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Mechanism of inhibition of contraction by cadmium in guinea-pig taenia coli

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Further evidence about the mechanism of the inhibition of contractions caused by cadmium ions (Cd^{2+}) in guinea-pig taenia coli has been sought. Cd^{2+} at a concentration of 0·5 mM completely inhibited the high-K (40 mM)-induced contraction within 3–5 min. Cd^{2+} did not shift the Ca^{2+}-induced concentration-response curve to the right in Ca^{2+}-free K^+ depolarized muscle, although it reduced the Ca^{2+} response size. The K^+-induced increase in tissue calcium content and ^{45}Ca uptake determined by the lanthanum method was significantly reduced in the presence of Cd^{2+} (0·5 mM) and the contractions of the glycerolated taenia coli were inhibited with increasing Cd^{2+} (0·001–0·5 mM). Muscle strips, incubated in a medium containing 0·5 mM Cd^{2+}, accumulated greater amounts of cadmium than within the extracellular space. It is suggested that the inhibitory action on tension produced by Cd^{2+} in taenia coli may result from the interference of calcium permeability at the cell membrane. There is the possibility that Cd^{2+} acts on the contractile system of the muscle.

It has been demonstrated that cadmium ions (Cd^{2+}) are a non-specific relaxant, and have inhibitory actions on contractions induced by agonists in cardiac (Toda 1973a), vascular (Perry et al 1967; Thind et al 1970; Toda 1973b), myometrial (Osa 1974), and intestinal (Osa 1974; Triggle et al 1975) muscles.

Osa (1974) showed that after application of Cd^{2+}, the contractile activity of the ileum induced by carbachol was suppressed, but its membrane depolarization effect was maintained in both the ileum and the myometrium preparations. Thus, Cd^{2+} induced dissociation of excitation-response events. This evidence suggests that the mechanism of contractile inhibition caused by Cd^{2+} in smooth muscles cannot be due simply to an electrical membrane activity. Toda (1973b) reported that the contractions obtained with calcium ions (Ca^{2+}) on the high-K treated aorta in a Ca^{2+}-free medium were inhibited by Cd^{2+}. Toda (1973b) considered that inhibition of the inward fluxes of Ca^{2+} appeared to be the major mechanism responsible for the inhibitory effect of Cd^{2+}.

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The present study was undertaken to examine the effects of Cd^{2+} on calcium movement in taenia coli cells, glycerolated muscle and on the respiration of mitochondria, in an attempt to further define the mechanism of the muscular inhibitory effects of Cd^{2+}.

MATERIALS AND METHODS

Strips of taenia coli were freshly dissected from male Hartley strain guinea-pigs, 400 g, and immersed in Tyrode solution bubbled with 95% O_2 and 5% CO_2 at 37 °C. The solution contained (mM): NaCl 136·8, KCl 2·7, $CaCl_2$ 2·5, $MgCl_2$ 1·0, $NaHCO_3$ 11·9 and glucose 5·5. The high-K (40 mM) medium was prepared by adding an appropriate amount of 2 M KCl solution to Tyrode solution. Cadmium ions as $CdCl_2$ were added to bathing medium.

Contractile responses were recorded isometrically by a strain-gauge transducer (Nihon-Kohden, RM-150) under 0·6 g resting tension.

To examine tissue calcium content of muscles, the strips were removed from the bath at the end of experiments. Then the strips were drawn across a sheet of filter paper (Toyo, No 4) to remove adhering solution, weighed and incinerated in a muffle furnace at 550 °C for 2 h. The samples were diluted in a solution containing 0·2% $SrCl_2$ and 0·4% ethylenediamine tetraacetic acid (EDTA) to eliminate interference with other cations and anions (Urakawa et al

1968). The calcium contents of the diluted sample were measured by an atomic absorption spectrophotometer (Hitachi, 207). Muscle segments in these determinations were approximately 20 mg in weight.

^{45}Ca uptake into the muscle was measured by a modified 'lanthanum (La) method' described by Karaki & Weiss (1979). The strips were exposed to a medium containing ^{45}Ca (5 μCi ml^{-1}, New England Nuclear) for the desired period of time, after which the strips were rinsed with a lanthanum solution ($LaCl_3$ 73, glucose 5·5 and Tris-HCl 11·9 mM) (pH 7·2) aerated with 100% O_2 at 1 °C for 50 min. After blotting, the strips were treated with solubilizer (Soluene TM-350, Packard) and the radioactivities were counted using a liquid scintillation spectrophotometer (Aloka, LSC-602).

For the tests of the effects of Cd^{2+} on contractile proteins, strips of taenia coli were placed in the glycerol solution which contained 50% (v/v) glycerol, 50 mM KCl, and 20 mM Tris-malate (pH 6·8) and were stored at 2 °C. After 24 h the strips were replaced in a fresh glycerol solution and stored at −15 °C for 6 days. In experiments, the glycerolated muscles were incubated in the solution containing 50 mM KCl and 20 mM Tris-malate (pH 6·8). The contractile responses were recorded isotonically. One end of the glycerolated muscle was fixed, while the other end was connected to a light lever with a magnification of 7:1 under a resting tension of 100 mg.

Measurements of the muscle uptake from external Cd^{2+} medium were also made. In these studies, strips were weighed after blotting and then transferred to a quartz cuvette and incinerated by a plasma asher (Yanagimoto Model LTA-4SN) for 10 h. The samples were diluted in 0.01 M HNO_3. The cadmium contents of the diluted sample were measured by an atomic absorption spectrophotometer (Hitachi, 207). As the melting point of cadmium is low (321 °C), the plasma asher apparatus was suitable for the incineration of muscle.

To measure alterations of extracellular space, [^{14}C]sorbitol (0·5 μCi ml^{-1}) was added 30 min before the end of each experiment. After the incubation period, the strips were treated with solubilizer and the radioactivities were counted using a liquid scintillation spectrophotometer.

Mitochondria were isolated from the liver of rats by the procedure of Myers & Slater (1957). All reactions were carried out in the solution containing 25 mM Tris-HCl buffer, 50 mM sucrose, 5 mM $MgCl_2$ and 5 mM KCl in a total volume of 6 ml at pH 7·4. Oxygen consumption was measured with the oxygen electrode (Yellow Spring Instrument Co, YSI 53) at 25 °C. Protein concentration in mitochondria was measured through the change in absorbance from 605 to 630 nm as a result of reduction of cytochrome a according to Higuchi et al (1976). The absorbance was measured using dual wavelength beam spectrophotometer (Hitachi, 356).

RESULTS

All muscle strips of taenia coli were equilibrated for 40 min in normal Tyrode solution. At the end of that time, the response of each muscle was tested by exposing it to high-K (40 mM). When muscle strips of taenia coli were treated with high-K, muscle tension increased, and then remained at a steady level of about 10 g. After the strips were incubated in a high-K medium for 30 min, Cd^{2+} was applied in various concentrations. At a concentration of 0·001 mM, Cd^{2+} caused a small decrease in tension (less than 15% after 60 min). The addition of 0·1 mM of Cd^{2+} caused a gradual decrease to the base line in 45–50 min. Cd^{2+} at 0·5 mM decreased the tension to the base line levels in 3–5 min (Fig. 1A). Fig. 1B shows the tension developed at 15 and 30 min after the Cd^{2+} application, plotted as a percentage of the maximal tension induced by the addition of high-K.

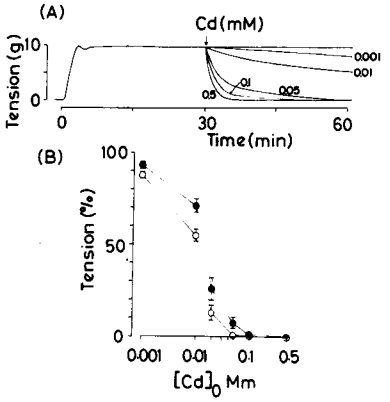

FIG. 1. Effects of Cd^{2+} on the tonic contraction induced by high-K (40 mM) medium in taenia coli. Cd^{2+} was applied 30 min after the addition of high-K. (A) Tension changes when 0·001, 0·01, 0·05, 0·1 or 0·5 mM $CdCl_2$ was applied. (B) Tension at 15 min (●) or 30 min (○) after Cd^{2+} application in various concentrations was calculated as per cent of maximal tension developed by high-K. Each point in this figure represents the mean of 20 experiments (mean ± s.e.).

The cumulative concentration-response curves for the contraction of smooth muscles could also be generally obtained by adding Ca^{2+} to a Ca^{2+}-free medium in depolarized muscle (Simonis et al 1971).

The strips were incubated in a Ca^{2+}-free isotonic 120 mM K medium for 30 min and then, again incubated in a Ca^{2+}-free isotonic 120 mM K medium containing 0·001, 0·005 or 0·01 mM of Cd^{2+} for 30 min. Cumulative concentration-response curves were obtained to $CaCl_2$ (0·1–20 mM) by increasing the Ca^{2+} concentration at 5 min intervals. The increasing concentrations of Cd^{2+} did not shift the concentration-response curves for Ca^{2+} to the right, but did cause a reduction in the maximum response (Fig. 2). The Ca^{2+}-induced contracture was inhibited by lower concentrations of Cd^{2+} than those required to reduce the resonse to high-K in a normal Ca^{2+} medium.

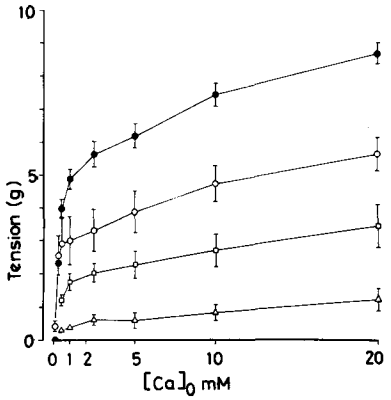

FIG. 2. Effect of Cd^{2+} on the Ca^{2+}-induced contracture in depolarized taenia coli. The concentration-tension-relationships were obtained by cumulative increasing in concentration of Ca^{2+} in Ca^{2+}-free isotonic 120 mM K medium containing three different concentrations of Cd^{2+}. Each point in this figure represents the mean of 12 experiments (mean ± s.e.). Control (●); cadmium 0·001 mM (○), 0·005 mM (□), and 0·01 mM (△).

To ascertain whether Cd^{2+} affects the calcium movements, tissue calcium and ^{45}Ca uptake measured by the 'La method' were determined in muscles.

It has been reported that high-K-induced contractions increase the tissue calcium content and net calcium uptake (Urakawa et al 1964; Karaki et al 1969). The tissue calcium content of taenia coli in a normal Tyrode solution was 2·25 ± 0·06 m mol kg^{-1} wet wt, (n = 8) (mean ± s.e.) and the value did not change significantly throughout the period of observation. In the presence of high-K, the tissue calcium increased during the first 30 min and was maintained at this high level. When 0·5 mM of Cd^{2+} was applied to the muscle 30 min after the addition of high-K, the tissue calcium decreased gradually (Fig. 3). In another series of experiments, the preparation was

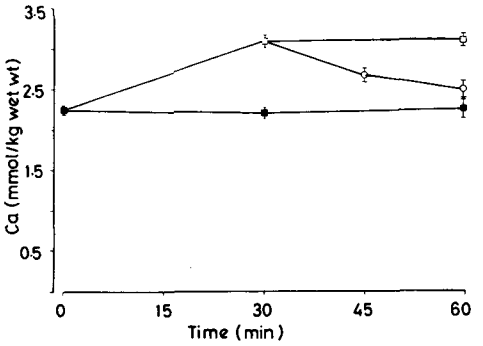

FIG. 3. Effect of Cd^{2+} on tissue calcium content in taenia coli. High-K was added at time 0 and 0·5 mM Cd^{2+} was added at time 30 min. Each point in this figure represents the mean 8–16 experiments (mean ± s.e.). Control (■); K, 40 mM (□); K, 40 mM + Cd, 0·5 mM (○).

pretreated with 0·5 mM of Cd^{2+} before the addition of high-K. In these conditions the tissue calcium content of taenia coli was not increased by the addition of high-K.

Since the affinity for La^{3+} at cell membranes of smooth muscle is much greater than for Ca^{2+}, La^{3+} would occupy the transport sites and thus displace Ca^{2+} from the cell membrane (Mayer et al 1972; Van Breemen et al 1973). The 'La method' was therefore used to measure calcium uptake into intestinal or vascular smooth muscle.

The ^{45}Ca uptake in normal medium reached a plateau phase within 20 min. A large increase in ^{45}Ca uptake was demonstrated after the addition of high-K (40 mM). The effect of high-K was then studied in muscles pretreated with 0·01 or 0·5 mM Cd^{2+} for 30 min before the addition of high-K. At 0·01 mM, Cd^{2+} caused a small decrease in ^{45}Ca uptake. Pretreatment with 0·5 mM Cd^{2+} completely inhibited the increase in ^{45}Ca uptake (Fig. 4).

To determine whether or not Cd^{2+} affected the contractile proteins in taenia coli, the effects of Cd^{2+} on the glycerolated taenia coli were studied. In the glycerolated taenia coli, tension is developed at 1×10^{-6} M Ca^{2+} and 5 mM Mg-ATP as described earlier (Nasu & Ishida 1975). Because the Cd^{2+} binding potency of ethylene glycol bis(β-aminoethylether) tetraacetic acid (EGTA) is higher than its Ca^{2+} binding potency, the EGTA- Ca EGTA buffer system could not be used to make a medium of low stabilized concentration of free ionized calcium. Therefore, the solution containing high concentrations of Ca^{2+} (1 mM, no EGTA) and 5 mM Mg-ATP was used to produce the contraction.

Fig. 5A illustrates some typical recordings of contractions of glycerolated taenia coli in the

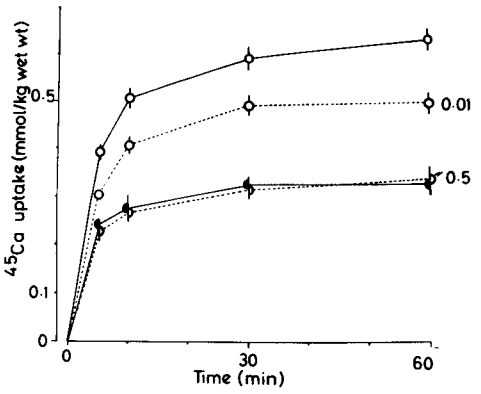

FIG. 4. ^{45}Ca uptake by taenia coli in normal medium (●), high-K medium (○) or high-K medium containing Cd^{2+} (---○---). High-K or ^{45}Ca was added at time 0. Cd^{2+} was applied at 30 min before the application of high-K. The accompanying number of each curve indicates the concentration of Cd^{2+} in the medium (mM). Each point on the curves represents the mean of 8–12 experiments.

presence of 1 mM of Ca^{2+} and 5 mM Mg-ATP containing various concentrations of Cd^{2+} (0·001, 0·01, 0·1 or 0·5 mM). The length of the muscle in its contracted state compared with that in its resting state was expressed (Fig. 5B). The % shortening was about 10 and 16% at 30 and 60 min following the application of 1 mM of Ca^{2+} and 5 mM Mg-ATP. The inhibition of the shortening in the glycerolated muscles was linear with increasing Cd^{2+} concentration, and a complete inhibition of the shortening occurred with Cd^{2+} pretreatment above 0·5 mM levels.

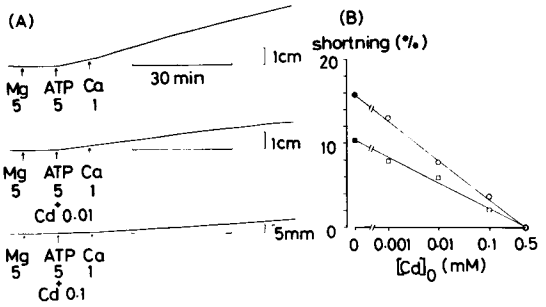

FIG. 5. Effects of Cd^{2+} on the glycerinated taenia coli. (A) Each curve is a tracing from an actual experiment. The media were replaced with the medium containing Mg^{2+}; Mg-ATP plus Cd^{2+}; or Mg-ATP, Cd^{2+} plus Ca^{2+}. The pH of each medium was adjusted to pH 6.8. (B) The % shortening in the presence of 1 mM Ca^{2+}, 5 mM Mg-ATP and Cd^{2+} in the various concentrations. The % shortening was calculated using the following equation; {the length of muscle at the time of 30 (□) or 60 (○) min incubation after application of Mg-ATP and Ca^{2+} in medium containing Cd^{2+} (0·001, 0·01, 0·1 or 0·5 mM)/the length of muscle in relaxed state} × 100.

To investigate the extent of Cd^{2+} binding to cells of taenia coli, the tissue uptake of cadmium was examined in a 0·5 mM Cd^{2+} medium over 90 min. The cadmium uptake by taenia coli reached an equilibrium level at 60 min (Fig. 6). On the other hand, the extracellular space in taenia coli was measured using [^{14}C]sorbitol as the indicator. Uptake of [^{14}C]sorbitol by taenia coli reaches a plateau within 10 min (Goodford & Leach 1966). [^{14}C]Sorbitol space was determined during the last 30 min in each experiment and the sorbitol space was expressed as the tissue/medium ratio. The [^{14}C]sorbitol space was 0·35 ± 0·01 (n = 6) in normal Tyrode solution and 0·35 ± 0·01 (n = 8) in Tyrode solution containing 0·5 mM of Cd^{2+}. On the assumption that extracellular space is saturated with the same concentration of substance as the external medium, the hatched part (0·5 mM of Cd^{2+} in external medium X 0·35 in extracellular space measured by [^{14}C]sorbitol) in Fig. 6 represents the Cd^{2+} amount that existed in the extracellular space of tissue cadmium.

The effects of Cd^{2+} on the respiration of mitochondria were tested by using rat liver mitochondria. The addition of 0·4 mM ADP to mitochondria suspended in a medium containing glutamate or succinate substrate, produced a sharp increase in the rate of oxygen uptake. Fig. 7A and B shows that 0·05 (data not shown) or 0·5 mM of Cd^{2+} inhibited the stage 3 respiration of mitochondria results from the addition of ADP in the presence of either glutamate or succinate substrate.

DISCUSSION

It has been reported that high-K-induced contracture in taenia coli is accompanied by an increase in the

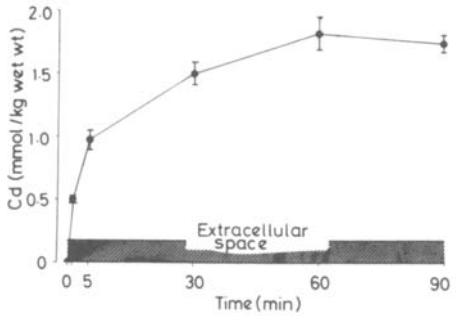

FIG. 6. Cadmium uptake in taenia coli. Cd^{2+} (0·5 mM) was added at time 0. Assuming that extracellular space is saturated with 0·5 mM Cd^{2+} equal to the external medium concentration, the hatched part of the figure represents Cd^{2+} present in the extracellular space. Each point in this figure represents the mean of 5–8 experiments (mean ± s.e.).

tissue calcium levels resulting from the increased net calcium uptake (Urakawa & Holland 1964; Karaki et al 1969) and ^{45}Ca uptake measured by 'La method' (Kishimoto et al 1980). We tested the effects of Cd^{2+} on tissue calcium content and ^{45}Ca uptake measured by the 'La method' to distinguish between the effects of Cd^{2+} on the overall changes in tissue calcium concentration and differential changes which may be produced on muscle proteins. When Cd^{2+} is added to the medium, the increased tissue calcium induced by high-K began to decrease, and this may indicate that Cd^{2+} inhibits net calcium uptake. Further, the increased ^{45}Ca uptake determined by the 'La method" resulting from application of high-K was progressively reduced by Cd^{2+} (0·01–0·5 mM) (Fig. 4). This fact suggests that the increased cellular Ca^{2+} concentration produced by high-K does not occur.

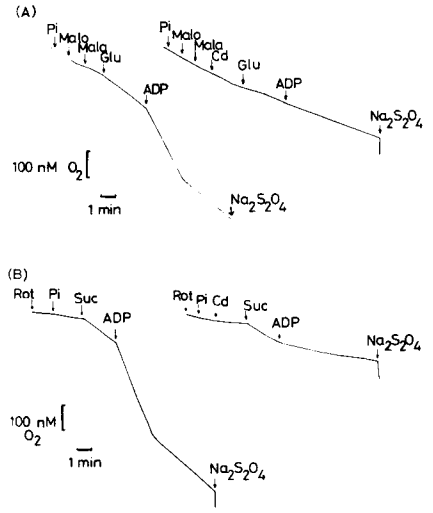

FIG. 7. (A) Effects of Cd^{2+} on rat liver mitochondrial respiration with glutamate as substrate. Malonate was used to inhibit the succinate dehydrogenase. Malate was used for carrier of glutamate (De Hann et al 1968). The mitochondria added to each vessel contained 1·53 mg protein ml^{-1}. Additions: 10 mM Pi, 8 mM malonate, 5 mM malate, 10 mM glutamate, 0·4 mM ADP and 0·5 mM Cd^{2+}. Each curve is a tracing from an actual experiment. (B) Effect of Cd^{2+} on mitochondrial respiration with succinate as substrate. Rotenone was used to block diphosphopyridine nucleotide-flavin-linked transport (Ernster et al 1963). The mitochondria added to each vessel contained 1·09 mg protein ml^{-1}. Additions: 1×10^{-6} M rotennone, 10 mM Pi, 10 mM succinate, 0·4 mM ADP and 0·5 mM Cd^{2+}.

On the other hand, Cd^{2+} did not shift the concentration-response to Ca^{2+} in Ca^{2+}-free KCl medium, but decreased the maximum responses in taenia coli of the guinea-pig. Spedding (1982) reported that the Ca^{2+} antagonist drug verapamil caused parallel displacement to the right of the concentration-responses curves to Ca^{2+} in taenia coli. However, the inhibition of oxidative phosphorylation by N_2 gas or 2,4-dinitrophenol antagonized Ca^{2+}-induced contractions but reduced the maximum responses (Spedding 1982). Thus, Cd^{2+} belongs to the group of non-specific spasmolytics such as dinitrophenol as judged by the effects of its action on Ca^{2+}-induced contraction in taenia coli. From this, it is postulated that Cd^{2+} may interfere at some level in the sequence of the contraction process besides the inhibition of Ca^{2+} influx at the cell membrane.

The inhibition of the rates of shortening in the glycerolated taenia coli were dependent on the Cd^{2+} concentration. The Cd^{2+} concentration required to inhibit contractions of intact and glycerolated muscle was similar. If Cd^{2+} can penetrate the cell membrane of taenia coli intact muscle, Cd^{2+} would be expected to have an inhibitory action on the contractile proteins in smooth muscle. Blum (1962) reported that the ATPase activity of myosin in skeletal muscle was inhibited by Cd^{2+}. There is no report whether or not Cd^{2+} inhibits the ATPase of the contractile proteins of smooth muscle.

There are few studies reported on the effects of Cd^{2+} on respiration in mitochondria separated from smooth muscle cells. Tsuda et al (1975, 1977) reported that the mitochondria from taenia coli of guinea-pig had a low respiratory control index, which may have resulted from damage in the isolation procedures (protease method) as the cell membrane of taenia coli is resistant to disruption. However, the quality of mitochondria obtained from taenia coli and rat liver preparations is essentially the same (Tsuda et al 1975, 1977). Therefore, the effect of Cd^{2+} on rat liver mitochondria was also examined. The addition of 0·5 mM of Cd^{2+}, which inhibited the high-K-induced contraction, also inhibited the ADP-stimulated oxidations of glutamate and succinate when used as substrates in the mitochondria. These observations indicate that Cd^{2+} inhibits electron transfer in the respiratory chain. Jacobs et al (1956) reported that Cd^{2+} inhibited the oxidation of succinate and citrate in rat liver mitochondria.

Cadmium uptake increased along with the duration of the Cd^{2+} incubation in taenia coli (Fig. 6). The cadmium uptake reached an equilibrium in 30-60 min. Incubation of taenia coli for 60 min in 0·5 mM of Cd^{2+} medium produced a tissue cadmium content of about 2·5 m mol kg^{-1} wet wt (1·82 mM in tissue cadmium content after 60 min incubation—0·5 mM in external Cd^{2+} concentration × 0·35

in extracellular space)/(1–0·35 in extracellular space) at equilibrium. The concentration of cadmium in the cells of taenia coli was therefore about 5 times higher (2·5 mM in cells/0·5 mM in external medium) than that of the external medium.

Moreover, we have tested the cellular distribution of cadmium in taenia coli to gain further evidence about the possible site of Cd^{2+} action. After the muscles were incubated in a medium containing 0·5 mM of Cd^{2+} to reach equilibrium levels (60 min), they were then immersed in a Cd^{2+} free normal medium. The tissue cadmium content was found to be reduced to about 43% of the original tissue cadmium levels. Also, when the muscles were rinsed in a medium containing EDTA, which did not penetrate the cell membrane of taenia coli (Brading & Jones 1969), only about 27% of the original level of tissue cadmium was retained (Nasu & Koshiba, unpublished data). These results do not therefore exclude the possibility that Cd^{2+} is able to penetrate the cell membrane of taenia coli.

It is suggested that the inhibitory action on tension produced by Cd^{2+} in taenia coli may result from interference with the calcium permeability at the cell membrane. Furthermore, there is the possibility that Cd^{2+} acts on the contractile system components such as the contractile proteins and mitochondria.

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