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Spasmolytic effect of cadmium and cadmium uptake in aorta

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1 The relationship between inhibition of tension by Cd^{2+} in aorta and the kinetics of cadmium uptake and efflux was studied. Cd^{2+} (0.01–0.5 mM) inhibited the contraction of aorta to high-K^+ (30 mM) in a dose-dependent manner.

2 The high-K^+-induced tension completely returned to control values after 60 min washing with a solution containing 5 mM disodium edetate (EDTA) or 5 mM cysteine, following a treatment with 0.5 mM Cd^{2+} for 30 min; after washing with normal medium only 15% of the control response returned.

3 Cadmium uptake increased with an increase of the Cd^{2+} concentration (0.01–0.5 mM).

4 Aortae preincubated with 0.5 mM Cd^{2+} for 60 min were washed subsequently with a medium containing 5 mM EDTA or 5 mM cysteine. About 5% of the original tissue cadmium was retained after washing with EDTA or cysteine.

5 It is suggested that Cd^{2+} binds chiefly to the surface membrane of aorta. It seems possible that the quantity bound is correlated with the degree of inhibition of tension.

Introduction

Previous work has indicated that cadmium ions (Cd^{2+}) administered orally or intraperitoneally produced hypertension in rats (Schroeder, 1964; Perry & Erlanger, 1974; Ohanian, Iwai, Leitl & Tuthill, 1978). The hypertension resulting from Cd^{2+} was reversed by injection of a chelator (Schroeder & Buckman, 1967). Schroeder & Buckman further noted that the chelator removed cadmium from the liver and kidney. However, the effects of a chelator on vascular smooth muscle have not been investigated.

Thind, Karreman, Stephan & Blackmore (1970) reported that aortic strips obtained from cadmium-hypertensive rabbits developed significantly lower tension than those from normotensive rabbits. Also, it has been demonstrated that Cd^{2+} inhibited noradrenaline, angiotensin- and high-K^+-induced contraction of isolated aorta in a non-specific manner (Thind, Stephan & Blakemore, 1970; Toda, 1973) and cysteine reversed this inhibition (Toda, 1973).

It is important to assess the site of action of Cd^{2+} in the aorta. Based on the experimental fact that the chelating agent, disodium edetate (EDTA), does not penetrate the cell membrane of smooth muscle (Brading & Jones, 1969), the effects of EDTA on tension and cadmium retention after Cd^{2+} treatment were investigated and compared with the action of the thiol agent, cysteine.

Methods

Albino male rabbits, weighing 2–3 kg, were killed by stunning and decapitation without anaesthesia. Aortae were removed and cut helically into strips of about 5×20 mm. The strips were immersed in Tyrode solution bubbled with 95% O_2 and 5% CO_2 at 37°C. The Tyrode 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. Phosphate-free Tyrode solution was used in order to avoid precipitation of cadmium phosphate. The pH of the solution was 7.3. For experiments using ethylenediamine tetraacetic acid (EDTA), a Ca^{2+}- and Mg^{2+}-free solution was prepared by omission of both $CaCl_2$ and $MgCl_2$ from the

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Tyrode solution. The high-K^+ (30 mM) was prepared by the addition of an appropriate amount of 3 M KCl solution. Cd^{2+} was added as a $CdCl_2$ solution directly to the bathing solution.

Contractile responses were recorded isometrically using a strain-guage transducer (Nihon Kohden, RM-150) and 2 g resting tension. The muscle strips were equilibrated for 60 min before the experiment.

To determine tissue cadmium concentration, the strips were removed from the bath at the end of experiment. After blotting on filter paper, strips were weighed, transferred to a quartz cuvette and incinerated by a plasma asher (Yanagimoto, Model LTA-4SN) for 10 h. As the melting point of cadmium is low (321°C), this apparatus was suitable for incineration of the muscle. The samples were dissolved in 0.01 M HNO_3 and Cd^{2+} content measured by an atomic absorption spectrophotometer (Hitachi, 207).

Results

Effects of Cd^{2+} on high-K^+-induced tension in aorta

When a high concentration of K^+ (30 mM) was applied to strips of aorta, a steady tension was produced of 2.2 ± 0.1 g ($n = 20$). After the aortae were incubated for 60 min in various concentrations of Cd^{2+} (0.01, 0.1 or 0.5 mM), high-K^+ was applied in the presence of Cd^{2+}. The contraction was expressed as a percentage of the maximum response in high-K^+. Cd^{2+}, at a concentration of 0.01 mM, caused a small decrease in tension (less than 5%), while 0.1 mM produced a $72 \pm 6\%$ ($n = 15$) relaxation of K^+-induced tension and 0.5 mM completely inhibited the tension.

In order to test whether washing has any effect on tension recovery after Cd^{2+} treatment, muscles were first incubated in 0.5 mM Cd^{2+} medium and then washed with normal medium, Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA or normal medium containing 5 mM cysteine for 60 min. After returning to normal medium, high-K^+ was applied and the

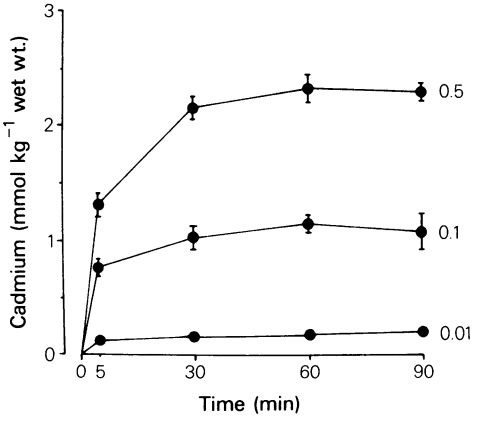

Figure 2 Time course of cadmium uptake by aorta. $CdCl_2$ was added at time 0 at the three concentrations (mM) indicated. Ordinate scale: tissue cadmium concentration (mmol kg^{-1} wet wt.). Abscissa scale: time (min). Each point is mean of from 10 to 12 experiments, s.e.means shown by vertical lines.

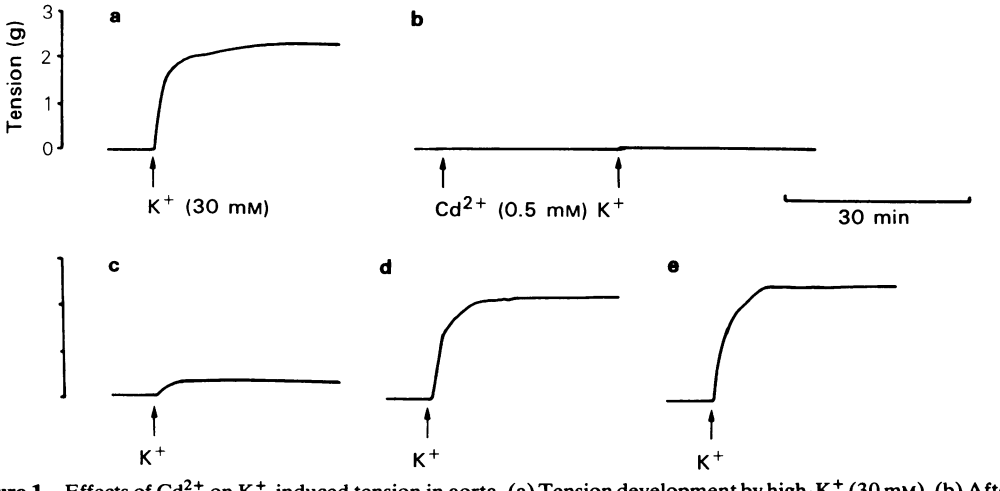

Figure 1 Effects of Cd^{2+} on K^+-induced tension in aorta. (a) Tension development by high-K^+ (30 mM). (b) After incubation for 30 min with 0.5 mM Cd^{2+}, high-K^+ was applied in the presence of 0.5 mM Cd^{2+}. After treatment with 0.5 mM Cd^{2+} for 30 min, the muscles were washed with (c) normal medium, (d) Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA or (e) normal medium containing 5 mM cysteine for 60 min; high-K^+ was applied after subsequent incubation for 60 min in normal medium.

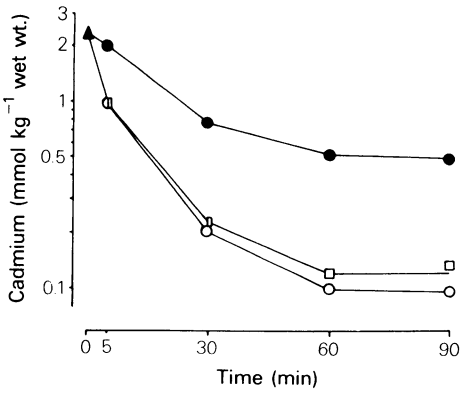

Figure 3 Cadmium efflux from aorta. Muscles were incubated with 0.5 mM Cd^{2+} for 60 min (▲) before washing. The muscles were washed with normal medium (●), Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA (□) or normal medium containing 5 mM cysteine (○). Ordinate scale: tissue cadmium concentration (mmol kg^{-1} wet wt.), logarithmic scale. Abscissa scale: time (min). Each point represents the mean of 10–12 experiments. Standard errors were less than the size of the symbol.

tension recorded. Responses were only 15% of controls after washing with normal medium. In comparison, high-K^+ responses returned to control values when muscles were washed with Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA or normal medium containing 5 mM cysteine (Figure 1).

Cadmium uptake by aorta at various Cd^{2+} concentrations

Aortae were incubated in a medium containing different Cd^{2+} concentrations (0.01, 0.1 or 0.5 mM). The cadmium uptake reached an equilibrium level which was dependent on Cd^{2+} concentration. In 0.5 mM Cd^{2+}, the time required to reach equilibrium was approx. 30 min (Figure 2). Cadmium uptake was 2.3 ± 0.1 mmol kg^{-1} wet wt. ($n = 12$) after incubation in 0.5 mM Cd^{2+} medium for 60 min.

Cadmium efflux

Strips of aortae were incubated in a 0.5 mM Cd^{2+} medium for 60 min and subsequently rinsed with normal medium. The cadmium content of the muscles reached equilibrium levels after 60 min in normal medium, about 80% of the tissue cadmium having been released. The effects of EDTA and cysteine on the retention of cadmium were studied. Only $5.0 \pm 0.3\%$ ($n = 12$) or $4.3 \pm 0.3\%$ ($n = 10$) of the initial cadmium content was retained after 60 min wash in Ca^{2+}- and Mg^{2+}-free medium containing 5 mM EDTA or normal medium containing 5 mM cysteine, respectively (Figure 3). There was no significant difference in the effects of EDTA and cysteine on retention of cadmium in the aorta. When the muscles were treated with medium containing 20 mM EDTA and subjected to the same experimental procedure, the results did not differ.

Discussion

These experiments have shown that Cd^{2+} (0.01–0.5 mM) inhibits contraction of aorta to high-K^+ in a dose-dependent manner and that cadmium uptake increases with increased Cd^{2+} concentration in the medium.

About 80% of tissue cadmium in aorta was released by washing in normal medium. The initial efflux may represent washout of Cd^{2+} from the extracellular space and exchangeable Cd^{2+} bound loosely to the surface membrane.

EDTA, which does not penetrate the cell membrane of smooth muscle (Brading & Jones, 1969) and whose action in aorta appears to be restricted to the cell membrane compartment (Weiss & Goodman, 1976) completely restored tension evoked by high-K^+ following treatment with 0.5 mM Cd^{2+}, EDTA also increased Cd^{2+} efflux and only 5% of the original tissue content remained after washing with EDTA. The results suggest that Cd^{2+} bind chiefly to the surface membrane of aorta with minimal penetration into the cell. It is known that Cd^{2+} suppress non-specifically responses of intestinal smooth muscle to several stimulant drugs (Schnieden & Small, 1971; Nasu, Koshiba, Mase & Ishida, 1983). The cadmium uptake by guinea-pig taenia coli reached an equilibrium level at 60 min; tissue content was 1.82 ± 0.13 mmol kg^{-1} (Nasu *et al*, 1983). When taenia coli were washed with medium containing EDTA following treatment with 0.5 mM Cd^{2+}, about 27% of the original content of tissue cadmium remained (Nasu & Koshiba, unpublished data). Thus, the fraction of total cadmium not eliminated by EDTA in the aorta was small in comparison with taenia coli.

Cysteine 5 mM following treatment with 0.5 mM Cd^{2+} also restored tension responses. Toda (1973) has already shown that cadmium inhibition of contractile responses to stimulatory agents in the aorta was antagonized by cysteine, glutathione and dithiothreitol. Only 4% of the original tissue cadmium remained after washing with cysteine. The thiol agent, cysteine, may displace Cd^{2+} from sulfhydryl groups in the cell membrane of the aorta.

K^+-induced contraction is caused mainly by increasing membrane permeability to Ca^{2+} as a result of depolarization in vascular smooth muscle (Karaki

& Weiss, 1979). Toda (1973) has shown that Cd^{2+} inhibited the Ca^{2+} response (induced by added Ca^{2+}) in Ca^{2+}-free, K^+-treated muscle. Furthermore, treatment with Cd^{2+} caused a significant decrease in tissue calcium content in aorta (Toda, Usui, Kimura & Itokawa, 1975). Thus, Cd^{2+} inhibit movement of Ca^{2+} into vascular smooth muscle. The effects of EDTA and cysteine described above on tension recovery and tissue cadmium suggest that surface bound Cd^{2+}, which can be displaced by these agents, can inhibit Ca^{2+} influx and inhibit contraction of the aorta.

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