Br. J. Pharmac. (1985), **85**, 471–479

Mode of stimulatory actions of cadmium ion on the mouse diaphragm

W.M. Fu & S.Y. Lin-Shiau

Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan

1 Effects of Cd^{2+} on the phrenic nerve-diaphragm preparation of the mouse varied markedly in media containing various Ca^{2+} concentrations. In normal 2.5 mM Ca^{2+} medium, Cd^{2+} inhibited acetylcholine release from nerve endings without appreciable effect on the muscle membrane. However, Cd^{2+} elicited stimulatory effects on the muscle membrane in low Ca^{2+} medium (10^{-3}–1 mM). These stimulatory effects included the induction of spontaneous contractions, augmentation of twitch responses to direct electrical stimulation and potentiation of the muscle contracture induced by acetylcholine, carbachol and high K^+. By contrast, caffeine contracture was not affected by Cd^{2+}.

2 Tetrodotoxin, procaine, cysteine and glycerol pretreatment abolished these stimulatory effects of Cd^{2+}. Moreover, changing the ionic composition of the bathing medium to one containing low Na^+, high K^+, high Mg^{2+} or high Ca^{2+} also antagonized these effects of Cd^{2+}. In contrast, low Mg^{2+} markedly potentiated the frequency of spontaneous contractions induced by Cd^{2+}.

3 (+)-Tubocurarine and β-bungarotoxin had no effect on Cd^{2+}-induced spontaneous contractions indicating that they may be myogenic rather than neurogenic in origin.

4 By use of conventional microelectrodes, it was found that Cd^{2+} not only depolarized the muscle membrane but also induced spontaneous action potentials at a high frequency (173 ± 17 Hz).

5 It is concluded that increased Na^+ permeability of the muscle membrane is the essential step bringing about spontaneous contractions. The binding of Cd^{2+} to –SH groups of the membrane is closely related to the induction of these effects.

Introduction

Cd^{2+} inhibits transmitter release from motor nerve endings without appreciable effect on skeletal muscle (Toda, 1976; Forshow, 1977; Fu & Lin-Shiau, 1978; Lin-Shiau & Fu, 1980). In inducing this effect, Cd^{2+} was the most potent among nine divalent cations tested (Fu & Lin-Shiau, 1978; Lin-Shiau & Fu, 1980). In addition, Cd^{2+} also inhibits the contraction of myocardium and aorta (Kleinfeld *et al.*, 1955; Kleinfeld & Stein, 1968; Thind *et al.*, 1970; Toda, 1973). Since cysteine and Ca^+ reversed the effects of Cd^{2+}, it was postulated that Cd^{2+} induces these effects by binding to the sulphhydryl group of the membrane and decreasing the transmembrane transport of Ca^{2+}.

On lowering the concentration of Ca^{2+} in the bathing medium, we accidentally discovered that Cd^{2+} stimulated the muscle instead of having an inhibitory action. In 0.25 mM Ca^{2+} medium, Cd^{2+} induced spontaneous contractions of the mouse diaphragm, augmented twitch responses to direct electrical stimulation and potentiated the responses to acetylcholine, carbachol and high K^+. In this paper, the mode of the stimulatory actions of Cd^{2+} is explored.

Methods

Mouse phrenic nerve-diaphragm preparation

Mice (NIH strain) of either sex, weighing 20–25g were used. The phrenic nerve-diaphragm preparation was isolated according to the method of Bülbring (1946). A modified Krebs solution was used of the following compositions (mM): NaCl 130.6, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 12.5 and glucose 11.1. Low Na^+ (55.5 mM) Krebs solutions were obtained by substituting either isosmotic sucrose or LiCl for NaCl. The concentration of $CaCl_2$ and $MgSO_4$ of Krebs solution was varied from 10^{-3} to 2.5 mM and from 10^{-3} to 3.6 mM, respectively, without any substitution, while KCl was substituted for NaCl in the concentration range of 2.4 mM to 14.4 mM. The pH of the solutions was 7.2–7.4. The nerve-diaphragm preparation was suspended in Krebs solution in a 10 ml organ bath which was constantly gassed with 95% O_2 + 5% CO_2 at 37 ± 0.5°C. The phrenic nerve was stimulated with supramaximal rectangular pulses

of 0.05 ms at a rate of 12 min^{-1}. In some experiments, direct and indirect stimulation to the muscle was alternately applied at a rate of 6 min^{-1}. The contraction was recorded isometrically with a force-displacement transducer (Grass FT. 03) on a Grass Model 7 polygraph.

Spontaneous contractions of the diaphragm were induced by adding 0.1 mM Cd^{2+} to the organ bath containing low Ca^{2+} (0.25 mM) Krebs solution. Effects of cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+), tetrodotoxin, procaine, cysteine and (+)-tubocurarine on the spontaneous contractions were tested either by pretreatment of the diaphragm before the addition of Cd^{2+} or after spontaneous contractions appeared. Acetylcholine and carbachol as well as high K^+ contracture of the diaphragm were monitored both before and after the addition of Cd^{2+}. The number of the experiments for each group was more than four and significance was assessed by using Student's t test. Data are shown as mean ± s.e.mean.

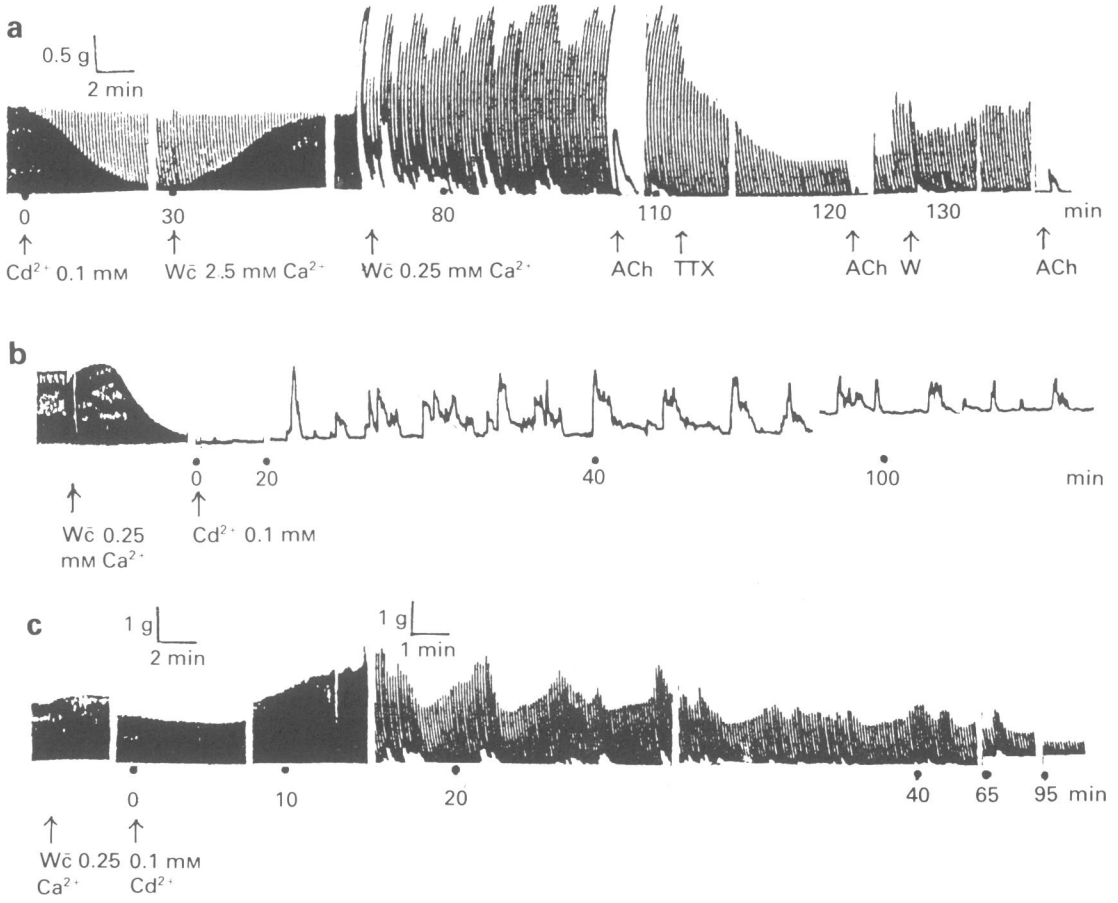

Figure 1 Spontaneous contractions of the mouse diaphragm and potentiation of twitch responses to direct stimulation induced by Cd^{2+}. (a) Alternate direct and indirect stimulation was applied to the mouse phrenic nerve-diaphragm preparation. Note that 0.1 mM Cd^{2+} reversibly inhibited twitch responses to indirect stimulation but those to direct stimulation remained unaltered. On lowering Ca^{2+} concentration from 2.5 to 0.25 mM in the presence of 0.1 mM Cd^{2+}, spontaneous contractions, potentiation of twitch responses to direct stimulation and acetylcholine (ACh 0.05 mM) contracture appeared, all of which were antagonized by tetrodotoxin (TTX 0.03 μM). (b) The mouse diaphragm was indirectly stimulated. In 0.25 mM Ca^{2+} medium, Cd^{2+} induced spontaneous contractions which lasted for more than 100 min. (c) The mouse diaphragm was directly stimulated. In 0.25 mM Ca^{2+} medium, Cd^{2+} potentiated twitch responses and induced spontaneous contractions which were self-limiting and declined in 95 min. Wc̄ denotes washout with the solution indicated.

Effect of Cd^{2+} on the glycerol pretreated diaphragm

The isolated diaphragm of the mouse was soaked in oxygenated modified Krebs solution containing 400 mM glycerol for 30 min and then washed repeatedly with modified Krebs solution until responses to indirect electrical stimulation and to 75 mM K^+ solution were abolished, which indicated that the transverse tubular systems of the muscle fibre were closed (Eisenberg & Eisenberg, 1968). Cd^{2+} (0.1 mM) was added to the detubulated muscle pretreated with 0.25 mM Ca^{2+} Krebs solution for 30 min. Spontaneous contractions induced by Cd^{2+} in the contralateral diaphragm without pretreatment with glycerol were simultaneously carried out as a control.

Intracellular microelectrode recording

The conventional microelectrode recording technique (Fatt & Katz, 1951) was adopted; glass microelectrodes filled with 3 M KCl and having 5–20 MΩ resistance were used. A WPI model FD223 preamplifier and Hitachi V-352 Oscilloscope were used. The mouse diaphragm was suspended in 10 ml of modified Krebs solution at 37 ± 0.5°C.

Materials

Tetrodotoxin thrice purified by crystallization was obtained from Sankyo, (+)-tubocurarine from Sigma and procaine from Abbott. α- and β-Bungarotoxin were isolated from the venom of *Bungarus multicinctus* by the method described by Lee *et al.* (1972).

Results

Effects of Cd^{2+} on the mouse diaphragm in low Ca^{2+} medium

In 2.5 mM Ca^{2+} Krebs solution, 0.1 mM Cd^{2+} reversibly inhibited twitch responses to indirect nerve stimulation without having an appreciable effect on those to direct muscle stimulation (Figure 1a). In low Ca^{2+} Krebs (0.25 mM), twitch responses to indirect stimulation diminished in 5 min (Figure 1b), while those to direct stimulation were reduced only slightly in 20 min and then persisted for more than 120 min (Figure 1a and c). Although 0.1 mM Cd^{2+} had no appreciable effect on twitch responses to direct stimulation in 2.5 mM Ca^{2+} medium, the twitch responses markedly increased following the incubation of the diaphragm with Cd^{2+} in the presence of 0.25 mM Ca^{2+} (Figure 1a). If the diaphragm was pretreated with 0.25 mM Ca^{2+} medium for 20 min and 0.1 mM Cd^{2+} then added, the twitch responses to direct stimulation gradually increased with a latent period of 9.3 ± 2.4 min ($n = 8$, Figure 1c). Twitch responses reached a peak tension of 241.7 ± 24.2% of control after the addition of Cd^{2+} for 23.0 ± 4.9 min and then gradually declined to 90 ± 13% of control tension after 90 min incubation with Cd^{2+} (Figure 1c). In addition to the increase in amplitude, Cd^{2+} also markedly prolonged the duration of a single contraction (Table 1). Cd^{2+} could also induce spontaneous contractions in low Ca^{2+} Krebs solution (Figure 1b). Like increased twitch responses to direct stimulation, spontaneous contractions induced by Cd^{2+} were self-limiting, persisting for 94.5 ± 8.2 min ($n = 11$).

Table 1 Effects of metal ions on twitch responses to direct electrical stimulation of mouse diaphragm

Metal ion	*Concentration* (mM)	*Twitch amplitude* (g)	*Duration of a single contraction* (s)	n
Control		0.95 ± 0.06	0.056 ± 0.003	21
Cd^{2+}	0.1	2.37 ± 0.16*	0.590 ± 0.060*	8
Mn^{2+}	0.8	0.75 ± 0.07	0.055 ± 0.002	4
Co^{2+}	0.9	0.77 ± 0.04	0.068 ± 0.004	4
Ni^{2+}	0.6	0.95 ± 0.01	0.059 ± 0.002	3
Zn^{2+}	0.1	2.40 ± 0.10*	0.252 ± 0.044*	8
Sr^{2+}	4	0.90 ± 0.02	0.060 ± 0.003	3
Cu^{2+}	0.01	0.71 ± 0.06*	0.044 ± 0.006	3
Ba^{2+}	0.2	0.23 ± 0.04*	0.058 ± 0.003	3
UO_2^{2+}	0.6	1.67 ± 0.24*	0.074 ± 0.003*	5

Responses of diaphragm pretreated with 14 μM (+)-tubocurarine were measured 20 min after the addition of metal ions in 0.25 mM Ca^{2+} Krebs solution.
The values are shown as means ± s.e.mean.
* $P = 0.05$ as compared with control.

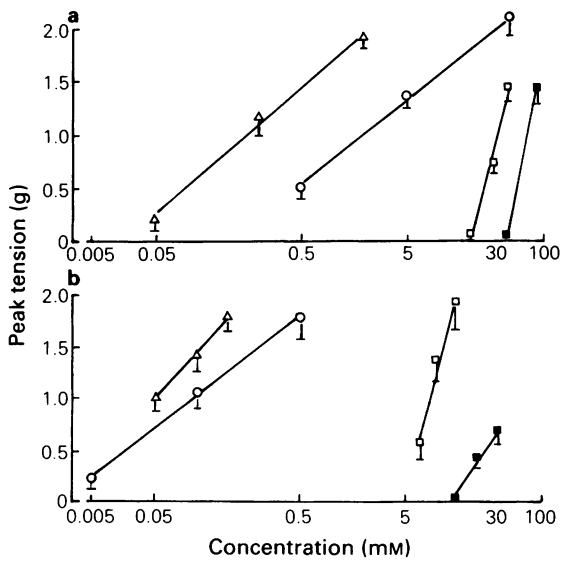

Figure 2 Potentiation by Cd^{2+} of contractures induced by acetylcholine (○), carbachol (△) and high K^+ (□) in the mouse diaphragm; (■) high K^+ without Cd^{2+}. In Krebs solution containing no Cd^{2+}, the mouse diaphragm had almost no response to high concentrations of acetylcholine (50 mM) and carbachol (3 mM), whether the concentration of Ca^{2+} was 2.5 mM (a) or 0.25 mM (b).

Potentiation by Cd^{2+} of contracture induced by acetylcholine, carbachol and high K^+

The mouse diaphragm was almost non-responsive to either acetylcholine or carbachol even at concentrations higher than 2 mM. Both acetylcholine (50 mM) and carbachol (2 mM) induced small tensions of 0.10 ± 0.02g and 0.11 ± 0.05g, respectively. However, treatment of mouse diaphragm with Cd^{2+} in 0.25 mM Ca^{2+} medium markedly enhanced responses to acetylcholine and carbachol (Figure 2b). In 2.5 mM Ca^{2+} medium, Cd^{2+} potentiated acetylcholine- and carbachol-contractures but to a lesser extent and did not induce spontaneous contractions (Figure 2a). In addition, potassium contracture of the diaphragm was also potentiated by Cd^{2+} (Figure 2a and b). In contrast, caffeine contracture of the diaphragm pretreated with 0.1 mM Cd^{2+} was 1.99 ± 0.40g, which was not significantly different from that of the control (2.15 ± 0.32g) in the absence of Cd^{2+} ($P > 0.05$, $n = 6$).

The diaphragm was pretreated with α-bungarotoxin (1 μg ml^{-1}) until neuromuscular transmission was completely abolished, when it was washed repeatedly with Krebs solution in order to remove free toxin. Subsequently, the application of Cd^{2+} no longer potentiated responses to acetylcholine and carbachol, although Cd^{2+} was still able to induce spontaneous contractions.

Effects of ions on the stimulatory actions of Cd^{2+}

As shown in Table 2, Ca^{2+} exerted an antagonistic effect on Cd^{2+}; the lower the Ca^{2+} concentration, the more the spontaneous contractions induced by 0.1 mM Cd^{2+}. In 2.5 mM Ca^{2+} medium, Cd^{2+} (0.1 mM or higher than 1 mM) did not induce spontaneous contractions at all. On lowering the Ca^{2+} concentration to 1 mM, a few small spontaneous contractions were induced only 30 min after pretreatment with 0.1 mM Cd^{2+}. On further decrease of Ca^{2+} concentration to 0.001–0.5 mM, more spontaneous contractions were induced by Cd^{2+}. Moreover, the latent period of spontaneous contractions was reduced and the frequency was increased on lowering Ca^{2+} concentration from 0.5 mM to 1 μM. In the diaphragm pretreated with 1 μM Ca^{2+} medium for 3 h, Cd^{2+} was still able to induce spontaneous contractions. On the other hand,

Table 2 Effect of Ca^{2+} and Mg^{2+} on Cd^{2+}-induced spontaneous contractions in the mouse diaphragm

Ca^{2+} (mM)	*Mg^{2+}* (mM)	n	*Latent period* (min)	*Duration* (min)	*Frequency* (times/30 min)
10^{-3}	1.2	6	19.1 ± 1.5*	75.0 ± 11.8	10.5 ± 0.5
0.25 (Control)	1.2	11	27.2 ± 3.8	94.5 ± 8.2	9.2 ± 1.4
0.5	1.2	4	30.00 ± 4.0	127.5 ± 12.5	4.5 ± 1.5*
1.0[a]	1.2	4	—	—	2*
2.5	1.2	5	—	—	0*
0.25	10^{-3}	5	17.1 ± 4.1*	110.0 ± 10.2	21.5 ± 1.5*
0.25	3.6	4	—	—	0*

Data are means ± s.e.mean.

[a] Spontaneous contractions were inhibited by 1 mM Ca^{2+}; after pretreatment with 0.1 mM Cd^{2+} for 30 min, only a few spontaneous contractions appeared on washing the mouse diaphragm with 1 mM Ca^{2+} solution.

* $P < 0.05$ as compared with control in Krebs solution containing 0.25 mM Ca^{2+} and 1.2 mM Mg^{2+}.

Table 3 Effect of ions and toxins on the stimulatory actions of Cd^{2+} in the mouse diaphragm

Effect	*Cd^{2+} alone*	*Pretreatment with* *Low Na^+*	*Low Mg^{2+}*	*TTX*	*Procaine*
Spontaneous contractions (frequency/30 min)	9.2 ± 1.4	0*	21.1 ± 1.4*	0*	0*
Twitches to direct stimulations (%)[a]	241.7 ± 24.2	88.6 ± 5.8*	359.5 ± 35.0*	108.0 ± 5.8*	100 ± 0*
Acetylcholine contracture (g)	1.59 ± 0.29	0.26 ± 0.21*	1.38 ± 0.22	0*	0*
Carbachol contracture (g)	1.89 ± 0.30	0.16 ± 0.11*	1.47 ± 0.33	0*	0*
High K^+ contracture (g)	2.29 ± 0.16	0.63 ± 0.22*	2.30 ± 0.28	0*	0*

The concentration of ions, toxin and drugs were: low Na^+ (55 mM), low Mg^{2+} (omitting $MgSO_4$), tetrodotoxin (TTX 0.03 μM), procaine (1mM), acetylcholine (0.05 mM), carbachol (0.02 mM) and high K^+ (9.8 mM).
[a] Amplitude of twitches to direct stimulation was calculated as percentage of that before the addition of Cd^{2+}.
* Significantly different from control ($P < 0.05$).

omitting Mg^{2+} from the bathing medium enhanced spontaneous contractions, while raising Mg^{2+} concentration from 1.2 mM to 3.6 mM abolished spontaneous contractions induced by Cd^{2+} (Table 2). Reduction of the Na^+ concentration from 143.1 mM to 55 mM also abolished spontaneous contractions (Table 3).

Potentiation of twitch responses to direct stimulation by Cd^{2+} was also abolished by low Na^+ but enhanced by low Mg^{2+} (Table 3). On the other hand, acetylcholine, carbachol and high K^+ contractures were inhibited by low Na^+ but were unaltered by low Mg^{2+} (Table 3).

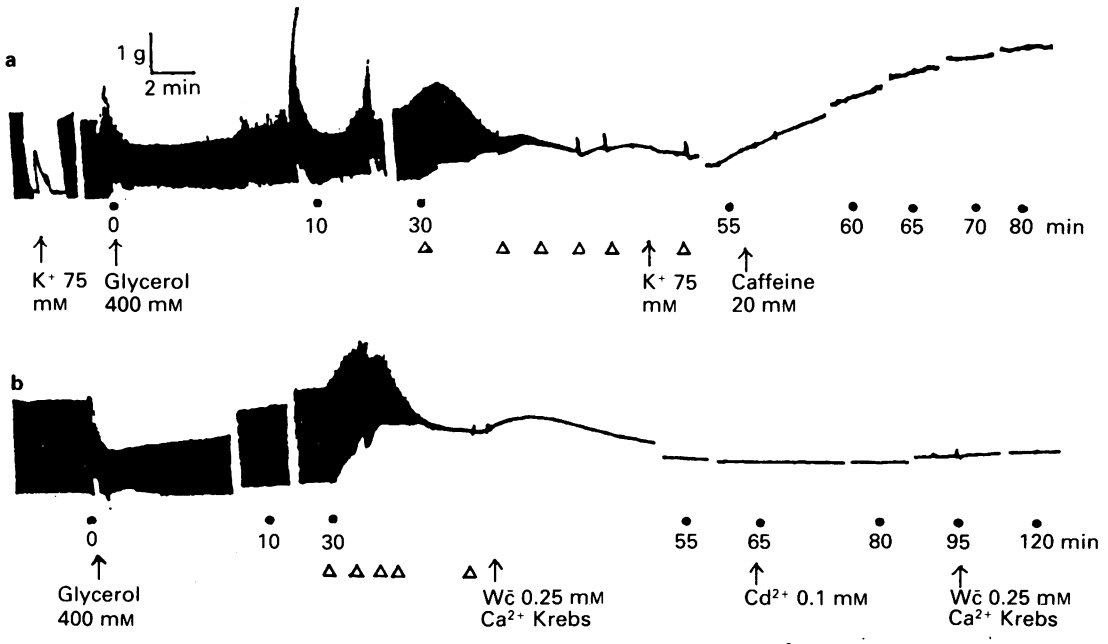

Figure 3 Inhibition by glycerol treatment of spontaneous contractions induced by Cd^{2+} in the mouse diaphragm. The mouse diaphragm was suspended in 2.5 mM Ca^{2+} Krebs solution and stimulated indirectly. (a) Glycerol treatment for 30 min and repeated washing with Krebs solution abolished K^+-contracture but only slightly reduced caffeine contracture. (b) Cd^{2+} failed to induce spontaneous contractions in the contralateral glycerol-treated diaphragm. Δ denotes washout with Krebs solution.

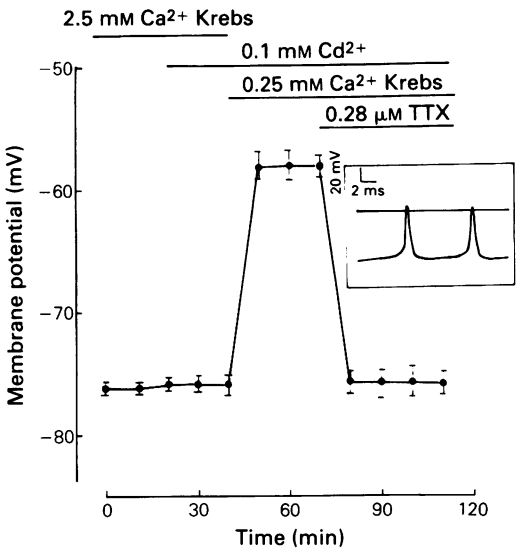

Figure 4 Effects of Cd^{2+} on the membrane potential of mouse diaphragm. The inset shows spontaneous action potentials induced by 0.1 mM Cd^{2+} in 0.25 mM Ca^{2+} Krebs solution.

Effects of toxins and glycerol treatment of the diaphragm on the stimulatory actions of Cd^{2+}

Both tetrodotoxin and procaine completely antagonized the stimulatory actions of Cd^{2+} (Figure 1a and Table 3). Cysteine (2.5 mM) abolished spontaneous contractions induced by Cd^{2+} ($n = 6$). Both (+)-tubocurarine (14 μM) and β-bungarotoxin (0.36 μM) which blocked neuromuscular transmission, did not affect the induction of spontaneous contractions by Cd^{2+} ($n = 4$). The glycerol-treated diaphragm was unresponsive to Cd^{2+} (Figure 3). Caffeine (20 mM) contracture was only partially inhibited by glycerol treatment; peak tension of the control diaphragm was 3.60 ± 0.32g ($n = 4$), while that of the glycerol-treated diaphragm was 2.10 ± 0.17g ($n = 6$).

Effects of Cd^{2+} on membrane potential of the mouse diaphragm

The resting membrane potential of the diaphragm was estimated to be 78.5 ± 0.6 mV and 77.8 ± 0.5 mV in 2.5 mM and 0.25 mM Ca^{2+} media, respectively ($n = 75$, obtained from 5 preparations). Occasionally, spontan-

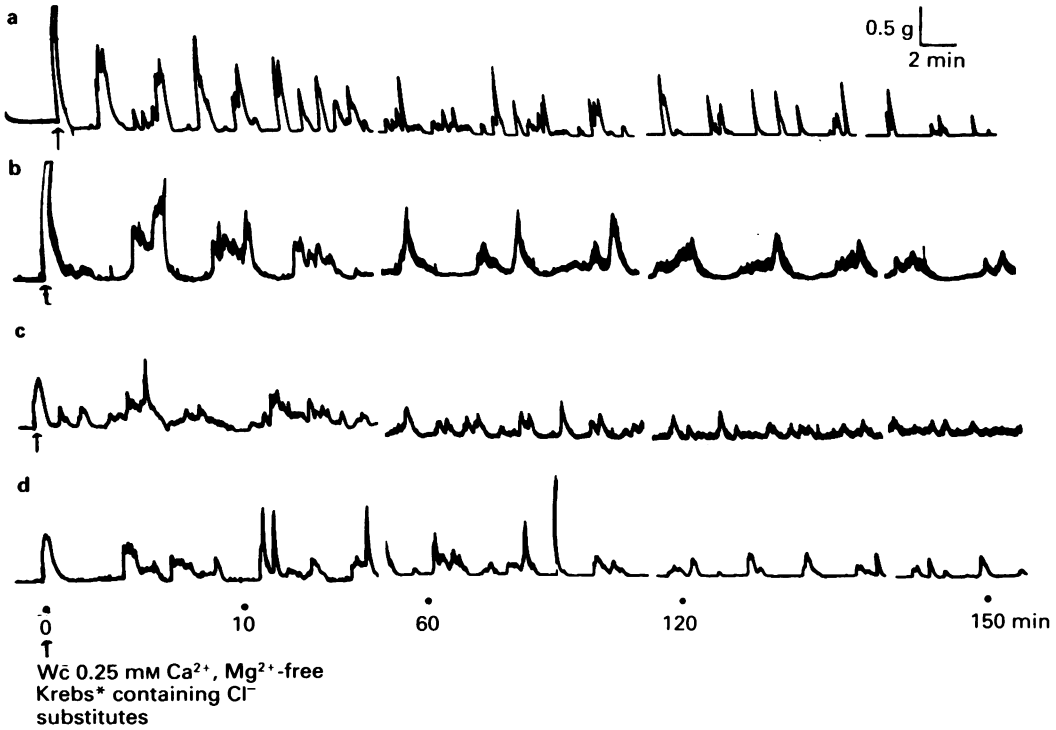

Figure 5 Spontaneous contractions induced by substances replacing chloride in mouse diaphragm. After preincubation of the preparation in Krebs for 30 min, the medium was replaced with 0.25 mM Ca^{2+}, Mg^{2+}-free-Krebs in which one third of NaCl was substituted with isosmotic sodium nitrate (a), sodium sulphate (b), sodium acetate (c) or sodium propionate (d).

eous action potentials were observed in some muscle fibres in low Ca^{2+} Krebs solution. After treatment with Cd^{2+} in low Ca^{2+} medium, the membrane was depolarized with 85% of muscle cells firing spontaneously with a frequency of 173 ± 13 Hz ($n = 30$, Figure 4), while 15% cells remained unaffected. Tetrodotoxin inhibited Cd^{2+}-induced spontaneous firing and depolarization (Figure 4). In normal Ca^{2+} medium, Cd^{2+} did not induce spontaneous contractions; however, spontaneous firing of action potentials and depolarization were observed in about 20% of cells.

Effects of chloride substituents on mouse diaphragm and their influence on the actions of Cd^{2+}

In low Ca^{2+} and Mg^{2+}-free medium, but without pretreatment with Cd^{2+}, substitution of one third of the sodium chloride with isotonic sodium salts of nonpenetrating anions such as nitrate, sulphate, acetate and propionate produced spontaneous contractions similar to those produced by Cd^{+} (Figure 5).

In low Ca^{2+} medium, the substitution of two thirds of the sodium chloride with sodium propionate inhibited the spontaneous contractions induced by Cd^{2+} ($n = 3$).

Effects of other metal ions on the mouse diaphragm

After the mouse diaphragm was pretreated with 0.25 mM Ca^{2+} Krebs solution for 20 min, various metal ions were then added. Among the nine metal ions tested, only UO_2^{2+}, Zn^{2+} and Cd^{2+} enhanced the contractile responses to direct stimulation, increasing them about 1–1.5 fold in amplitude, while Zn^{2+} and Cd^{2+} prolonged the duration of a single contraction three and nine times, respectively (Table 1). None of these cations, excepting Cd^{2+}, induced spontaneous contractions.

Discussion

Cd^{2+} inhibits acetylcholine release from motor nerve endings without appreciable effect on the skeletal muscle (Toda, 1976; Forshaw, 1977; Fu & Lin-Shiau, 1978; Lin-Shiau & Fu, 1980). Unlike skeletal muscle, the myocardium and aorta are sensitive to the inhibitory action of Cd^{2+} (Kleinfeld *et al.*, 1955; Kleinfeld & Stein, 1968; Thind *et al.*, 1970; Toda, 1973). Also, Cd^{2+} can inhibit Ca^{2+} channels in an axon of *Aplysia* (Horn, 1978). In this paper, we found that Cd^{2+} elicited stimulatory effects in the mouse diaphragm in low Ca^{2+} medium just as in chick biventer cervicis muscle (Fu & Lin-Shiau, 1982).

Spontaneous contractions or fibrillation of the skeletal muscle could be induced by the following methods: (1) Increase in Na^{+} permeability of the muscle cells, with for example, scorpion toxin (isolated from *Androctonus australis*) and crotamine (isolated from *Crotalus terrificus*), induced irregular, spontaneous contractions of high amplitude in the mouse diaphragm or chick biventer cervicis muscle (Lin-Shiau *et al.*, 1975; Chang & Tseng, 1978). (2) Substitution of the majority of chloride ion with impermeant anions (e.g. methane sulphate) induced spontaneous contractions in the rat diaphragm (Rüdel & Senges, 1972). (3) Reduction of Ca^{2+} and K^{+} concentration to one twentieth of normal elicited regular spontaneous contractions of low amplitude in frog skeletal muscle (Bülbring *et al.*, 1956). (4) Divalent cations such as UO_2^{2+} or Ba^{2+} induced muscle fibrillation by releasing acetylcholine from motor nerve terminals (Lin-Shiau *et al.*, 1979; Fu & Lin-Shiau, 1978; Lin-Shiau & Fu, 1980). On the other hand, Ba^{2+} induced muscle fibrillation in Cl^{-}-free medium by decreasing membrane K^{+} conductance (Sperelakis *et al.*, 1967). (5) Denervated muscle fibres displayed fibrillation because of spontaneous action potentials (Purves & Sakmann, 1974; Smith & Thesleff, 1976). In this paper, we found that Cd^{2+} induced most frequently rhythmic spontaneous contractions of high amplitude (about 1g). It is interesting that among nine metal ions tested, Cd^{2+} was the only one capable of inducing spontaneous contractions of the mouse diaphragm.

The possible mechanisms of the stimulatory effects of Cd^{2+} were investigated by use of several specific inhibitors, change of ionic composition of the bathing medium and electrophysiological studies. Both (+)-tubocurarine and α-bungarotoxin, which block postsynaptic acetylcholine response and β-bungarotoxin, which specifically blocks acetylcholine release from nerve terminals (Chang *et al.*, 1973), did not affect Cd^{2+}-induced spontaneous contractions. Therefore, the effects of Cd^{2+} are myogenic. In the case of skeletal muscle, it is generally agreed that Ca^{2+} released from the sarcoplasmic reticulum are contractile activators (Caputo, 1978). However, extracellular Ca^{2+} seem to be involved in excitation-contraction coupling (Lüttgau & Spiecker, 1979). Although in the absence of extracellular Ca^{2+}, resting membrane potential is unchanged, the inactivation process is accelerated and tension cannot be maintained during a K^{+} contracture or tetanic stimulation. Maybe this characteristic of low Ca^{2+} solutions enabled Cd^{2+} to elicit spontaneous contractions one by one instead of a sustained contracture. It was found that Cd^{2+} not only depolarized the muscle membrane but also induced spontaneous action potentials of high frequency. Low Na^{+}, procaine and tetrodotoxin abolished the effects of Cd^{2+}. It appears that increasing the Na^{+} permeability of the muscle membrane is an essential step in bringing about spontaneous contractions. However, we are not sure whether increased sodium permeability is the primary

action of Cd^{2+}, since no depolarization was observed in muscle fibres showing no spontaneous action potentials. Probably, tetrodotoxin increased the membrane potential by inhibiting conduction of spontaneous action potentials. On the other hand, the substitution of chloride with poorly-permeating anions (sulphate, acetate, nitrate or propionate) produced effects similar to that of Cd^{2+}, i.e. spontaneous contractions, increased contractile responses to direct stimulation and enhanced contracture responses to acetylcholine and carbachol (some data not shown). Furthermore, low chloride medium also inhibited the effects of Cd^{2+}. Whether the lowering of chloride permeability is responsible for the effects of Cd^{2+} needs further investigation.

The failure of Cd^{2+} to induce spontaneous contractions in the diaphragm pretreated with glycerol suggests the importance of transverse tubules in the initiation of spontaneous contractions by Cd^{2+}. On the other hand, cysteine abolished spontaneous contractions induced by Cd^{2+} suggesting that the binding of Cd^{2+} to $-SH$ groups of the membrane is closely related to its effect.

In low Ca^{2+} medium, Cd^{2+} potentiated acetylcholine and carbachol-induced contractures. After treatment of the preparation with α-bungarotoxin which binds irreversibly with acetylcholine receptors, the addition of acetylcholine and carbachol did not elicit any contracture in spite of the presence of Cd^{2+}. Therefore, it appears that Cd^{2+} does not increase acetylcholine receptor number or expose any acetylcholine receptors buried in the muscle membrane. Since Cd^{2+} does not affect acetylcholinesterase activity (Hedlund *et al.*, 1979), the effects of Cd^{2+} were probably due to increase in excitability of the muscle membrane. In line with this contention, Cd^{2+} also enhanced K^+-contracture. These effects of Cd^{2+} could be elicited not only in low Ca^{2+} medium but also in normal Ca^{2+} medium. Electrophysiological studies showed that spontaneous action potentials were induced in about 20% of muscle fibres in normal Ca^{2+} medium.

Intracellular recording of action potentials indicated that Cd^{2+} allowed a single direct stimulation to induce many action potentials (data not shown). This may explain the potentiating actions of Cd^{2+} on responses to direct stimulation, since Cd^{2+} apparently converted a single stimulation into a tetanic stimulation.

In conclusion, in low Ca^{2+} medium, Cd^{2+} induced spontaneous contractions, increased twitch responses to direct stimulation and potentiated contracture induced by acetylcholine, carbachol and high K^+, probably by increasing the excitability of the muscle membrane.

References

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmac.*, **1**, 38–61.

BÜLBRING, E., HOLMAN, M. & LÜLLMANN, H. (1956). Effects of calcium deficiency on striated muscle of the frog. *J. Physiol.*, **133**, 101–117.

CAPUTO, C. (1978). Excitation and contraction processes in muscle. *A. Rev. Biophys. Bioeng.*, **7**, 63–83.

CHANG, C.C., CHEN, T.F. & LEE, C.Y. (1973). Studies on the presynaptic effect of β-bungarotoxin on neuromuscular transmission. *J. Pharmac. exp. Ther.*, **184**, 339–345.

CHANG, C.C. & TSENG, K.H. (1978). Effect of crotamine, a toxin of South American rattle-snake venom on the sodium channel of murine skeletal muscle. *Br. J. Pharmac.*, **63**, 551–559.

EISENBERG, B. & EISENBERG, R.S. (1968). Selective disruption of the sarcotubular system in frog sartorius muscle. A quantitative study with exogenous peroxidase as a marker. *J. cell. Biol.*, **39**, 451–467.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol.*, **115**, 320–370.

FORSHAW, P.J. (1977). The inhibitory effect of cadmium on neuromuscular transmission in the rat. *Eur. J. Pharmac.*, **42**, 371–377.

FU, W.M. & LIN-SHIAU, S.Y. (1978). Effects of heavy metals on the neuromuscular transmission of the mouse diaphragm. *Proc. Natl. Sci. Council ROC.*, **2**, 127–134.

FU, W.M. & LIN-SHIAU, S.Y. (1982). Studies on spontaneous contractions of chick biventer cervicis muscles induced by cadmium ion. *J. Formosan Med. Ass.*, **81**, 910–920.

HEDLUND, B., GAMARRA, M. & BARTFAI, T. (1979). Inhibition of striatal muscarinic receptors *in vivo* by cadmium. *Brain Res.*, **168**, 216–218.

HORN, R. (1978). Propagating calcium spikes in an eaxon of *Aplysia*. *J. Physiol.*, **281**, 513–534.

KLEINFELD, M., GREEN, H., STEIN, E. & MAGIN, J. (1955). Effect of the cadmium ion on the electrical and mechanical activity of the frog heart. *Am. J. Physiol.*, **181**, 35–38.

KLEINFELD, M. & STEIN, E. (1969). Action of divalent cations on membrane potentials and contractility in rat atrium. *Am. J. Physiol.*, **215**, 593–599.

LEE, C.Y., CHANG, S.L., KAU, S.T. & LU, S.H. (1972). Chromatographic separation of the venom of *Bungarus Multicinctus* and characterization of its components. *J. Chromatogr.*, **72**, 71–82.

LIN-SHIAU, S.Y. & FU, W.M. (1980). Effects of divalent cations on neuromuscular transmission of chick biventer cervicis muscle. *Eur. J. Pharmac.*, **64**, 259–269.

LIN-SHIAU, S.Y., FU, W.M. & LEE, C.Y. (1979). Effects of uranyl ion on neuromuscular transmission of chick biventer cervicis muscle. *Archs. int. Pharmacodyn.*, **241**, 332–343.

LIN-SHIAU, S.Y., TSENG, W.C. & LEE, C.Y. (1975). Pharmacology of scorpion toxin II in the skeletal muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **289**, 359–368.

LÜTTGAU, H.CH. & SPIECKER, W. (1979). The effects of calcium deprivation upon mechanical and electrophysiological parameters in skeletal muscle fibres of the frog. *J. Physiol.*, **296,** 411–429.

PURVES, D. & SAKMANN, B. (1974). Membrane properties underlying spontaneous activity of denervated muscle fibres. *J. Physiol.*, **239,** 125–153.

RÜDEL, R. & SENGES, J. (1972). Mammalian skeletal muscle: reduced chloride conductance in drug-induced myotonia and induction of myotonia by low-chloride solution. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **274,** 337–347.

SMITH, J.W. & THESLEFF, S. (1976). Spontaneous activity in denervated mouse diaphragm muscle. *J. Physiol.*, **257,** 171–186.

SPERELAKIS, N., SCHNEIDER, M.F. & HARRIS, E.J. (1967). Decreased K^+ conductance produced by Ba^{2+} in frog sartorius fibres. *J. Gen. Physiol.*, **50,** 1565–1583.

THIND, G.S., STEPHAN, K.F. & BLAKEMORE, W.S. (1970). Inhibition of vasopressor resonse by Cd. *Am. J. Physiol.*, **219,** 577–583.

TODA, B. (1973). Influence of cadmium ions on contractile response of isolated aortas to stimulatory agents. *Am. J. Physiol.*, **225,** 350–355.

TODA, B. (1976). Neuromuscular blocking action of cadmium and manganese in isolated frog striated muscle. *Eur. J. Pharmac.*, **40,** 67–75.

(Received October 24, 1984.
Revised December 13, 1984.
Accepted January 14, 1985.)