INHIBITORY EFFECT OF CADMIUM ON VITAMIN D-STIMULATED CALCIUM TRANSPORT IN RAT DUODENUM IN VITRO

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Summary In vitro effect of cadmium on vitamin D-stimulated calcium transport in the rat was studied using the everted gut sac technique. Calcium transport was significantly inhibited by the addition of cadmium to the mucosal incubation medium. Furthermore, the kinetic analysis of inhibitory effect of cadmium on calcium transport revealed that the type of inhibition was competitive. The apparent “$K_m$” and “$V_{max}$” value for calcium was 1.0 mM, and 2.86 µmoles ml⁻¹·hr⁻¹, respectively. An apparent “$K_i$” for cadmium was 0.02 mM.

Cadmium is one of the highly toxic trace metals and it has been studied as a serious problem of environmental pollution. Symptoms of chronic poisoning by this metal include various forms of skeletal deformation (1-3), suggesting interference with calcium metabolism by cadmium.

Recent evidence has shown that vitamin D-stimulated calcium transport is repressed in cadmium exposed rats (4, 5).

In order to obtain further information on the inhibitory effect of cadmium on intestinal calcium transport, the in vitro effect of the metal on vitamin D-stimulated calcium transport was studied kinetically using the everted gut sac technique.

METHOD

Animals. Female, albino rats of the Wistar strain, weighing 40–50 g were raised on a vitamin D-deficient diet (Ca: 0.002%, P: 0.3%) (6) for 3 weeks. Food and distilled water were given ad libitum. Each animal received 100 IU of vitamin D₃ orally 24 hr
before sacrifice. Animals were fasted for 18 hr before sacrifice. One hundred units of vitamin D₃ was dissolved in 0.1 ml of propylene glycol: ethanol solution (9:1).

**Intestinal calcium transport.** Calcium transport was studied by the everted gut sac technique essentially according to the method of MARTIN and DELUCA (7). The standard mucosal and serosal incubation medium had the following composition: 30 mM Tris-Cl buffer (pH 7.4), 125 mM NaCl, 10 mM fructose, 0.25 mM CaCl₂ and 10,000 cpm/ml ⁴⁵CaCl₂. Some modifications were adapted for the incubation medium. The effect of cadmium and magnesium on calcium transport was studied under the condition of various concentrations of CdCl₂ or MgCl₂ (0–2.5 mM) being added to the standard mucosal medium. For the kinetic study of calcium transport, the initial CaCl₂ concentration in the serosal medium was zero and that in the mucosal medium was changed from 0.25 to 2.5 mM in the absence or in the presence of CdCl₂ (0.025 or 0.05 mM). After the incubation, the radioactivity of serosal and mucosal medium was measured using a Packard Tri-Carb liquid scintillation spectrometer model 3380. Aliquots (50 µl) from serosal and mucosal medium were placed on a glass fiber filter (GF/A Whatman, 2.1 cm), dried and placed in counting vials containing 10 ml of the scintillation fluid. The scintillation fluid contained 0.4% Omnifluor in toluene. Results were expressed as either the radioactivity ratio of serosal fluid to mucosal fluid (S/M ratio) or μmoles Ca transferred/ml serosal medium/hr.

**Chemicals.** ⁴⁵Ca (16.9 mCi/mg Ca) and Omnifluor were purchased from New England Nuclear Co., USA and vitamin D₃ was kindly provided by Eisai Co., Ltd., Tokyo, Japan. All other reagents were analytical grade.

**RESULTS**

Using the gut sac technique, the *in vitro* effect of cadmium on vitamin D-stimulated calcium transport was observed in the rats, which were raised on vitamin D-deficient and low calcium diet for 3 weeks and which received 100 IU of vitamin D₃ before sacrifice. In the absence of CdCl₂ in the mucosal medium, vitamin D₃ stimulated calcium transport significantly, however, the vitamin D-stimulated calcium transport was obviously repressed in the presence of 0.025 mM of CdCl₂. Moreover, as the concentration of CdCl₂ increased, the inhibitory effect increased (Fig. 1).

On the other hand, Mg²⁺, as an essential metal ion, had a little effect on the calcium transport at the concentration of MgCl₂ less than 0.25 mM, although MgCl₂ also showed inhibitory effect on vitamin D-stimulated calcium transport at 2.5 mM (Fig. 1).

These results suggest that cadmium results in a specific and direct inhibitory effect on calcium transport. Therefore, it is likely that cadmium interferes with calcium metabolism by competing for the same cellular site of the calcium active transport process as that stimulated by vitamin D. Thus the kinetic study for
Fig. 1. Effect of cadmium and magnesium on the vitamin D-stimulated calcium transport in vitro. Calcium transport was studied in the rats which were raised on vitamin D-deficient diet (Ca: 0.002%, P: 0.3%) for 3 weeks followed by the dose of 100 IU of vitamin D$_3$ 24 hr before sacrifice. The mucosal medium consisted of 0.25 mM CaCl$_2$, containing $^{45}$Ca (10,000 cpm/ml) and various concentration of CdCl$_2$ or MgCl$_2$. The serosal medium was without CdCl$_2$ or MgCl$_2$. Incubation was carried out at 37°C for 2 hr. Each point represents the mean ± S.E. of 5 rats. -●-, in the presence of CdCl$_2$; -△-, in the presence of MgCl$_2$.

Fig. 2. Time course of vitamin D-stimulated calcium transport. Calcium transport was studied by the mucosal medium containing 0.5 mM CaCl$_2$ ($^{45}$Ca, 10,000 cpm/ml). The initial serosal medium was minus Ca and $^{45}$Ca from mucosal medium. Incubation was carried out at 37°C for 0.5, 1, 2 and 3 hr. Each point represents the mean ± S.E. of 5 rats. -●-, Ca concentration in serosal medium; -○-, Ca concentration in mucosal medium.

inhibition of cadmium on calcium transport was undertaken. For this study, Ca and $^{45}$Ca were free from the initial serosal medium and calcium transport activity was expressed as μmoles Ca transferred to 1 ml of serosal medium per hour.

Linear transport of calcium was observed for 2 hr of incubation (Fig. 2).
Fig. 3. Saturation analysis of vitamin D-stimulated calcium transport. Mucosal medium consisted of various concentration of CaCl\(_2\) with \(^{45}\)Ca (10,000 cpm/ml). Initial serosal medium was minus Ca and \(^{45}\)Ca from mucosal medium. Incubation was conducted at 37°C for 1 hr. Each point represents the mean ± S.E. of 5 rats.

Fig. 4. Kinetic analysis of inhibitory effect of cadmium on vitamin D-stimulated calcium transport. Mucosal medium consisted of various concentration of Ca in the absence (−○−) or in the presence of Cd (0.025 mM CdCl\(_2\) (−△−)), and 0.05 mM CdCl\(_2\) (−△−). Initial serosal medium was free from Ca and Cd. Incubation was carried out at 37°C for 1 hr. The plots are according to the method of Lineweaver-Burk. The abscissa represents 1/S where S is the concentration of Ca in the initial mucosal medium. The ordinate represents 1/V where V is the amount of Ca transferred/ml/hr. Each point represents the mean of 5 rats.

Therefore, the following study on calcium transport was carried out for 1 hr as an incubation period.

Changes in calcium transport related to its various concentration of calcium in the mucosal medium (0.25–2.5 mM) is shown in Fig. 3. The rate of transferred calcium to the serosal medium showed a hyperbolic curve, suggesting that calcium transport from mucosal medium to serosal medium by everted gut sac was an active transport. In such case, we could derive an apparent "K\(_{m}\)" and "V\(_{max}\)" values from double reciprocal plots in an analogy with the Michaelis-Menten equation for enzyme (δ). This treatment of the data is also useful in determining whether the inhibition of active transport of calcium by cadmium is competitive or non-competitive.

Consequently, to investigate the type of the inhibition by cadmium, vitamin D-stimulated calcium transport was studied by the media which consisted of various concentration of calcium (0.25–2.5 mM) in the absence or in the presence of cadmium (0.025 or 0.05 mM).

At the concentration of cadmium between 0.025–0.05 mM, double reciprocal plots were characterized by straight lines of differing slopes intersecting at a common intercept on 1/V axis, and not altered by the presence of any concentration of
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Cadmium. However, due to the presence of cadmium, the apparent “$K_m$” for calcium became greater than the “$K_m$” in the absence of cadmium (Fig. 4). In such case, the type of the inhibition by cadmium will be defined as competitive, namely, it was reversed by increasing calcium concentration in the mucosal medium.

The apparent “$K_m$” and “$V_{max}$” values for calcium were calculated as 1.0 mM and 2.86 μmoles/ml/hr from Fig. 4. The apparent “$K_i$” value for cadmium was 0.02 mM.

DISCUSSION

In the present study, the effect of cadmium on vitamin D-stimulated calcium transport was observed in the rat using everted gut sac technique in vitro.

SUDA et al. showed that dietary cadmium had an inhibitory effect on vitamin D-stimulated calcium transport in intestine from rats adapted to low calcium diet (4). Recently, we also demonstrated that vitamin D-stimulated calcium transport in rat intestine was inhibited by dietary cadmium. Furthermore, the inhibitory effect by dietary cadmium was more evident in the rats raised on low calcium diet than those on normal calcium diet (5). These observations suggested that cadmium might interfere the cellular site of calcium transport.

As little as 0.025 mM cadmium added to the mucosal incubation medium produced a reduction in the calcium transport ratio which was stimulated by vitamin D$_3$. However, magnesium did not show any significant effect on calcium transport below 0.25 mM (Fig. 1). This suggests that cadmium has a specific and direct effect on calcium transport.

Furthermore, kinetic study of the effect of cadmium on calcium transport in vitro revealed that cadmium competed same site for calcium transport at the concentration of CdCl$_2$ 0.025–0.05 mM, namely, the type of inhibition by cadmium was competitive in nature.

However, HAMILTON and SMITH reported that calcium uptake by intestinal slices was inhibited by cadmium and the type of inhibition was non-competitive (9). In their study, the concentration of CdCl$_2$ was 0.5 and 1.0 mM which caused a complete inhibition of vitamin D-stimulated active calcium transport in our study (Fig. 1). Therefore, it is suggested that a low concentration of cadmium shows the inhibitory effect on calcium transport via competition with the site of calcium transport. This inhibition will be reversed by increasing the concentration of calcium. This results in the reduction of the availability of dietary calcium for absorption, especially in rats raised on a low calcium diet.

On the other hand, a high concentration of cadmium might show its inhibitory effect via a nonreversible effect on intestinal brush border membranes. This will be observed as a non-competitive type of inhibition.

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