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Calcium Antagonism by Cobalt Ions on Contraction of Guinea-pig Taenia Coli

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Abstract—In guinea-pig taenia coli, cobalt ions (Co^{2+}) inhibited the tonic response to a highly concentrated K^+ solution (high-K^+, 40 mM) more strongly than the phasic response. Co^{2+} displaced Ca^{2+} concentration–response curves to the right, inhibited the increase in tissue calcium content caused by high-K^+, and inhibited Ca^{2+} binding at low affinity sites more than at high affinity sites. After treatment with Co^{2+}, the tonic tension caused by high-K^+ was not restored by a wash with normal medium, but it was restored by a wash with EDTA. The cobalt remaining in muscles was almost completely eliminated after a 20–30 min wash with EDTA. The results suggest that Co^{2+} binds chiefly to the surface membrane of taenia coli. Co^{2+} probably reduced the tension in response to high-K^+ mainly by inhibiting Ca^{2+} influx rather than by affecting Ca^{2+} release.

Heavy metal ions, including cobalt (Co^{2+}), cadmium (Cd^{2+}), mercury (Hg^{2+}) and manganese (Mn^{2+}) ions, are often called inorganic Ca^{2+}-channel antagonists (Schramm & Towart 1985). Previous reports have indicated that Co^{2+} suppresses contractile responses of the ileum (Little et al 1985) and vein (Feletou et al 1986; Sutter et al 1988). Moreover, Co^{2+} has been found to block the inward current in guinea-pig taenia coli muscle (Inomata & Kao 1976; Ganitkevich et al 1986) and in rat uterine muscle (Jmari et al 1987). Co^{2+} has also been found to inhibit the binding of a Ca^{2+}-channel antagonist, nitrendipine, to a microsomal fraction from intestinal muscle (Ehlert & Roeske 1982; Luchowski et al 1984). These data indicate that Co^{2+} has a great affinity for Ca^{2+} channels in the cell membranes of smooth muscle. In contrast, previously published data suggest that Cd^{2+} and Hg^{2+} have inhibitory effects within taenia coli cells, and also inhibit Ca^{2+} influx at the cell membrane (Nasu et al 1983, 1984; Nasu & Koshiba 1984, 1985). Thus, the present study was designed to determine if the inhibition of K^+-induced tension by Co^{2+} can be completely explained by inhibition of Ca^{2+} influx through the cell membrane in taenia coli.

Materials and Methods

Taenia coli

Strips of taenia coli were dissected from male Hartley strain guinea-pigs, 400 g, and were immersed in modified Tyrode solution bubbled with 100% O_2 at 37°C. The solution contained (mM): NaCl 123·7, KCl 2·7, $CaCl_2$ 2·5, $MgCl_2$ 1·0, tris (hydroxymethyl) aminomethane 25 and glucose 5·5. The pH of the solution was adjusted to 7·4 with HCl at 37°C. High-K^+ solution (40 mM K^+) was prepared by adding an appropriate amount of 2 M KCl to Tyrode solution. Cobalt ion, as $CoCl_2 \cdot 6H_2O$, was added to the bath solution.

The muscle strips were suspended at a resting tension of 0·6 g and allowed to equilibrate for 40 min. Isometric contraction of the muscle was measured by a strain gauge transducer (Nihon Kohden, RM-6000).

Tissue calcium and La^{3+}-resistant residual ^{45}Ca uptake

To examine muscle tissue calcium, the strips were removed from the bath at the end of the experiments. The strips were then drawn across a sheet of filter paper to remove any remaining bath solution, weighed and transferred to a quartz cuvette with 0·1 mL $HClO_4$ and heated 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 from other cations and anions (Urakawa et al 1968). The calcium contents of the diluted samples were measured with an atomic absorption spectrophotometer (Hitachi 308) and were compared with known amounts of Ca^{2+} which were measured at the same time.

^{45}Ca uptake into the muscle was measured by a modification of the lanthanum method described by Karaki & Weiss (1979). The muscles were exposed to 0·03 or 3 mM Ca^{2+} + ^{45}Ca (5 μCi mL^{-1}, New England Nuclear), K^+ (40 mM) medium containing 0·3 or 1 mM Co^{2+}, for 60 min, after which they were rinsed with a lanthanum solution ($LaCl_3$ 73, glucose 5·5 and Tris-HCl 11·9 mM, pH 7·4), which was aerated with 100% O_2 at 1°C for 50 min. The strips were blotted and then treated with a solubilizer (Soluene TM-350, Packard) and the radioactivities were counted with a liquid scintillation spectrophotometer (Aloka, LSC-602).

Tissue cobalt

To determine tissue cobalt concentration in muscles, the strips were removed from the bath after incubation in a medium containing an appropriate amount of Co^{2+}. They were blotted on filter paper, then weighed and heated in a muffle furnace at 550°C for 2 h. The samples were dissolved in water and the Co^{2+} concentrations were measured with an atomic absorption spectrophotometer. Because the melting point (1493°C) and boiling point (3100°C) of cobalt are very high, the Co^{2+} was almost completely recovered, even when the furnace was set at 550°C.

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

Tissue ATP concentration

The tissue ATP concentration was measured by the method of Ishida et al (1984). The muscles were removed from the bath at the end of each experiment and boiled for 5 min in test tubes containing 1 mL of water. The ATP concentration in the extracts was measured with a luminometer (Lumac M1070) using a luciferine–luciferase reagent.

Results

Effects of Co^{2+} on high-K^+-induced contraction

When strips of taenia coli were treated with the high-K^+ (hypertonic, 40 mM) medium, the phasic tension was followed by a tonic contraction sustained at a level of about 8·5 g. After the muscles were incubated in the high-K^+ medium for 30 min, Co^{2+} was applied at various concentrations. At 0·1 mM, Co^{2+} caused a small decrease in tension. The addition of 0·3 mM Co^{2+} caused a gradual decrease in tension, to about 8% of the original value after 90 min. Co^{2+} at 1 mM decreased the tension almost to the baseline level within 30 min (Fig. 1a, b).

The effects of preincubation with Co^{2+} on tension induced by the high-K^+ solution were also examined. After 60 min of incubation with 0·3 and 0·7 mM Co^{2+}, 63 ± 4 (n=8) and 48 ± 3%, respectively, of the phasic response to the high-K^+ solution remained, but the tonic response decreased to 19 ± 2 and 8 ± 2% of the control, respectively (Fig. 2). The phasic response to K^+ was less affected by Co^{2+} than the tonic response, although the beginning of the phasic response was slightly slowed in the presence of Co^{2+}. However, 1 mM Co^{2+} nearly abolished both responses.

Effect of Co^{2+} on the Ca^{2+}-induced contraction

The effects of Co^{2+} on the Ca^{2+}-induced responses in depolarized muscles were examined. The strips were incubated in a Ca^{2+}-free (isotonic 120 mM K^+) medium for 30 min, and were then incubated in a medium containing Co^{2+} for 30 min. Cumulative concentration–response curves were obtained by increasing the Ca^{2+} concentration (0·03–30 mM) at 5 min intervals. Co^{2+} at 0·1 or 0·2 mM displaced the concentration–response curves to the right and did not lower the response at 30 mM Ca^{2+}. In the presence of 0·3 and 1 mM Co^{2+}, the curves were displaced further to the right, but the responses to 30 mM Ca^{2+} were lower. Higher concentrations of Ca^{2+} could not be tested because they would be greater than the solubility of $CaCl_2$ in the medium (Fig. 3).

Effects of Co^{2+} on tissue calcium and La^{3+}-resistant residual ^{45}Ca uptake

When 0·3 or 1 mM Co^{2+} was added, the increase in tissue calcium content caused by the high-K^+ (40 mM) solution was inhibited (Fig. 4a).

To determine the effects of Co^{2+} on the high and low affinity binding sites of Ca^{2+}, muscles were incubated in 0·03 or 3 mM Ca^{2+} (+^{45}Ca), 40 mM K^+ medium containing Co^{2+} for 60 min and were washed with a La^{3+} solution at 1°C for 50 min. The low concentration of Co^{2+} (0·3 mM) inhibited the Ca^{2+} binding at low affinity sites more than high affinity sites. However, 1 mM Co^{2+} inhibited binding at both types of sites (Fig. 4b, c).

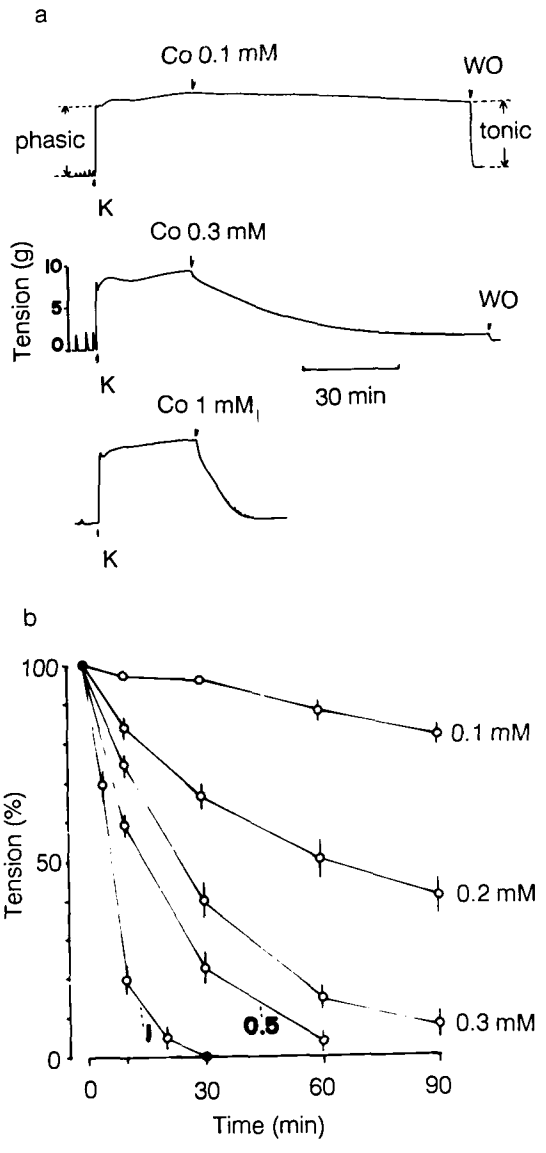

FIG. 1. Effects of Co^{2+} on high-K^+ (hypertonic, 40 mM)-induced contraction in taenia coli. $CoCl_2$ was added 30 min after the high-K^+ solution. (a) Changes in tension caused by 0·1, 0·3 and 1 mM Co^{2+}. (b) The tensions after application of various concentrations of Co^{2+} were calculated as percentages of the maximal tonic tension in response to the high-K^+ solution. Each point represents 10–12 experiments (mean ± s.e.). The two phases of the phasic and tonic contraction to high-K^+ solution are indicated on the figure. WO = wash-out.

Effects of washing on tension recovery after Co^{2+} treatment

To determine if washing has any effect on the recovery of the tension after Co^{2+} treatment, muscles were incubated in medium containing Co^{2+} (0·3–1 mM) for 160 min as shown in Fig. 2 and then washed with normal medium for 60 min. The wash completely restored the phasic response to the high-K^+ solution but the tonic response was still inhibited (Fig. 2).

The effects of EDTA on tension recovery after Co^{2+} treatment were also studied. Muscles were first incubated in 1 mM Co^{2+} medium for 60 min and then washed with Ca^{2+}- and Mg^{2+}-free medium containing 0·5 mM EDTA for 45 min. They were returned to the normal medium for 60 min, and then high-K^+ was added. After a small contraction, the tonic response to K^+ was completely restored (Fig. 5).

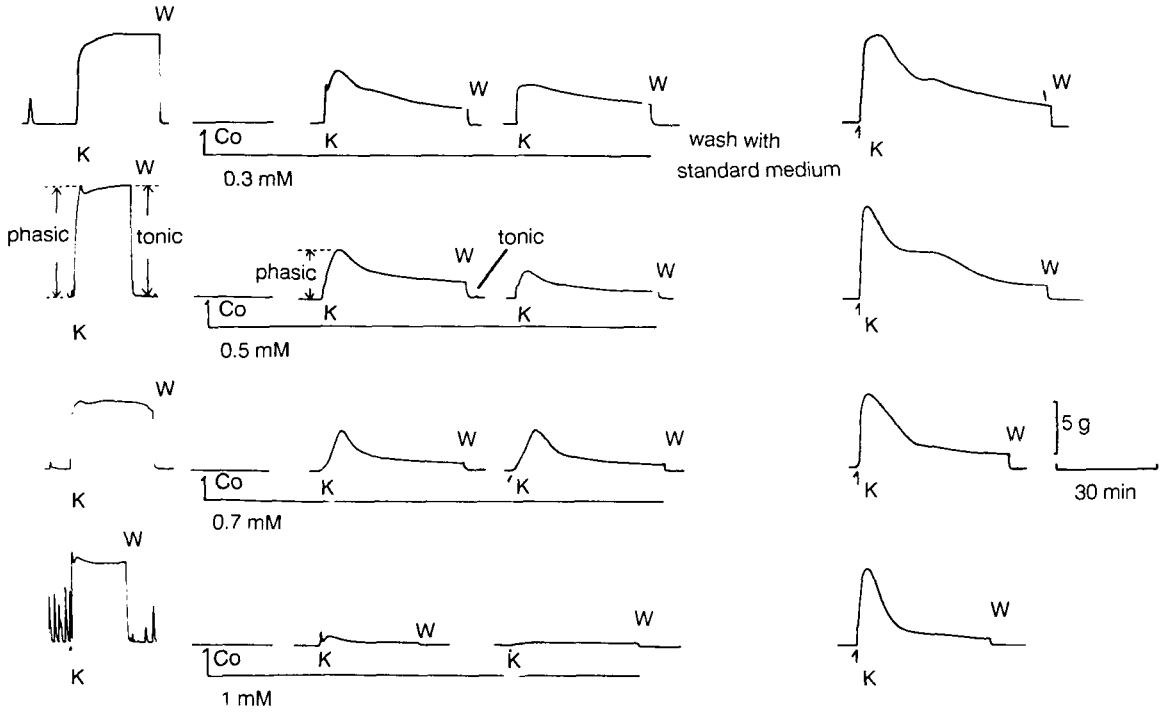

FIG. 2. The effects of preincubation with Co^{2+} on responses to the high-K^+ solution and the effects of a wash (W) with normal medium on recovery of the high-K^+ induced response after Co^{2+} treatment in taenia coli. In each row, the first response is a control. Muscles were preincubated for 60 min in Co^{2+} medium before the addition of the high-K^+ solution. Following incubation in the high-K^+ medium containing Co^{2+} for 40 min, the muscles were washed with medium containing Co^{2+} for 20 min. The high-K^+ solution was then reapplied in the presence of Co^{2+}. After 40 min, the muscles were washed with normal medium for 60 min. Then the high-K^+ medium was added. The tensions in response to each concentration of Co^{2+} were recorded from different strips.

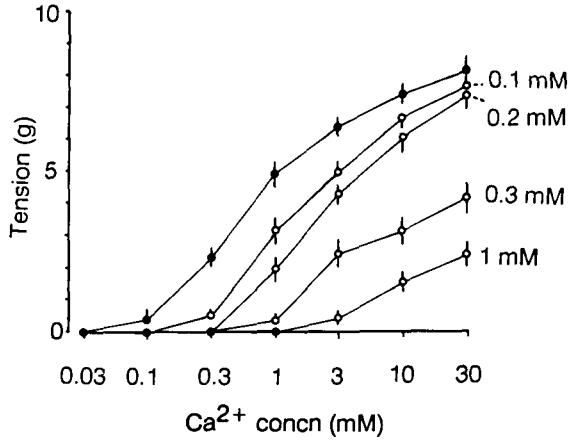

FIG. 3. Effects of Co^{2+} on the Ca^{2+}-induced contraction in depolarized taenia coli. The concentration-tension relationships were obtained by increasing the concentration of Ca^{2+} in isotonic 120 mM K^+ medium containing Co^{2+}. The number near each curve represents the concentration of Co^{2+} in the medium. Each point represents 10 experiments (mean ± s.e.).

Cobalt uptake and efflux

The cobalt uptake by taenia coli stabilized after 60 min, and the equilibrium level depended on the external Co^{2+} concentration (Fig. 6).

To study the change in cobalt efflux, the muscles were incubated in 0·5 or 1 mM Co^{2+} medium for 60 min and then washed with normal medium or Ca^{2+}- and Mg^{2+}-free medium containing 0·5 mM EDTA. The amount of cobalt remaining in the muscles reached an equilibrium after 30 min in the normal medium. In contrast, the cobalt retained in muscles was nearly eliminated by a 20–30 min wash with EDTA (Fig. 7).

The [^{14}C]sorbitol space, expressed as the tissue/medium ratio, was 0·34 ± 0·02 (n = 8) in normal medium and this space is not changed in 0·5 or 1 mM Co^{2+} medium.

Effects of Co^{2+} on tissue ATP concentration

Co^{2+} (1 mM) did not significantly affect the tissue ATP concentration (1·58 ± 0·14 mmol (kg wet wt)$^{-1}$, n = 12) of taenia coli in the high-K^+ medium over 30 min.

Discussion

Low concentrations (0·1–0·2 mM) of Co^{2+} shifted the Ca^{2+} concentration–response curves to the right in a KCl medium in taenia coli. The inhibition of tension by the low concentrations of Co^{2+} was completely antagonized by increasing the external Ca^{2+} concentration. However, metabolic inhibitors, N_2 gas and 2,4-dinitrophenol (Spedding 1982) and heavy metal ions, Cd^{2+} and Hg^{2+} (Nasu et al 1983, 1984) did not shift the Ca^{2+} concentration–response curves to the right, but reduced the maximal response in taenia coli.

In taenia coli stimulated with K^+ (40–60 mM), the tonic response is caused by the increase in tissue calcium which results from increased net calcium uptake (Urakawa & Holland 1964; Karaki et al 1969). The Ca^{2+}-channel antagonists verapamil (Karaki et al 1984) and D-600 (Nasu &

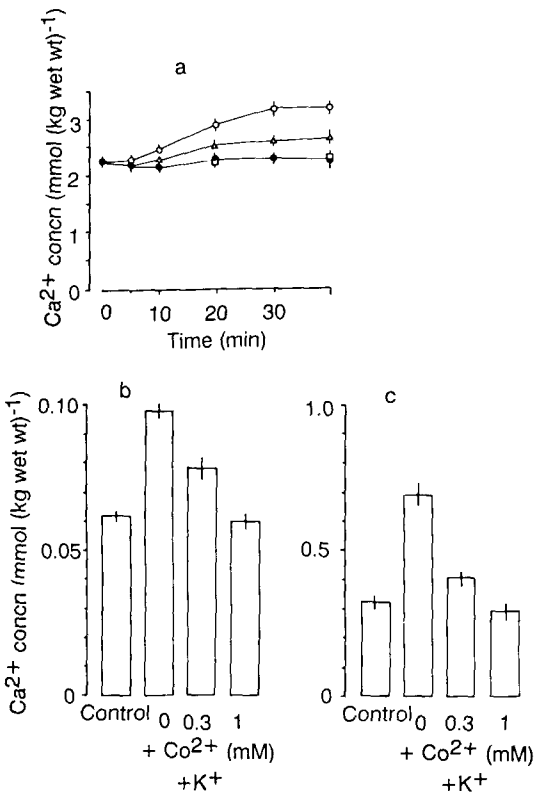

FIG. 4. (a) Effects of Co^{2+} on tissue calcium content. Co^{2+} (0·3, 1 mM) was added 60 min before the addition of the high-K^+. Control (●), 40 mM K^+ (○), 0·3 mM (△) or 1 mM (□)+40 mM K^+. (b) The effects of Co^{2+} on La^{3+}-resistant uptake of high and low affinity Ca^{2+} in taenia coli. Muscles were incubated in 0·03 (b) or 3 mM (c) Ca^{2+} (+^{45}Ca), K^+ (40 mM) medium containing 0·3 or 1 mM Co^{2+} for 60 min, and were washed in a La^{3+} (73 mM) medium at 1 C for 50 min.

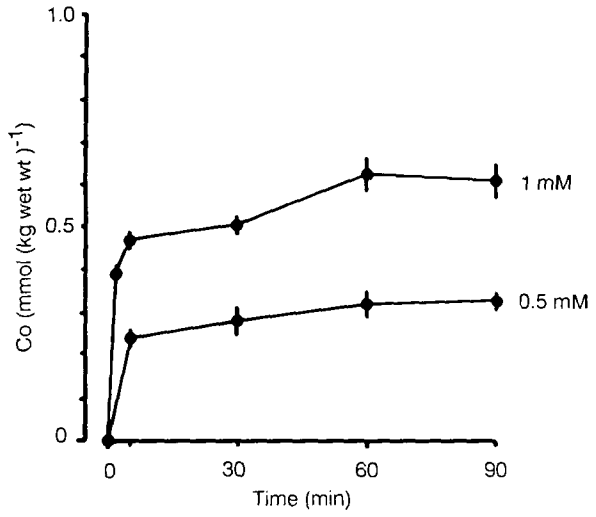

FIG. 6. Time course of cobalt uptake by taenia coli. $CoCl_2$ was added at time 0 at the concentration indicated. Each point represents 10–12 experiments (mean ± s.e.).

Ishida 1990) inhibited the tonic response more than the phasic response in taenia coli. Furthermore, D-600 also inhibited the La^{3+}-resistant ^{45}Ca uptake more at low affinity sites than at high affinity sites during K^+-induced contraction in taenia coli (Nasu & Ishida 1990). Thus, in taenia coli, the phasic response to K^+ can be attributed to release of Ca^{2+} at a high affinity site in the cell membrane, and tonic response can be attributed to increased inward movement of Ca^{2+} from extracellular fluid. We found that low concentrations (<0·7 mM) of Co^{2+} reduced the tonic contraction and had little effect on the phasic response, but with higher concentrations (≧1 mM), both responses were inhibited by Co^{2+}, while K^+-induced increase in tissue calcium concentration was inhibited by about 75 and 100% by 0·3 and 1 mM Co^{2+}, respectively. Our results show that a low concentration (0·3 mM) of Co^{2+} reduced the increase in La^{3+}-resistant uptake of low affinity Ca^{2+} more than the uptake of high affinity Ca^{2+} during K^+-induced contraction. However, a high concentration (1 mM) of Co^{2+} inhibited both high and low affinity Ca^{2+} binding. This may mean that the lower concentration of Co^{2+} reduced K^+-induced tension by inhibiting Ca^{2+} influx through voltage-dependent slow channels, rather than by any effect on Ca^{2+} release. However, at higher concentrations, it is probable that Co^{2+} inhibits both Ca^{2+} release and Ca^{2+} influx.

With Co^{2+} continuously present (<0·7 mM) after the first exposure to K^+, the muscles were washed with normal medium (+Co^{2+}) and then reincubated in a high-K^+ medium. The phasic response appeared on the second stimulation with K^+ (Fig. 2), which suggests that Ca^{2+} is stored and is available for the phasic response even when Co^{2+} is continuously present.

To my knowledge, no one has previously described cobalt uptake and efflux in smooth muscle. In the present study, the tissue/medium Co^{2+} concentration ratios at equilibrium in taenia coli were about 0·6 with 0·5 or 1 mM Co^{2+} in the

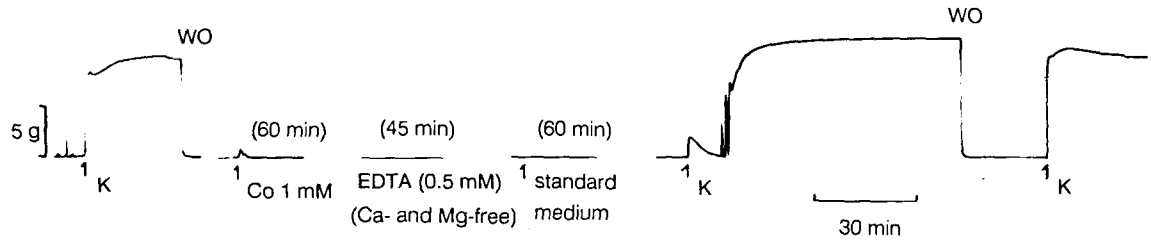

FIG. 5. Effect of a wash (WO) with EDTA medium on recovery of the response to a high-K^+ solution after Co^{2+} treatment in taenia coli. After treatment with 1 mM Co^{2+} for 60 min, muscles were washed with Ca^{2+}- and Mg^{2+}-free medium containing 0·5 mM EDTA for 45 min. The high-K^+ solution was applied after the muscles were incubated for 60 min in normal medium.

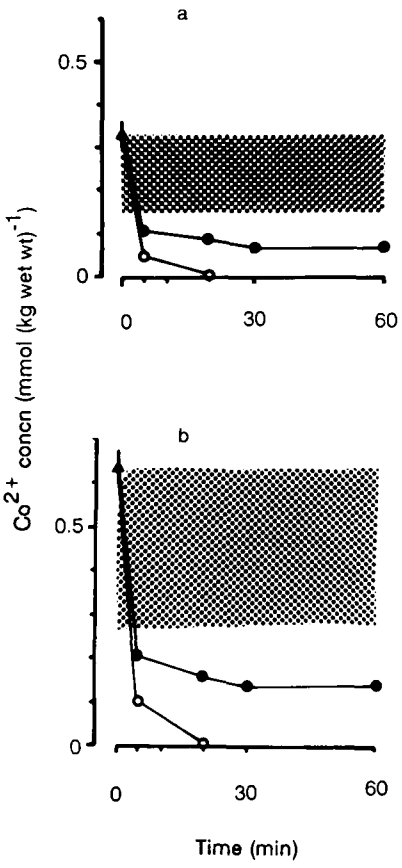

FIG. 7. Cobalt efflux from taenia coli. Muscles were incubated with 0·5 mM (a) or 1 mM (b) Co^{2+} medium for 60 min (▲) before the wash. The muscles were washed with normal medium (●) or with Ca^{2+}- and Mg^{2+}-free medium containing 0·5 mM EDTA (○). Each point represents the mean of 10 experiments. Error bars are not shown when the standard errors were less than the size of the symbol. If we assume that extracellular space is saturated with 0·5 or 1 mM Co^{2+} equal to the extracellular medium concentration incubated, the dotted areas represent Co^{2+} which had been present in the extracellular space.

medium. This ratio is remarkably lower than that of cadmium (Nasu & Koshiba 1985) in taenia coli. If we assume that the extracellular space was saturated with the same concentration of substrate as the external medium, then the taenia coli muscles accumulated more cobalt than the extracellular space (tissue/medium ratio 0·34), as determined by [^{14}C]sorbitol.

The inhibition by Co^{2+} of the phasic response to K^+ was completely restored after the wash with normal medium. In contrast, Co^{2+}-induced inhibition of the tonic response to K^+ was not entirely reversed by a wash with normal medium. When muscles were washed with normal medium after treatment with 1 mM Co^{2+} for 60 min, the tissue cobalt concentration reached an equilibrium after 30 min and about 25% of the initial tissue cobalt concentration remained. After the wash with normal medium, all of the cobalt in the extracellular space (0·5 or 1 mM Co^{2+} in external medium $\times$0·34 in the extracellular space) (represented by the dotted areas in Fig. 7) was probably washed out, which may have left a small amount of cobalt bound loosely to the cell membrane.

The inhibition caused by the high concentration (1 mM) of Co^{2+} was also completely restored by a wash with EDTA medium. In addition, the cobalt fraction after Co^{2+} treatment was completely eliminated by the medium containing non-penetrating chelator, EDTA (Brading & Jones 1969) (Fig. 7). This indicates that Co^{2+} binds to the cell membrane of taenia coli and does not penetrate it.

However, when the taenia coli muscle was exposed to 0·5 mM Cd^{2+} for more than 30 min, the total cadmium uptake and the cadmium compartment which was not eliminated by EDTA increased, and K^+-induced tension was not restored by a wash with EDTA medium. Moreover, the size of the fraction of total cadmium not eliminated by EDTA depends on temperature and on aerobic metabolism (Nasu 1985). The cadmium not eliminated by EDTA may accumulate in the intracellular compartment, where EDTA has no effect. Thus the kinetics of cobalt uptake and efflux were different from those of cadmium in taenia coli.

The contraction of skinned taenia coli muscle was not affected by pretreatment with 1 mM Co^{2+} (data not shown), which agrees with the study of Little et al (1985) in guinea-pig ileal longitudinal muscle, but it can be inhibited by Cd^{2+} (Nasu et al 1983). Furthermore, at a concentration which completely inhibited high-K^+-induced tension in intact muscle (1 mM), Co^{2+} had no effect on the tissue ATP concentration in taenia coli, but Cd^{2+} decreased the tissue ATP concentration (Nasu & Ishida 1990). This suggests that Co^{2+} does not affect contractile proteins or energy metabolism.

In summary, we suggest that Co^{2+} binds chiefly to the surface membrane of taenia coli. It is probable that Co^{2+} reduced K^+-induced tension mainly by inhibition of Ca^{2+} influx, rather than by affecting Ca^{2+} release. Cobalt ions may selectively inhibit Ca^{2+} channels opened during sustained depolarization in response to K^+, without affecting intracellular processes. However, at higher concentrations (1 mM), Co^{2+} abolished both the phasic and the tonic responses to K^+, by inhibiting both Ca^{2+} release and Ca^{2+} influx.

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