MODIFICATION BY CADMIUM IONS OF $^{45}$CALCIUM UPTAKE BY ISOLATED RABBIT AORTAE

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Abstract—Treatment with 0.5 mM Cd++ suppressed the K+ (30 mM)-stimulated uptake and influx of $^{45}$Ca and abolished the K+-induced contraction in helical strips of rabbit aortae. The rapid and slow efflux of $^{45}$Ca previously accumulated was not appreciably affected by Cd++. It is concluded that interference with the transmembrane influx of Ca++ is a major mechanism of Cd++ action.

Cadmium ions (Cd++) applied in vitro to aortic strips produce decreased responsiveness to norepinephrine, epinephrine, histamine, angiotensin II, Ba++ and K+ (1-3). Such an inhibition may be associated with an interference with the Ca++ influx across the cell membrane for the following reasons: (a) The attenuation by Cd++ of the effect of K+ on aortic strips is greater than that of the effect of the amines and peptide (3), (b) Ca++-induced aortic contraction is abolished by Cd++ (3), (c) Atrial contraction is suppressed by Cd++ in concentrations insufficient to significantly alter the maximum rate of rise of action potentials, resting membrane potentials and action potential amplitudes (4, 5), (d) The Cd++-induced inhibition is reversed by excess Ca++. However, direct evidence is not available concerning the Cd++-induced inhibition of inward movement of Ca++. Thus, the present study was undertaken to clarify the effect of Cd++ on the uptake and influx of $^{45}$Ca in isolated rabbit aortae in relation to the suppression of K+-induced persistent contractions.

Materials and Methods

Under ether anesthesia, albino rabbits of either sex, weighing 2.0 to 2.4 kg, were sacrificed by bleeding from the common carotid arteries. The thoracic aorta was rapidly removed and placed in a Tris buffered solution (TS) composed of (mM): NaCl, 160; KCl, 4.6; CaCl$_2$, 1.5; MgCl$_2$, 2.0; dextrose, 10; Tris, 5.0. The pH of the solution was adjusted to 7.4. The solution was gassed with O$_2$ and was maintained at 37°C. The isolated aorta was then cleaned and helically cut into strips, approximately 20 mm long (for experiments on contractility), and transversely cut into pieces weighing approximately 25 mg (for the assay of $^{45}$Ca). The preparations were allowed to equilibrate for 90 to 120 min in the TS before the experiments were begun. During the equilibration period, the solution was replaced every 15 to 20 min with fresh solutions.

Experiments on contractility: Aortic strips were vertically fixed between hooks under a resting tension of 2 g in the muscle bath containing the TS. The hook anchoring the upper end of the strip was connected to the
lever of a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo). Isometric contractions were recorded on an ink-writing oscillograph. K⁺ in a concentration of 30 mM was added directly to the bathing media. Cd²⁺ was added simultaneously with K⁺.

**Experiments on ⁴⁵Ca uptake:** Aortic pieces were placed in 2 ml TS, TS containing 30 mM K⁺, TS containing 0.5 mM Cd²⁺, or TS containing 30 mM K⁺ and 0.5 mM Cd²⁺. These solutions were labelled with 2.5 μCi/ml ⁴⁵Ca. After 5 to 120 min incubation, the pieces were removed from the radioactive solution, blotted gently with filter paper, dipped for 4 to 5 sec, shaken in 40 ml volumes of non-radioactive solution, and blotted. This procedure was performed with four sequential beakers, and the muscles were weighed and ashed. Each ash was dissolved in 1 ml of 1 mM LaCl₃ (6) and in 0.1 M acetic acid. 13 ml of Bray's solution was added, and the radioactivity of the solution mixture was counted by a Packard Tri-Carb liquid scintillation counter.

**Experiments on ⁴⁵Ca efflux:** Some aortic pieces were allowed to accumulate ⁴⁵Ca for 60 min from labelled TS at 37°C. After the blotting and dipping procedures described above, 2 aortic pieces obtained from the same rabbits were placed in different tubes containing 2 ml of TS and TS containing 0.5 mM Cd²⁺. The radioactivity of the solution removed from the tubes was determined. The whole solution was evaporated to dryness prior to counting. At the end of experiments, the pieces were blotted, weighed and ashed as performed in the uptake experiments, and the radioactivity was counted.

The results shown in the text and figures are expressed as mean values±S.E.M. Statistical analyses were made using the Student's t-test.

**Results**

Isolated rabbit aortae exposed for 5, 10 and 30 min to ⁴⁵Ca at 37°C accumulated the labelled Ca to the extent of 88.1±1.7, 99.2±3.6 and 108.3±4.0 nmol/cm² surface area, respectively (N=12). Treatment with 0.5 mM Cd²⁺ reduced the ⁴⁵Ca uptake to 67.8±1.9, 79.3±2.6 and 83.8±3.2 nmol/cm², respectively (N=12); this uptake was markedly attenuated by treatment with 0.5 mM Cd²⁺. Time courses of the ⁴⁵Ca uptake by isolated aortae under control conditions and in response to 30 mM K⁺ in the absence and presence of Cd²⁺ (0.5 mM) are demon-

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**Fig. 1.** Time-dependent ⁴⁵Ca uptake and contraction of isolated rabbit aortae incubated in control and experimental media. Each value represents the mean±S.E.M. of 12 aortic pieces (upper panel) and 11 aortic strips (lower panel) incubated in the Tris buffered solution containing 30 mM K⁺ (filled circles) or containing 30 mM K⁺ +0.5 mM Cd²⁺ (open circles).
Cd-INDUCED INHIBITION OF Ca UPTAKE

Fig. 2. Effects of Cd\(^{+2}\) on \(^{45}\)Ca efflux from rabbit aortic pieces to non-radioactive solutions. The desaturation curves were obtained from mean values of 3 experiments. Closed and open triangles represent the results obtained in the absence and presence of 0.5 mM Cd\(^{+2}\).

![Graph](image)

...strated in Fig. 1, upper panel. Cd\(^{+2}\) significantly attenuated the uptake (P<0.001). Under the same experimental conditions, aortic strips responded to 30 mM K\(^+\) with a persistent contraction, which was abolished by simultaneous treatment with 0.5 mM Cd\(^{+2}\) (Fig. 1, lower panel).

Mean values of the Ca\(^{+2}\) influx during 5 min of exposure to control media and those containing excess K\(^+\), Cd\(^{+2}\) and Cd\(^{+2}\)+excess K\(^+\) were 294±5.8, 350±7.3, 226±6.3 (significantly different from 294±5.8, P<0.001) and 235±6.0 pmol/sec/cm\(^2\) (P<0.001 vs. 350±7.3), respectively (N=12).

Treatment with 0.5 mM Cd\(^{+2}\) did not appreciably alter the rapid and slow phase of \(^{45}\)Ca efflux from isolated aortae. The results are summarized in Fig. 2.

Discussion

Treatment with 0.5 mM Cd\(^{+2}\) abolished the persistent contraction of rabbit aortic strips induced by 30 mM K\(^+\) and the increased uptake of \(^{45}\)Ca by isolated aortae following the addition of K\(^+\) and attenuated the \(^{45}\)Ca influx during the first 5 min. On the other hand, \(^{45}\)Ca accumulated during an incubation period was liberated into non-radioactive solutions at a similar rate in the presence of Cd\(^{+2}\). Extracellular space measured with \(^{14}\)C-inulin is not significantly altered by treatment with 0.5 mM Cd\(^{+2}\) (7). Therefore, the suppression by Cd\(^{+2}\) of K\(^+\)-induced contractions appears to be associated with an inhibition of influxes of Ca\(^{+2}\) across the membrane of aortic smooth muscle cells.

The present findings support the hypothesis that a suppression by Cd\(^{+2}\) of atrial contractility (5), sinoatrial electrical activity (8), aortic contraction induced by K\(^+\) and Ca\(^{+2}\) (3) and skeletal muscle twitch induced by motor nerve stimulation (9), and an acceleration of early repolarization in atrial action potentials (4, 5) are due to an interference with the slow inward current carried by Ca\(^{+2}\) and possibly by Na\(^+\).

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