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Action of Mercury on Sugar Transport across Rat Small Intestine, *in vivo*

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Absorption of galactose from *in vivo* perfused rat jejunum was inhibited by 0.1 - 0.5 mM Hg^{2+}. A few minutes' delay was required for maximal inhibition values. The effects remained after saline solution washing but were in part reversed by EDTA and in higher proportion by dithioerythritol. Absorption inhibition could be ascribed to impairment of the sugar-Na phlorizin-sensitive cotransport component. The passive apparently diffusional component that remains under 0.5 mM phlorizin and absorption of L-sorbose were unaffected by the metal. Hg action is explained as due to its binding to thiol and perhaps other chemical groups of proteins, at different depths in the membrane, which are directly or indirectly related to the sugar transport system.

Key words: Mercury, Sugar transport, Small intestine, Rat.

The inibitory action of Hg^{2+} on sugar transport has been well known for a long time (13) from reports on erythrocytes (11, 26) and rat diaphragm (4). Intestinal absorption of glucose and galactose in rats, *in vivo*, decreased markedly by Hg, an effect ascribed to fixation of the metal to the epithelium (16). Similar observations were made *in vitro* on amino acid and sugar transport in rabbit ileum (6, 23, 24), on glucose transport in chick and fish intestine (14, 15), and in alanine and glucose transport in bullfrog intestine (25). Glucose transport inhibition has also been shown in vesicles of brush-border membranes from rabbit intestine (9, 10).

Hg can be slowly absorbed from the digestive tract (1, 3, 21), the entry and retention of the metal in the intestinal mucosa being an easier and faster process than its further transfer to blood (8, 22). Binding of Hg to different chemical prevailing thiol groups of membrane and intracellular proteins is involved in its intestinal absorption (5, 12, 24). In vesicle preparations, the Hg binding to thiol groups is very stable and takes place at different depths in the membrane (9, 10).

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The present paper studies *in vivo* the inhibition of sugar intestinal absorption and shows that only the phlorizin-sensitive transport component is affected, whilst the apparently diffusional one remains unmodified. This transport inhibition does not disappear by washing the mucosa with saline solution, but is reverted in some proportion by EDTA and to a greater extent by dithioerythritol (DTE) treatment.

Materials and Methods

In vivo experiments were made in anaesthetized Wistar rats by the PONZ *et al.* (17) method. Cannulated segments of jejunum of about 20 cm in length, were perfused at 5.6 ml/min rate with a 150 mM NaCl solution which contained sugar, $HgCl_2$ or other compounds as indicated in the experiments, adjusted to 6.5 pH. Absorption periods were 1 min long for D-glucose and of 5 min for L-sorbose. The experimental procedures, compounds used and sugar determinations were as specified elsewhere (14, 20). The $HgCl_2$ was from Merck.

Results

For each animal, control absorption of 1 mM galactose was first determined along several successive 1 min periods, then in the presence of $HgCl_2$. The latter was studied by washing the lumen with saline solution between an absorption period and the next one, or under uninterrupted perfusion but taking perfusate fractions every minute.

As fig. 1 shows, addition of 0.5 mM Hg did not significantly modify galactose absorption in the first period, but inhibitions up to 35-45% were seen in the following ones. Washing with saline solution after a period with Hg, did not improve the next absorption without the metal. With 1 or 5 mM Hg, galactose absorption was a little lower but some epithelial desquamation was noticed.

When the absorption in the presence of Hg was followed along the course of time (fig. 2), a progressive inhibition could be

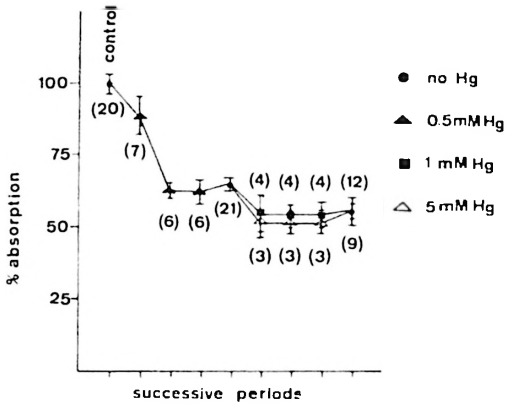

Fig. 1. *Effects of Hg^{2+} on the intestinal absorption of 1 mM D-galactose without previous exposure to Hg solution.*
Absorption periods of 1 min duration. Washing with saline solution between each period and the next one. Mean values in percent of control ± SE. Data number in parenthesis.

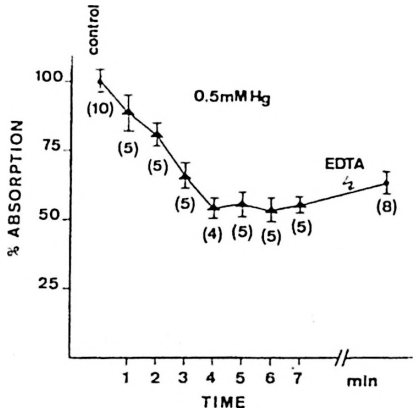

Fig. 2. *Time course of the inhibition of 1 mM D-galactose absorption by 0.5 mM Hg and partial reversion by EDTA.*
Absorption was measured along 7 minutes under continued perfusion. Mean values in percent of control ± SE. Data number in parenthesis.

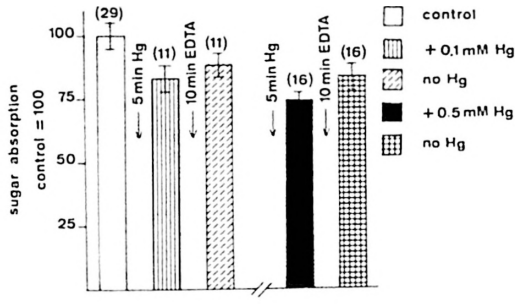

Fig. 3. *Effects of Hg on galactose absorption (1st period), after 5 min exposure of the mucosa to the metal, and partial reversion by EDTA.*
Mean values in percent of control ± SE. Data number in parenthesis.

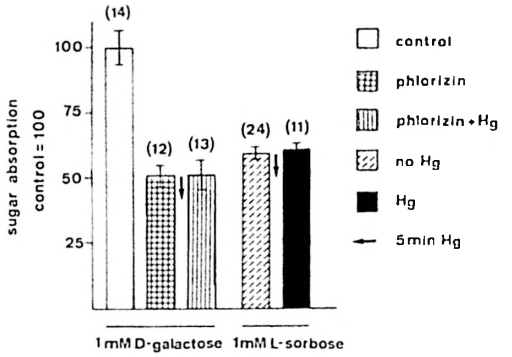

Fig. 4. *Ineffectiveness of 0.5 mM Hg on apparently diffusional absorption of sugars by rat jejunum.*
Mean values ± SE, referred in percent of control galactose absorption. Data number in parenthesis. Lack of Hg action on 1 mM D-galactose absorption in the presence of 0.5 mM phlorizin and on 1 mM L-sorbose absorption.

shown during about 4 minutes. Washing the lumen with a 10 mM EDTA containing saline solution led to a slightly lower inhibition.

In other experiments, after evaluation of control absorption, the lumen was exposed for 5 min to a sugar free, Hg containing, saline solution, followed by the measurement of galactose absorption also in the presence of Hg. With this procedure (fig. 3) the inhibitory action of Hg was stronger, and could already be observed in the first absorption period, where it reached 17% with 0.1 mM Hg and 25% with 0.5 mM Hg. On the third period the inhibition attained 40-45% with 0.5 mM Hg (not represented in the figure). EDTA treatment again reduced that inhibition partially.

Some animals were used to see the effects of Hg on the apparently diffusional absorption of galactose which remains when sugar transport is canceled by 0.5 mM phlorizin. Fig. 4 shows that Hg did not affect this passive absorption of sugar. On the other hand, absorption of 1 mM L-sorbose, which crosses the intestinal wall by simple diffusion because it does not share the classic sugar-Na cotransport or the D-fructose carrier, was also unaffected by Hg (fig. 4).

Table I shows the effects of 0.5 mM Hg on the absorption of different concentrations of galactose and their partial

Table I. *Inhibition of intestinal absorption of D-galactose by Hg^{2+} and partial reversion by DTE.*
Perfusion of rat jejunum *in vivo* at 5.6 ml/min, absorption periods of 1 min duration. Mean values ± SE in nmoles · cm^{-1} · min^{-1}. Inhibitions in percent. Data number in parenthesis.

D-GALACTOSE (mM)	CONTROL	Exposure to 0.5 mMHg	0.5 mMHg	10 mM DTE	No Hg
0.5	11.7 ± 0.8 (12)	+	6.16 ± 0.6 (10) 47.3%	+	8.30 ± 0.7 (11) 29%
1	17.4 ± 0.9 (11)	+	9.36 ± 0.7 (11) 46.1%	+	13.33 ± 1.0 (12) 23.7%
5	83.5 ± 7.2 (12)	+	58,4 ± 8 (10) 30%	+	71.0 ± 7.1 (10) 14.9%

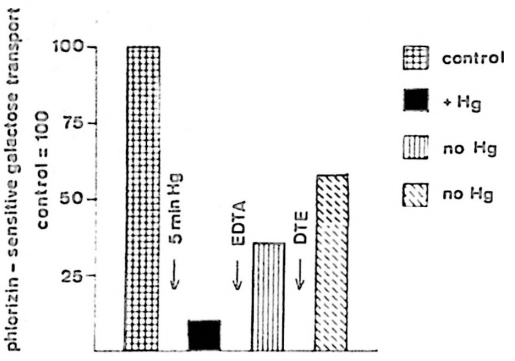

Fig. 5. *Effects of 0.5 mM Hg on the phlorizin-sensitive transport component of 1 mM D-galactose absorption by rat jejunum and their partial reversibility by 10 mM EDTA or DTE.*
Mean values referred in percent of control transport.

reversion by 10 mM DTE treatment. At all sugar concentrations absorption inhibition was observed. After washing the lumen with DTE containing saline solution for 10 min, the inhibition was diminished by about 38-50%, indicating that DTE is a more effective restoring agent than EDTA.

Fig. 5 shows the effects of Hg on the phlorizin-sensitive transport component of galactose absorption, and their partial reversibility by EDTA or DTE. Values for this transport component have been calculated from the aforementioned experiments, computing the differences between the absorption in the absence and in the presence of 0.5 mM phlorizin. It can be observed that 0.5 mM Hg was a very strong (about 90%) inhibitor of galactose transport, and that this transport inhibition came down to 65% or to 42% after EDTA and DTE treatment respectively.

Discussion

Present results corroborate previuos reports on the inhibitory effects of Hg on intestinal absorption of sugars (9, 10, 14-16, 24, 25). Those *in vivo* for rat intestine (16) had been obtained with very high sugar concentrations (300 mM) and without considering the Na role in sugar transport.

The inhibitory action of Hg (19), as that of Cu (20), is not instantaneously exerted as it was the case with Cd, but requires some delay to reach maximal values. On addition of 0.5 mM Hg, the absorption inhibition was not significant on the first minute, but it was on the following ones, progressively increasing for 4-5 minutes. However, after 5 min exposure of the mucosa to Hg the inhibition appeared as early as the 1st minute, even with 0.1 mM Hg, while maximal inhibition values were found in 2 or 3 minutes time.

Absorption inhibition by Hg was not reduced by washing the lumen with saline solution, but a partial reversion was obtained by EDTA and a more effective one by dithioerythritol.

The impairment of galactose absorption by Hg can be ascribed to an exclusive inhibition of the phlorizin-sensitive transport component since, after the entire suppression of this component by 0.5 mM phlorizin, the remaining apparently diffusional type passive component (2, 7, 18) was not affected by the metal. This assertion is corroborated by the fact that absorption of a non-transported sugar such as L-sorbose was not inhibited by 0.5 mM Hg. Under the assumption of an exclusive inhibition of the transport component, 0.5 mM Hg could be estimated to diminish galactose transport by 90%.

The relatively short time in which Hg exerts its inhibitory action makes possible to disregard any general toxic effect of the metal on the organism, since the access of Hg from the intestinal lumen to the circulatory system is very slow (8, 22). Moreover, with much longer experimental times, Hg action had been proved not to exist on an intestinal segment different from that directly exposed to the

metal (16). Therefore, the inhibition of sugar transport must be due to the action of Hg on the epithelium that is exposed to it, as a consequence of its fixation to proteins (1, 3, 5, 9, 10, 12, 21, 24).

Some minutes delay, which is required for Hg to display maximal inhibitory effects, suggests that at least part of the involved binding of the metal takes place with chemical groups of proteins not immediately accessible to the metal.

The weak recovery of sugar transport after EDTA treatment may be explained by the high stability of the Hg-protein complexes or by the very scarce penetration of EDTA into the enterocyte membranes. The inhibitory effects of Hg are better reverted by dithioerythritol, a well known SH-group protector, which enters cell membranes easily (9, 10), a result that very strongly suggests the involvement of thiol groups in the Hg binding. As about 50% of the inhibitory action still remains after DTE treatment, a part of the mercaptide bonds rests unsolved or bonds other than these cannot be excluded.

In any case, the present results obtained from *in vivo* experiments agree with those reported from *in vitro* experiments with brush border vesicle preparations (9, 10) and lead to similar interpretations. The inhibition of sugar transport by Hg seems to be explained by the binding of the metal to membrane proteins which pertain to the Na-sugar cotransport phlorizin-sensitive system or are in some way related to the function of this system.

Resumen

La absorción de galactosa por el yeyuno de rata *in vivo* se inhibe por Hg^{2+} 0,1-0,5 mM. Para alcanzar la inhibición máxima se requiere transcurran algunos minutos. Los efectos persisten después de lavado con solución salina, pero son reversibilizados en parte por EDTA y en mayor proporción por ditioeritritol. La inhibición de la absorción puede atribuirse a perturbación del componente de cotransporte de azúcar y Na sensible a la florricina. El componente pasivo aparentemente difusional que permanece en presencia de florricina 0,5 mM y la absorción de L-sorbosa no se afectan por el Hg. La acción del Hg se explica como resultado de su unión a grupos tiol y quizás otros de proteínas a diferente profundidad en la membrana, que estén directa o indirectamente relacionados con el sistema de transporte de azúcar.

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