Mechanism of manganese-induced contraction in ileal longitudinal muscle of guinea-pig

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(Received: May 31, 1986)

Abstract

The mechanism of manganese (Mn^{2+})-induced contraction was studied in isolated ileal longitudinal muscle of guinea-pig. $MnCl_2$ (1×10^{-6} to 5×10^{-4} M) caused a transient contraction which subsided within approximately 6 min of application. The contraction was reproducible and dependent on the concentration. The dose-response curve was bell-shaped. A maximal response was observed at concentration of 5×10^{-5} M. The contractile effect was inhibited to some degree at 20° C or by tetrodotoxin (0.1 μg/ml), hyoscine (0.1 μg/ml) or hexamethonium (10 μg/ml), but completely inhibited by Ca^{2+}-removal from the medium.

Mn^{2+} increased the output of [^{14}C]-acetylcholine biosynthesized from [^{14}C]-choline by the preparation depending on the concentration. The increase terminated within the first 6 min and was reduced by tetrodotoxin (0.1 μg/ml) or by removal of Ca^{2+} from the medium. Both the contractile and transmitter releasing effects of Mn^{2+} were dependent on the concentration of external Ca^{2+}. Strontium ions were able to replace Ca^{2+} for Mn^{2+} induced transmitter release.

It is suggested that Mn^{2+} contracts ileal longitudinal muscle through a release of cholinergic transmitter from the parasympathetic nerve terminals, which is dependent on external Ca^{2+}. It also has a smaller hyoscine-resistant contractile effect, presumably due to a direct action on smooth muscle cells.

Introduction

Manganese ions (Mn^{2+}) are known to have an inhibitory action on mechanical response in smooth muscle preparations by various spasmogens[6, 13, 21]. Of heavy metal ions, cobalt ions, nickel ions, copper ions, zinc ions and Mn^{2+} are reported to produce a contraction in guinea-pig ileal muscle preparations[4, 19, 20]. Cadmium ions (Cd^{2+}) also have been found to have a similar action on smooth muscle, and it has been suggested that they act by increasing

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release of endogenous acetylcholine from cholinergic nerve terminals[1].

The present investigation was carried out to elucidate the effect of Mn^{2+} on the mechanical response in ileal longitudinal muscle of guinea-pig. The effect of Mn^{2+} on the release of transmitter from ileal longitudinal muscle preparations was also investigated in relation to external Ca^{2+} concentration.

Materials and Methods

Ileal longitudinal muscle (ILM) was isolated from male guinea-pigs of the Hartley strain (average weight 300g) by the methods described by PATON[14, 16]. The longitudinal muscle layer was separated from the underlying circular muscle by stroking it away from its mesenteric attachment not only at the upper end but along the whole length (10 to 15 cm) of the portion of ileum beginning 10 cm above the ileo-caecal junction. ILM was fixed vertically between two hooks on a holder and set up under, a loading tension of 0.1g in a 20 m*l* organ bath containing Tyrode solution with the following composition (mM): Na 148.7, K 2.7, Ca 2.5, Mg 1.0, Cl 143.8, HCO_3 11.9, SO_4 1.35 and glucose 5.5. The solution was gassed with a mixture of 5% CO_2 and 95% O_2 for at least 1 hr before use and also during the experiment, and kept at pH 7.2 under oxygenation and 37° C. The mechanical response was recorded isotonically by an isotonic transducer (Natsume Ltd).

Acetylcholine (ACh) output from cholinergic nerves of ILM was determined by means of radioassay of [^{14}C]-ACh biosynthesized from [^{14}C]-choline. ILM was incubated with [^{14}C]-choline (3×10^{-5} M, specific activity, 67 mCi/mM) for 60 min in Tyrode solution containing physostigmine (Phys, 1×10^{-7} M). For the next 60 min, the incubated ILM was washed 6 times at 10 min intervals with 50 m*l* of [^{14}C]-choline free solution containing Phys. After washing, the incubated ILM remained for 6 min in 3 m*l* of Phys-Tyrode solution, and the external medium was collected for counting. Pretreatments with tetrodotoxin (TTX) or low Ca^{2+} and replacement of Ca^{2+} with strontium ions (Sr^{2+}) were performed during the 60 min washing period and the following 6 min incubation period. Mn^{2+} was applied during the 6 min incubation period. [^{14}C]-ACh in the medium was identified by chromatography and determined by a method similar to that described by POTTER[17], and its content was presented as d min^{-1} g^{-1} wet weight of ILM.

Drugs used were: manganese chloride (Kokusan Chemicals); tetrodotoxin (Sankyo); hyoscine hydrobromide, hexamethonium chloride, choline chloride and physostigmine sulphate (Wako Pure Chemicals); acetylcholine chloride (Sigma); ^{14}C-choline chloride (NEC-141, choline chloride, [methyl-^{14}C], 1 mCi/3.0 mg in 5.0 m*l* ethanol solution, New England Nuclear Corp.)

Results

Effect of Mn^{2+} on mechanical response in ileal longitudinal muscle of guinea-pig: Guinea-pig ILM used in these experiments maintained a stable contractile activity in hypertonic 40 mM potassium chloride solution (H40K) for

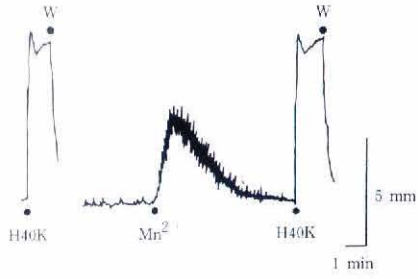

Fig. 1 Effects of Mn^{2+} on mechanical response and hypertonic potassium chloride solution (H40K)-induced contraction in ileal longitudinal muscle of guinea-pig. Mn^{2+}, manganese chloride (1×10^{-5} M); W, preparation washed. This concentration of Mn^{2+} did not reduce the response to H40K (cf. Fig. 2)

at least 4 hr. Mn^{2+} (5×10^{-5} M) caused a transient contraction which subsided within 6 min (Fig. 1). The transient contraction reached a maximal level within the first 1 to 2 min. The effect of Mn^{2+} was reproducible and dependent on its concentration. Dose-response curve to Mn^{2+} is presented in Fig. 2. The contractile effect of Mn^{2+} is presented as a percentage of the maximal level of the H40K-induced contraction in normal Tyrode solution. The Mn^{2+}-induced contraction appeared at a concentration of 1×10^{-6} M and reached a peak with 5×10^{-5} M. However, the contractile response decreased at concentrations over 1×10^{-4} M. The dose-response curve was bell-shaped. Mn^{2+} in higher concentrations ranging from 1×10^{-4} to 1×10^{-3} M, reduced the H40K-induced contraction depending on its concentrations.

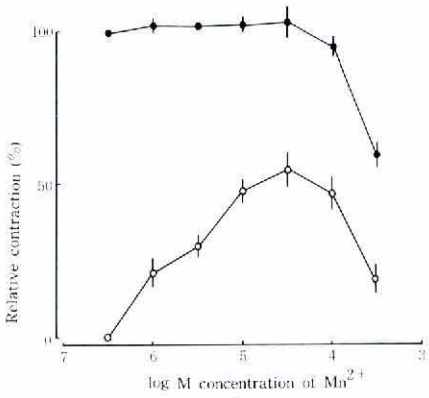

Fig. 2 Dose-response curve to Mn^{2+} (○) for effects on mechanical response and effects of Mn^{2+} (●) on hypertonic 40 mM potassium chloride (H 40 K)-induced contraction in ileal longitudinal muscle of guinea-pig. All the contractile effects of the agents are expressed as a percentage of the maximal response induced by H 40 K in normal Tyrode solution; vertical bars show s. e. mean of 10 experiments.

Effects of lowering temperature and neural or cholinoceptor blockade on Mn^{2+}-contraction: At 20°C, the Mn^{2+}-induced contraction was completely inhibited (Table 1). Lowering the temperature also significantly reduced the H40K-contraction to 75±2.1% of control ($P<0.05$), but did not affect the contraction induced by ACh (5×10^{-8} M). TTX (0.1 μg/ml) significantly reduced the Mn^{2+}-contraction to 11±5.7% ($P<0.01$), but did not affect the H40K- and ACh-contraction. The Mn^{2+}-contraction was significantly reduced to 64±2.5% ($P<0.01$) in the presence of hexamethonium (C_6, 10 μg/ml) which did not affect the H40K- and ACh-contractions. Hyoscine (0.1 μg/ml) completely inhibited the ACh-contraction and significantly reduced the Mn^{2+}-contraction to 35+4.5% ($P<0.01$), but did not affect the

Table 1 Effects of lowering temperature (20°C), tetrodotoxin (TTX, 0.1 μg/ml), hexamethonium (C_6, 10 μg/ml), hyoscine (0.1 μg/ml) and Ca^{2+}-removal from the medium on muscle contraction induced by Mn^{2+} (1×10^{-5} M), acetylcholine (ACh, 5×10^{-8} M) or hypertonic 40 mM potassium chloride solution (H40K) in guinea-pig ileal longitudinal muscle.

Treatment	Contraction to each stimulant relative to control response (%)		
	Mn^{2+}	ACh	H40K
20°C	Complete block	103±1.5	75±2.1*
TTX	11±5.7**	102±2.7	98±2.4
C_6	64±2.5**	99±1.3	95±3.6
Hyoscine	35±4.5**	Complete block	97±2.5
Ca^{2+}-removal	Complete block	Complete block	Complete block

All the treatments with inhibitors were applied 30 min before addition of stimulants. Each value represents the mean±s.e. for 8 experiments. The tonic component of the ACh-contraction was measured. *Significantly different from control, $p<0.05$. **Significantly different from control, $p<0.01$.

H40K-contraction. Higher concentration of hyoscine (10 $\mu g/ml$) reduced the Mn^{2+}-contraction to 29±3.0% ($P < 0.01$, N=6). Under a resting tension of 0.1 g, removal of Ca^{2+} from the medium reduced spontaneous mechanical activity, and Ca^{2+} readdition (2.5 mM) restored the spontaneous activity. Removal of Ca^{2+} from the medium completely inhibited the Mn^{2+}- and H40K-contraction and the tonic component of the ACh-contraction. A simultaneous application of Mn^{2+} (5×10^{-5} M) and Ca^{2+} (2.5 mM) restored the Mn^{2+}-contraction.

Effect of Mn^{2+} on [^{14}C]-acetylcholine output from ileal longitudinal muscle: As the neural and cholinoceptor blocking agents inhibited Mn^{2+}-contraction, the effect of Mn^{2+} on [^{14}C]-ACh output from ILM was examined. ILM loaded with [^{14}C]-choline was incubated with or without Mn^{2+} (5×10^{-5} M) for 6, 12, or 24 min. The amount of [^{14}C]-ACh output from ILM is expressed as d min^{-1} g^{-1} wet weight or tissue (Fig. 3). [^{14}C]-ACh output increased linearly with increase in incubation period in the control solution. Mn^{2+} increased the [^{14}C]-ACh output and caused a parallel shift of the curve to the left (Fig. 3). The increased amount of [^{14}C]-ACh released by Mn^{2+} was almost the same in each incubation period. This shows that Mn^{2+}-induced increase in transmitter release terminates within the first

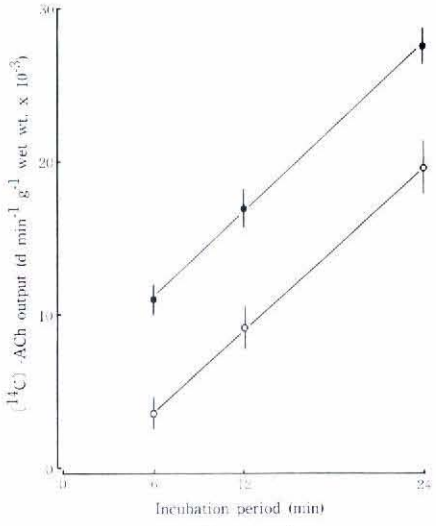

Fig. 3 Effects of Mn^{2+} on [^{14}C]-acetylcholine ([^{14}C]-ACh) output-incubation period curve in ileal longitudinal muscle of guinea-pig. In an experiment determining [^{14}C]-ACh output, 10 perparations were incubated simultaneously, and 4 experiments were carried out; (○) control; (●) in the presence of Mn^{2+} (1×10^{-5} M); vertical bars show s. e. mean of 4 experiments.

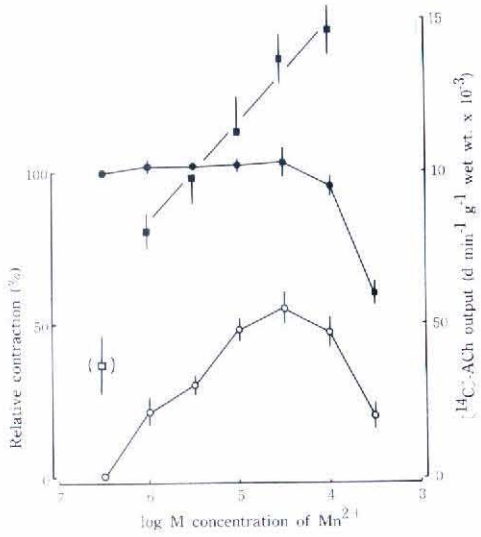

Fig. 4 Dose-response curve to Mn^{2+} for the effects on [^{14}C]-acetylcholine ([^{14}C]-ACh) output (■), on mechanical response (○), and on hypertonic potassium chloride (H 40 K)-induced contraction (●) in ileal longitudinal muscle of guinea-pig. In an experiment determining [^{14}C]-ACh output, 10 preparations were incubated simultaneously, and 4 experiments were carried out; (□) control output of [^{14}C]-ACh for 6 min incubation period. For the results of [^{14}C]-ACh output, vertical bars show s. e. mean of 4 experiments; in the results of mechanical response, vertical bars show s. e. mean of 10 experiments.

6 min. This is consistent with the time course of the Mn^{2+}-contraction.

Fig. 4 shows the effect of Mn^{2+} on the average [^{14}C]-ACh output for 6 min incubation period. Mn^{2+} in concentrations ranging from 1×10^{-6} to 1×10^{-4} M increased the output of [^{14}C]-ACh depending on the concentration. The [^{14}C]-ACh output was increased by Mn^{2+} in concentrations which inhibited the contractions induced by Mn^{2+} itself and H40K (data not shown). It seems that the ACh releasing effect of Mn^{2+} can be dissociated from the inhibitory effect of this agent on muscle contraction.

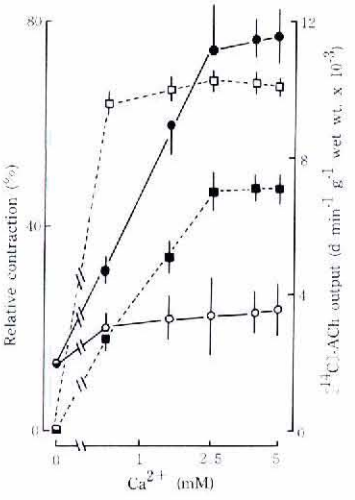

Fig. 5 Effects of external Ca^{2+} concentration on spontaneous output of [^{14}C]-acetylcholine ([^{14}C]-ACh) (○), on Mn^{2+}-induced increase in [^{14}C]-ACh output (●), on Mn^{2+}-induced contraction (■) and on ACh-induced contraction (□) in ileal longitudinal muscle of guinea-pig. In an experiment determining [^{14}C]-ACh output, 10 preparations were simultaneously incubated, and 4 experiments were carried out; for the results of [^{14}C]-ACh output, vertical bars show s.e. mean of 4 experiments; for the results of mechanical response, vertical bars show s.e. mean of 10 experiments. The tonic component of the ACh-contraction was measured.

Effects of tetrodotoxin, external Ca^{2+} or Sr^{2+} on Mn^{2+}-induced acetylcholine release: Pretreatment with TTX completely inhibited the ACh releasing effect of Mn^{2+}. This effect of TTX was consistent with its inhibitory effect on the Mn^{2+}-contraction.

Fig. 5 shows the effects of external Ca^{2+} concentration on the Mn^{2+}-induced increase in [^{14}C]-ACh output and also on the contractions induced by Mn^{2+} and ACh (5×10^{-8}M). Removal of Ca^{2+} decreased the spontaneous resting [^{14}C]-ACh output to approximately half that of the control. [^{14}C]-ACh output maintained a steady level in the presence of Ca^{2+} ranging from 0.5 to 5.0 mM. Removal of Ca^{2+} from the medium completely inhibited the ACh releasing effect of Mn^{2+}. However, in the presence of Ca^{2+} (0.5 to 2.5 mM), Mn^{2+} linearly increased the [^{14}C]-ACh output depending on Ca^{2+} concentration. This effect of Mn^{2+} reached a maximal level at 2.5 mM Ca^{2+}. The Ca^{2+} dependency of the Mn^{2+}-induced increase in [^{14}C]-ACh output was consistent with the Mn^{2+}-contraction depending on external Ca^{2+} concentration. The tonic component of the ACh-contraction maintained a steady level in concentrations of Ca^{2+} over 0.5 mM.

Equimolar replacement of external Ca^{2+} with Sr^{2+} slightly decreased the [^{14}C]-ACh output but this was not statistically significant. Although Mn^{2+} increased the [^{14}C]-ACh output approximately three fold above the control output in normal Tyrode solution, this effect was reduced to an approximately two fold increase by the replacement with Sr^{2+}. It seems that Sr^{2+} can replace Ca^{2+} in the Mn^{2+}-induced increase in transmitter release, but that Sr^{2+} is less effective.

Discussion

We observed that Mn^{2+} produced a transient contraction in ILM. This effect of Mn^{2+} was inhibited to some degree by lowering the

temperature or by the application of TTX, hexamethonium or hyoscine which suggests that Mn^{2+} has its main effects on the cholinergic innervation of the ILM. This was confirmed by experiments in which the effects of Mn^{2+} on transmitter release were examined. The contraction and the increase in transmitter release induced by Mn^{2+} were transient and subsided within the first 6 min, suggesting the possibility that the contraction is brought about by the transient increase in transmitter release. A small part (approximately 30%) of the Mn^{2+}-induced contraction was resistant to hyoscine (0.1 or 1 $\mu g/ml$) which completely inhibited the ACh-contraction. It is possible that Mn^{2+} acts directly on smooth muscle. We have already demonstrated that the Cd^{2+}-induced contraction is evoked by an increase in the output of endogenous ACh from ILM[1]. The mode of Mn^{2+}-induced contraction was similar to that of Cd^{2+}-induced contraction.

The dose-response curve of the effect of Mn^{2+} on the mechanical response was bell-shaped. In higher concentration, Mn^{2+} reduced the contraction induced by itself, ACh or H40K. Nevertheless, Mn^{2+} increased ACh release. Mn^{2+} has been shown to inhibit muscle contraction in intestinal smooth muscle by inhibiting Ca^{2+} influx[2,13]. The inhibitory effect of Mn^{2+} on the mechanical response observed in the present experiments is also possibly due to its direct action of inhibiting Ca^{2+} influx essential for the contractile machinery.

TTX inhibited the ACh releasing effect of Mn^{2+}. TTX is known to inhibit nerve excitability by reducing Na spikes[12], while not inhibiting action potential generation at the motor nerve terminal[19]. However, no information concerning the action of TTX on nerve terminals of the parasympathetic system is available[15], and the inhibitory effect of TTX on the transmitter releasing action of Mn^{2+} cannot be explained from the present data. Spontaneous resting ACh output was not significantly reduced by TTX. This is inconsistent with a finding by PATON et al.[15] which was obtained with a 100 fold higher concentration of Phys than in the present experiments. ACh release has been reported to be promoted by Phys itself[3]. At higher concentrations of Phys, spontaneous release of ACh may be maintained at a higher level which would be sensitive to TTX.

The Mn^{2+}-induced contraction was dependent on the external Ca^{2+} concentration. Extracellular Ca^{2+} is well known to be essential for transmitter release from motor nerve terminals[9] and parasympathetic nerve terminals[7,15] and for muscle contraction. It seems that Mn^{2+} cannot replace Ca^{2+} in its effect on transmitter release. The present experiments indicated that the ACh-contraction was maintained at a steady level in the presence of Ca^{2+} ranging from 0.5 to 5.0 mM, suggesting that the response of ILM to ACh is almost constant in these concentrations of Ca^{2+}. It is, therefore, possible that the Ca^{2+}-dependency of the Mn^{2+}-contraction is due to the Mn^{2+}-induced increase in transmitter release. The contractile effect and the transmitter releasing effect of Mn^{2+} were maintained at a maximal level in external Ca^{2+} concentrations above 2.5 mM, suggesting the possibility that Mn^{2+} fully activates the process of transmitter release at these Ca^{2+} levels. According to the Ca^{2+} hypothesis proposed by KATZ et al.[8], ACh release from the cholinergic site is mediated by an influx of external Ca^{2+}. Based on the Ca^{2+} hypothesis the Ca^{2+}-dependency of the transmitter releasing effect of Mn^{2+} suggests the possibility that Mn^{2+} increases the transmembrane influx of Ca^{2+} at the axon terminals.

It has been reported that Mn^{2+} inhibits Ca^{2+} influx through the membrane of the motor nerve terminals[24] and intestinal smooth muscle[2,13]. The inconsistency between this

information and the present data suggests the possibility that the nerve terminal of the parasympathetic system responds to Ca^{2+} differently from other tissues. Cd^{2+} also has been known to inhibit Ca^{2+} influx through the membrane of the motor nerve terminals[5, 18, 24, 27] and of vascular[22, 23, 25] and intestinal[26] smooth muscles. The same suggestion has been made for Cd^{2+} too[1].

From these results, it is suggested that Mn^{2+} contracts ileal longitudinal muscle mainly by increasing ACh release from a cholinergic site depending on external Ca^{2+}, possibly through an increase in Ca^{2+} influx.

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モルモット回腸縦走筋のマンガンによる収縮

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摘　要

マンガン（Mn^{2+}, 1×10^{-6}～5×10^{-4} M）はその単独適用により，モルモット回腸縦走筋に約6分間の一過性の収縮を誘起した。この収縮反応には再現性および濃度依存性が認められた。用量反応曲線はベル型を示し，最大反応は 5×10^{-5} M で観察された。

Mn^{2+}（1×10^{-5} M）による収縮は，低温（20°C），tetrodotoxin（0.1 μg/ml），hyoscine（0.1 μg/ml）および hexamethonium（10 μg/ml）により抑制され，外液からのカルシウム（Ca^{2+}）の除去により完全に抑制された。

Mn^{2+} は，回腸縦走筋において ^{14}C-choline から生合成された ^{14}C-acetylcholine（^{14}C-ACh）の放出を，濃度に依存して増加した。この増加は6分以内に終了し，tetrodotoxin（0.1 μg/ml）および外液からの Ca^{2+} の除去により抑制された。Mn^{2+} による収縮反応と ^{14}C-ACh の放出は，外液の Ca^{2+} 濃度に依存した。^{14}C-ACh の Mn^{2+} による放出促進に関して，ストロンチウム（2.5 mM）は Ca^{2+} を部分的に代替した。

以上の成績から，回腸縦走筋の Mn^{2+} による収縮

は，副交感神経終末からの内因性 ACh の遊離促進に起因すると考えられる。さらに，この作用は細胞外の Ca^{2+} の動態に関連すると思われる。

帯大研報 I, **15−1** (1986) 9〜17.