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MECHANISM OF CADMIUM-INDUCED CONTRACTION IN ILEAL LONGITUDINAL MUSCLE OF GUINEA-PIG

FUMITOSHI ASAI, MASAKAZU NISHIMURA, EIKI SATOH & NORIMOTO URAKAWA

Department of Veterinary Pharmacology, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

1 The mechanism of cadmium (Cd^{2+})-induced contraction was studied in isolated ileal longitudinal muscle of guinea-pig.

2 $CdCl_2$ (1×10^{-8} to 1×10^{-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 concentrations of 5×10^{-6} to 1×10^{-5} M.

3 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.

4 Cd^{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.

5 Both the contractile and transmitter releasing effects of Cd^{2+} were dependent on the concentration of external Ca^{2+}. Strontium ions were able to replace Ca^{2+} for Cd^{2+}-induced transmitter release.

6 It is suggested that Cd^{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

Cadmium ions (Cd^{2+}) are known to have an inhibitory action on cholinergic transmission in motor nerve-skeletal muscle preparations (Toda, 1976; Forshaw, 1977; Lin-Shiau & Fu, 1980; Satoh, Asai, Itoh, Nishimura & Urakawa, 1981) and in the parasympathetic nervous system (Hayashi & Takayama, 1978). Of heavy metal ions, cobalt, nickel, copper and zinc ions are reported to produce a contraction in guinea-pig ileal muscle strip (Eichler & Lippert, 1966). Manganese ions (Mn^{2+}) also have been found to have a similar action on smooth muscle, and it has been suggested that they act by increasing release of endogenous acetylcholine from cholinergic nerve terminals (Schnieden & Weston, 1968; 1969).

The present experiments were carried out to elucidate the effects of Cd^{2+} on the mechanical response and on acetylcholine output in ileal longitudinal muscle of the guinea-pig.

Methods

Ileal longitudinal muscle (ILM) was isolated from male guinea-pigs of the Hartley strain (average weight 300 g) by the methods described by Paton & Zar (1968) and Paton & Vizi (1969). 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 0.1 g in a 20 ml 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 h before use and also during the experiment, and kept at pH 7.2 under

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oxygenation and at 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 ml of [^{14}C]-choline-free solution containing Phys. After washing, the incubated ILM remained for 6 min in 3 ml of Phys-Tyrode solution, and the external medium was collected for counting. Pretreatment 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. Cd^{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 (1970), and its content was presented as d min^{-1} g^{-1} wet weight of ILM.

Drugs used were: cadmium chloride and 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 ml ethanol solution, New England Nuclear Corp.).

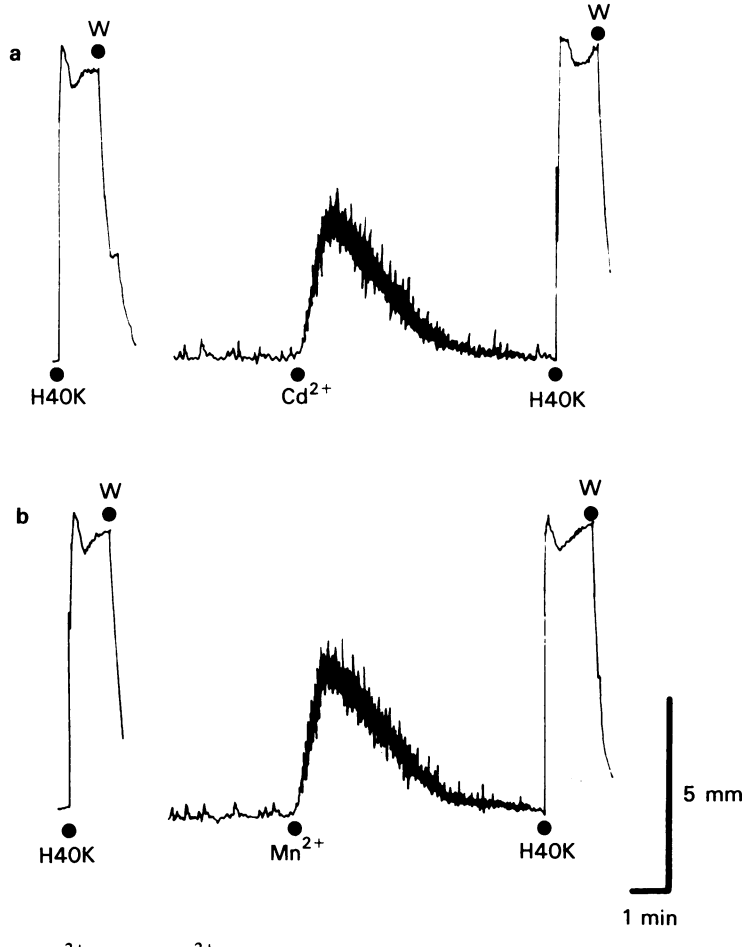

Figure 1 Effects of Cd^{2+} (a) and Mn^{2+} (b) on mechanical response and on hypertonic potassium chloride solution (H40K)-induced contraction in ileal longitudinal muscle of guinea-pig. Cd^{2+}, cadmium chloride (5×10^{-7} M); Mn^{2+}, manganese chloride (5×10^{-5} M); W, preparation washed. These concentrations of Cd^{2+} and Mn^{2+} did not reduce the response to H40K (cf. Figure 2).

Results

Effect of Cd^{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 at least 4 h or more. Cd^{2+} (5×10^{-7} M) caused a transient contraction which subsided within 6 min (Figure 1a). The transient contraction reached a maximal level within the first 1 to 2 min. Mn^{2+} (5×10^{-5} M) also produced muscle contraction (Figure 1b). The Mn^{2+}-contraction mimicked a Cd^{2+}-induced one. However, a much higher concentration of Mn^{2+} (approximately 100 fold that of Cd^{2+}) was necessary to produce a contractile effect equivalent to that of Cd^{2+} (5×10^{-7} M). These effects of Cd^{2+} and Mn^{2+} were reproducible and dependent on their concentrations. Dose-response curves to Cd^{2+} and Mn^{2+} are presented in Figure 2. The contractile effects of both ions are presented as a percentage of the maximal level of the H40K-induced contraction in normal Tyrode solution. The Cd^{2+}-induced contraction appeared at a concentration of 1×10^{-8} M and reached a steady level with 5×10^{-6} to 1×10^{-5} M. However, the contractile response decreased at concentrations over 10^{-5} M. The dose-response curve was bell-shaped. The Mn^{2+}-induced contraction appeared at 1×10^{-6} M and reached a steady level with 5×10^{-5} M. At concentrations of Mn^{2+} over 5×10^{-5} M, the contractile response declined as in the case of Cd^{2+}. Cd^{2+} and Mn^{2+} in higher concentrations ranging from 5×10^{-6} to 1×10^{-4} M and from 1×10^{-4} to 1×10^{-3} M, respectively, reduced the H40K-induced contraction depending on their concentrations (Figures 1 and 2).

Effects of lowering temperature and neural or cholinoceptor blockade on Cd^{2+}-contraction

AT 20°C, the Cd^{2+}-induced contraction was completely inhibited (Table 1). Lowering the temperature also significantly reduced the H40K-contraction to $77 \pm 2.5\%$ 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 Cd^{2+}-contraction to $12 \pm 7.8\%$ ($P < 0.01$), but did not affect the H40K- and ACh-contractions. The Cd^{2+}-contraction was significantly reduced to $67 \pm 3.8\%$ ($P < 0.01$) 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 Cd^{2+}-contraction to $33 \pm 3.0\%$ ($P < 0.01$), but did not affect the H40K-contraction. Higher concentrations of hyoscine (10 μg/ml) reduced the Cd^{2+}-

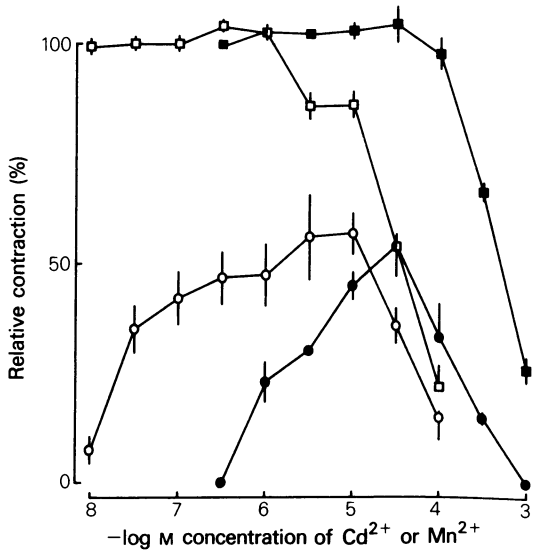

Figure 2 Dose-response curve to Cd^{2+} (○) and Mn^{2+} (●) for effects on mechanical response and effects of Cd^{2+} (□) and Mn^{2+} (■) on hypertonic 40 mM potassium chloride (H40K)-induced contraction in ileal longitudinal muscle of guinea-pig. All the contractile effects of the agents are expressed as a percentage of the maximal response induced by H40K in normal Tyrode solution; vertical bars show s.e.mean of 8 experiments.

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 Cd^{2+} (5×10^{-7} M), acetylcholine (ACh, 5×10^{-8} M) or hypertonic 40 mM potassium chloride solution (H40K) in guinea-pig ileal longitudinal muscle

	Contraction to each stimulant (%) relative to control response		
	Cd^{2+}	ACh	H40K
20°C	Complete block	104 ± 1.8	77 ± 2.5*
TTX	12 ± 7.8**	104 ± 2.2	99 ± 1.0
C_6	67 ± 3.8**	97 ± 2.0	94 ± 2.0
Hyoscine	33 ± 3.0**	Complete block	98 ± 1.8
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, $P < 0.01$

contraction to 30 ± 2.5% ($P < 0.01$, $n = 6$). 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 Cd^{2+}- and H40K-contractions and the tonic component of the ACh-contraction. A simultaneous application of Cd^{2+} (5×10^{-7} M) and Ca^{2+} (2.5 mM) restored the Cd^{2+}-contraction.

Effect of Cd^{2+} on [^{14}C]-acetylcholine output from ileal longitudinal muscle

As the neural and cholinoceptor blocking agents inhibited Cd^{2+}-contraction, the effect of Cd^{2+} on [^{14}C]-ACh output from ILM was examined. ILM loaded with [^{14}C]-choline was incubated with or without Cd^{2+} (5×10^{-7} 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 of tissue (Figure 3). [^{14}C]-ACh output increased linearly with increase in incubation period in the control solution. Cd^{2+} increased the [^{14}C]-ACh output and caused a parallel shift of the curve to the left (Figure 3). The increased amount of [^{14}C]-ACh released by Cd^{2+} was almost the same in each incubation period. This shows that the Cd^{2+}-induced increase in transmitter release terminates within the first 6 min. This is consistent with the time course of the Cd^{2+}-contraction.

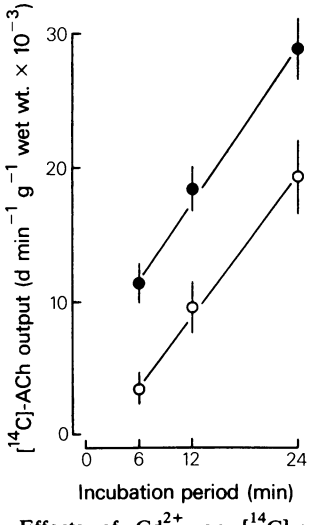

Figure 3 Effects of Cd^{2+} on [^{14}C]-acetylcholine ([^{14}C]-ACh) output-incubation period curve in ileal longitudinal muscle of guinea-pig. In an experiment determining [^{14}C]-ACh output, 15 preparations were incubated simultaneously, and 3 experiments were carried out; (○) control; (●) in the presence of Cd^{2+} (5×10^{-7} M); vertical bars show s.e.mean of 3 experiments.

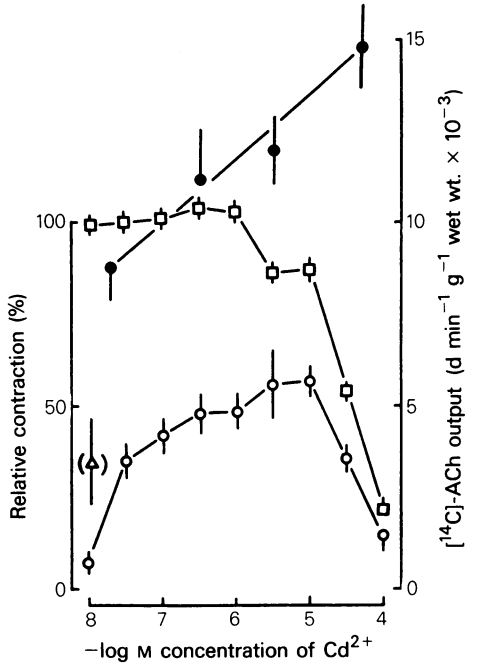

Figure 4 Dose-response curve to Cd^{2+} for the effects on [^{14}C]-acetylcholine ([^{14}C]-ACh) output (●), on mechanical response (○), and on hypertonic potassium chloride (H40K)-induced contraction (□) in ileal longitudinal muscle of guinea-pig. In an experiment determining [^{14}C]-ACh output, 15 preparations were incubated simultaneously, and 3 experiments were carried out; (△) control output of [^{14}C]-ACh for 6 min incubation period. For results of [^{14}C]-ACh output, vertical bars show s.e.mean of 3 experiments; in the results of mechanical response, vertical bars show s.e.mean of 8 experiments.

Figure 4 shows the effect of Cd^{2+} on the average [^{14}C]-ACh output for a 6 min incubation period. Cd^{2+} in concentrations ranging from 2.5×10^{-8} to 7.5×10^{-5} M increased the output of [^{14}C]-ACh depending on the concentration. The [^{14}C]-ACh output was increased by Cd^{2+} in concentrations which inhibited the contractions induced by Cd^{2+} itself and H40K. It seems that the ACh releasing effect of Cd^{2+} can be dissociated from the inhibitory effect of this agent on muscle contraction.

Effects of tetrodotoxin external Ca^{2+} and Sr^{2+} on Cd^{2+}-induced acetylcholine release

TTX (0.1 μg/ml) caused a slight but non-significant reduction in the control output of [^{14}C]-ACh (cf. Paton, Vizi & Zar, 1971). Pretreatment with TTX completely inhibited the ACh releasing effect of Cd^{2+}. This effect of TTX was consistent with its inhibitory effect on the Cd^{2+}-contraction.

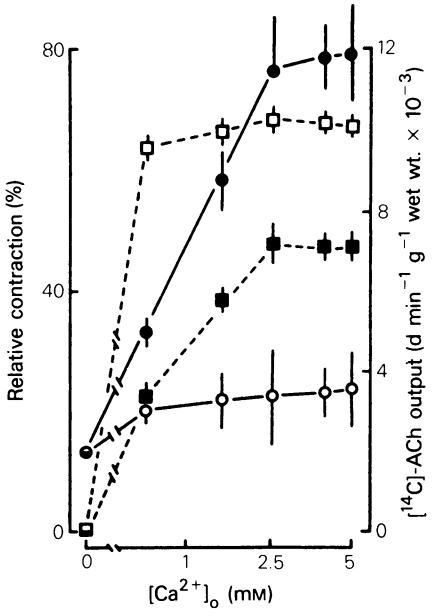

Figure 5 Effects of external Ca^{2+} concentration on spontaneous output of [^{14}C]-acetylcholine ([^{14}C]-ACh) (○), on Cd^{2+}-induced increase in [^{14}C]-ACh output (●), on Cd^{2+}-induced contraction (■) and on ACh-induced contraction (□) in ileal longitudinal muscle of guinea-pig. In an experiment determining [^{14}C]-ACh output, 15 preparations were simultaneously incubated, and 3 experiments were carried out; for the results of [^{14}C]-ACh output, vertical bars show s.e.mean of 3 experiments; for results of mechanical response, vertical bars show s.e.mean of 8 experiments. The tonic component of the ACh-contraction was measured.

Figure 5 shows the effects of external Ca^{2+} concentration on the Cd^{2+}-induced increase in [^{14}C]-ACh output and also on the contractions induced by Cd^{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 Cd^{2+}. However, in the presence of Ca^{2+} (0.5 to 2.5 mM), Cd^{2+} linearly increased the [^{14}C]-ACh output depending on Ca^{2+} concentration. This effect of Cd^{2+} reached a maximal level at 2.5 mM Ca^{2+}. The Ca^{2+} dependency of the Cd^{2+}-induced increase in [^{14}C]-ACh output was consistent with the Cd^{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 Cd^{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 Cd^{2+}-induced increase in transmitter release, but that Sr^{2+} is less effective.

Discussion

The present experiments indicated that Cd^{2+} produced a transient contraction in ILM. This effect of Cd^{2+} was inhibited to some degree by lowering the temperature or by the application of TTX, hexamethonium or hyoscine which suggests that Cd^{2+} has its main effects on the cholinergic innervation of the ILM. This was confirmed by experiments in which the effects of Cd^{2+} on transmitter release were examined. The contraction and the increase in transmitter release induced by Cd^{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 Cd^{2+}-induced contraction was resistant to hyoscine (0.1 or 1 μg/ml) which completely inhibited the ACh-contraction. It is possible that Cd^{2+} acts directly on smooth muscle. The Cd^{2+}-induced contraction was similar to Mn^{2+}-induced contraction. It has been suggested that the Mn^{2+}-contraction is evoked by an increase in the output of endogenous ACh from the ileum (Schnieden & Weston, 1968; 1969). The effect of Cd^{2+} on the mechanical activity of ILM was not distinguished pharmacologically from that to Mn^{2+}. This indicates that Cd^{2+} acts on the cholinergic system by a mechanism similar to Mn^{2+}. Both the contractile effect and the ACh releasing effect of Mn^{2+} have been shown to be inhibited by TTX (Schnieden & Weston, 1968; 1969). These authors did not mention the mechanism inhibited by TTX.

The dose-response curve of the effect of Cd^{2+} on the mechanical response was bell-shaped. In higher concentrations, Cd^{2+} reduced the contraction induced by itself, ACh or H40K. Nevertheless, Cd^{2+} increased ACh release. Cd^{2+} has been shown to inhibit muscle contraction in vascular (Thind, Stephan & Blakemore, 1970; Toda, 1973; Toda, Usui, Kimura & Itokawa, 1975) and intestinal (Triggle, Grant & Triggle, 1975) smooth muscle by inhibiting Ca^{2+} influx. The inhibitory effect of Cd^{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 Cd^{2+}. TTX is known to inhibit nerve excitability by reducing Na spikes (Narahashi, Moore & Scott, 1964),

while not inhibiting action potential generation at the motor nerve terminal (Katz & Miledi, 1967b). However, no information concerning the action of TTX on nerve terminals of the parasympathetic system is available (Paton *et al.*, 1971), and the inhibitory effect of TTX on the transmitter releasing action of Cd^{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.* (1971) 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 (Carlyle, 1963). At higher concentrations of Phys, spontaneous release of ACh may be maintained at a higher level which would be sensitive to TTX.

The Cd^{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 (Katz & Miledi, 1967a) and parasympathetic nerve terminals (Johnson, 1963; Paton *et al.*, 1971) and for muscle contraction. It seems that Cd^{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 Cd^{2+}-contraction is due to the Cd^{2+}-induced increase in transmitter release. The contractile effect and the transmitter releasing effect of Cd^{2+} were maintained at a maximal level in external Ca^{2+} concentrations above 2.5 mM, suggesting the possibility that Cd^{2+} fully activates the process of transmitter release at these Ca^{2+} levels. According to the Ca^{2+} hypothesis proposed by Katz & Miledi (1965), 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 Cd^{2+} suggests the possibility that Cd^{2+} increases the transmembrane influx of Ca^{2+} at the axon terminals.

On the other hand, Cd^{2+} has been reported to inhibit Ca^{2+} influx through the membrane of the motor nerve terminals (Toda, 1976; Usui & Toda, 1976; Forshaw, 1977; Satoh *et al.*, 1981) and of vascular (Thind *et al.*, 1970; Toda, 1973; Toda *et al.*, 1975) and intestinal (Triggle *et al.*, 1975) smooth muscles. The inconsistency between this information and the present data suggests the possibility that the nerve terminal of the parasympathetic system reponds to Ca^{2+} differently from other tissues.

From the results, it is suggested that Cd^{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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