}-loaded preparations repeatedly induced by 10 μM norepinephrine in Ca^{2+}-free medium. After Mn^{2+} loading for 90 min, norepinephrine was applied for 10 min every 40 min. (●) control; (○) after the 1st contraction induced in Ca^{2+}-free medium, Mn^{2+} (2.1 mM) was applied to the medium to prevent leakage of intracellular Mn^{2+}. The applied Mn^{2+} remained in the medium during the resting periods throughout this experiment to avoid accelerating Mn^{2+} uptake by norepinephrine. Each tonic contraction is expressed as a percentage of the tonic component of the contraction in the Mn^{2+}-loaded preparations before Ca^{2+} elimination. The abscissa represents the incubation time (h) in Ca^{2+}-free medium. Vertical bars show S.E.M. ((●) $n = 8$; (○) $n = 8$).

was reversed and the norepinephrine-induced contractions were augmented with each repeated stimulation (Fig. 3). The manganese contents after the 6-h incubation in Ca^{2+}-free medium with and without extracellular Mn^{2+} were 1.81 ± 0.15 ($n = 6$) and 1.18 ± 0.12 mmol/kg wet weight of tissue ($n = 6$).

3.4. Effects of ryanodine and caffeine on the contractions induced by norepinephrine

Caffeine (50 mM) induced a small twitch contraction followed by a slight relaxation and did not affect the following norepinephrine-induced contraction in the Mn^{2+}-non-loaded preparations (Fig. 4A). After 3 min of exposure to ryanodine (3 μM) plus caffeine, without which ryanodine had no effect in the guinea pig vas deferens, the development of the 2nd phasic component of the norepinephrine-induced contraction was delayed, and the initial phasic and tonic components were reduced to 61.8 ± 13.4% and 60.2 ± 4.3% of each component of the contraction before exposure to ryanodine ($n = 12$, Fig. 4B).

Similar to the norepinephrine-induced contractions, caffeine-induced twitch contractions were remarkably potentiated after Mn^{2+} loading. In the Mn^{2+}-loaded preparations, caffeine induced a twitch contraction after a 7-min incubation in Ca^{2+}-free medium, although the magnitude was 44.0 ± 4.9% of the caffeine-induced

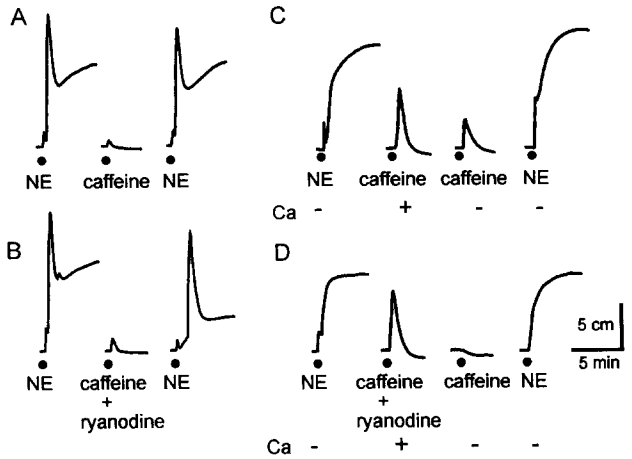

Fig. 4. The effects of 3 μM ryanodine on the contractions induced by 10 μM norepinephrine (NE) and 50 mM caffeine. Caffeine was applied for 3 min hypertonically. Ryanodine was applied simultaneously with caffeine for 3 min (B, D). Contractions were evoked every 40 min. A and B: Traces of contractions in the Mn^{2+}-non-loaded preparations in the presence of extracellular Ca^{2+}. C and D: Traces of contractions in the Mn^{2+}-loaded preparations in the presence (+) and absence (−) of extracellular Ca^{2+}. When the contractions induced without extracellular Ca^{2+} were recorded, the preparations were incubated in Ca^{2+}-free medium for 7 min. Norepinephrine or caffeine was then added. After washout of the stimulants, the preparations were incubated in normal medium for a further 25 min. A–D were from different preparations.

contraction in normal medium ($n = 6$, Fig. 4C). Ryanodine applied with caffeine had little effect on the tonic component of the norepinephrine-induced contraction in Ca^{2+}-free medium, but abolished both the caffeine-induced contraction and the initial phasic component of the norepinephrine-induced contraction in Ca^{2+}-free medium (Fig. 4D). The effects of ryanodine on acetylcholine- and norepinephrine-induced contractions in the Mn^{2+}-loaded preparations were the same (data not shown).

3.5. Mn^{2+}-dependent contractions induced in the Ca^{2+}-depleted preparations

After incubation of preparations in Ca^{2+}-free medium for 40 min, norepinephrine (10 μM) and high K^+ (100 mM) induced no contractions. In the Ca^{2+}-depleted preparations, high K^+ induced a small tonic contraction in the presence of extracellular Mn^{2+} (2.1 mM). This contraction gradually increased with repeated stimulation, although the magnitude of the 6th K^+-induced contraction, which was the end of the Mn^{2+} loading procedure in Ca^{2+}-free medium, was not more than 20% of the tonic component of the norepinephrine-induced contraction in normal medium before Mn^{2+}-loading (Fig. 5). The manganese content of these Ca^{2+}-depleted preparations was 4.36 ± 0.27 mmol/kg wet weight of tissue ($n = 6$). In these Ca^{2+}-depleted Mn^{2+}-loaded preparations, norepinephrine induced tonic contractions, but not the initial and 2nd phasic components in Ca^{2+}-free medium without extracellular Mn^{2+} (Fig. 5). The magnitude of the tonic contractions was $102.3 \pm 3.2\%$ ($n = 14$) of the tonic component of the norepinephrine-induced contraction in the normal medium before Mn^{2+} loading. These tonic contractions returned to the resting level within a few minutes after washout of the norepinephrine. This was similar to the norepinephrine-induced contractions in normal medium before Mn^{2+} loading. The norepinephrine-induced tonic contraction was reproducible at least 6 times without either Ca^{2+} or Mn^{2+} in the medium, when it was evoked every 30 min.

4. Discussion

In the guinea pig isolated vas deferens, the contractions induced by norepinephrine (10 μM), acetyl-

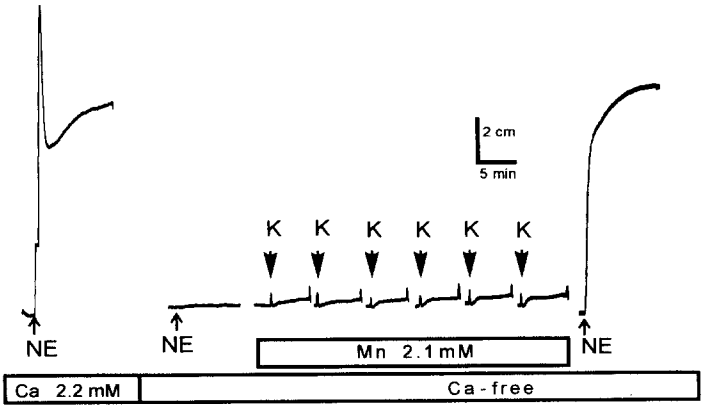

Fig. 5. Mn^{2+}-dependent tonic contractions induced by 100 mM K^+ (K) and 10 μM norepinephrine (NE) in the Ca^{2+}-depleted preparations. K^+ was applied for 5 min every 15 min in the presence of extracellular Mn^{2+} after the depletion of Ca^{2+}. Peaks recorded at both the start and the end of the K^+-induced contractions were artifacts caused by changing the medium. Norepinephrine was applied for 10 min.

choline (10 μM) and high K^+ (100 mM) were highly dependent on extracellular Ca^{2+} and were abolished within 20 min after removal of Ca^{2+} from the medium. However, in the Mn^{2+}-loaded preparations, the rates of decrease of these contractions in a Ca^{2+}-free medium were much slower. It is notable that the tonic components of the norepinephrine- and acetylcholine-, but not the high-K^+-induced contractions, were extremely resistant to Ca^{2+} removal in the Mn^{2+}-loaded preparations. When Mn^{2+} loading lasted 135 min, the tonic components remained at about 80% of the control contraction even after a 60-min incubation in Ca^{2+}-free medium while the tonic component of the K^+-induced contraction was abolished. These results suggested that receptor-activating stimulants induced and/or maintained tonic components through different mechanisms from those responsible for the tonic components of the K^+-induced contractions.

When the preparations loaded with less manganese, by shortening exposure time to Mn^{2+}, were stimulated by norepinephrine, the augmented tonic component in normal medium and the remaining tonic component in Ca^{2+}-free medium were smaller than those in the preparations loaded with more manganese. The elimination of extracellular Ca^{2+} abolished the initial phasic component and remarkably reduced the tonic component in these preparations, which suggested that Ca^{2+} contributed to the augmented contractions. However, the tonic component in these preparations could be produced repeatedly during the 6-h incubation in Ca^{2+}-free medium, though its magnitude was gradually reduced. This time-dependent decrease in the tonic component was accompanied by a reduction in the manganese content. Extracellular Mn^{2+} reversed the decrease of the contraction and inhibited the manganese reduction. Thus, the magnitude of the Ca^{2+} removal-resistant tonic component was dependent on the manganese level in the preparations.

We suggested that, in the guinea pig vas deferens, intracellularly accumulated manganese increased the cytoplasmic Ca^{2+} concentration by inhibiting the sequestration and/or extrusion of the intracellular Ca^{2+} and augmented the contractions in a concentration-dependent manner (Ushijima and Gomi, 1991). As shown here, the transient contraction induced by caffeine, which releases Ca^{2+} from the sarcoplasmic reticulum, was noticeably augmented after Mn^{2+} loading. The augmentation was decreased in Ca^{2+}-free medium and was abolished by ryanodine. These results provided evidence that Ca^{2+} released from intracellular stores mediates the augmenting effect of Mn^{2+}. In the Mn^{2+}-non-loaded vas deferens of the guinea pig, the initial phasic components of norepinephrine- and acetylcholine-induced contractions were dependent on the Ca^{2+} released from the ryanodine-sensitive Ca^{2+} store (Tsunobuchi and Gomi, 1990a). Similar to the caffeine-induced contractions, the augmented initial phasic components of these receptor-mediated contractions were abolished by ryanodine and by incubation in Ca^{2+}-free medium. Thus, it was confirmed that intracellularly accumulated manganese augmented the initial phasic components by increasing the availability of Ca^{2+} released from the ryanodine-sensitive Ca^{2+} store.

In contrast to the initial phasic components, the augmented tonic components remaining in Ca^{2+}-free medium were insensitive to ryanodine. Together with this fact, the pronounced resistance to removal of extracellular Ca^{2+} of the tonic components of the receptor-mediated contractions indicated that the augmenting mechanism of Mn^{2+} for the tonic component may be different from that for the initial phasic component. After the disappearance of norepinephrine-induced contractions in Ca^{2+}-free medium, Mn^{2+} loading restored norepinephrine-induced tonic contractions without an initial phasic component in the absence of extracellular Ca^{2+} and Mn^{2+}. This tonic contraction was reproducible in Ca^{2+}-free medium for several hours, and the magnitude was comparable to that of the tonic component of the norepinephrine-induced contraction of the non-loaded preparation in normal medium. These results also indicated that Mn^{2+} can support the activation of the contractile apparatus.

Although K^+ is the most potent stimulator of Mn^{2+} influx though L-type Ca^{2+} channels (Tsunobuchi and Gomi, 1990b), repetitive application of high K^+ during Mn^{2+} loading of Ca^{2+}-depleted preparations could not induce a contraction like that induced by norepinephrine. Thus, Mn^{2+} may preferentially interact with a receptor-linked contractile system. In this study, high K^+ was added to Ca^{2+}-free medium containing Mn^{2+}. Under these conditions, the amount of neurotransmitters released by high K^+ is considerably lower than that in normal medium (Drapeau and Nachshen, 1984). Therefore, if the K^+-induced contraction was mediated by the released neurotransmitters in Ca^{2+}-free medium containing Mn^{2+}, the magnitude would not conflict with the above notion of Mn^{2+} specificity. The possibility however remains that intracellular Mn^{2+} increased the availability of Ca^{2+} released by norepinephrine from ryanodine-insensitive Ca^{2+} stores that were not depleted. If so, sufficient Ca^{2+} would have to be repeatedly released to produce a tonic component with a magnitude similar to that of the control contraction. To account for the ability of Mn^{2+} to sustain this contraction, Mn^{2+} would also have to completely inhibit all of the normal Ca^{2+}-buffering and Ca^{2+}-extrusion mechanisms. However, the contractions induced in the Mn^{2+}-loaded preparations were restored completely by eliminating the stimulant as fast as those in the Mn^{2+}-non-loaded ones. In addition, the initial phasic components and high-K^+-induced con-

tractions in the Mn^{2+}-loaded preparations decreased depending on the incubation time in Ca^{2+}-free medium. The residual ^{45}Ca in both the Mn^{2+}-loaded and non-loaded preparations was reduced in Ca^{2+}-free medium. These results suggest that the buffering or extrusion processes are not completely inhibited by intracellular Mn^{2+}. Therefore, it is likely that Mn^{2+} can support the activation of the contractile apparatus and sustain the contractions when receptor-mediated signal pathways are activated. Although Mn^{2+} can activate calmodulin and substitute for Ca^{2+} in the calmodulin signaling system (for review, see Raos, 1990; Savineau et al., 1988), the selectivity of Mn^{2+} for receptor-mediated signal pathways cannot be explained by simple substitution in the activation of the calmodulin-myosin light chain kinase system. The pathway affected by Mn^{2+} remains to be clarified.

In conclusion, this study confirmed that the increase in Ca^{2+} availability is partly responsible for the augmenting effect of intracellular Mn^{2+}. The results also suggested that Mn^{2+} contributes to contractile mechanisms, independently of Ca^{2+}. The most important finding in this study was that Mn^{2+} may support receptor-mediated contractions more preferentially than those induced by high K^+ in the guinea pig isolated vas deferens. This suggests that receptor-activating stimulants induce tonic components through different mechanisms from those activated by K^+. The primary site of the action of intracellular Mn^{2+} seems to be specific for developing and/or maintaining tonic contractions by receptor-activating stimulants. Clarifying the mechanism by which Mn^{2+} can activate smooth muscle may provide useful insight into the regulation of smooth muscle contraction.

References

Anderson, N.C., F. Ramon and A. Snyder, 1971, Studies on calcium and sodium in uterine smooth muscle excitation under current clamp and voltage clamp conditions, J. Gen. Physiol. 58, 322.

Delahayes, J.F., 1975, Depolarization-induced movement of Mn^{2+} across the cell membrane in the guinea pig myocardium. Its effect on the mechanical response, Circ. Res. 36, 713.

Drapeau, P. and D.A. Nachshen, 1984, Manganese fluxes and manganese-dependent neurotransmitter release in presynaptic nerve endings isolated from rat brain, J. Physiol. 348, 493.

Hoar, P.E. and W.G.L. Kerrick, 1988, Mn^{2+} activates skinned smooth muscle cells in the absence of myosin light chain phosphorylation, Pflüg. Arch. 412, 225.

Itoh, T., H. Kuriyama and T. Nanjo, 1982, Effects of calcium and manganese ions on mechanical properties of intact and skinned muscles from the guinea-pig stomach, J. Physiol. 333, 555.

Jamieson, D.D., R.J. Quinn and A. Le Coutier, 1983, Antagonism by manganese of isoprenaline dilatation of the guinea-pig isolated trachea, Clin. Exp. Pharmacol. Physiol. 10, 511.

Lategan, T.W. and A.F. Brading, 1988, Contractile effects of manganese on taenia of guinea pig cecum, Am. J. Physiol. 254, G489.

Milanov, M.P. and I.N. Stoyanov, 1982, A possibility of substituting Ca^{2+} by Ba^{2+} or Mn^{2+} during the contractile processes of complex stomach smooth muscles, Gen. Pharmacol. 13, 511.

Nonomura, Y., Y. Hotta and H. Ohashi, 1966, Tetrodotoxin and manganese ions: effects on electrical activity and tension in taenia coli of guinea pig, Science 152, 97.

Osa, T., 1974, Modification of the mechanical response of the smooth muscles of pregnant mouse myometrium and guinea pig ileum by cadmium and manganese ions, Jpn. J. Physiol. 24, 101.

Raos, N., 1990, Binding of metal cations to calmodulin, Period. Biol. 92, 341.

Sakai, K. and M. Uchida, 1981, Manganese-induced contraction in K^+-depolarized rat uterine smooth muscle, Gen. Pharmacol. 12, 41.

Sakai, K., T. Yamaguchi, S. Morita and M. Uchida, 1983, Agonist-induced contraction of rat myometrium in Ca-free solution containing Mn, Gen. Pharmacol. 14, 391.

Savineau, J.P., J. Mironneau and C. Mironneau, 1988, Contractile properties of chemically skinned fibers from pregnant rat myometrium: existence of an internal Ca-store, Pflüg. Arch. 411, 296.

Shibata, S., 1969, Effects of Mn on ^{45}Ca content and potassium-induced contraction of the aortic strip, Can. J. Physiol. Pharmacol. 47, 827.

Shimodan, M. and S. Sunano, 1981, The initiation of phasic and tonic contraction by potassium and the effect of calcium, multivalent cations and Ca-antagonist on potassium contracture in guinea-pig vas deferens, Jpn. J. Physiol. 31, 15.

Sunano, S., 1984, The effects of Ca antagonists, manganese and lanthanum on cooling-induced contracture of depolarized vas deferens, Jpn. J. Pharmacol. 34, 51.

Tsunobuchi, H. and Y. Gomi, 1990a, Effects of ryanodine and nifedipine on contractions induced by norepinephrine, acetylcholine, and potassium in the isolated vas deferens of the guinea pig, J. Pharmacobio-Dyn. 13, 574.

Tsunobuchi, H. and Y. Gomi, 1990b, The uptake of manganese induced by agonists in the isolated vas deferens of the guinea pig, J. Pharmacobio-Dyn. 13, 285.

Ushijima, H.T. and Y. Gomi, 1991, Tachyphylaxis to the inhibitory effect of manganese on potassium-induced contractions in the isolated vas deferens of the guinea pig, J. Pharmacobio-Dyn. 14, 194.

Ushijima, H.T. and Y. Gomi, 1995, Mechanism of tachyphylaxis on the inhibitory effect of manganese on agonist-induced contractions in the isolated vas deferens of the guinea pig, Biol. Pharm. Bull. 18, 246.