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Inhibition of Sugar Active Transport across Rat Intestine *in vivo* by Cadmium

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Galactose absorption by rat jejunum perfused *in vivo* is inhibited by 0.5 mM Cd^{2+}. This effect is explained by impairment of the phlorizin-sensitive sugar transport system, as Cd does not modify the absorption of L-sorbose or that of galactose in the presence of 0.5 mM phlorizin. Cd inhibition is observed as early as in the 1st minute, does not increase by previous exposure of the mucosa to the metal and does not decrease after washing with saline solution, but it is entirely reversed by EDTA or dithioerythritol. Results agree with a Cd^{2+} binding to HS- groups of membrane proteins at the brush border, appertaining or functionally related to the phlorizin-sensitive and Na^+ dependent transport system for sugars.

Key words: Sugar transport, Cadmium, Intestinal absorption.

It has been reported that Cd^{2+} inhibits D-glucose absorption in rat small intestine *in vivo* (15), and in fish intestine (20, 21) as well as intestinal transport of amino acids in bull-frog (26). Though in short porportion (13, 14), cadmium can be absorbed across the intestine by a paracellular (1, 12) or transcellular (4, 8) pathway, with considerable mucosal retention (2, 8, 24) distributed among a wide spectrum of cell constituents including proteins at the luminal membrane (18, 19, 25) and in cytosol (3, 23, 27). Cadmium can bind to proteins by thiol groups (7, 22).

The present paper aimed at a better understanding of the action mechanism of Cd^{2+} on sugar absorption. Cd inhibition of galactose absorption has been studied in rat small intestine *in vivo*, and explained by the binding of the metal to thiol groups of proteins related to the function of the phlorizin-sensitive sugar transport systems.

Materials and Methods

Male and female Wistar rats, 125-200 g body weight, were used. *In vivo* intestinal absorption was measured by PONZ *et*

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al. technique (16), with *in situ* cannulated jejunum segments of 15-20 cm in length, perfused at 5.6 ml/min rate. The perfusion solution contained 150 mM NaCl with sugar, cadmium or other compounds at the concentrations specified in the experiments, and was always adjusted to pH 6.5.

For galactose experiments, single pass perfusion and 1 min absorption periods were adopted. Absorbed sugar was calculated as the difference in sugar contents of the solution at the entry and the exit of the jejunum, and expressed in micromoles per cm length per minute (16).

For sorbose absorption experiments, the solution was recycled through the jejunum for 5 min periods.

Determination of galactose was made by radioactivity measure of the labelled sugar with a liquid scintillation counter, and that of sorbose by the ROE method (5, 17).

D-galactose transport by the phlorizin-sensitive system was evaluated as the difference between absorption in control conditions and that in the presence of 0.5 mM phlorizin (6).

D-(1-^{14}C) galactose (Amersham), D-galactose (Merck), L-sorbose (Sigma), phlorizin (Sigma), $CdCl_2$ (Merck), ethylendiaminetetra-acetate (EDTA, Merck) and dithioerythritol (DTE, Sigma) were utilized.

Results

In some experiments, several successive absorption periods were made on each animal to find the control values for galactose absorption, then another series was conducted with Cd^{2+} added to the sugar solution, followed by another series with metal-free solution. The absorption of 1 mM galactose decreased about 25% to 30% when the sugar solution contained 0.5 mM cadmium (fig. 1 A). With 5 mM $CdCl_2$, some epithelial desquamation as well as a slightly higher inhibition was observed.

Cadmium effect was tested as early as the first 1 min period after the metal addition, and did not significantly increase along the successive ones with the added metal. Moreover, galactose absorption remained inhibited in a similar proportion when the perfusion solution contained no cadmium, provided this element had been present in the preceding perfusion periods. This fact was produced in spite of abundant washing of the jejunum lumen with saline solution.

The inhibition by Cd was also tested with a range of galactose concentrations between 0.5 mM and 10 mM, the percent decrease being inferior when 5 mM or 10 mM galactose was used than with the lower concentrations (table I).

In other experiments, after some control absorption periods the lumen was exposed for 5 min to a saline solution with 0.5 mM Cd, and then the galactose absorption with the same Cd concentration was measured along 9 minutes. Later on, the jejunum segment was thoroughly washed with 10 mM EDTA in saline solution and finally galactose absorption from Cd-free solutions was determined. Fig. 1-B shows that the inhibitory effect of Cd did not appreciably increase by previous exposure of the mucosa to the metal, nor was it modified along the 9 minute continuous perfusion of Cd containing galactose solution. On the other hand, washing with EDTA entirely reversed the Cd inhibition, restoring galactose absorption to its control values.

These results suggested that cadmium inhibited galactose absorption through its rapid fixation to easily accessible chemical groups at the luminal membrane, EDTA being able to restore absorption by sequestering the metal.

To find out if thiol groups were involved in the Cd action, control absorption periods were first made in some animals, then other ones were conducted in

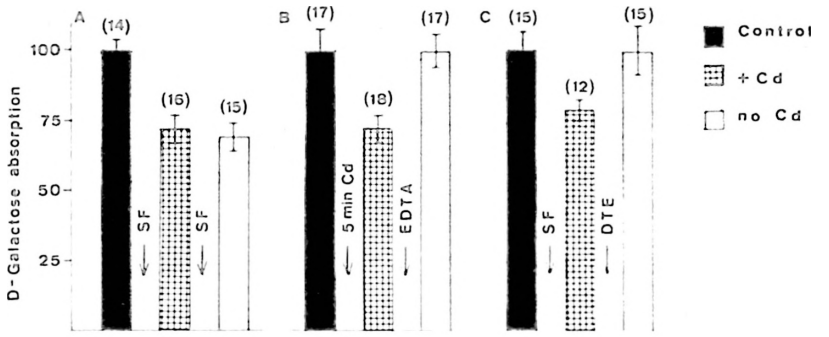

Fig. 1. *Effects of 0.5 mM Cd on 1 mM D-galactose absorption by rat jejunum.*
Mean values ± SE, referred in percent of control. Data number in parenthesis. A) Persistence of the inhibition by Cd after SF washing. B) and C), Cd inhibition is reversed by 10 mM EDTA or DTE respectively. SF, 150 mM NaCl solution.

the presence of 0.5 mM Cd, subsequently, the lumen was perfused for 10 min with 10 mM dithioerythritol in saline solution and lastly galactose absorption in the absence of Cd was measured. Fig. 1-C shows that Cd inhibition of galactose absorption completely disappeared after DTE treatment, conferring strong support to the involvement of HS-groups in Cd action.

Intestinal sugar absorption *in vivo* includes a mediated transport component and an apparently diffusional one (6). Experiments were carried out to discover if cadmium affected the component of the galactose absorption that remained after the phlorizin-sensitive transport system for sugars had been blocked by 0.5 mM phlorizin. Fig. 2 shows that 0.5 mM Cd did not produce any effect under these

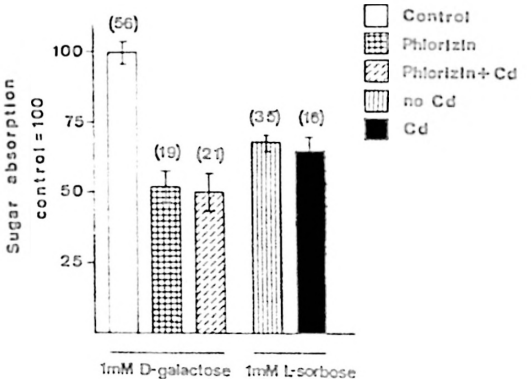

Fig. 2. *Absence of effect of 0.5 mM Cd on apparently diffusional absorption of sugars by rat intestine.*
Mean values ± SE, referred in percent of control D-galactose absorption. Data number in parenthesis. Lack of Cd action on 1 mM galactose absorption in the presence of 0.5 mM phlorizin, and on 1 mM L-sorbose absorption.

Table I. *Intestinal absorption of D-Galactose at several different concentrations in the perfusion solution, in the absence or in the presence of 0.5 mM Cd^{2+}*
Mean values ± SE, in nanomoles per cm per minute. Data number in parenthesis.

	D-GALACTOSE (mM)			
	0.5	1	5	10
Control	13.3 ± 0.8 (14)	18.82 ± 0.65 (86)	89.2 ± 7 (15)	158.7 ± 8 (44)
Cd^{2+} 0.5 mM	10.0 ± 0.6 (14)	13.69 ± 0.71 (30)	74.3 ± 6 (14)	140.2 ± 7 (28)
Inhibition (%)	24.8	27.22	16.7	11.6

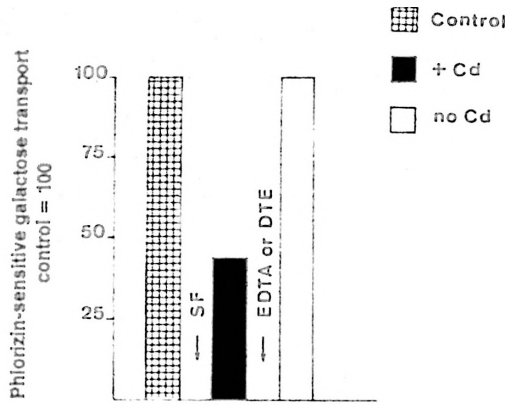

Fig. 3. *Effects of 0.5 mM Cd on the phlorizin-sensitive transport component of 1 mM D-galactose absorption by rat jejunum and their reversibility by 10 mM EDTA or DTE.*
Mean values referred in percent of control transport.

conditions, which points to a lack of Cd action on passive non-mediated sugar absorption.

L-sorbose was taken as an example of a non-transportable sugar, absorbed at a very low rate across the intestine, which is a lineal function of the luminal concentration. In sorbose absorption experiments, the perfusion solution was recycled along 5 min periods. As fig. 2 shows, 0.5 mM Cd did not modify the sorbose absorption rate, a fact consistent with the observed lack of actions on galactose absorption when the transport component was excluded by 0.5 mM phlorizin.

Cadmium inhibition of galactose absorption seems, therefore, to be restricted to impairment of the phlorizin-sensitive sugar transport system. As the component of galactose absorption corresponding to the use of this transport system could be evaluated from the difference between galactose absorption in the absence and in the presence of 0.5 mM phlorizin, the aforementioned changes in absorption shown in fig. 1 were referred exclusively to sugar transport changes in fig. 3. Under this assumption, it could be seen that a 52% inhibition of galactose transport was produced by 0.5 mM Cd, which could be entirely reverted by washing the mucosa with 10 mM EDTA or 10 mM DTE.

Discussion

Luminal Cd at 0.5 mM concentration decreased galactose absorption by rat jejunum *in vivo*, which is in agreement with previous observations for glucose absorption in rat (15) and fish (20), but in discordance with the lack of action in bull-frog intestine (26). This last paper on amphibian intestine was based on transport evaluation by transmural PD measures, and reported an apparently competitive inhibition of amino acid transport and lack of effect on glucose transport, a surprising observation that would require further explanation.

The inhibitory effect of cadmium was observed as early as the first 1 min absorption period, did not increase by previous exposure of the mucosa to the metal for 5 min, or along the perfusion time, and it did not disappear by thoroughly washing the mucosa with saline solution. These results suggest that Cd exerts its inhibitory action on galactose absorption by fixation to chemical groups at the luminal surface of the epithelium, which are easily accessible to the metal from the lumen. The binding of Cd to those groups was not solved by washing with saline solution; sugar absorption, however, returned to the control values by washing the lumen with the well known metal sequestering agent, EDTA, or with thiol group protector DTE. Consequently, the binding of Cd related to the galactose absorption inhibition has to involve HS-groups of proteins.

The rapidity of the Cd action, the lack of inhibition increase after previous exposure or along the perfusion time, and its

complete reversibility by EDTA, which is a non or very scarcely penetrating substance (9, 10), make it possible to affirm that the thiol groups responsible for the effects on absorption belong to membrane proteins of the enterocyte brush border.

The lack of Cd inhibitory effects on the absorption of galactose when 0.5 mM phlorizin was present, and on the absorption of sorbose, reveals that Cd inhibits precisely the phlorizin-sensitive transport system for sugars, without affecting the apparently diffusional component of absorption. If the Cd action is referred to an exclusive impairment of the transport component, an inhibition of 52% is found.

The inhibitory action of Cd ions on galactose absorption by rat jejunum can, therefore, be understood by their binding to chemical groups of membrane proteins at the enterocyte brush border. These groups would prevailingly be thiol groups, located in places easily accessible to the metal from the lumen, and belonging or being functionally related to phlorizin-sensitive and Na^+ dependent transport system for glucose, galactose and other sugars, in such a way that sugar transport becomes seriously impaired, whilst the non carrier mediated passive absorption is not affected.

Resumen

La absorción de galactosa por yeyuno de rata perfundido *in vivo* se inhibe por la presencia de Cd^{2+} 0,5 mM. Este efecto se explica por alteración del transporte de azúcar sensible a la florricina, ya que el Cd no modifica la absorción de galactosa cuando está presente la florricina (0,5 mM), ni afecta a la absorción de L-sorbosa. La inhibición por Cd se observa ya en el primer minuto, no aumenta por exposición previa de la mucosa al metal, no disminuye por lavado con solución salina, pero desaparece por lavado con EDTA o con ditioeritritol. Los resultados sugieren la unión del Cd a grupos -SH de proteínas de membrana del borde en cepillo, que pertenecen al sistema de transporte de azúcares dependiente de Na^+ y sensibles a la florricina, o que tienen relación funcional con él.

Palabras clave: Transporte de azúcares, Cadmio, Absorción intestinal.

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