REVISTA ESPAÑOLA DE FISIOLOGIA, 44 (2), 121-126, 1988

Inhibition by Cadmium of D-Galactose and L-Phenylalanine Transport by Rat Intestine *in vitro*

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(Received on October 2, 1987)

A. LUGEA, A. BARBER and F. PONZ. *Inhibition by Cadmium of D-Galactose and L-Phenylalanine Transport by Rat Intestine* in vitro. Rev. esp. Fisiol., 44 (2), 121-126, 1988.

The effect of cadmium ($CdCl_2$) on galactose and phenylalanine uptake by rat everted intestinal rings has been studied. The rings were preincubated (15 min) and incubated (5 min) in the presence of Cd. Galactose uptake (from 0.5 mM to 10 mM) was inhibited by 0.5 mM Cd about 25 %. Only the phlorizin-dependent galactose transport was affected by cadmium, being a non-competitive type inhibition. A 15 min washing with saline solution significantly reduced the cadmium induced inhibition, which was practically reversed by washing with 5 mM EDTA. The uptake of 0.5 mM phenylalanine was not affected by 0.5 mM Cd but it was depressed by 1 mM Cd. Such inhibition was exerted on the sodium-dependent phenylalanine transport. Washing with 5 mM EDTA diminished only slightly the inhibition of the transport by cadmium. It is suggested that the inhibition of intestinal transport of galactose and phenylalanine by cadmium may be due to its reversible interaction with metal-binding ligands, possibly sulfhydryl groups, related to the luminal transport systems.

Key words: Cadmium, D-Galactose, Intestinal transport, Phenylalanine.

Cadmium is a heavy metal non-essential to living organisms and highly toxic (1, 11), although with varying degrees of tolerance according to the species (2). It is slowly absorbed across the intestine by a not well established apparently saturable process (4). Its slow turnover causes it to accumulate in the organisms, bound mostly to metalothioneins, cysteine rich proteins which could play a role in detoxification or storage (5, 22). Cadmium toxicity has been related to its great capacity for binding to membrane and intracellular ligands, basically sulfhydryl groups (13, 20), being even able to replace essential cations such as Ca^{2+} or Zn^{2+} (9).

Inhibition of intestinal absorption of sugars and amino acids by Cd^{2+} has been observed in amphibians (21), teleosts (15-17) and mammals (8, 19). Works carried out in our laboratory showed that cadmium inhibits galactose transport in *in vivo* rat intestine (12, 14).

In the present paper the effect of Cd on

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the transport of galactose and phenylalanine by rat intestine has been studied *in vitro*.

Materials and Methods

The influx of D-galactose and L-phenylalanine into rings of everted rat jejunum was determined by the tissue accumulation method (3). Albino Wistar rats of either sex, weighing 100-250 g were fasted for about 20 h with water *ad libitum* prior to the experiments. Under urethane anaesthesia (1.25 g/kg, s. i.), a jejunum segment of about 20 cm in length was removed, washed free of intestinal contents with ice cold physiological solution and everted. Three rings of everted intestine (about 0.5 cm in length and 25-30 mg in weight) were incubated in each erlenmeyer flask for 5 min at 37° C with shaking in 10 ml oxygen-saturated incubation medium containing D-galactose or L-phenylalanine. After incubation, the rings were rapidly removed, washed in ice-cold solution, gently blotted on wet filter paper, weighed and suspended in 0.5 ml 0.1 N HNO_3 overnight in cold storage. Aliquot samples were taken, added to 1.6 ml of scintillation fluid and their radioactivity was measured and related to that of the medium. The results are expressed as substrate uptake in nmoles × 100 mg^{-1} wet weight.

The saline solution contained, in mM: 140 NaCl, 5.6 KCl, 3 $CaCl_2$, 1.4 $MgSO_4$. 7 H_2O, 6.1 Tris, and 4.9 HCl, the pH being 7.4. When cadmium was added ($CdCl_2$), the solution was adjusted at the same pH. In Na^+-free solution, Tris osmotically substituted for Na^+. Most of the experiments included a 15 min preincubation period in the same solution without the substrate, in the presence or absence of Cd.

All reagents were of analytical grade. Radioactive tracers D-(1-^{14}C) galactose (55.7 μCi/mmole) and L-(u-^{14}C) phenylalanine (504 mCi/mmol) were from Amersham.

Results

Effects of Cd on D-galactose uptake. — Initial experiments of 5 min incubation periods showed that 0.5 mM Cd had no effect on 1 mM galactose uptake, and that 5 mM Cd causes a turbidness, attributable to tissue disorganization. Consequently, it was decided to preincubate for 15 min with 0.5 mM Cd and maintain this concentration during the incubation for 5 min with the substrate. Galactose concentration in the medium ranged from 0.5 to 10 mM. Galactose uptake by the tissue was clearly inhibited by Cd (fig. 1). When the galactose transport was totally inhibited by 0.5 mM phlorizin, the kinetics became lineal (fig. 1), as corresponding to an apparently diffusional process, the influx being not affected by preincubation and incubation of the tissue with 0.5 mM Cd. The Cd effect could be attributed, therefore, to a disturbance of the phlorizin-sensitive sugar transport system. If the mediated sugar transport is

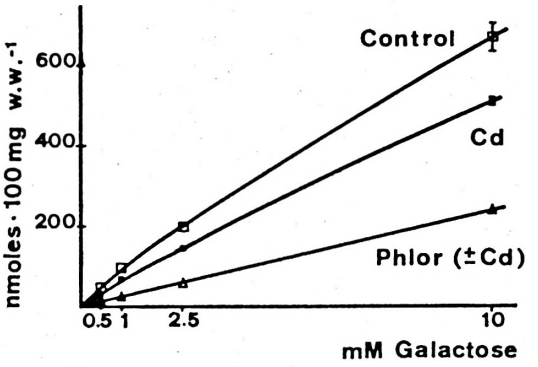

Fig. 1. *Influence of 0.5 mM cadmium on D-galactose uptake by rat intestinal rings. The tissue was preincubated (15 min) and incubated (5 min) in the presence of Cd.*

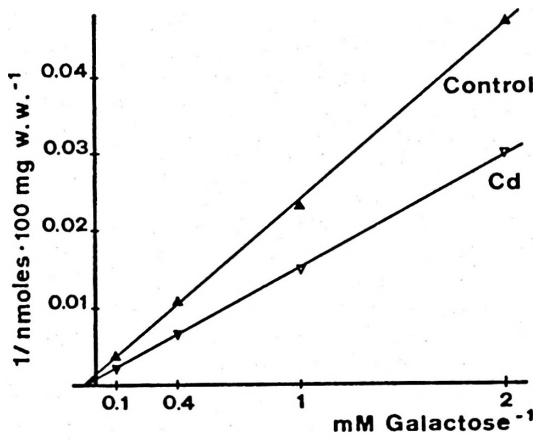

Fig. 2. *Lineaweaver-Burk plot of the inhibition of D-galactose transport by cadmium in rat intestine.*

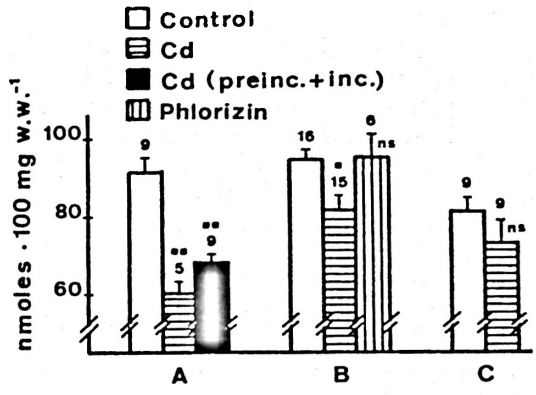

Fig. 3. *Reversibility of the inhibition of 1 mM galactose uptake by 0.5 mM Cd^{2+}.*
After the 15 min preincubation period without (control) or with the inhibitor (Cd^{2+} or phlorizin), the rings were incubated with galactose: A) without intermediate washing, B) after saline solution washing and C) after washing in the presence of 5 mM EDTA. Vertical bars denote SE of the mean for the number of separate determinations indicated at the top of the column. ** $p < 0.001$; * $p < 0.01$; n.s. non significant.

estimated as the difference between the uptake values in the absence and presence of 0.5 mM phlorizin, the effect of Cd on the transport can be appreciated. In this way, the inhibition of galactose transport by Cd seems to be of a non-competitive type (fig. 2).

Reversibility of the inhibitory effect of Cd on galactose uptake. — In some experiments, after preincubation of the intestinal rings with 0.5 mM Cd, they were washed three times for 5 min in saline solution in the absence or presence of 5 mM EDTA, before incubation with the sugar in medium without Cd. The reversibility of the inhibition induced by preincubation with 0.5 mM phlorizin was also studied in the same way for comparative purpose.

Results (fig. 3) show that washings with saline solution bear no effect on galactose entry, whereas they reduce it somewhat when they are carried out in the presence of EDTA. Washings with saline solution after preincubation with Cd improve sugar influx appreciably, although without reaching control values; if preincubation has been carried out with phlorizin, the washings cause every inhibition to disappear. However, if the washings are carried out in the presense of 5 mM EDTA, sugar influx after preincubation with Cd is similar to the control one. Thus, while the inhibition by phlorizin disappears entirely by saline washing, the inhibition by Cd is only partially reversed. Washing with EDTA almost completely reversed the inhibition by Cd.

Effects of Cd on phenylalanine uptake. — The effect of Cd at 0.5, 1 and 2.5 mM concentrations on 0.5 mM Phe (fig. 4) has been tested. The lowest concentration does not affect the amino acid influx for 5 min even when the tissue was preincubated for 15 min in the presence of the metal. With 1 mM Cd, inhibition is obtained only in experiments with preincubation; with 2.5 mM, both with and without preincubation, an inhibition

of similar value to that observed with 1 mM is produced.

Preincubation with 1 mM Cd inhibited 0.5 mM phenylalanine influx about 35 % relative to control values (fig. 5). This inhibition may be attributed to impairment of the amino acid transport, since when the entry by transport is annulled by high concentrations (40 mM) of its competitive inhibitor, methionine, Cd does not modify the apparently diffusional residual influx into the tissue. If the effect of Cd is exclusively referred to the Phe transport process, a 50 % inhibition is obtained.

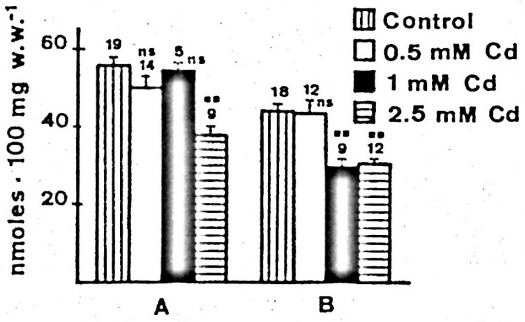

Fig. 4. *Effect of various cadmium concentrations on 0.5 mM Phe uptake by rat intestinal rings.* A) Without preincubation with $CdCl_2$ and B) in the presence of cadmium during both preincubation and incubation periods. Legends as in figure 3.

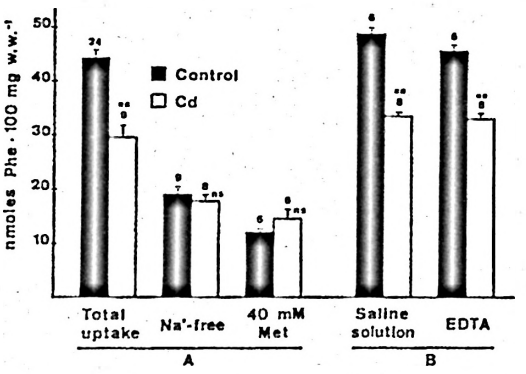

Fig. 5. *Inhibition of 0.5 mM Phe uptake by 1 mM cadmium in various experimental conditions.* A) Without intermediate washing. B) After the preincubation period with Cd^{2+}, the rings were washed with saline solution alone or with EDTA prior to the incubation with Phe. Legends as in figure 3.

When the intestinal rings are incubated in Na^+-free medium, amino acid uptake is markedly lower (about 40 %) than in the presence of the cation, but it is still somewhat higher than in the presence of 40 mM methionine. This phenylalanine influx in Na^+-absence is not affected by preincubation with 1 or 2.5 mM Cd.

Contrary to what happened with galactose, the inhibition of Phe uptake by preincubation with 1 mM Cd is not reversed by washing with saline solution, even in the presence of 5 mM EDTA.

Discussion

Present results show that Cd inhibits both galactose and Phe uptake by rings of rat intestine *in vitro*.

The inhibition of galactose influx into the jejunum rings during 5 min incubation by 0.5 mM Cd requires preincubation (15 min) of the tissue with the metal. Inhibition of the galactose transport by 0.5 mM Cd in perfused rat intestine *in vivo* (14) was observed 1 min after the metal addition and did not increase by previous exposure of the mucosa to Cd.

To inhibit 0.5 mM Phe uptake in similar proportion to galactose uptake, higher Cd concentrations are necessary: 1 mM if there was preincubation with the metal, and 2.5 mM if Cd is present only during the incubation period. Results suggest that the maximum inhibition by Cd requires a certain minimal metal concentration and time of action on the tissue, which may diminish if its concentration increases. In the teleosts *Heteropneustes fossilis* (15) intestine, with 1 h long experimental times, no inhibition differences of 40 mM glucose or fructose absorption

were appreciated when Cd concentration ranged between 0.01 and 1 mM. There has recently been reported also inhibition of histidine absorption by Cd in rat intestine *in vivo* (8).

Experiments reveal that when the active transport of galactose is inhibited by phlorizin and that of phenylalanine by high concentrations of methionine, Cd does not affect the sugar or the amino acid residual influxes into the tissue. Cd, therefore, seems to exclusively inhibit the transport component. These results coincide qualitatively with those observed *in vivo* (14) on galactose transport and with the lack of inhibition of L-sorbose absorption by Cd.

Phe transport measured in the absence of Na^+ from the incubation medium is not affected by Cd, which suggests that the inhibition by the metal is exerted on the Na^+-dependent transport systems. Although an indirect Cd action cannot be excluded by inhibition of the Na^+, K^+, ATP-ase (20), the duration of the *in vitro* and *in vivo* experiments on one hand, and the high reversibility by EDTA treatment on the other, seem to suggest that inhibition is due to a more direct non competitive action in the brush border.

Since the -SH groups are fundamental in sugar and amino acid intestinal transport (6, 7, 10, 18, 19), it seems likely that inhibition by Cd derives from its interaction with sulfhydryl groups linked to the transport systems.

A non-competitive type inhibition of sugar and amino acid transport by Hg^{2+} has been found in guinea pig intestine (19) and bullfrog (21). It is nevertheless surprising that in the latter species, Cd does not affect glucose transport and inhibits competitively that of Ala and Gly (21).

Washing with physiological solution diminished the inhibition by 0.5 mM Cd on galactose uptake significantly from 36 to 14 %, and it even disappeared by washing with 5 mM EDTA. Nevertheless the inhibition of Phe transport by 1 mM Cd was not reversed even by EDTA treatment. Perhaps the lower sensitivity and reversibility in the case of amino acid transport may be related to a lesser accessibility of the ligands responsible for the inhibition by Cd in the Phe transport system with respect to that of galactose.

The inhibition of the galactose transport in rat intestine *in vivo* (14) by 0.5 mM Cd is entirely reversed by both EDTA and dithioerythritol, an agent which separates specifically the metals from their complexes with -SH groups. All these findings fit favorably with the present results *in vitro*.

Resumen

Se estudia el efecto del Cd sobre la penetración de galactosa y de fenilalanina en anillos de intestino evertido de rata. Los preparados se preincuban (15 min) e incuban (5 min) en presencia de $CdCl_2$. El Cd 0,5 mM inhibe un 25 % la penetración de D-galactosa 0,5-10 mM. La inhibición sólo se ejerce sobre el componente del transporte sensible a la florricina y es de tipo no competitivo. El efecto del Cd disminuye apreciablemente por lavado de 15 min con solución salina y desaparece prácticamente tras lavado con EDTA 5 mM. La entrada de fenilalanina 0,5 mM no se modifica por Cd 0,5 mM, pero se reduce por Cd 1 mM. La inhibición se ejerce sobre el sistema de transporte dependiente de Na^+. El lavado con EDTA 5 mM disminuye sólo ligeramente la inhibición por Cd sobre el transporte de fenilalanina. Los resultados sugieren que la inhibición por Cd puede deberse a interacción reversible con ligandos, posiblemente grupos sulfhidrilo, de los sistemas de transporte de la membrana luminal.

Palabras clave: Cadmio, D-galactosa, Transporte intestinal, Fenilalanina.

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